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Digital Transformation for Sustainable Future - Agriculture 4.0: A review

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ABSTRACT
In the last few years, while the COVID-19 pandemic affects food supply chains around the world, the agriculture sector also has faced many global problems, such as global warming, environmental pollution, climate change, and weather disasters. It has known that technological opportunities are available for human beings to get out of these predicaments, solving the interconnections between food-water-energy-climate nexus, and achieving agricultural transformation from traditional to digital.

The aim of this review is to gain holistic solutions in a systematic view, based on water-energy-food resources to agricultural digital transformation that will play role in sustainable development. The transition from primitive to digital is given with road maps covering agricultural and industrial revolutions at four stages on timeline. Digital agriculture combined under precision agriculture and Agriculture 4.0 are handled based on domains covering monitoring, control, prediction, and logistics. Digital technologies are explained with application examples such as the Internet of Things (IoT), cloud computing, big data, artificial intelligence, decision support systems, etc. Wearable sensor technologies, real-time monitoring systems tracking whole conditions of animals in livestock, the IoT-based irrigation and fertilization systems that help enhance the efficiency of irrigation processes and minimize water and fertilizer losses in agricultural fields and greenhouses, blockchain-based electronic agriculture, and solutions based on drones and robotics that reduce herbicide and pesticide use are handled systematically. Moreover, renewable energy technologies to be provided synergy between technologies such as agrivoltaics and aquavoltaics combining food and energy production in rural are explained, besides solar-powered pivot and drip irrigation systems and environmental monitoring systems. As a result, for a sustainable future, technological innovations that increase crop productivity and improve crop quality, protect the environment, provide efficient resource use and decrease input costs can help us facing in agriculture of today overwhelm many the economic, social, and environmental challenges.

Keywords: Smart farming, Digital transformation, Sustainable development, Blockchain, Artificial intelligence in agriculture, Information technologies

1. Introduction

Agriculture producing food, feed, fibers, fuel, and raw materials by cultivating plants and breeding livestock is one of the oldest and most vital sources in the civilization of human beings and in the economic growth of each country that forms the backbone. The latest FAO report (FAO 2017a) emphasized that the world's population will be near 10 billion by 2050, and agriculture will be a more deeply key role each day for the continuation of life and socio-economic development. It has been predicted that increases in food, water and energy demands will be 60% (FAO 2012), 55% (OECD 2012) and 50% (EIA 2019) by 2050, respectively.

At global level, while consuming approximately 30% of the total energy in during agri-food production, this causes nearly one third of GHG emissions (FAO 2020; Platis et al. 2019). Global food systems are responsible for about 25% to 42% of all global greenhouse gas (GHG) emissions emitted during production, processing, transportation, packaging, consuming, and disposing of food according to a study carried out for the years 1990-2015 (Crippa et al. 2021).

While agriculture is the pillar of food security, it is the largest user of freshwater that affects energy security (Tian et al. 2018). Global projections indicate that the demand for clean water, energy, and food will increase significantly in the coming years (Hoff 2011).

Today, agriculture accounts for 70% of global freshwater use and 34% of land use, putting unsustainable pressure on our natural resources (Lee 2019). Also, water use is 80-90% in the agri-food sector including irrigation (FAO 2016; Bundschuh et al. 2107). But irrigation only amounts can reach as much as 95 percent in some developing countries (Mohtar & Daher 2016; FAO 2017b).

Water plays a key role in socioeconomic development that strongly connects food and energy (Daher et al. 2019). Industry consumes 22% of water resources, most of which is used for cooling thermal processes in electricity generation (EIA 2016). If action is not taken now, such expanding gap between resource supply and demand would be further occurs deep problems related to continuous population growth, climate change and extreme climatic events, socio-economic development.

Both agricultural activities and production of food consume at large amounts of water and energy (Roidt & Avellán 2019). Therefore, water, energy, and food (WEF) are three strategic resources for the sustainable development of national economies (Purwanto et al. 2019). It is becoming increasingly difficult to ensure sufficient WEF with the impact of climate change, population growth, changing diets, urbanization, and aging (Zhang et al. 2018; Alcon et al. 2019).

Agriculture is also a major source of water pollution from nutrients, pesticides, and other contaminants, which if unmanaged can lead to significant social, economic, and environmental costs (FAO 2017). In the recent decades, agriculture is beset by two major discrepancies: the first conflict is between resources supply and demand, while the second conflict is between economic development and environmental protection (Yue & Guo 2021).

In recent years, global climate change has caused many irregular and extreme weather events in many parts of the world (Li et al. 2021). As a result of global warming, intense drought, severe hurricanes and storms, and extreme floods have begun to be experienced intensely in different parts of the world (FAO 2021). In addition, the success of crop and animal production varies depending on the short and long-term complex environmental effects of global warming and climate change (Hatfield et al. 2011). In many places of the world, plants are negatively affecting by climate conditions that are very hot or very cold, and wet or dry (Hatfield et al. 2015; FAO 2019). Rising seed, fertilizer prices and wages, epidemic and vegetative diseases, trade wars, flash floods, heatwaves, and other weather changes negatively affect agriculture. To meet these challenges and meet the demands of the future, countries need to develop policies and programs in a sustainable way.

In a report published by UNECE (2021), it was emphasized that demographic, economic, social, and climatic changes, were all exerting increasing pressure on natural resources, including through a seemingly ever-growing global demand for energy, food, and water that threatens the ecosystems. As a solution, the key to shifting towards sustainable development was highlighted that lies in the strategic decisions to be taken regarding natural resources, which need to be better valued and more responsibly managed.

Among literature studies reviewed, no article has been found that analyzes in view of systematic and holistic related to digital transition and transformation for a sustainable future in agriculture. Most of the literature focuses on individual integration of Agriculture 4.0 technologies and their applications in different sectors. This review aims to close the study gap with a systematic approach, analyzing digital technologies in agriculture for a sustainable future based on “Energy-Water-Food” Nexus.

The rest of this paper is organized as follows. Section 2 gives the development roadmap of industrial revolutions and agricultural revolutions. Section 3 introduces sustainable food and agriculture, water-energy-food nexus, and digital transformation concepts. Section 4 discusses precision agriculture and Agriculture 4.0 topics under the smart farming heading, covering novel technologies, digital transition and challenges. Section 5 introduces renewable energy and environmental technologies, new combined solutions; and discussion and conclusions are presented in the 6th and 7th sections, respectively.

2. Agricultural and Industrial Revolutions

The expression Industry 4.0 was used to point out new tsunami of technological progress that was defined in 2015 by Schwab as the “fourth industrial revolution” (Schwab 2015). While the first industrial revolution in the 18th century was characterized by transition from an agricultural economy to an industrial economy, the second industrial revolution covered from water and steam power to electric power (Adebanjo et al. 2020).

The evolution of agriculture, from the primitive level to the advanced level of today, took place gradually over time. Figure 1 shows together schematic timelines of both industrial revolutions and agricultural revolutions. Technological developments in agriculture are divided into 4 long-term and named the transformation from Agriculture 1.0 to Agriculture 4.0 (Liu et al. 2021).

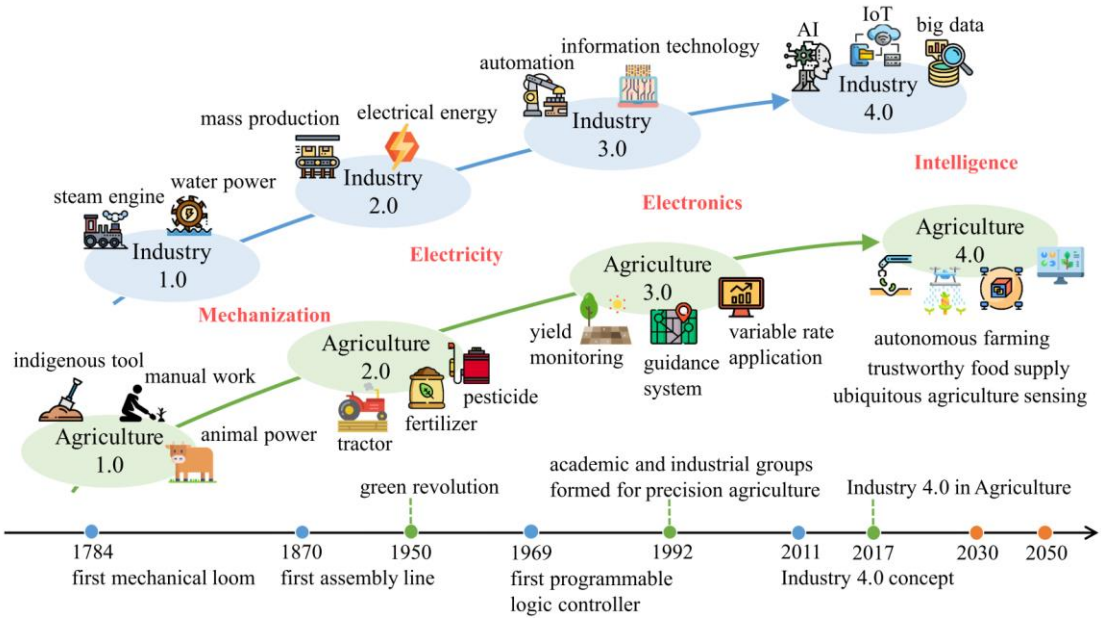

Figure 1- Time lines of industrial revolutions and agricultural revolutions (Liu et al. 2021)

Period that is conventional agriculture practices from antique ages when farmers mostly used domestic hand tools for cultivation mainly relying on manpower and animal forces to the end of the 19th century is accepted as Agriculture 1.0.

Agriculture 2.0 is referred as the period that is the increase food production and reduce hand jobs between 1784 and almost 1870, when agricultural machinery was used for soil tillage, sowing, weeding, irrigation, and harvesting (Liu et al. 2021).

After passing from the main steam power to oil and gas power, the industry 2.0 period of the 20th century started. Due to developments in the energy and transportation sectors, agricultural products were started shipping to long distances. Resulting, far communities connected together and new agriculture markets emerged that farmers can sell their produce (Liu et al. 2021). The first assembly line that significantly increased efficiency and productivity was set up for mass production (Zhai et al. 2020). The first agricultural mass production was applied to meat production in livestock. All these developments provided making for large-scale intensive animal farming.

"The Green Revolution" era, namely Agriculture 2.0, is the phase of farming that began in the late 1950s when new agronomic management practices, new synthetic pesticides, and fertilizers were used in agricultural fields and various machines were manually operated by farmers (Zambon et al. 2019). Thus, yields, productivity, and returns increased at all levels (CEMA 2017a). Although increased productivity and improved efficiency were achieved thanks to mechanization, all of them, overconsumption of fossil fuels, water, and chemicals caused the destruction of the environment. Indeed, still today, mankind continues the devastations with effects so great that it changes the climate in many parts of the world. Furthermore, the first programmable logic controller (PLC) was used in industry in 1969, depending on electronics and on information technology (Yülek 2018).

Agriculture 3.0, called also "Precision Farming" emerged thanks to advances carried out on computing and electronics. These developments provided saving on energy in machinery use of and saving on the water in irrigation, and reducing in use of chemicals in the field and, thanks to increasing operational performance of agricultural systems (Ahmad & Nabi 2021). According to the definition of the International Society of Precision Agriculture (PA), "Precision Agriculture is a management strategy that gathers, processes, and analyses temporal, spatial, and individual data and combines it with other information to guide site, plant, or animal-specific management decisions to improve resource efficiency, productivity, quality, profitability and sustainability of agricultural production." (ISPA 2021).

Although agriculture has been affected from every era of industrial revolutions, it should be noted the digital transformation in the agriculture sector started with PA. Today, the agricultural sector is experiencing a new revolution, called Agriculture 4.0 from with the affecting of digital technologies also in "Industry 4.0" entered our life in 2011.

3. Sustainability for Future

United Nations (UNs) called for a universal action plan related to Sustainable Development Goals (SDGs) adopted on 25 September 2015 to end poverty, protect the planet, and improve the lives of everyone, everywhere. The 17 goals are part of the 2030 Agenda for Sustainable Development. This agenda sets out a 15-year (2016-2030) plan to achieve the goals. These were

planned to help measure progress in each of the 193 countries that agreed to work towards achieving the goals. SDGs 17 targets are illustrated holistically in Figure 2 (United Nations 2015).

As highlighted in report prepared by The World in 2050 initiative, governments that signed the 2030 agenda of the Sustainable Development Goals (SDGs) and the Paris Agreement on Climate Change are calling for profound transformations that will require complementary actions from civil society, science and business (TWI2050 2018).

Figure 2- Sustainable Development Goals numbered according to icons accepted by United Nations (United Nations 2015)

Setting off to carry out the activities in The World in 2050 initiative, Sachs et al. (2019) presented six SDG transformations to achieve SDG targets. Among which, Transformation 4 has taken attention as one of the key parts covering sustainable food, land, water, and oceans. Transformation 6 covers the digital revolution for sustainable development comes forward as a foremost force that makes a difference, also it has been also pointed out as the Fourth Industrial Revolution.

In Trends in Biotechnology published in 2021, it emphasized that biotechnology to be coupled with digital technologies will play a key role in transforming current land-use systems in the future (Goh et al. 2021).

3.1. Sustainable food and agriculture

The FAO has established five basic principles that will provide to become increasingly productive and sustainable, building a production system that works in favour of the ecosystem, satisfying human needs to achieve 2030 SDG targets. FAO's five basic principles are: (1) Increasing productivity, employment, and value addition in food systems, (2) Protecting and enhancing natural resources, (3) Improving livelihoods and fostering inclusive economic growth, (4) Enhancing the resilience of people, communities, and ecosystems, and (5) Adapting governance to new challenges (FAO 2018).

Moreover, the 20 practical and interconnected actions integrated by FAO (2018) are prepared for decision-makers to provide transformations related to the 2030 Agenda SDGs.

The 2030 SDGs comprise the aims related to food (SDG 2), water (SDG 6), energy (SDG 7), climate change (SDG 13), and ecosystem (SDG 15). Policies and programs managers to be developed by managers will sign managing of resources in future in the way a more sustainable one. Nevertheless, there are many operational difficulties. Holistic systematic analyses are needed to obtain comprehensive solutions covering problems related to the water-energy-food-climate. Future farming will be built on four main pillars are follows (1) Improving productivity, (2) Rational use of resources, (3) Adaptation to climate change, and (4) Avoidance of food waste (FAO 2018).

3.2. Water-Energy-Food Nexus

Energy, water and food security have been identified as three of the key resources in achieving the United Nations' sustainable development goals (Babatunde et al. 2019). Therefore, the water-energy-food nexus has been proposed as a conceptual tool for achieving sustainable development. Many developing countries has faced many challenges to meet the demands increasing for healthy food, clean water and green energy due to the climate change. An effective solution can only be achieved through sustainable use of vital resources such as land, water, and energy (Rasul & Sharma 2016).

Water, energy, and food are resources that are unavoidably connected with each other. Impacts on only one resource can also cause changes in the other ones. The increasing global population, energy demands, freshwater resources, and food supply emphasize the significance of this topic. The Water-Food-Energy (WEF) Nexus concept was introduced by Hoff in the World Economic Forum held in the Bonn in 2011 to take measures pointing out the problems the world may face and to address the links between water and economic development (Hoff 2011). In recent years, the WEF nexus has become an independent technical term due to its growing popularity. The interdependent and complex interactions of these three basic resources are called as the water-energy-food nexus (Zhang and Vesselinov 2016; Bieber et al. 2018). Solutions of challenges concerning food, water, and energy have to be planned to meet current and future socio-economic demands at local, regional, national, and global scales for sustainable development (Bazilian et al. 2011; FAO 2014; Howells et al. 2013; IRENA 2015; Cevher 2019; Namany et al. 2021). Figure 3 shows connections water-energy-food nexus, interactions among WEF.

There are technological solutions based on nexus models for rural and urban fields (Lehmann 2018), sensor applications (Abegaz et al. 2018), renewable energy (IRENA 2015), and greenhouse monitoring (Asolkar & Bhadade 2015). Moreover, if knowing the interconnections between the three elements of the Nexus, it can be determined which resource should be shared in what proportion for sustainable production, how taking temporal and spatial strategical decisions. (Tian et al. 2018; Zhang et al. 2018; Ravar et al. 2020, Norouzi & Kalantari 2020; Keyhanpour et al. 2021; Yue et al. 2021).

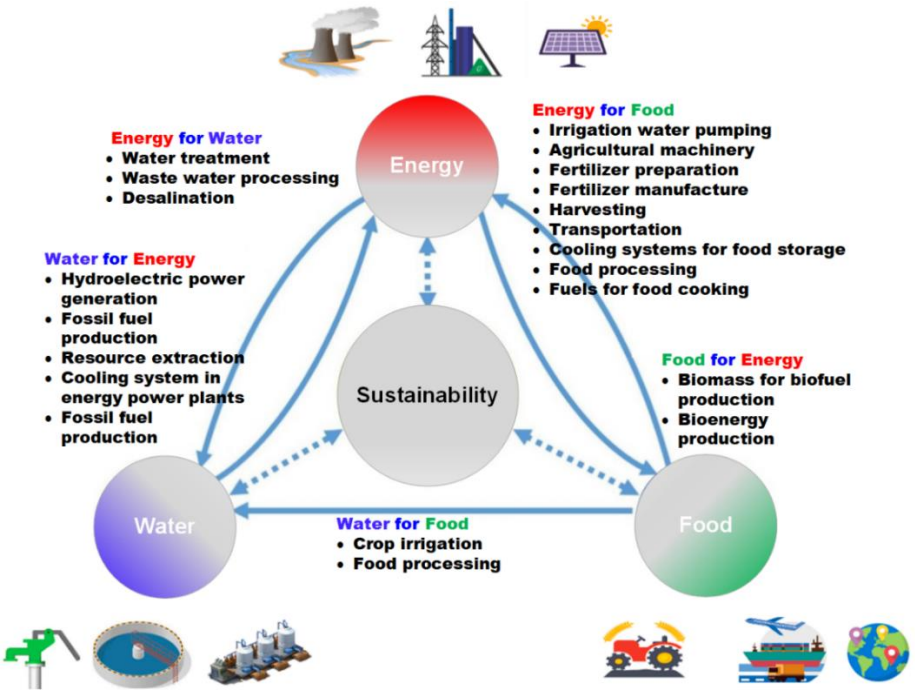

Figure 3- Water-energy-food nexus, interactions among WEF (modified from Bieber 2018).

3.3. Digital Transformation

According to FAO and World Bank reports, digital transformation can be used to solve challenges that are faced in agriculture and in rural areas (WB 2019; Trendov et al. 2019). Thanks to a well-planned transition towards smart farming (Klerkx & Rose 2020), this transformation can contribute to agri-food systems (Klerkx and Begemann 2020). Digital transformation is a concept that defines the process of finding solutions to social and sectoral needs with the integration of digital technologies, and accordingly the development and change of workflows and culture (ElMassaha & Mohieldin 2020). Digitalization is defined as the socio-technical process providing with using digital innovations. In the agricultural sector, numerous notions have emerged to define different forms of digitalization in agri-food production systems (Klerkx et al. 2019).

These are Precision Farming (McBratney et al. 2005; Aubert et al. 2012; Eastwood et al. 2017), Smart Farming (Wolfert et al. 2017), Agriculture 4.0 (Rose & Chilvers 2018; De Clercq et al. 2018; Zambon et al. 2019; Zhai et al. 2020; Raj et al. 2021; Rijswijk et al. 2021; Rose et al. 2021). Moreover, synonyms of Agriculture 4.0 are “Numerical Agriculture” (Agriculture Numérique) in France (Klerkx et al. 2019), “Smart Farming” in many countries of the European Union, “Digital Agriculture” in Australia and New Zealand (Keogh & Henry 2016; Shepherd et al. 2018; Fielke et al. 2020; Fleming et al. 2021). These concepts have defined as the "Digital Agricultural Revolution" by Food and Agriculture Organization (FAO) (Trendov et al. 2019)

According to CEMA (2018), Digital Farming is the transformation from Precision Farming to end to end connectable, knowledge-based farm production systems. CEMA explains that Digital Farming has a similar structure to Industry 4.0. However, there are many aspects of production parameters in agriculture that are different from industry. Agriculture is mostly

defined with environmental and biological factors, and therefore its physical specifications should be basically considered (CEMA 2017a; 2017b).

Lioutas et al. (2021) presented the beneficial and negative aspects of digitalization. They also indicated the necessity to develop new approaches based on science in terms of societal impacts in the evolution of digital agriculture.

Pylianidis et al. (2021) reported a review on digital twins in agriculture in the period 2017-2020. They defined the unique characteristics of agricultural digital twins and pointed out that digitalization will provide multiple gains in terms of productivity and sustainability to the agricultural sector.

Fielke et al. (2019) explained that more comprehensive effects due to agricultural innovation and digitalization and pointed out that the main themes covering socio-technical dimensions of digitalization in agricultural production practices are connectivity, transparency, and governance.

Four key technologies that will be an active role in the future of digital farming are (1) vision and sensors, (2) automation and robotics (3) digitization and big data, (4) biological actors. At the heart of all these key technologies is the achievement of high efficiency in connectivity (Berger 2019).

3.3.1. Connectivity

Agricultural digital transformation is one of the topics that have political priority on a global scale (WB 2017; WB 2019; Trendov et al. 2019). According to the European Commission Report (EC 2017), one of the targets of the EU is to connect farmers end to end to the digital economy to succeed in the sustainable evolving of food and farming.

The digital connectivity in agricultural knowledge networks and agro-food value exchange will increase using novel tools such as digital hardware and software (Fielke et al. 2019). As shown in Figure 4, all three of these technologies-sensors, robotic automation, and digital data work together and are enabled by adequate connectivity as well as advances in edge computing and the cloud. At the heart of all these key technologies is the achievement of high efficiency in connectivity (Berger 2019).

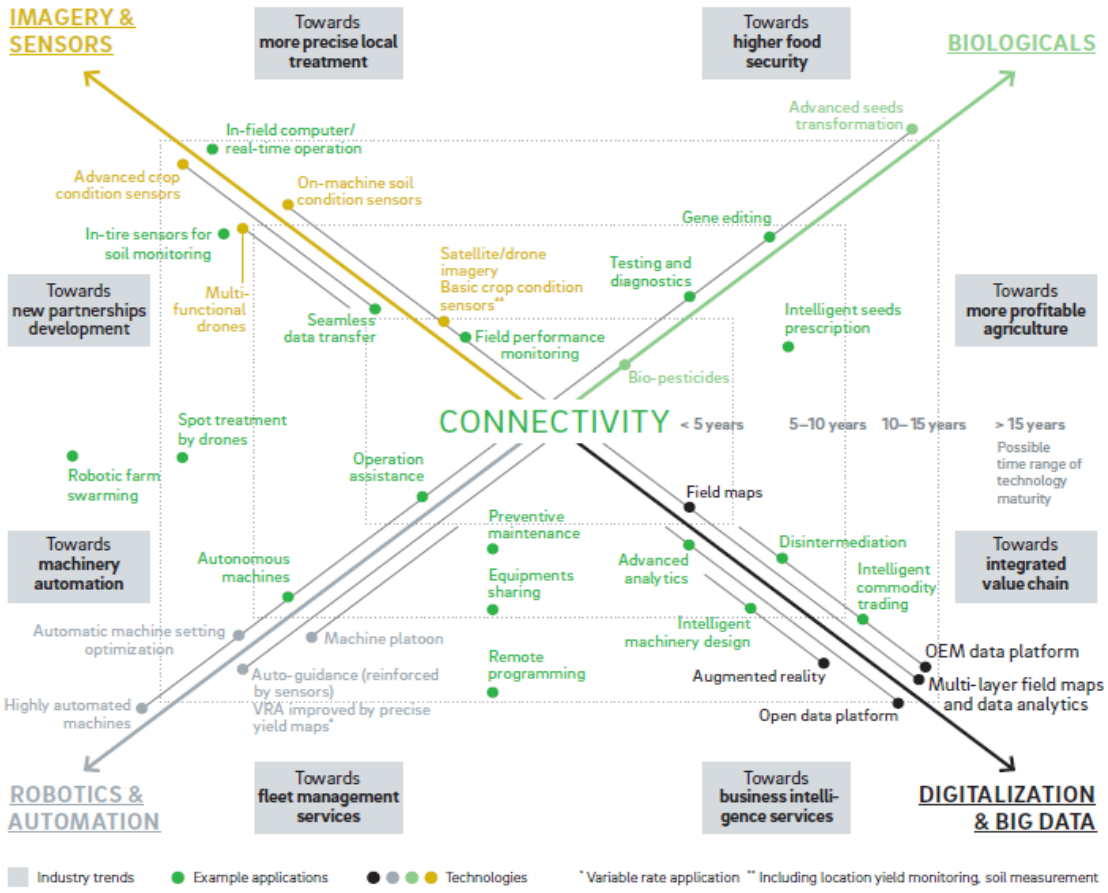

Figure 4- Four key technology levers to drive improvements to the farm economy and shape a new agricultural ecosystem - Agricultural industry technology map (Berger 2019)

3.3.2. Transparency

Transparency in agricultural practices is important for all stakeholders in the agricultural sector including consumers, and regulators, in terms of deep connectivity and informational interaction between them (Fielke et al. 2019). Agricultural knowledge and advice networks digitalized will be crucial in terms of the transformation of agricultural innovation (Wolfert et al. 2017). Digital information flows transfer with transparency to everywhere will provide trust between farmers themselves and interactions with technologies (Kelton et al. 2008; Sligo & Massey 2007). Transparency is need due to providing informational interaction with extensive connectivity between them (Wolfert et al. 2017).

3.3.3. Governance

It is known that challenges appear in balancing the priorities in terms of sustainable development in the agriculture sector as agri-food systems digitalize. In order to solve these, planning between public and private organizations will require (Carolan 2018; Regan 2019), considering socio-ethical factors to meet public and private goals in the agricultural sector (Fielke et al. 2019). Past experiences in agricultural decision support systems have seen that without the contributions of all stakeholders in the agricultural sector including social systems it will not be a sufficient solution (Fielke et al. 2018; Klerkx et al. 2017).

4. Smart Farming

Improvements in both hardware and software technology open a new chapter in agriculture, especially when it comes to sensors, biologicals, robotic & automation, and digital data. PA is a part of smart farming technologies including agricultural management, information systems, and automation and robotics (Balafoutis et al. 2020; Klerkx et al. 2019; Rose & Chilvers 2018).

4.1. Precision Agriculture

PA is the management of spatial and temporal variability through using advanced technologies to precisely match agricultural inputs to increase economic gains and lessen environmental impacts.

PA not only offers solutions to address many of the problems posed, exacerbated and exposed by this global crisis, it also has the potential to be an invaluable tool for rebuilding and strengthening the efficient and sustainable food systems of tomorrow. Prior to PA, farmers homogeneously managed their inputs such as seed, fertilizer, pesticide and irrigation despite existing within-field variability in soil properties, crop stress and crop yield. Beginning in the 1990s, after the emergence of PA, farmers were able to divide their heterogeneous areas into smaller units that were relatively homogeneous. Knowing all the historical data of an area can help growers adopt a PA approach and adjust their input to benefit their land, increase profitability and reduce their overall environmental impact. PA offers the use of satellite technologies that allow real-time management of crops, fields and animals (Talepbour et al. 2015).

In the light of the developments it has shown from the emergence of PA to the present day, it develops agricultural management by making use of various perspectives. These perspectives are listed below.

a) *Agronomic perspective*: considering the real needs of the crop in the regulation of cultural practices (for example; better fertilization management).
b) *Technical perspective*: establishing better time management at farm level (for example; planning of agricultural activities).
c) *Environmental perspective:* reducing agricultural effects (to more accurately estimate the soil's need for nitrogen to limit nitrogen flow).
d) *Economic perspective*: increasing productivity or reducing the amount of input, increasing the efficiency of the yield (for example; making nitrogen fertilization application at low cost).

Recently, various technologies related to PA have developed technologies like GPS systems, yield mapping, smart sensors, and automatic steering systems. As expected, these systems were developed for the farmer to increase yields, save nutrients and so as to make efficient sensing and decision support systems that can increase profitability on the farm and reduce negative environmental impact. PA supports reduced tillage and other cost and labor-saving novel technologies. Along with the latest developments in semi-autonomous systems and agricultural robots in this process, it seems that the process has accelerated among farmers. Figure 5 shows the connections between different technical systems and sensors, especially from geolocation and sensing systems to decision support, variable-rate operation, and route planning among emerging technologies in PA (Pedersen 2017).

Frontier technologies used in PA for long time have four main phases that span almost all agricultural applications. These four main stages and related technologies (Balafoutis et al. 2020);

1. *Guidance technologies*- These include all types of automatic guidance for tractors and self-propelled agricultural machinery such as driver assistance, controlled traffic in agriculture, developed based on hardware and software.
2. *Information management*- Measurement is essential for accurate farming and better control results. Measurement systems consist of sensors that can be mounted on ground-based stations and attached to aerial or satellite platforms. They collect spatial information including soil mapping, canopy mapping, and yield mapping, also soil moisture mapping. Knowledge management will enable farmers to perform PA more efficiently and comfortably. If the connection between the digital and the physical farm is not made right, there can always be a shortage of precision farming among farmers.
3. *Application technologies*- These involve technologies such as variable rate irrigation and variable rate application technologies for nutrients, crop protection agents, irrigation, seeding, and precision weed control, developed based on hardware and software.
4. *Data analysis technologies*- These are used for the analysis of data gathered through data collection. Related technologies are:

   - Management zone definition
   - Decision support systems (DSS)
   - Farm management information systems (FMS)

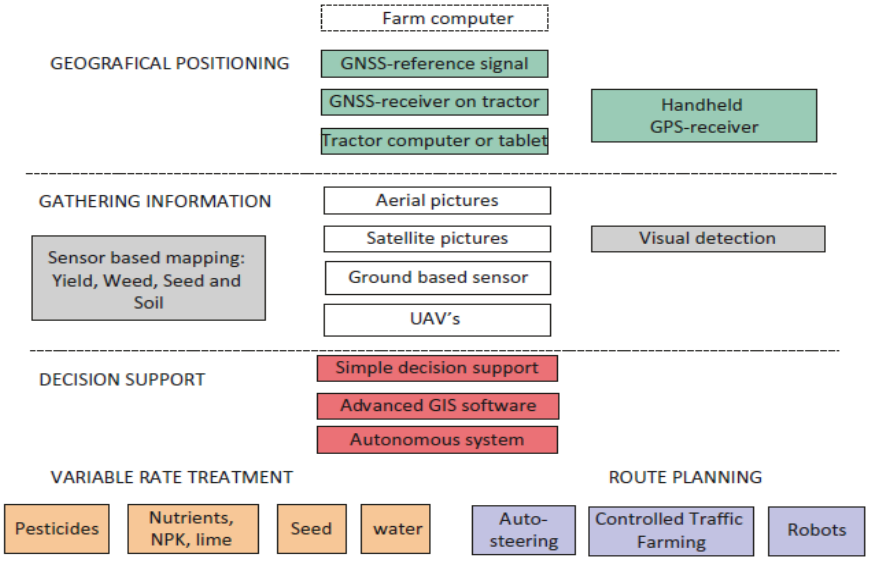

Figure 5- Major digital systems used in precision agriculture (Pedersen 2017)

International experiences and survey studies show that Aided Steering Systems (ASS) tend to be the first technologies adopted by farmers, due to their relative simplicity and the tangible benefits in terms of reduced fuel consumption and inputs as well as increased labour productivity (less driver fatigue, working during night, etc.) (Say et al. 2017). Recording technologies are increasingly available through remote sensing from satellites and drones even though their use by individual farmers is still limited. Field sensors are increasingly being deployed in higher value crops such as orchards and vegetables but also in field crops, to support irrigation management and for early warning of diseases. Reacting technologies such as VRAs (Variable Rate Applications) are being adopted more slowly, as they are most complex requiring proper recording technologies as well as sufficient intra-field heterogeneity to be profitable for farmers. Evidence in Turkey suggests a similar pattern: As such, farmers are less convinced about their profitability and require more sensitization, training and support (Say et al. 2017).

Satellite technology has also shown significant progress for imaging applications among PA technologies. For example, remote sensing (RS) is used successfully to detect variability in soil and crop conditions and to match inputs such as water, seed, and fertilizer via variable rate technology. Currently, satellite imaging technology is more suitable for large areas due to its cost effectiveness in comparison to drone-based imaging. However, with a number of high-precision satellite launches being planned, that precision capabilities will improve significantly to enable usage for medium-sized and small fields.

Drone-based imaging in PA has evolved rapidly over the last few years. Current drone imaging favours usage of smaller farms where satellite imagery is not cost effective. With the improvements in satellite technology described above, however, drone-based imaging may be limited to certain conditions such as heavy cloud cover or difficult terrain. With regards to precision spraying, however, drones may prove to be a significant advantage as payload capacity continues to increase.

Lastly, farm management systems are another interesting and developing area in the PA ecosystem. They assist farmers and land managers in achieving higher yields and less waste through smarter resource management and greater reduction of input

costs. Current farm management systems use predictive algorithms to issue warnings on imminent pest attacks and other key risks affecting farm productivity.

4.2. Agriculture 4.0

The agricultural sector sets sail towards a new era of agriculture called "Agriculture 4.0" thanks to emerging novel digital technologies. Agriculture 4.0 finds solution to agricultural and environmental problems thanks to digitalization, automation, and artificial intelligence adjusted to various agricultural technologies from crop and livestock production, to weeding, to pest control, and also to harvest.

Most studies related to new digital technologies are carried out on topics, such as Information and Communications Technologies (ICT), Internet of Things (IoT), Cloud Computing (CC) Big Data Analytics (BDA), robotics, Machine Learning (ML), Deep Learning (DL), Artificial Intelligence (AI), Unmanned Aerial Vehicles (UAVs), Unmanned Ground Vehicles (UGVs), and Blockchain. There are many literatures covering various applications used in Agriculture 4.0 in the past few years: ICT (Lezoche et al. 2020; Moysiadis et al. 2021); WSN (Ojha et al. 2015; Kochhar & Kumar 2019), IoT (Sinha & Dhanalakshmi 2022; Zhang et al. 2021; Sadowski & Spachos. 2020; Lin et al. 2020; Tzounis et al. 2017; Ayaz et al. 2019; Kim et al. 2018; García et al. 2020; Kour & Arora 2020; Marolia et al. 2021; Tao et al. 2021), Big Data (Newton et al. 2020; Lezoche et al. 2020; Moysiadis et al. 2021; Wolfert et al. 2017), UAV technology (Moysiadis et al. 2021; Kim et al. 2018; Boursianis et al. 2020), and Artificial Intelligence (Moysiadis et al. 2021; Jha et al. 2019), Machine Learning (Moysiadis et al. 2021; Liakos et al. 2018), Blockchain (Chen et al. 2020; Bera et al. 2022; GHD and AgThentic 2018), Automation & Robotics (Aceto et al. 2019; Carolan 2019). Furthermore, other smart farming topics including 3D Printing, Synthetic Biology, Gene Editing, Nano Materials have been researched by GHD & AgThentic (2018).

Nonetheless, Agriculture 4.0 paradigm occurs five core technologies covering sensors and robotics, Internet of Things, cloud computing, data analytics, decision support system. Figure 6 shows core technologies and connections among them. (1) Sensors and robotics fulfill sensing and actuating functions, according to the needs of the system. (2) IoT provides connectivity based on protocol and network for data communication. (3) Cloud computing is responsible for data storage and processing. (4) Data analytics involves big data and AI and ML-based algorithms for data analysis. (5) Decision support system helps to provide data visualization, guidance functions, and user interaction (Araújo et al. 2021).

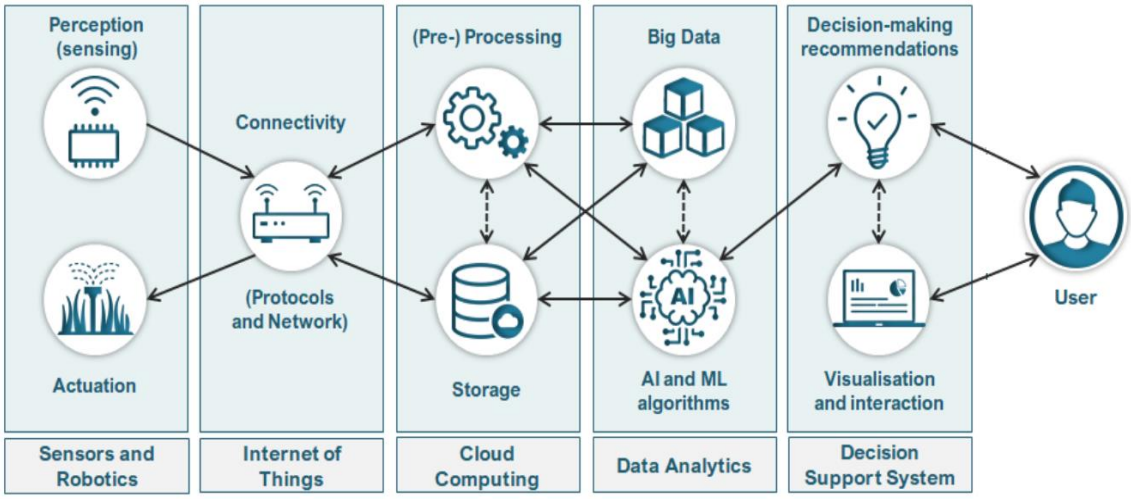

Figure 6- Agriculture 4.0 core technologies and connections (Araújo et al. 2021)

4.2.1. Novel digital technologies

Industry 4.0 has the potential to transform the production capabilities of whole industries, including the agricultural fields. Connectivity is the cornerstone of this transformation and IoT is a key enabling technology that is increasingly part of agricultural equipment. Technologies, such as IoT, Edge Computing, AI, robotics, 5G, blockchain, and supercomputing, all have the potential to make agriculture more efficient, sustainable, and competitive. Some definitions related to the advanced digital technologies given by European Commission are following (EC 2021a):

The Internet of Things (IoT) refers to the network of smart, interconnected devices and services that are capable of sensing or even listening to requests. IoT is an aggregation of endpoints that are uniquely identifiable and that communicate bi-directionally over a network using some form of automated connectivity. The IoT enables based on networked sensors to remotely connect, track and manage products, systems, and grids.

Cloud computing includes the use of tools and applications such as data storage, servers, databases, and software based on a network of remote servers through the Internet. Cloud computing services enable users to store files and applications in a virtual place or the cloud and access all the data via the Internet. Public cloud services are available on public networks and open to a widely unlimited cosmos of potential users, designed for a market, not a single enterprise.

Big Data is a term describing the continuous increase in data, and the technologies needed to collect, store, manage and analyze it. It is a complex and multidimensional phenomenon, impacting people, processes, and technology. Big Data is usually characterized by "four Vs ": Volume (size of the data sets), Velocity (high speed of data processing), Variety (different types and sources of data used), and Veracity (high quality of analyzed data).

Artificial Intelligence (AI) is a term used to define machines achieving human-like cognitive functions such as learning, understanding, reasoning, or interacting. It comprises different forms of cognition and meaning understanding (e.g. Speech recognition, Natural Language processing) and human interaction (e.g. signal sensing, smart control, simulators). In terms of its technology base AI is a very heterogeneous field. While some aspects like sensors, chips, robots as well as certain applications like autonomous driving, logistics, or medical instruments refer to hardware components, a relevant part of AI is rooted in algorithms and software.

Agricultural robots can be used for crop monitoring and plant phenotyping, yield estimation, soil sampling, smart irrigation, smart spraying, dairy milking, sorting tasks, disease detection, weed and pest control, planting, harvesting, environmental monitoring and pruning. UAVs and UGVs are robots that are used both in the air and on land for agricultural applications.

Blockchain is a shared, digital, immutable ledger that facilitates the process of recording transactions and tracking assets in a business network. Shared ledgers technology allows new transactions to be added to an existing chain of transactions using a secure, digital or cryptographic signature. Assets can be tangible (house, car, cash, land) or intangible (intellectual property, patents, copyrights, brand). Blockchain protocols aggregate, validate, and relay transactions within the blockchain network. Almost anything of value can be tracked and transacted on a blockchain network, reducing risk and reducing all costs involved in the business. Blockchain is ideal for presenting information as it provides instantaneous, shared, and completely transparent information stored in a non-modifiable ledger that can only be accessed by authorized network members. A blockchain network can track orders, payments, accounts, production, and much more. As members share a single view of the truth, all the details of a transaction can be seen from start to finish; thus, creating new efficiencies and opportunities as well as greater confidence.

Industrial Biotechnology is the application domain of biotechnology for the industry in the production and processing of chemicals, materials, and fuels. This technology involves the practices related to using microorganisms or enzymes to generate industrially useful products in a more efficient way (e.g. less energy use, or fewer by-products) without conventional petrochemical processes.

Nanotechnology is an umbrella term that covers from the design to structural application and production, to devices, and systems by controlling shape and size at a nanometer scale. Nanotechnology has revolutionary the potential for the development of smart nano and micro-devices and systems in fields such as agriculture, healthcare, energy, environment, and manufacturing. Nanotechnology use in agriculture allows smart solutions for nutrients, pesticides, and genetic materials for improved soil fertility and soil protection, thanks to better stress tolerance. Nano-based sensors can be used for monitoring whole factors that affect productivity in smart farming. Moreover, nanotechnology can be used in post-harvest food processing and packaging to reduce food contamination and waste.

Photonics is a multidisciplinary field related to light including energy generation, detection, and process management, devices such as electronic components, and photodiodes, lasers, and LEDs. It allows the technological basis for the economic conversion of sunlight to electricity for solar-powered systems.

4.2.2. Agriculture 4.0 applications

Agriculture 4.0 can be applied at four main groups as showed in Figure 7: (a) monitoring; (b) control, (c) prediction, and (d) logistics (Araújo et al. 2021).

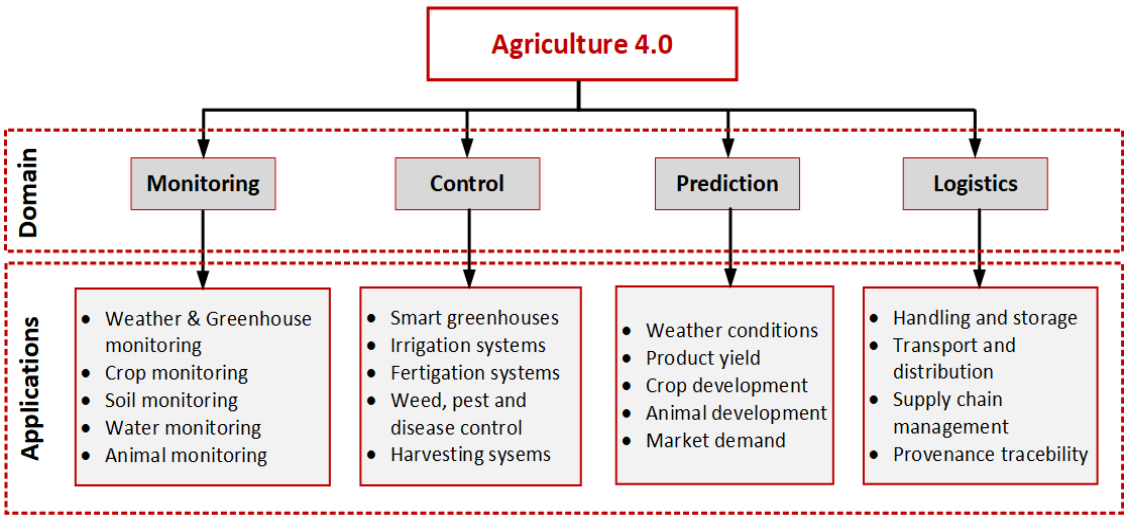

Figure 7- Domains and applications in Agriculture 4.0 (Araújo et al. 2021)

Monitoring- Automatic monitoring is the first step to passing to Agriculture 4.0. Smart monitoring systems have potential changing the game rule thanks to real-time data collected from the field and advanced data analytics tools providing the success of agricultural management. Monitoring allows farmers to get smart and fast decisions and perform timely interventions, to increase productivity in agriculture, save time and costs and protect the environment (Araújo et al. 2021).

Monitoring include usually applications as follows:

- Weather monitoring (air temperature and humidity, rainfall, wind direction, wind velocity, atmospheric pressure, solar radiation, etc.).
- GHG monitoring (temperature, GHG emissions- CO_2, CH_4, N_2O, SO_2).
- Crop monitoring (NDVI) and soil monitoring (temperature, moisture, electrical conductivity, pH value, and nutrient content) for agricultural management.
- Water monitoring (temperature, conductivity, pH, salinity, turbidity, specific chemical compounds, dissolved oxygen content) for irrigation systems, aquaculture or aquaponics (aquaculture + hydroponics) for management.

Control- While information is managed in one way in the monitoring applications, the control applications use a bidirectional information line. Commonly, an IoT-control system works together automatic monitoring system, which employs IoT sensors to collect necessary data and to transfer them for storage and then processing. Control applications are the following (Araújo et al. 2021):

- Irrigation Systems (sensors, actuators such as water pumps, and solenoid valves) for irrigation management.
- Fertilization and Fertigation for optimum fertilization.
- Weed, Pest and Disease Control for many problems like a waste of chemicals, increased costs, and pest resistance to chemicals, environmental pollution, and contamination of agricultural products.

Prediction- Predicting function in Agriculture 4.0 is used in decision-making support for the optimization in the management process. Monitoring and documentation are vital processes since it is used real-time and historical data to improve exact analytical methods in predicting actual events. Prediction applications are the following (Araújo et al. 2021):

- Forecasting Weather Conditions
- Crop Development and Yield Estimation
- Forecasting Market Demand

Logistics- It is known that, in recent years, consumers have been more concerned about how the agri-food products bought are produced, handled, packaged, stored, and distributed. Agriculture 4.0 provides more efficient management and transparency by increasing efficiency in logistics, addressing food safety and security, traceability and food authentication, decreasing intrinsic risks, and complying with certifications and regulations (Araújo et al. 2021).

4.2.3. Examples from digital agriculture

Several application examples related to domains such as monitoring, control, prediction, and logistics of smart farming are given below.

Smart Livestock Farming (SLF) is one of applications of PA that supports real-time monitoring of productions, health, and welfare of livestock, and to ensure optimal yield, to increase the management capacity of animals. A variety of sensors and actuators and decision-making tools, and IoT technologies are used in SLF (Cadero et al. 2018). Electronic identification systems such as ear tags, ruminal boluses, and sub-cutaneous radio-frequency identification; on-animal sensors like accelerometers, global positioning systems, and social activity loggers; and stationary management systems, milking parlor, and related technologies and flock management software are used in SLF (Vaintrub et al. 2021). Newton et al. (2020) examined a case study related to the dairy milk recording system under Australian conditions, using big data analytics based on individual cow and herd performance information, such as milk production, lactation, and breeding records. They studied farm decision support using big data and explored various factors and processes along with farmer engagement. Knight (2020) reviewed the current status of wearable sensor technologies in livestock farming that could revolutionize dairy farming; explained the important difficulties, and emphasized the advantages need for animal wellbeing, profitability. Sensors are split into three categories: (1) "*At Cow*" sensors, (2) "*Near Cow*" sensors, and (3) "*From Cow*" sensors. Wearable sensors can be placed by swallowing or inserting into cows. Figure 8 shows wearable sensor technologies developed for cows. While "*At Cow*" sensors at the red zone are used to measure acceleration, temperature, heart rate, and pH, "*Near Cow*" sensors at the blue zone are used to analyze video and sound information, as well as climate analysis, feed analysis, and GPS, these are designed to be make interaction with the cloud. "*From Cow*" sensors at the green zone are employed to monitor products and wastes like milk, hair, saliva, sweat, nasal secretion, breath, and faeces taken from the cow. Zhang et al. (2021) pointed out that SLF has important potential thanks to intelligent technologies such as environmental control, disease early warning, precise feeding, and remote diagnosis. They discussed the framework and technical characteristics of wearable Internet of Things (W-IoT), the advantages, difficulties, and expectations of the W-IoT in farm animals (Figure 9).

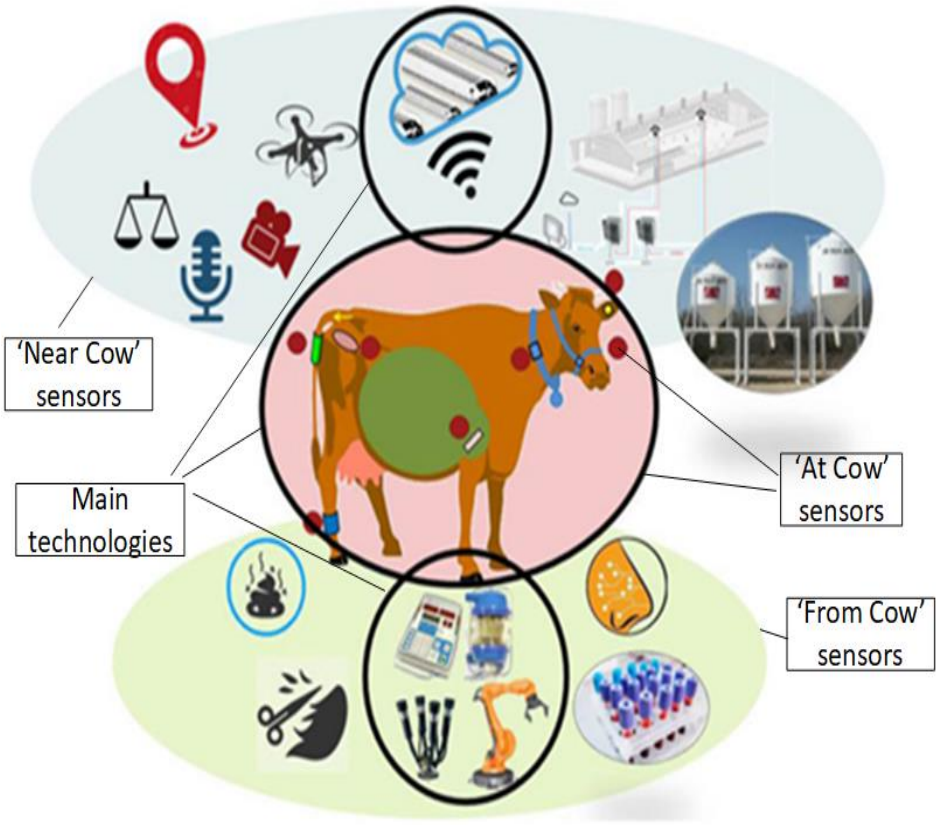

Figure 8- Overview of sensor technologies associated with dairy animals for cows (modified from Knight 2020)

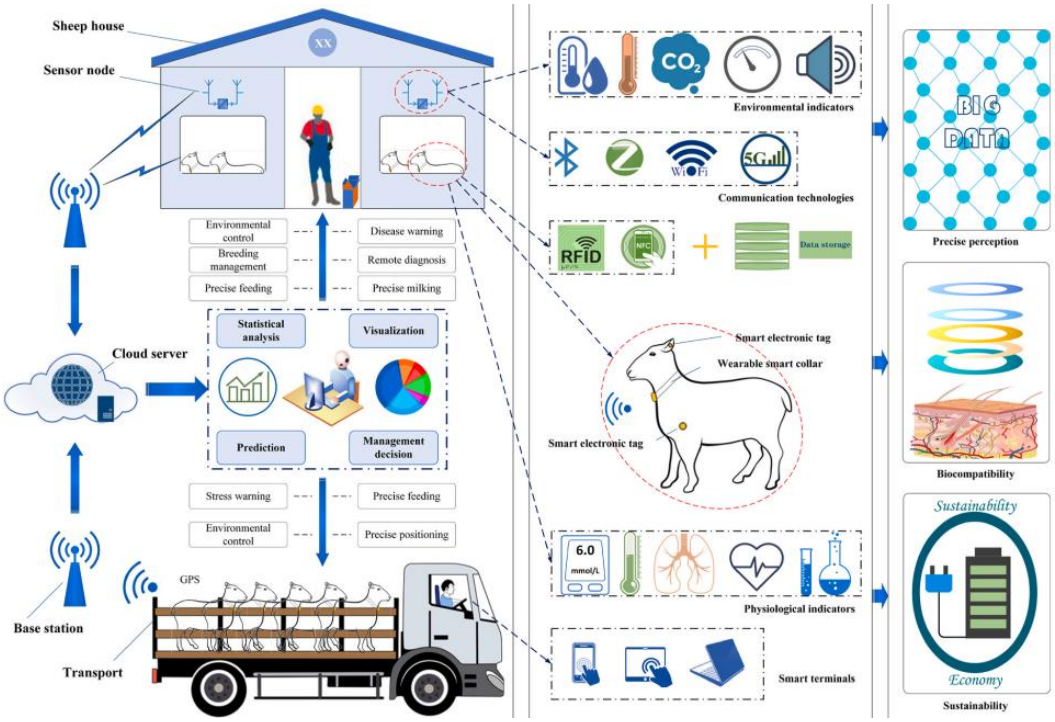

Figure 9- Wearable IoT system for SLF (Zhang et al. 2021)

Smart greenhouses enable farmers to cultivate crops with minimum human interference. Climatic conditions such as temperature, humidity, luminosity, and soil moisture are continuously monitored inside a greenhouse. Variations in these conditions will trigger automated actions. These actions will then evaluate the changes and implement corrective actions to maintain optimal conditions for plant growth.

Zamora-Izquierdo et al. (2019) proposed a flexible platform for greenhouses that used soilless culture with full recirculation. The system that obtained saving of more than 30% in water consumption and up to 80% in some nutrients has low-cost hardware and a three-tier open-source software platform at local, edge, and cloud planes.

Liao et al. (2017) developed an IoT-based system including three subsystems like environmental monitoring system, a wireless imaging platform, and a host analysis platform, shown in Figure 10. In an orchid greenhouse, environmental sensors that measure temperature, relative humidity, and illumination, and cameras capture the images of orchids were used. The IoT-based environmental monitoring system consists of 12 WSN, and a gateway based on ZigBee. While WSNs are responsible for measuring environmental parameters, the gateway is used to organize the sensor readings sent by the WSNs and transmit them to the host via Wi-Fi. The IoT-based wireless imaging platform is built upon a Raspberry Pi single-board computer with cameras. Sensor data and camera images are saved in the host platform.

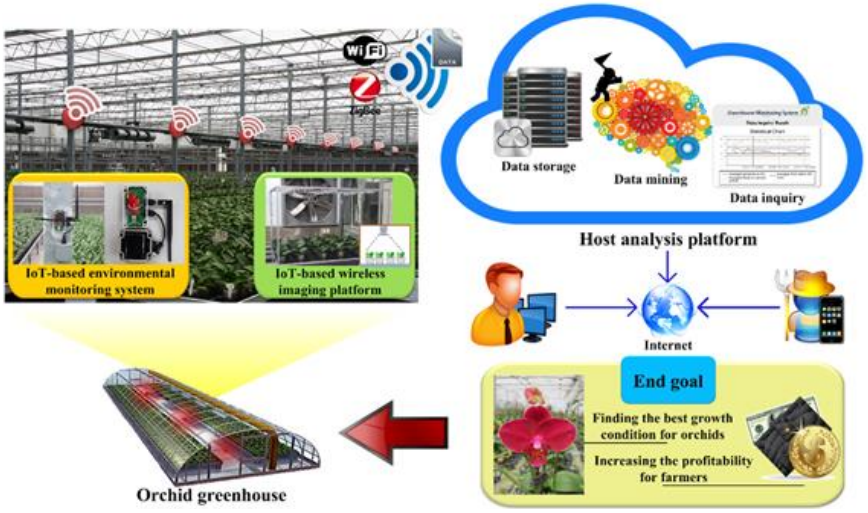

Figure 10- Overall conceptual diagram of IoT-based monitoring system for greenhouses (Liao et al. 2017)

Lin et al. (2020) developed a framework using a linear programming model maximizing economic profits and environmental benefits for the IoT-based irrigation and fertilization system in which both long-term and short-term planning are considered as illustrated in Figure 11.

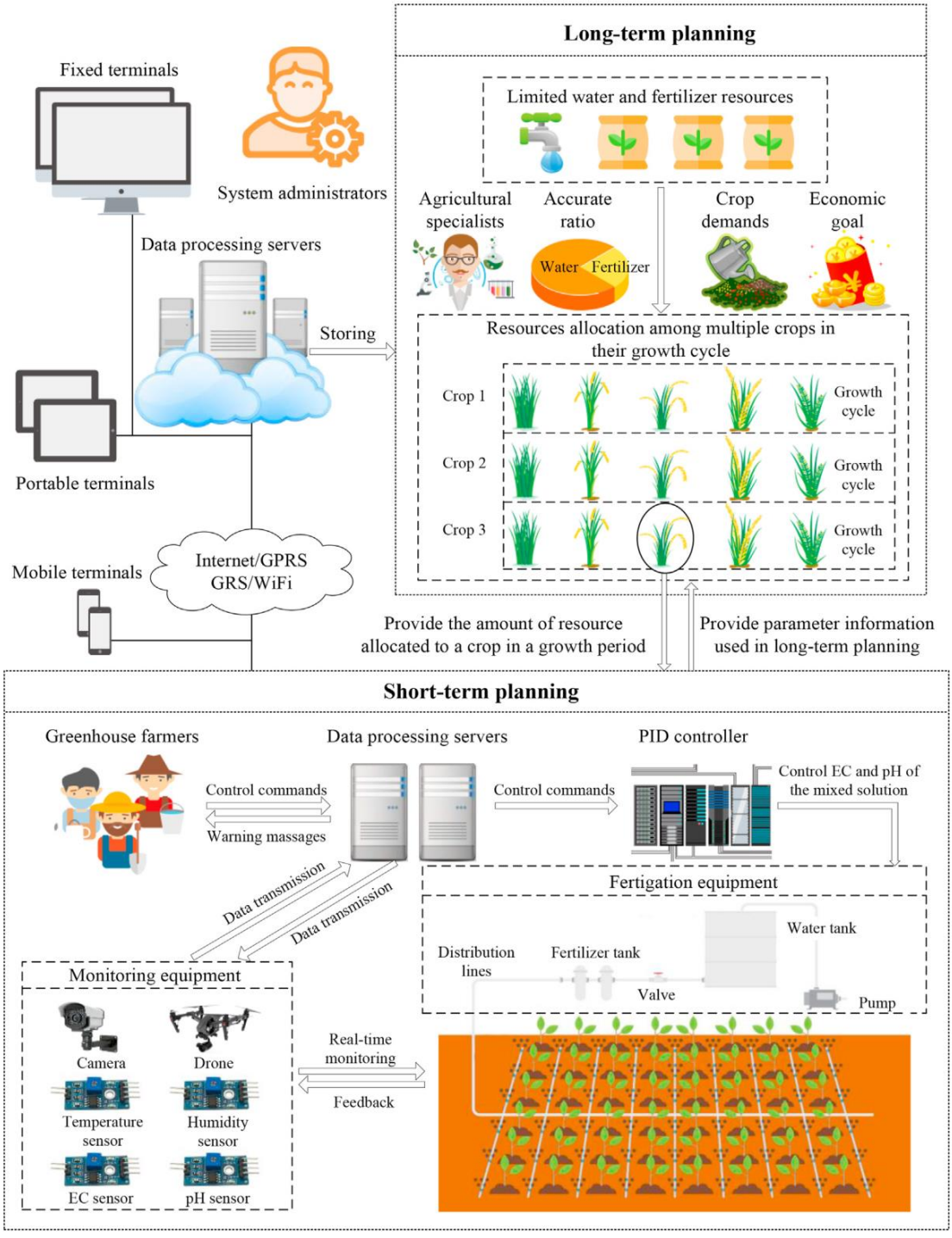

Figure 11- IoT-based smart fertigation management system (Lin et al. 2020)

Roy et al. (2021) designed an IoT system according to a dynamic irrigation scheduling called "AgriSens" for efficient water management of irrigated crop fields. The AgriSens enables real-time, automatic irrigation, as well as remote manual irrigation for different growth phases. IoT helps not only enhance the efficiency of irrigation processes but also minimize water losses. In order to calculate the precise needs for water, sensors that measure various parameters, such as relative humidity, soil moisture, temperature, and light intensity, need. IoT-based smart irrigation systems with such a mechanism can contribute to higher irrigation efficiency. Benyezza et al. (2021) proposed a block diagram of the example irrigation system, which is composed of three main parts, as shown in Figure 12: data processing, cloud, and communication part.

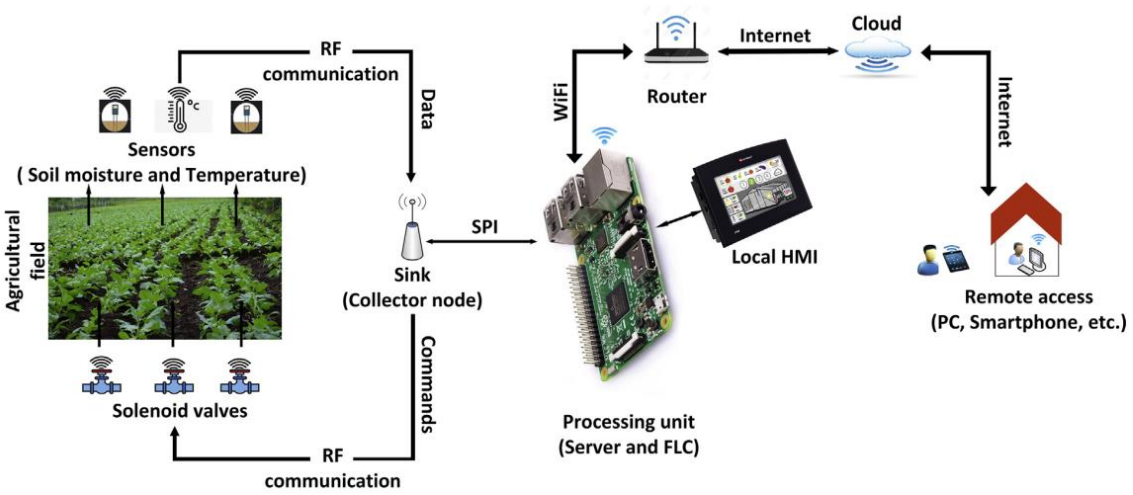

Figure 12- Block diagram of the example irrigation system (Benyezza et al. 2021)

Chen et al. (2020) proposed a framework called "blockchain-based electronic agriculture" on its development and challenges of Ecological Farm in the Beijing Liuminying. According to this framework, the whole circular agricultural model of the ecological farm into the blockchain network automatically handles and uploads data through several types of smart devices, which expands the information set that can be used for sharing. This innovation provides a powerful agriculture model, and presents a reliable path for reaching "digital agricultural". As shown in Figure 13, Blockchain-based applications can be used along with IoT smart devices and drones to monitor agricultural environments (Bera et al. 2022).

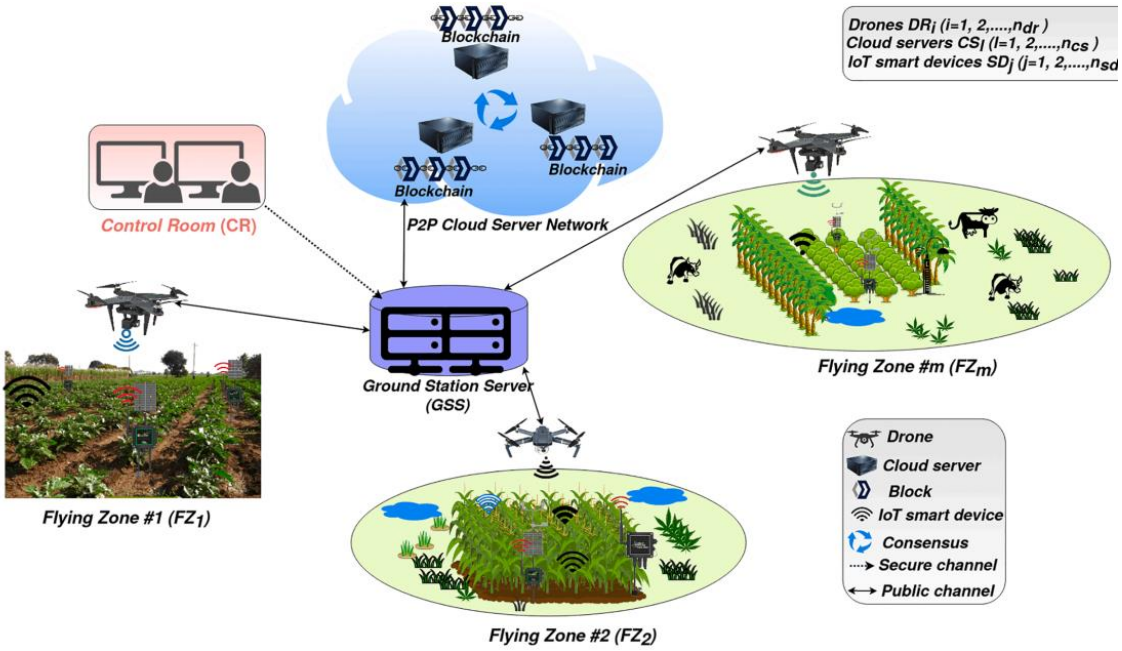

Figure 13- Blockchain-IoT enabled agriculture using drones (Bera et al. 2022)

The European Commission has set up large-scale projects to initiate the digitization of agriculture in Europe with approximately €1 billion of EU funding under the Horizon 2020 program (EC 2021b). Several examples of these projects are given below:

ATLAS project is agricultural interoperability & analysis system, also being an open digital service platform for agricultural applications. The goal of ATLAS is the development of an open interoperability network for agricultural applications and to build up a sustainable ecosystem for innovative data-driven agriculture. The platform enables the flexible combination of agricultural machinery, sensor systems, and data analysis tools to overcome the lack of interoperability. This allows farmers to increase their productivity in a sustainable way. The ATLAS platform is built around a data exchange network using a uniform application program interface (ATLAS 2019).

IoF2020 investigates the potential of the IoT technologies in the European food and farming industry both precision farming and smart farming (IoF2020 2017). IoT-driven smart farming enables farmers to optimize their operating tasks to boost crop yields and to reduce the costs of inputs such as water, fertilizer, insecticides, and herbicides.

DEMETER project is a large-scale deployment of farmer-driven, interoperable smart farming-IoT based platforms, delivered through a series of 20 pilots across 18 countries (15 EU countries). Involving 60 partners, DEMETER adopts a multi-actor approach across the value chain (demand and supply), with 25 deployment sites, 6000 farmers and over 38000 devices and sensors being deployed (DEMETER 2019).

Some of EU's robotics projects are Vinbot, MARS, GRAPE, VINEROBOT, ROMI and Flourish projects (Moysiadis et al. 2021). Vinbot aimed operations crop monitoring using devices like laser sensors, cameras and the navigation system that is based on a hybrid reactive/GPS (VINBOT 2017). MARS subproject was designed for the development of a mobile agricultural robot, which had the ability to cooperate and work in swarms (Figure 14a). GRAPE subproject targeted to develop a UGV able to perform semi-autonomous vineyard monitoring and farming tasks (Figure 14b). VINEROBOT aimed to develop a novel UGV capable of monitoring grape growth and factors like grape yield, vegetative growth, water stress, and grape composition in covering vineyard management (Figure 14c). ROMI project is developed for a UGV performing tasks such as weeding, crop monitoring, and collecting specific information on any individual plant (ROMI 2020). Flourish project is developed for weed control with UGV robots shown in Figure in 14d using data taken from UAVs (Flourish 2018).

Figure 14- EU's robotics projects (a) MARS, (b) GRAPE, (c) VINEROBOT and (d) Flourish (Moysiadis et al. 2021)

4.2.4. Digital transition and challenges

Although the many gains that the success of Agriculture 4.0 could lead, there are still various challenges that need to be handled for providing a successful digital transformation. The main challenges to be considered in Agriculture 4.0 are split into five main levels. Those challenges are related to device, data, network, application, and the system according to levels. Challenges at device levels are usually related to a harsh field environment, power consumption, and cost issues. Data levels are mostly linked to quality, availability, privacy, integrity, and interpretability issues. Facing at network levels are mostly network size, latency and throughput, transmission range, propagation losses, and interoperability issues. Emerging of during applications are usually real-time use, context-awareness, cyber-security, modularity, and reliability problems. Problems that emerged in the system are scalability, flexibility, robustness, complexity, affordability, and continuous improvement issues (Araújo et al. 2021).

As noted earlier (besides the small scale of land and low level of mechanization), farmers face significant challenges in the transition to smart farming as they try to adopt sensors, biologicals, robotic automation, and digital data from third-party providers. Farmers are traditionally conservative and adopt new technology solely for its usefulness rather than its technological appeal. As a result, compelling applications must be significantly deep to deal with the farmer's specific situation, while also relying on integrated solutions that make data and insights easy and seamless.

There are two basic stages in smart farming that should be taken into account for the transition: (1) The pre-processing stage and (2) the post-processing stage. The pre-processing phase covers market trends geographical conditions and soil properties of the land, seeds choosing, and the land preparation for the smart farming system. The post-processing stage is employed machine vision techniques for disease and weed identification while using smart technologies in irrigation and harvesting. For traditional agricultural players, this transition is not easy. As the industry moves from products to outcome-based solutions and worksite optimization, many organizations face the conflict of traditional platform or product-based organizations versus the needs of the farmer and marketplace. As a result, smart farming is undergoing a rapid transformation from both a product and a service perspective. Technological disruptions, business model transformations, and organizational changes are all creating an exciting but challenging opportunity.

5. Renewable Energy and Environmental Technologies

Renewable energy technologies have the potential to provide synergy between water, energy, and food; and also, the optimum mix is available for three sectors. Solar-based pumping solutions offer a cost-effective alternative to the grid- or diesel-based irrigation pumps. Renewable energy and rainwater harvesting technologies can be combined with a nexus system. Renewable

energy technologies such as solar energy, wind energy, and bioenergy can be integrated into agriculture in villages and cities. If coupling an energy storage system with renewable energy technologies, the excess energy generated can be stored to use in the future. As an alternative solution to the Nexus approach, new applications where photovoltaic panels are combined can be used in field and horticulture, and aquaculture.

5.1. Agrivoltaics

Agrivoltaic systems are PV technologies installed at a certain height from the ground, making it together integrating agriculture and solar energy. Agrivoltaic systems can provide both preserve agricultural land and advantage crop production by increasing water use efficiency and decreasing water stress (Agostini et al. 2021; Allardyce et al. 2017). Sun'Agri agrivoltaic system driven by artificial intelligence in Piolenc provides the vines shaded by the pivoting solar panels continue to grow and show a reduced need for water thanks to the reduction in evapotranspiration (pv magazine 2020). However, these positive attributes need comprehensive environmental and economic analysis of agrivoltaic systems. Figure 15 shows various applications of agrivoltaic systems.

Figure 15- Agrivoltaic systems: (a, b) Agrovoltaics a solar innovation and triple usage of solar energy (Agrovoltaics 2021), (c) Sun'Agri's agrivoltaic experiment in Piolenc, France built on vineyards (pv magazine 2020), (d) Agrivoltaics on a greenhouse roof. Swissradies Kerzers, Switzerland (Allardyce et al. 2017)

5.2. Aquavoltaics

Aquavoltaics (AquaPV) is a concept emerged with combining electricity production and aquaculture. AquaPVs floating on the water body can lessen water losses preventing evaporation by up to 70–85% due to covering the water. The aquavoltaics technology enables electricity to be generated and aquaculture to be carried out in the same area, and thus significantly improves overall productivity per unit area compared to conventional land use. Several projects and studies are carried out to verify positive and negative aspects in terms of ecosystem and the technical and economic feasibility of dual-use in Aquavoltaics (Pringle et al. 2017, Fraunhofer 2021; Solaripedia 2021; PV TECH 2021). Figure 16 shows various applications related to aquavoltaic and floatvoltaic systems.

Figure 16- Aquavoltaic systems: (a) Floating PV plant on Dutch lake (PV TECH 2020), (b) Floating solar power system located in California's Napa Valley region (Solaripedia 2021), (c) India's largest floating solar plant (pv magazine 2021)

5.3. Solar-powered monitoring system

Industrial development including agriculture has caused pollution of natural resources such as water, air, soil, food. GHG emissions, heavy metals, pesticides, and other chemicals that pollute terrestrial and aquatic ecosystems pose, and also unbalanced water use in all sectors a threat to the health of animals and humans, so our future.

A nexus approach based on water-energy-food given in this study supports the transition to a green economy to solve whole those problems. The agricultural nexus planning combined with digital technologies can be used to solve challenges related to water, energy, and food including the environment. In this context, topics including overall agricultural management can be handled for the environment (water, soil, air – whole ecosystem), optimum water use, and efficient energy use.

5.3.1. Crop and soil monitoring

Crop cultivation and any form of life on earth are inevitably dependent on sunlight, and today, thanks to modern technologies emerging, Sun offers a bigger source with the possibility to harness the energy from it all around the year. Not only does the development of the crop with energy coming from the sun but supports the gathering of precious information about the soil, air, water, and weather conditions themselves.

Solar-powered plug-and-play wireless field sensors provide farmers with instant access to data on their soil properties and crop health. This hardware is a device IoT-based, low-power, long-range, and adaptable based on farm size. A farm monitoring system shown in Figure 17 is a network of smart sensors driven by powerful AI-based crop intelligence and farm management software to aid farmers across the globe with instant access to soil and crop health. It is designed to help farmers accomplish high crop productivity and high-profit margins (Solarvibes 2021).

Figure 17- Solar-powered Plug & Play Farm Monitoring System (Solarvibes 2021)

5.3.2. Environmental monitoring

Climate change is due to emissions of greenhouse gases (GHGs) that have been greatly affecting agricultural production. The side effects of climate change result in frequent experiences of droughts, floods, and extreme weather conditions. Data-driven IoT systems are have monitoring and control functions including reducing energy and fuel consumption, generating renewable energy on-site, and performing closed-loop measurements of carbon consumption and waste. It is seen that IoT-powered smart services and industry could account for up to 10 percent carbon reduction by increasing efficiency and eliminating reliance on disposable materials in both the public and private sectors.

Monitoring systems can assist farmers in monitoring and improving the quality to avoid degradation of soil. They allow for the monitoring of a number of physical, chemical, and biological properties such as texture, water-holding capacity, and absorption rate. Soil monitoring can help to minimize problems, such as erosion, densification, salinization, acidification, and pollution due to toxic elements (Yurtseven et al. 2018).

Pesticides and several other agrochemicals are used widely in agriculture. These chemicals that affect in way serious and permanent ecological balance are harmful to human and animal welfare, causing substantial damage to complete ecosystems. IoT-based smart devices, such as WSNs, drones, robots, enable farmers to substantially decrease the use of pesticides. Advanced IoT-based pests and insect control allow precise tracking, simulation, disease prediction, and therefore is more efficient than traditional pest control calendars or prescripts (Raj et al. 2021). The IoT-based systems with ML, DL, and image processing can be used in preventing fruit diseases, diagnosing and preventing infection in agricultural products. Studies have seen that chemical pollution can prevent using big data, AI, ML technologies. Wastewater from industry can be used in agricultural production; Water polluted with heavy metals threatens our food (paddy, greenhouse cultivation, fish production, etc.) and our health. In recent years, it is known that cancer and many diseases that adversely affect the immune system are caused by excessive agricultural spraying and industrial wastewater. Excessive use of pesticides in fields, vineyards, gardens, and greenhouses pollutes air, soil, and water resources. In other words, excessive spraying, irrigation, fertilization harm the entire ecosystem in an unsustainable way. All of this should be traced back to the source where the problem started; all data should be forwarded to the center and evaluated; monitoring and control mechanisms should be developed.

PA has not only the potential to reduce greenhouse gas emissions but also having a positive effect on-farm productivity and the economy. These technologies cover whole farming applications such as variable-rate spraying, irrigation planting, fertilization, and weeding (Balofoutis et al. 2017). Both driver assistance (Machine Guidance) and Variable Rate Technologies (VRT) contribute to climate change mitigation by reducing fuel and input use at given yields. Based on a literature review, the EU classifies GHG reduction potential of different Precision Agricultural Technologies (PATs) as it follows (Table 1):

Table 1- Greenhouse Gas (GHG) reduction potential of different PATs (Balofoutis et al. 2017; EC 2019)

PAT	*GHG reduction potential*
Nutrient application (VRT)	5
Irrigation (VRT)	3
Controlled Traffic Farming	2
Machine Guidance	2
Pesticide Application (VRT)	2
Planting/Seeding (VRT)	1
Weeding (Precision physical)	1

Variable-rate nutrient application has the highest GHG emission reduction potential amongst PA technologies, as nitrogen fertilizer is the main source of N_2O that is the most influential GHG emitted from farming activities. Its impact on global warming is 298 times higher than that of CO_2 (Soto et al. 2019). Variable-rate irrigation has a dual impact. (1) decreasing the energy need for water pumping from the water source, (2) reducing of the GHG emissions by applying the optimum irrigation scheduling. On the other hand, applications such as controlled traffic in agriculture, machine guidance, VRT-pesticide use have GHG reduction potential. Moreover, all-natural resources (water, air, soil) have threatened due to chemical contaminants, overwatering, over-spraying, over-fertilizing.

5.3.3. Water monitoring

Water Quality Index (WQI) that developed to measure water quality is determined through different water quality parameters, such as pH, turbidity, temperature, chloride, electrical conductivity (EC), dissolved oxygen (DO), biological oxygen demand (BOD), and chemical oxygen demand (COD), etc. (Ahmed et al. 2020). Smart water management requires the integration of systems and a complex of measures to monitor, control and regulate the usage and quality of water resources as well as maintain the associated equipment (sensors, actuators, pipes, pumps, etc.). Smart water systems based on the combination of the IoT, big data, and AI technologies can help stop water scarcity from happening and remove the damage the incautious usage of water resources has already caused.

In rural areas shown in Figure 18, solar-powered wireless air - water - soil monitoring stations can be used to monitor environmental variables at vineyards, orchards, fields, fish farms, greenhouses, basis on the energy - food - water nexus at real-time.

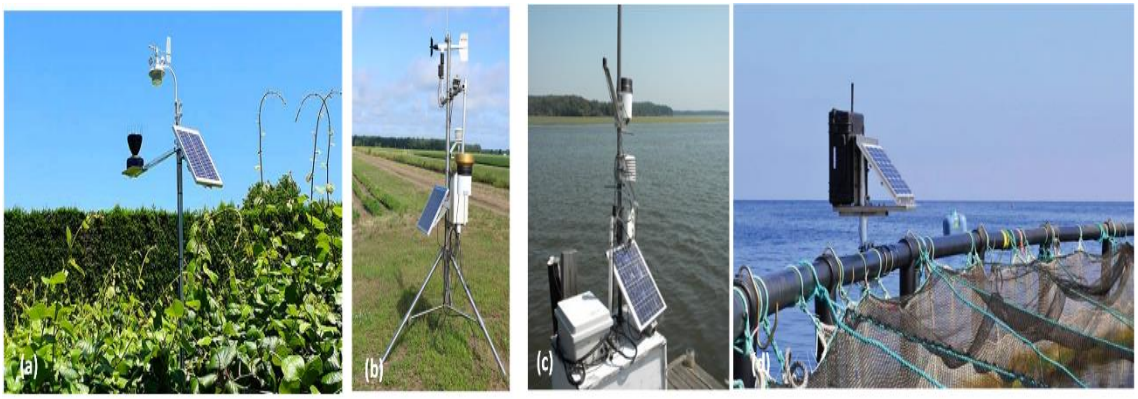

Figure 18- Solar-powered wireless-weather-soil-water stations (a) vineyard-orchard, (b) arable field, (c) water source, (d) aquaculture monitoring

5.4. Solar-powered pivot irrigation system

In a desert in Africa, a large pivot system with a radius of 479 meters has used to irrigate 50 hectares of desert. The fuel costs of operating this system are very high. Water is being supplied through an artificial canal from the Nile. At this point, Alfalfa is grown as feeding hay and harvested 11 times each year with the harvest season exceeding 300 days. Until 2013, a 135-kW diesel generator ran 22 hours per day to power the system. An alternative power source had to be found in this off-grid. Following feasibility analysis, a solar-powered system, using suitable pumps to replace the diesel-powered generator and AC pumps were installed. While all motors required to move the pivot now are powered with solar energy, the pivot speed is automatically controlled proportional to water flow. Figure 19 shows solar-powered center pivot irrigation system that is installed by Lorentz. In 11 hours, the pivot completes one 360 degrees turn, providing the required 3300 m^3 of water per day for partial irrigation (Lorentz 2014). This energy transformation not only no longer decreasing energy costs, but also greatly reducing carbon emissions.

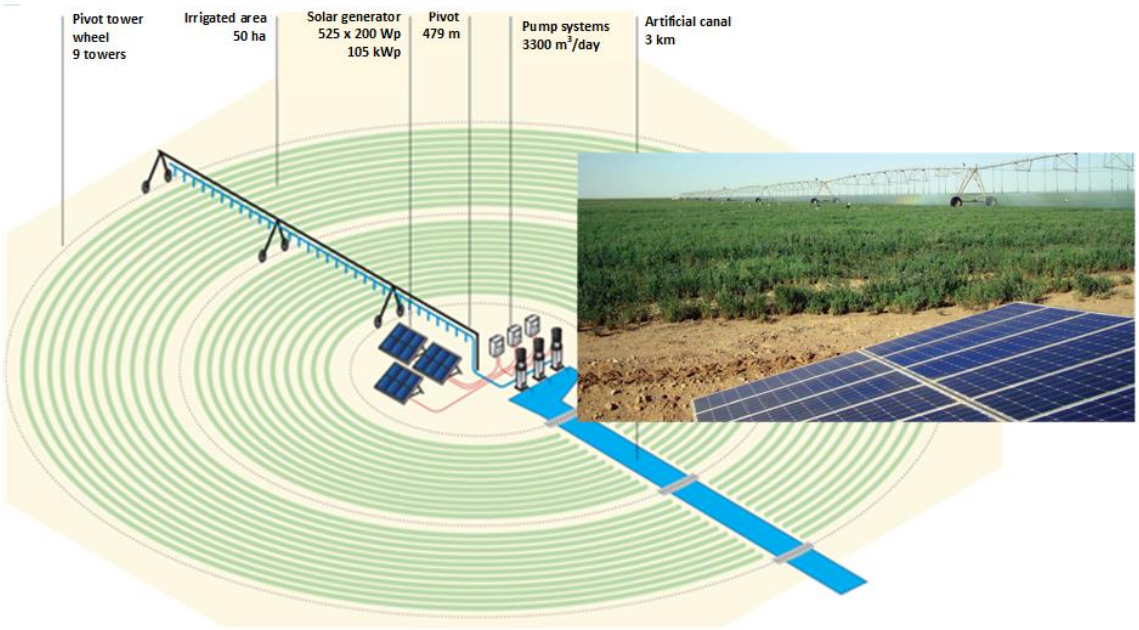

Figure 19- Solar water pumping for center pivot irrigation (Lorentz 2014)

5.5. Solar-powered drip irrigation system

In a valley in Chile, a drip system has used to irrigate 30 ha of vines planned like shown in the Figure 20. The 2.5 km pipeline with diameter of 140 mm, from the water source to two reservoirs, one of which is 40000 m^3 and the other is 15000 m^3, which is at 61 meters high was lay pipe. Submersible solar pump system was chosen being capable of delivering flows of up to 62 cubic meters per hour and to be operate at a maximum head of 90 meters. Power supply was provided with 16.56 kW_p of photovoltaic modules installed on 6 tracked arrays. System design performance is taken as volume pumped 134000 m^3 peak flow rate per year, 500 m^3 per day, so with an annual average of 10 L/s.

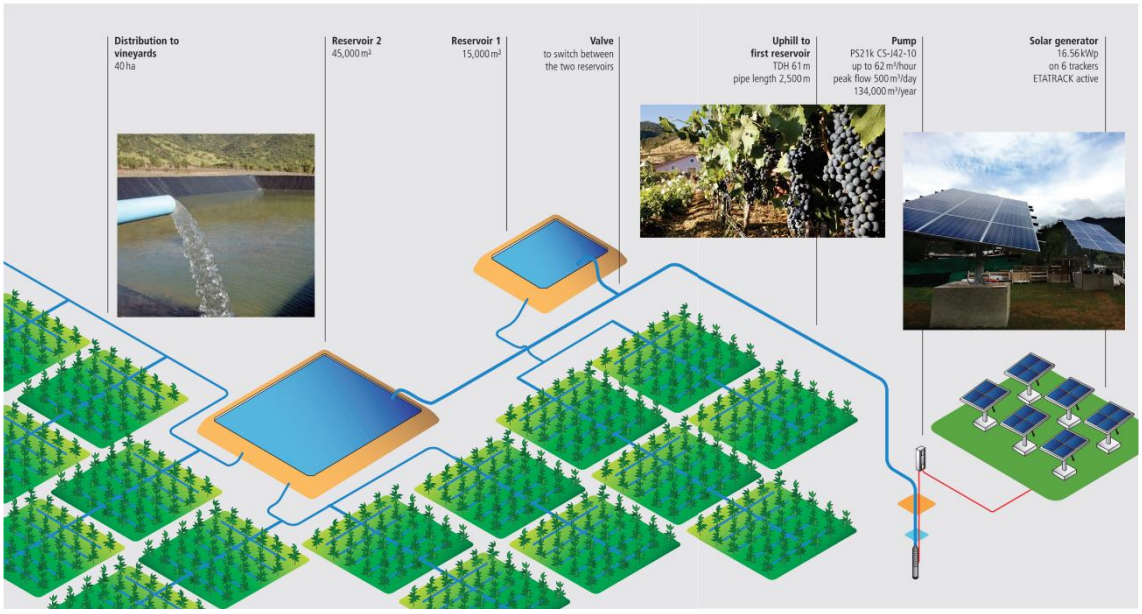

Figure 20- Solar-powered vineyard irrigation system (Lorentz 2012)

5.6. Integrated Food Energy Systems (IFES)

Food security and sustainable agriculture are the two main objectives of agriculture production process and these objectives can only be achieved by changing the farming practices from fossil-based energy generation to renewable energy based farming. Renewable energy technologies to be able to use in farming are solar energy, wind energy, geothermal energy, and biomass energy. IFES is a farming system model designed to combine, intensify, and hence improve the simultaneous production of food and energy through the sustainable use of biomass; achieved in two ways: 1. Combining the production of food and fuel feedstock

on the same land, e.g. inter-cropping, agroforestry, or agro-pastoral systems, 2. Using the by-products/residues of one production system i.e. 'closed loop' or 'zero waste' systems. Hence, energy-smart solutions can include simultaneously producing food and energy as a means of achieving sustainable production systems in rural communities as shown in Figure 21 (Sims & Flammini 2014).

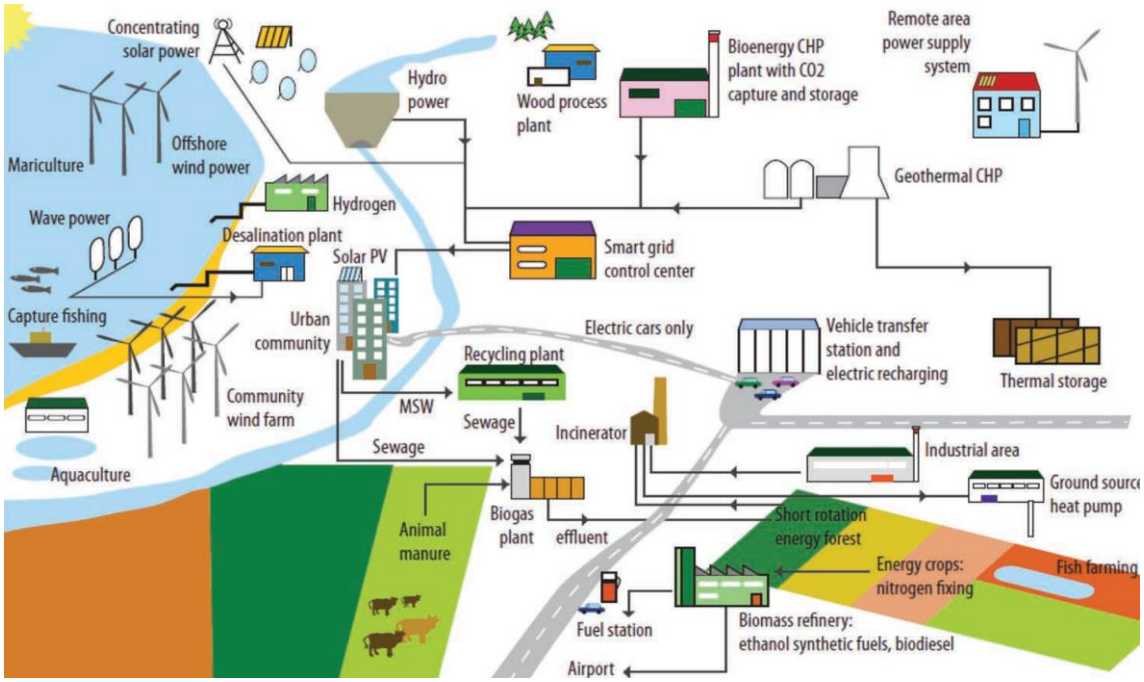

Figure 21- Integrated food energy system (IFES) (Sims & Flammini 2014)

6. Discussion

In this review, innovative digital technologies to be able to use in agri-food systems are discussed based on the water-energy-food nexus for sustainable development. The agriculture sector has a social-economic structure that is always a risky sector, where variable inputs are used to grow very different products such as food and feed, directly affected by weather conditions. Agricultural activities, unlike industry, are carried out in large, open areas like fields, vineyards, and orchards, or closed areas like greenhouses, barns, poultry houses, and mushroom houses. Today, it is necessary to switch from labor-intensive production to technology-intensive production for successful and sustainable agricultural production in all its processes.

Since the areas used in agricultural production are limited, a productivity increase in agricultural production should be ensured. While input costs can be reduced, production yield and quality can be increased using precision farming (Berger 2019; Wolfert et al. 2017) and smart farming (Balafoutis et al. 2019; Boursianis et al. 2019) technologies with variable rate applications, developed applications saving the environment.

The driving factor of smart agriculture is to meet the demand for more food to increase yields, optimize interdependent resources of energy, water, and land, and provide sustainable urbanization. Water and energy are two finite resources that must reach access to more people and so ensuring everyone has access to a reliable supply are crucial to human survival and sustainable development (EC 2017; FAO 2017a; WB 2019).

The agri-food value chain consumes 30 percent of the world's available energy (FAO 2020). Energy is needed in the production of crops, fish, livestock, and forestry products, food storage and processing, food transport and distribution and, as well as, in food preparation (FAO 2012). Although the availability of fossil fuels has made a significant contribution to feeding the world, these energy sources are finite and, in general, environmentally problematic. As discussed also in this study, renewable energy technologies are key to food security and a climate-friendly, sustainable transformation of agri-food systems to produce more and better-quality food. Data-driven systems, IoT-backed sensors keep an eye on consumption patterns and provide insights into inefficient areas which in turn help to analyze energy consumption, usage, and pattern. These solutions can be utilized to manage and optimize energy consumption patterns by taking complete control of energy data at the most fundamental and granular level.

FAO supports digital technologies, innovative ideas with high potential for impact in food and agriculture, for transforming digital solutions and services into global public goods. FAO aims to explore the responsible application and adoption of existing

and frontier technologies, design and new services, tools and approaches to empower rural households and inspire youth entrepreneurship in food and agriculture (Santos Valle & Kienzle 2020).

The farming sector is moving towards a new paradigm that is recalled Agriculture 4.0 that integrates a number of innovations in order to produce agricultural products. Agriculture has become a strategic sector that needs to be given more importance due to reasons such as climate imbalances caused by global warming, an increase in the world population, and the gradual decrease of usable agricultural resources. Today, when traditional farming methods are no longer sufficient, countries have to focus on practices that increase productivity and reduce costs with Agriculture 4.0 practices. Smart Agriculture or Agriculture 4.0 is used to point out the digitalization of production as in Industry 4.0. Indeed, modern technology is available that will make agriculture more environmentally friendly and efficient. Technical improvements in the agriculture sector can be carried out to optimize production efficiency, and improve quality, minimize carbon footprint; and can be reduced production-associated risks.

Agricultural applications that are from crop production to harvesting, from weather monitoring to prediction, irrigation, weeding, fertigation, pest control, and logistics are rapidly evolving towards a new digital cosmos covering technologies such as IoT, cloud computing, big data, automation, and artificial intelligence.

7. Conclusions

This study targets to obtain holistic solutions in a systematic view, basis on Water-Energy-Food nexus to agricultural digital transformation that will support achieving a sustainable future. Digitalization is a technology accessing unexploited data pools to valuable information, providing transformation to potential benefits for society and the environment if programming smartly and efficiently among data sets.

The water-food-energy resources are at the center of sustainable development. The development of smart systems interconnected can generate unique opportunities to effectively solve the challenges targeted in United Nations' Sustainable Development Goals. It can be emphasized that smart technologies can be able to use as tools for game-rule changing, if setting their systematic integrations, the food-water-energy nexus will benefit sustainable development. The strategical use of three essential sources for future generations will provide invaluable solutions listed here: (1) sustainable food production; (2) access to fresh and safe water; and (3) renewable energy generation and usage. Human beings have two inevitable missions to fulfill sustainable development. The first one is to solve the interconnections between food - water - energy - climate nexus. The second one is to develop smarter machines and systems achieving agricultural transformation from traditional to digital to improve efficiency, decrease input costs and resource use, and protect the environment.

In the new digital era starting with the Industry 4.0 revolution, the production capabilities of all industries thanks to innovations emerged, including agriculture will have to transform in order to feed the rapidly growing population of the world.

Smart agriculture is real-time farm management providing a high degree of automation, and data-driven intelligent decision-making to improve productivity, save natural resources. Digital technologies can support farmers' production of safe, sustainable, and quality food. Technological innovations can help us overwhelm many of the economic, social, and environmental challenges that agriculture is facing today. Novel technologies like the internet of things, cloud computing, big data, artificial intelligence, drones, and robotics provide making processes more efficient and drive the creation of new products and services and streamline agricultural operations.

The driven technology of Agriculture 4.0 is the IoT that provides the ability to securely connect end-to-end to anything, to anywhere. In IoT, numerous wireless sensor network deployed to the field can be used along with cloud technologies. Big data gathered site-specific are transformed into meaningful data set to be used in decision support systems for smart applications. The captured information in the field can be examined by computational techniques to reveal trends and patterns, and interactive information on human behaviors, environment, and experiences can be transformed into efficient actions.

By using smart agriculture technologies and data-driven techniques, it will be possible to monitor in real-time how water, chemicals, and energy are used for the production of healthy food and animal feed, and what environmental effects the applied agricultural activity may cause. However, this requires the development of national and international policies, the implementation of strategies, and the control and supervision of processes.

Developed, there are many smart farming technologies including decision support system, yield monitoring, smart irrigation, smart spraying, management of crop disease, crop monitoring, soil monitoring, treatment of pests with robots, intelligent harvesting according to maturity, traceability from farm to fork, logistics, as well as e-marketing. Although innovation is often conceived as techno-centric, the rise of Agriculture 4.0 thinking needs to consider also environmental and social sustainability perspectives. However, challenges related to power, performance, and security are obstacles to seamless connectivity.

Thanks to developments in recent years, it is seen that digital technologies and data-driven techniques have the potential to transform agriculture more precisely, efficiently, and sustainably. Furthermore, while providing greater transparency for

consumers on how food is produced, decision-making support has helped farmers in many practices, in terms of environmental and agricultural performance. Beyond farming, while digital technologies make the job more attractive to new generations, they offer many opportunities to reduce problems related to remoteness and improve access to services.

Although data-driven technologies play a key role at all stages of the production of food and feed in agri-food systems of the future, none of this can happen without enough energy and water from farm to fork. Being clean and sustainable the energy used in agriculture, not chemically polluted of the water is vital to access to fresh water and clean energy everyone, and for a better reconstructing in the aftermath of the COVID-19 pandemic.

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The performance of *Trichogramma* (Hymenoptera: Trichogrammatidae) Parasitoids Feeding on Honey Sources

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ABSTRACT

The effectiveness of parasitoids as organism for biological control is at times limited by food sources. Most of parasitoids rely on carbohydrate foods to enhance their longevity and reproductive capacity. Honey is the miraculous product of honey bees and is naturally delicious. A preliminary study was conducted on the use of different kinds of honey as food sources for the *Trichogramma* species. The value of honey as a food source for *Trichogramma* was evaluated by testing the influence of several honey diets on parasitism and longevity in the laboratory. The most commonly available honey is made from a variety of flowers, pine, citrus, chestnut, sunflower, and cotton. *Trichogramma* females fed on chestnut and sunflower honey parasitized relatively more than when fed on citrus honey. *Trichogramma* females fed on flower, sunflower, and citrus honey survived drastically longer than females fed on chestnut and pine honey. These results showed that supplying sugar, sunflower, and flower honey to *Trichogramma* resulted in greater longevity and total fecundity. Overall, feeding *Trichogramma* females on different kinds of honey had a negligible effect on parasitization but did certainly affect longevity. Of the *Trichogramma* species evaluated, *T. brassicae* appears to be the most suitable parasitoids regarding high parasitization and longer life span.

Keywords: Fecundity, Honey, Longevity, Parasitization, *Trichogramma*

1. Introduction

There are many beneficial insect species that can be used in biological control. Among these beneficial insects, parasitoids are a very important group. The *Trichogramma* species in this group is of great importance in biological control. As a favorable method for reducing egg hatching and consequent harm caused by larvae, the augmentative release of lab-reared trichogrammatid egg parasitoids has been used (Smith 1996). The production of the *Trichogramma* species in the laboratory is cheap and easy. In many countries, these parasitoids are produced in bulk and are used against various pests, especially Lepidoptera (Hassan 1993; van Lenteren 2012).

The efficiency of parasitoids as organism for biological control is sometimes limited by food sources, and the availability of food plays a major role in determining the effectiveness of parasitoids as control agents. The *Trichogramma species* is fed with nectar and pollen in nature (Wellinga & Wysoki 1989), and these foods are known to be effective on the longevity of parasitoids (Hohmann et al. 1989; Shearer & Atanassov 2004), parasitism (Somchoudhury & Dutt 1988; Shearer & Atanassov 2004) and adult sex ratio. These natural food sources are replaced by honey and sucrose in the laboratory. Many studies have reported that feeding *Trichogramma* adults significantly increases fecundity and longevity in the laboratory (Ashley & Gonzales 1974; Bourarach & Hawlitzky 1989).

Honey contains many substances and consists of sugar, proteins, vitamins, and minerals (Alavarez-Suarez et al. 2009). It also covers antimicrobials and antioxidants (Echingo & Takenaka 1974; Martos et al. 2000; Gheldof et al. 2002). The use of extra food sources to improve biological control services for parasitoids and predators has been extensively discussed (Heimpel & Jervis 2005). It was indicated that the providing of food in the field can help parasitoids maintain their sugar resources and increase fecundity and parasitism (Tena et al. 2015).

Despite the significance and valuable information that laboratory work provides in selecting potential candidates for biological control, the real value of these studies is still being discussed (Waage 1990; Jervis & Kidd 1992). These studies show the potential value of adult foods, particularly when compared to the *Trichogramma* spp. In this study, we compared the suitability of the various types of honey as food sources consumed by *Trichogramma* spp. The goal of this research was to find

out if different food obtainability would likely be a significant point in the field's success by evaluating the response to a honey diet under laboratory conditions.

2. Material and Methods

2.1. E. kuehniella Rearing Procedure

Mediterranean flour moths (MFM), *Ephestia kuehniella Zeller* 1879 (Lepidoptera: Pyralidae) has been reared in our laboratory since 2015. The MFM were reared on a mixture of wheat flour, wheat germ and yeast (Marec et al. 1999). While rearing, the moth cultures were kept in a rearing room maintained at 27±1 °C and 70±5% RH under 16 h light followed by 8 h darkness (16 L: 8 D). For obtaining host eggs, new emerged adults of MFM were obtained from stock culture and placed in plastic jars with screen bottoms. The eggs were collected, sifted to remove parts of insects and frass and placed in Petri dishes.

2.2. Trichogramma spp. Rearing Procedure

Trichogramma spp. were permanently reared on MFM eggs in the laboratory in a room maintained at 24±1 °C, 70± 5% r.h, and 14:10 (L:D). *Trichogramma evanescens* Westwood 1833, *Trichogramma brassicae* Bezdenko 1968, *and Trichogramma pintoi* Vogelé 1982 (Hymenoptera: Trichogrammatidae) used in the experiments were obtained from the Department of Plant Protection of the Faculty of Agriculture of Ankara University. Parasitoid cultures were started by using individual females on eggs of *E. kuehniella* and kept in glass vials (8 by 2 cm) in the same room. The females tested were 2 to 3 hours old, fed with honey, mated and not contacted with the host eggs prior to testing. Fresh eggs (less than 24 hours old) of MFM were glued on pieces of white cardboard (2 by 5 cm) and put into the vessels (8 by 2 cm). These eggs were presented at the same time to a single female of the *Trichogramma* species for parasitization for 24 hours and placed in the environmental chamber and then were discarded.

2.3. Feeding tests

Experimental Design: Eight food options (A: No food, B: Flower, C: Pine, D: Citrus, E: Chestnut, F: Sunflower, G: Cotton and H: Sugar) were used for dietary treatments. The honey sources and sugar were regularly given to females wasps and unfed wasps were used as a control.

Each honey and the other treatments (no food and sugar) were compared as food for *Trichogramma* spp females. Flower, pine, citrus, chestnut, sunflower, and cotton honeys were obtained from the Faculty of Agriculture, Erciyes University. Each food was tested in three independent experiments in glass tubes (8 by 2 cm), namely with diluted honey, with sugar, and with no food only. In tests with honey, a small piece of cotton moistened with the dilute honey was placed into each glass test tube. The food was renewed every second day.

To test the ability (parasitization, longevity) of individuals of each of the three *Trichogramma* species reared on different honeys, approximately 50 *E. kuehniella* fresh eggs glued on a cardboard was place in glass vials (10 cm x 3 cm) and were separately offered to a single, newly emerged (0-24 h) and mated *Trichogramma* female of each species for one day. After plugged with a ventilated stopper, the vials were then placed in a randomized block trial design with 10-12 replicates. The females of each *Trichogramma* species were removed after this time of exposure and the number of parasitized eggs was counted. Longevity was determined by counting the number of adults each day until death. The food source was replaced daily unless otherwise indicated. Then each vial was examined carefully to determine the female longevity, parasitization, adult development, and sex ratio.

Data were analyzed using one-way variance analysis (ANOVA) with food sources being used as a factor and the number of parasitized eggs, number of total emerged adults and number of total females as dependent variables (PROC GLM) (SPSS 1999). The homogeneity of the variance was evaluated with the Levene's test. Before statistical analysis, all data were transformed into the square roots; Tukey-HSD was used as a means of separation when significant differences occurred (SPSS 1999). The life table statistics were calculated using data from adult survival studies. The mean survival rates were calculated using the Kaplan-Meier survival analysis (Kaplan & Meier 1958). Differences in survival rates were compared with Breslow method (Generalized Wilcoxon) (Gehan 1965).

3. Results and Discussion

3.1. Fecundity

All of the honey had nutritional qualities and there is a potential to rear this insect on honey. Reproductive success in the *Trichogramma* species varied slightly when fed on different honey, but fecundity was not affected markedly (F, df, P>0.05). The effects of food on the parasitization of *T. pintoi* are shown in Figure 1. The mean fecundity (total offspring) or parasitism was between 29.0 and 36.7 per female; the highest values for honey F (Sunflower) were not significantly different from the other kinds of honey (F = 1.162, df =7, P>0.05). In *T. evanescens*, the mean fecundity was between 25.1 and 28.8 per female; the

highest values for honey C (Pine) were not significantly different from the other kinds of honey (F= 0.289, df =7, P>0.05) (Figure 2). The number of parasitized hosts decreased to a comparatively constant level from honey C (Pine), to honey D (Citrus), and to honey B (Flowers).

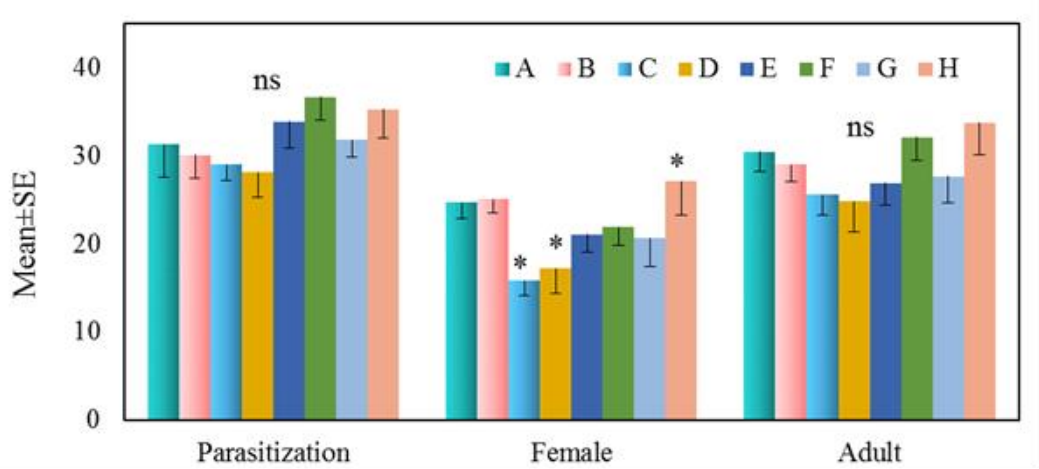

Figure 1- The mean number of parasitization of *T. pintoi* when reared on food sources. The asterisks in the columns indicate that there was significant difference at a 5% probability level by the Tukey test, ns: non-significant differences. (A: No food, B: Flowers, C: Pine, D: Citrus, E: Chestnut, F: Sunflower, G: Cotton, and H: Sugar)

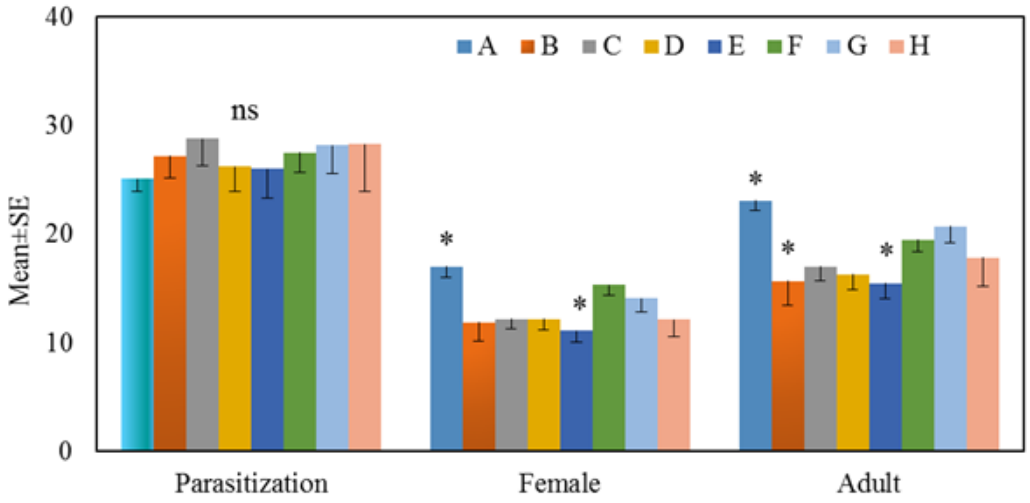

Figure 2- The mean number for parasitization of *T. evanescens* when reared on food sources. The asterisks in the columns indicate that there was significant difference at a 5% probability level by the Tukey test, ns: non-significant differences. (A: No food, B: Flowers, C: Pine, D: Citrus, E: Chestnut, F: Sunflower, G: Cotton and H: Sugar)

Regarding *T. brassicae*, the parasitization is shown in Figure 3. The number of offspring produced by *T. brassicae* females not differed significantly with the feeding treatment (F = 1.903, df =7, P>0.05). There is no difference since P>0.05). The mean fecundity was between 29.5 and 39.4 per female for honey D (Citrus) and A (No food), respectively. A large difference was found in fecundity among females. Wasps had the highest parasitization when fed on D (Citrus) and A (No food) and the lowest in the other feeding treatments, particularly in G (Cotton) honey and in H (Sugar). The mean fecundity of the females, provided with honey for a day and then allowed to oviposit for one day, was similar to unfed females. The number of parasitized eggs (Figure 3) indicated that *T. brassicae* is more fecund than *T. evanescens* and *T. pintoi*.

Reproductive success in the *Trichogramma* species varied only slightly when fed on different kinds of honey, the mean fecundity or parasitism not being significantly different. It was found that when the *T. euproctidis* females were fed on artificial foods and flowers nectars, the females fed with sucrose, honey and dog-fennel had higher parasitization than other nutrients (Tuncbilek et al. 2012). These results are consistent with other studies that addressed the fecundity of *Trichogramma* where sugar was supplied and showed change or no increase in parasitism (Heimpel & Jervis 2005; Wade et al. 2008).

Contrary to our findings, many researchers have indicated that an increase in fecundity can happen when parasitoid wasps fed with different foods (Ashley & Gonzalez 1974; Leatemia et al. 1995; Shearer & Atanassov 2004). These findings recommend

that habitat management should provide a continuous supply of sugar or nectars to parasitoids. The significance of sugar feeding could clearly be greater in the field than expected from the laboratory, where parasitoids are not so active and are maintained under adequate conditions (Steppuhn & Wackers 2004; Winkler et al. 2006). However, owing to the tendency of the *Trichogramma* spp to be pro-ovigenic the availability of food will likely not always increase fecundity. It is much more probable that adults will survive long enough to place appropriate hosts for their eggs (Harvey et al. 2012).

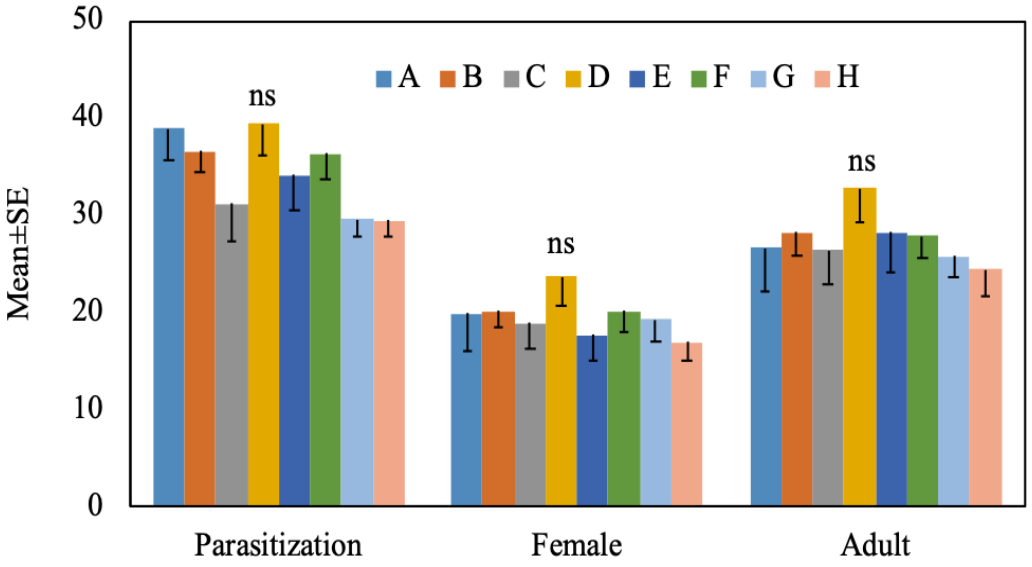

Figure 3- The mean number parasitization of *T. brassicae* when reared on food sources. The asterisks in the columns indicate that there was significant difference at a 5% probability level by the Tukey test, ns: non-significant differences. (A: No food, B: Flower, C: Pine, D: Citrus, E: Chestnut, F: Sunflower, G: Cotton and H: Sugar)

3.2. Female emergence

The number of female emergence for *Trichogramma pintoi* was greater on the diet H (Sugar). It was significantly different from honey C (Pine) and D (Citrus) ($F = 2.463$, df =7, $P<0.05$) (Figure 1). There was a large variation in the results of the kinds of honey for female emergencies. Results were similar to the values observed for other honey and are not significantly different ($P>0.05$). In *T. evanescens*, the female emergence was greater on diet A (No food) (17.0). It was significantly different from the chestnut honey diet ($F = 2.965$, df =7, $P<0.05$) (Figure 2). Regarding *T. brassicae*, the female emergence was greater on the diet D (Citrus) (23.7). It was not significantly different from the other honey (Citrus) ($F = 0.598$, df =7, $P>0.05$) (Figure 3). Our findings support the study of Lundgren et al. (2002), who performed quality evaluation experiments for commercially reared *Trichogramma* spp, and found that the percentage emergence increased when *Trichogramma* adults received honey.

3.3. Adult emergence

Adult emergence of *T. pintoi* was greater with diet H (Sugar) (33.8), although it was not significantly different from honey C (Pine) or from D (Citrus) ($F = 1.285$, df =7, $P>0.05$) (Figure 1). Results were similar to the values observed for other honeys and are not significantly different, though marginally more adults were produced for wasps fed on honey from flowers, sunflowers, and sugar ($P>0.05$). In *T. evanescens*, the adult emergence was greater with diet A (No food) (23.1) It was significantly different from honey B (Flower) and E (Chestnut) ($F = 3.022$, df =7, $P<0.05$) (Figure 2). Results were similar to the values observed for other honey and are not significantly different, though slightly more adults were obtained for wasps fed on sunflower and, cotton honey and no food ($P>0.05$). The daily emergence trend was very comparable to the amount of daily parasitized eggs. These results accord with our previous study (Çınar et al. 2015).

Regarding *T. brassicae*, the adult emergence was a little bit greater with diet D (Citrus honey) (32.8). It was not significantly different from the other honey ($F = 0.593$, df =7, $P>0.05$) (Figure 3). Results were similar to the values observed for other honeys and are not significantly different, even though slightly more adults were produced for wasps that were fed with flower, chestnut, and sunflower ($P>0.05$). The number and sex ratio of progeny emerging from hosts parasitized by *Trichogramma* females fed on different kinds of honey did differ significantly. The sex ratio (female/male) of *Trichogramma* was affected by honey sources. The sex ratio varied from 1.86 to 3.76; 2.12 to 3.42 and 1.67 to 2.97 for *T. pintoi*, *T. evanescens*, and *T. brassicae*, respectively. *T. evanescens* had a more significantly increased sex ratio than *T. pintoi.*

There has been extensive discussion of the use of additional food sources in agriculture to enhance biological control facilities for parasitoids and predators (Heimpel & Jervis 2005). The provision of food in the field was reported to help parasitoids maintain their sugar resources and raise fecundity and parasitism (Tena et al. 2015).

3.4. Longevity

Longevity in *Trichogramma* varied significantly when fed on different kinds of honey. Honey types provided to *T. pintoi* females significantly affected longevity. The highest longevity was recorded (6.91±0.8 days) on sugar. The shortest longevity was recorded on no food (2,00±0.0 days) (Figure 4). The longevity of parasitoids that were maintained with sugar was marginally higher than those kept on chestnut honey and no food.

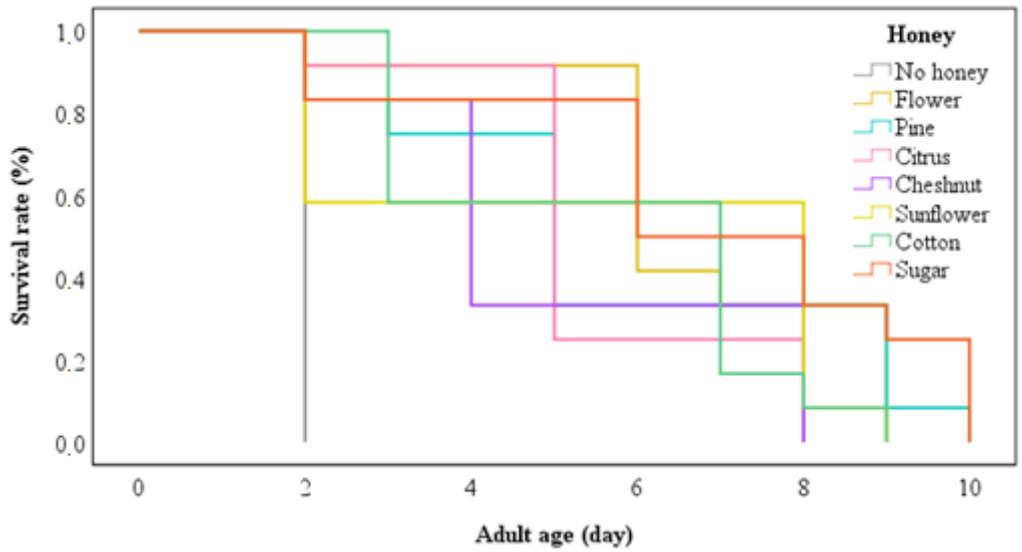

Figure 4- The survival rate of *T. pintoi* fed on different kinds of food sources (n=12), Kaplan-Meier curves of survivorship in days for *T. pintoi* females fed on different kinds of honey

The longevity of *T. evanescens* provided with sunflower, flower, and cotton honey was significantly different than those given no honey (χ^2 = 41.06, df =6, P<0.001). Parasitoids lived a longer period of time when they were fed on sunflower honey than cotton, and pine honey (Figure 5). The highest longevity was recorded (6.00±0.8 days) on sunflower honey, whereas the shortest longevity was recorded on no food (3.25±0.1 days) when they had no access to honey. The shortest longevity was recorded on no food (3.25±0.1 days). The longevity of parasitoids that were maintained with sugar was marginally higher than those kept on chestnut honey and no food.

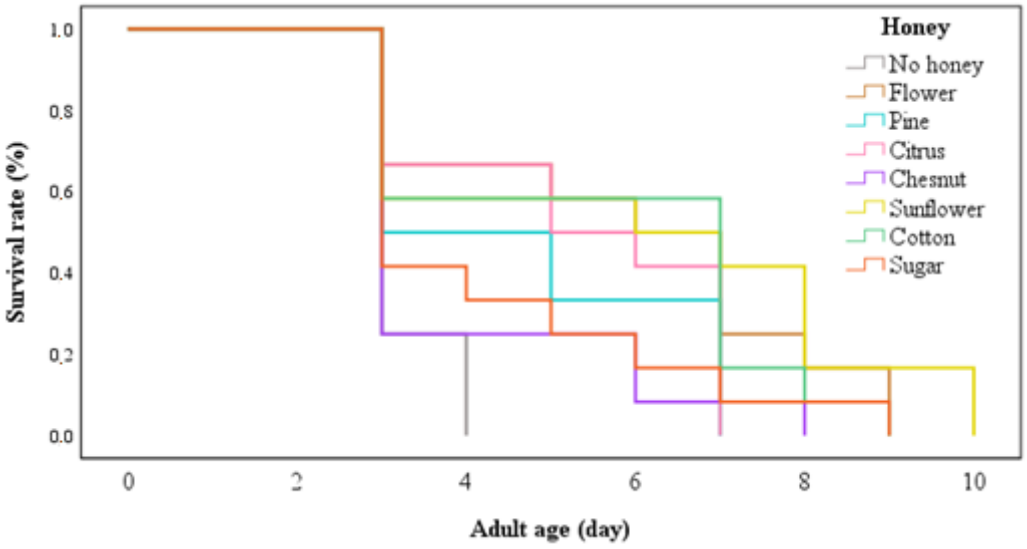

Figure 5- The survival rate of *T. evanescens* fed on different kinds of food sources (n=12)

Honey types provided to *T. brassiace* females significantly affected longevity. The highest longevity was recorded (8.71±1.2 days) on sugar. The shortest longevity was recorded on no food (1.83±0.3 days) (Figure 6). The longevity of parasitoids that were maintained with sugar was marginally higher than those kept on sunflower, pine honey, and no food. The longevity of *T. brassiace* provided with pine honey and no food was significantly different than those given other honey (χ^2= 52.45, df= 6, P < 0.001) (Figure 6). The *Trichogramma* male was not observed.

The survival curves of adult females of *T. pintoi* and *T. evanescens* (Figures 4 and 5) showed similar time patterns. The maximum female life expectancy was 10 days for *T. pintoi* and *T. evanescens*, and 15 days for *T. brassiace*. In spite of the similarity of the survival curves (*T. brassicae*), some minor differences could be observed (Figure 6). Although the availability of honey has clearly affected the life span of all three of the *Trichogramma* species, it did not markedly increase fecundity.

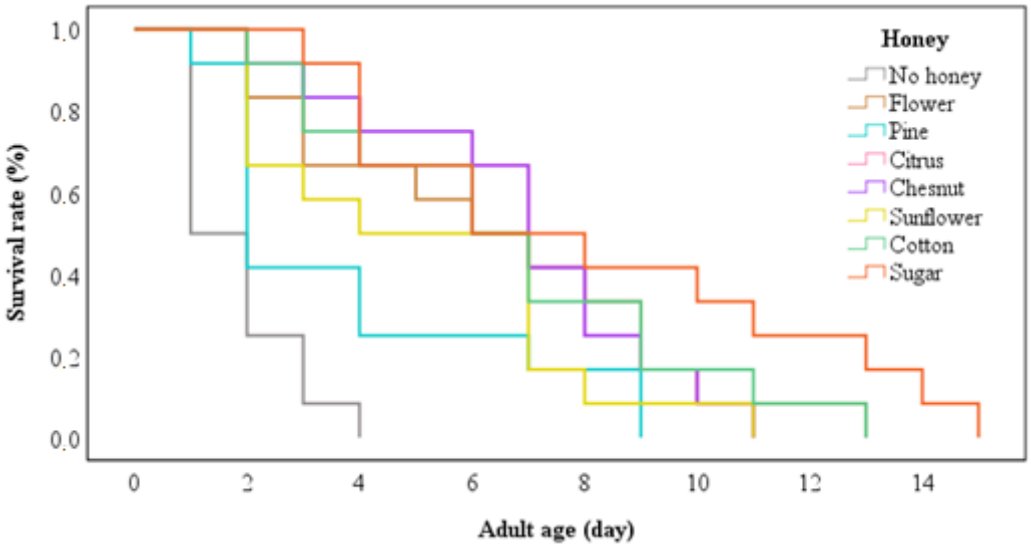

Figure 6- The survival rate of *T. brassiace* fed on different kinds of food sources (n=12)

The findings of the study indicated that the various kinds of honey had impressive effects on longevity in the *Trichogramma* species. There was a dramatic decrease in longevity when wasps were reared on chestnut honey. A similar amount of offspring was yielded within each species, however, the wasps had different longevity on the eight kinds of food examined. An earlier study (Wäckers 2001) observed a major shift in sugar quality in parasitoid *C. glomerata* longevity. There were important inter-specific differences in earlier experiments when wasps were fed honey (Harvey 2008). In a previous study it was found that a major shift in sugar quality had a major impact on the longevity of the parasitoid *C. glomerate* (Wäckers 2001).

Our study provides new insight into the biology of *Trichogramma* spp which can be useful concerning the ability to use *T. pintoi*, *T. evanescens* and *T. brassicae* species in biological control. However, further testing should be done before a decision is made on which species is the appropriate agent for control of pests. Therefore, we conclude that almost all honey is adequate for rearing *Trichogramma*, except for chestnut, based on the biological parameters of parasitization, adult emergence, and female longevity. Overall, feeding *Trichogramma* on various honey had a slight effect on parasitization, but greater effect on longevity. Of the species of *Trichogramma* assessed, *T. brassicae* appears to be the most appropriate parasitoids for high parasitization and longer life. Further studies should target the efficiency of resource use in adult parasitoids derived from different diets in order to establish possible relations to certain life histories and reproductive strategies for these insects. In the same way, the approaches to mass rearing of these wasps for biological control and the unraveling of trait-mediated evolutionary constraints could be strengthened. It was concluded that different honey sources are effective on female longevity in the rearing of the *Trichogramma* species. In the current study, some honeys have been shown to be a more adequate food to maintain the development and reproduction of *Trichogramma* species.

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Detection of Metabolite Content in Local Bitter White Lupin Seeds (*Lupinus albus* L.) and Acaricidal and Insecticidal Effect of its Seed Extract

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ABSTRACT

This study investigated the acaricidal and insecticidal effect of local lupin *(Lupinus albus* L. Fabaceae) seed extract against *Tetranychus urticae* Koch (Acari: Tetranychidae), *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae), *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) and metabolite content of seeds. In tests for *T. urticae* leaf-disk bioassay was employed. Contrarily, two µL of the *L. albus* extract were topically applied using a micro-applicator on *C. maculatus* and *P. interpunctella.* In the assays of *T. urticae* and *P. interpunctella*, the concentrations of 0.78, 1.56, 3.12, 6.25, 12.5, 25% (w/w) of the plant extracts were used. Furthermore, the concentrations of 0.625, 1.25, 2.50, 5, 10% (w/w) were applied to *C. maculatus*. Mortality data was collected 24, 48 and 72 hours after application. In the results, *L. albus* extract was found to be quite effective to *C. maculatus* adults with LD_{50} of 7.26, 1.21 and 0.55% after 24, 48 and 72 hours, respectively. Moreover, lupin extract was effective to *T. urticae* adults with LC_{50} values of 4.03, 3.15 and 2.73% for the same durations. *L. albus* extract was showed low insecticidal effect against the larvae of *P. interpunctella*.

In seeds metabolite content were detected, which contained 686.99 mg GAE / 100 mg total phenol, 22.06 mg QE / 100 mg total flavonoid, DPPH 26.04 mg TE / 100 g having antioxidant activity. The bitter taste of stem and seeds of the plant is due to their metabolite content which has a toxic effect.

In conclusion, results indicated that lupin seed has a high secondary metabolites content and also its extract had the high toxic effect against *T. urticae* and *C. maculatus.*

Keywords: *Callosobruchus maculatus, Tetranychus urticae, Plodia interpunctella; Lupinus albus* extract, Metabolite content

1. Introduction

Pests have a negative effect on the growth of culture plants grown in greenhouses and fields, resulting in the loss of stored products. *Tetranychus urticae Koch* (Acarina: Tetranychidae) is a major polyphagous pest that damages many agricultural crops both in the field and in greenhouses.

Callosobruchus maculatus F. (Coleoptera: Chrysomelidae*)* is a major pest especially for legumes and are able to survive both in the storages and in the field.

Plodia interpunctella (Hubner) (Lepidoptera: Pyralidae) is a major pest for grain and grain products with dried fruits being the main ones, as well as for processed and non-processed products like milk powder, corn flour, wheat flour.
Synthetic chemical pesticides have been used widely in pest management. Potential risks posed by synthetic chemical pesticides for mammals, consumer concerns regarding insecticide residues, and increased resistance of pest populations against insecticides, have encouraged researchers to discover new approaches for pest management. The approach of using plants for insecticide production has been brought up by different researchers (Gökçe et al. 2007; Erdoğan et al. 2012; Elma 2014; Ali et al. 2017). Given the destructive nature of synthetic chemical insecticides, it is critical to develop safe and environment friendly resources for better and safer pest management.

White lupin (*Lupinus albus* L.) is a species of the genus Lupinus in the family Leguminosae (Duranti et al. 2008). This plant is named termis, acibakla or termiye in Turkey and a local population of bitter white lupin is conventionally cultivated around lakes region.

The lupin plants have a secondary metabolite in its seeds and stem (Oomah et. al. 2006; Reinhard et al. 2006). They can deter a number of herbivores (nematodes, aphids, caterpillars, beetles, locusts, snails) (Gegear et al. 2007). Deterrent or toxic effects

of alkaloid such as sparteine, lupanine and cytisine against phytophagous insects have been evaluated (Wink 1992; Bermudez-Torres et al. 2009).

In previous studies, *L. albus* seed extract has not been tested against *C. maculatus*, *T. urticae* and *Plodia interpunctella* yet. The aim of this study was to determine of metabolite content of lupin seed and evaluating the insecticidal and acaricidal activities of its extracts against *C. maculatus*, *T. urticae* and *Plodia interpunctella.*

2. Material and Methods

2.1. Plant material

Lupin seeds (local *L. albus* landrace) that had been cultivated in the Deştiğin/Doğanhisar district (37° 59′ N, 31° 25′ E) of Konya, Turkey, in 2012, were obtained from a local seller. After this transaction, the lupin seeds were desiccated and they were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff as well as immature, broken seeds.

2.2. Metabolite content in seeds

Three analyzes in this section were performed in 2019 with the procurement of services in the Laboratory of Food Institute of TÜBİTAK-Marmara Research Center.

2.2.1. Total phenolic compound (TPC)

TPC was measured determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton & Rossi (1965). Briefly, 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of fruit juice. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 0.2 to 4 mg L^{-1}.

2.2.2. Estimation total flavonoid

Total flavonoid contents of lupin seed (mature) were estimated by using the aluminium nitrate colorimetric method as described by Ashokkumar et al. (2010). Quercetin was used to make the calibration curve.

2.2.3. Total antioxidant activity

The free radical scavenging effects of the methanol extracts were estimated according to the method of Blois (1958) with minor modifications. Antioxidant activity was found by "DPPH free radical scavenging activity method" and the results were given as "Trolox equivalent antioxidant capacity (TEAC)".

2.3. Plant extraction

Plant extracts were prepared as described by Gökçe et al. (2007). The dried grains were ground using a grinder (Retsch SM100) and 50-gram portions were transposed into a glass jar. Then 500 mL methanol (Merck 99.5%) was supplemented. The jar was covered with aluminum foil and were left in room temperature for 6 days. At the end of this period, plant suspension was sieved using a filter paper and the liquid part was extracted and methanol was left to evaporate at low pressure at a temperature of 42 °C using Rotary Evaporator (Heidolp-VAP Precision). Then lupin extract was diluted using purified water and six concentrations (0.78, 1.56, 3.12, 6.25, 12.5, 25% w/w) were prepared for *T. urticae* and *P. interpunctella* and five concentrations (0.625, 1.25, 2.5, 5, 10 % w/w) were prepared for *C. maculatus.*

2.4. Growing the insect cultures

Callosobruchus maculatus and *P. interpunctella* were obtained from the stock culture rearing in the climate cabinet that operates under dark situations at a temperature of 28±1 °C and at 55±5% RH. On the other hand, *T. urticae* red form was reproduced on bean (*Phaseolus vulgaris*) obtained from the stock culture in the laboratory of the Plant Protection Department (the Faculty of Agriculture of Selcuk University) and grown at a temperature of 28±1 °C, and at 65±5% RH, under a 16:8 h (L: D) photoperiod.

2.5. Toxic effect experiment

In the experiment, the newly emerged adults (1 day old) of *C. maculatus* and 3rd- 4th-period larvae of *P. interpunctella* were used. Each concentration of the extract was applied at two µL/insect using a micro-applicator on the dorsal part of *C. maculatus* and on the dorsal abdomen of *P. interpunctella* larvae. Prior to experiment, *C. maculatus* adult and *P. interpunctella* larvae were

immobilized by storing them for 5-10 minutes at a temperature of 2 °C. The adults that completed the dripping application were placed in petri dishes and the petri dishes were held in climate cabinet until the end of the experiments. Experiment was carried out using 3 repetitions in random parcels and for each repetition, 20 adults in each petri dish were used. Only water was used for controls. Mortality data was recorded 24, 48 and 72 hours after the experiment. In the experiments for *T. urticae* Erdoğan et al's (2012) method was adapted. *T. urticae* adults were transferred into bean leaf discs placed in Petri dishes with a layer of wet cotton of 3 cm diameter inside. Leaf discs were dipped for ten seconds into the lupin extract and water (control) and then allowed to dry for 30 min. Then *T. urticae* adults were transferred to leaf disc in the petri dish. Twenty adults were transferred to leaf discs. Each experimental unit (0.78, 1.56, 3.12, 6.25, 12.5, 25% w/w-) consisted of a glass petri dish (10 cm x 2 cm) containing three leaf discs and 60 adults. Insect mortality from Petri dishes assays was appraised after 24, 48 and 72 hours of exposure.

2.6. Data analysis

All mortalities data were corrected for control mortality using "Abbott's formula" (Abbott 1987).

$$\text{Abbotts formula} = [(A-B)/A] \times 100 \quad (1)$$

Where; A: Live insect in control after treatment (%); B, live insect in treated after treatment (%). Bioassay results were analyzed using Polo-PC probit package program (LeOra Software, 1987), and confidence intervals were determined with LD_{50}/LC_{50} and LD_{90}/LC_{90} values.

3. Results and Discussion

3.1. Metabolite content of lupin seeds

The lupin plants have a secondary metabolite in its seeds and stem, which gives it bitterness. These metabolites include alkaloids, phenols, flavonoids, antioxidants etc. According to result of analysis, local lupin seeds contain 686.99 mg GAE / 100 mg total phenol, 22.06 mg QE / 100 mg total flavonoid, DPPH 26.04 mg TE / 100 g antioxidant activity (Table 1). As can be seen here, it is seen that lupin seed has high total phenol content (TPC), flavonoid and antioxidant activity.

Table 1- Antioxidant activity, phenolic and flavonoid content in lupin seeds

Content	*mg 100g^{-1}*
Total phenolic	686.99 Gallic acid equivalent
Total flavonoid	22.06 Quercetin equivalent
Total antioxidant capacity	26.04 Trolax equivalent

According to Siger et al. (2012), total phenolic content (TPC) ranged between 4.92 and 7.31 mg g^{-1} dry material for cvs. Butan (*L. albus*) and Parys (*L. luteus*), respectively. TPC of the boiled legumes varied between 11.8 and 25.9 mg GAE/100 g, simple polyphenols varied from 0.32 to 2.4 mg 100 g^{-1} (Kalogeropoulos et al. 2010). *L. luteus* of TPC was 14.56 mg GAE/g dry material in 6 day-long germination process provides samples (Buszewski et al. 2019). Karamac et al. (2018) white lupin and wild forms compared the phenolic content and antioxidant capacities who was found 4.36-7.24 mg GAE/g dry matter total phenolic ingredient.

Oomah et al. (2006), eight lupin (*Lupinus angustifolius* L.) genotypes grown at four locations in south central Alberta in 2004 were analysed for phenolic components and antioxidant activity obtained by a photochemiluminescence assay. Phenolic content in cultivars changed between 11.9 and 14.7 mg catechin equivalent, 4.15 and 4.95 mg routine equivalent g $^{(-1)}$ lupin for TFC and flavonoid contents, respectively. Lupin cultivars displayed weak antioxidant activity based on water-soluble substances (ACW) of 0.54 to 1.07 mu mole Antioxidant capacities TE/g with lag time varied from 70 to 153 s and an antioxidant index of 6.7 to 14.5 and 1.9 to 3.3 mu mole TEAC/g based on analyses of lipid-soluble substances (ACL). In lupin genotypes, antioxidant activity was not correlated with seeds phenolic contents. Lupin, the reduced alkaloid content induced the sensibility for aphid invasion (Philippi et al. 2016).

According to these results, the local population has more phenols, flavonoids and antioxidants capacity than other genotypes. These results are the first report of the local population.

3.2. Acaricidal effect of lupin seed extract on Tetranychus urticae Koch

Lupin seed extract resulted in death at a rate of over 95% in all time of exposure at 25% and 12.5% concentrations. Especially at 25% concentration, 100% death rate was observed for all test periods (Table 2).

Table 2- Mortality percent of lupin seed extract against *Tetranychus urticae* Koch adults at different concentrations and time of exposure

Mortality (% ± SE)			
Concentrations (% w/w)	*Time of exposure (h)*		
	24	*48*	*72*
25	100±0.00	100±0.00	100±0.00
12.50	96.43±2.92	98.18±1.48	100±0.00
6.25	67.86±4.37	72.73±4.45	75.00±1.57
3.12	49.99±3.86	54.55±3.93	57.69±4.15
1.56	32.14±3.86	36.37±1.48	42.31±8.16
0.78	12.50±1.46	27.28±3.93	28.85±4.15

The LC_{50} and LC_{90} values of lupin extract against the *T. urticae* are shown in Table 3. Lupin seed extract was found effective on *T. urticae* adults after 24, 48 and 72 hours with LC_{50} of 4.03%, 3.15% and 2.73%, respectively.

Table 3- LC_{50} and LC_{90} values and fiducial limits of lupin seed extract against *Tetranychus urticae* at different times

Time of exposure (h)	*n*[a]	*Slope±SE*	*LC_{50} (Fiducial limit)*[b]	*LC_{90} (Fiducial limit)*[b]	*Heterogeneity*	*χ^2*
24	360	0.227±0.029	4.039 (3.13-4.94)	9.676 (8.30-11.79)	0.86	13.72
48	360	0.220±0.031	3.155 (2.12-4.08)	8.981 (7.57-11.25)	0.41	6.48
72	360	0.240±0.033	2.733 (1.59-3.68)	8.076 (6.72-10.37)	0.49	7.77

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Even though there are several studies on *T. urticae* regarding plant-based compounds prepared using different plants, no study carried out using Lupine seed extract has been observed in the literature. In studies regarding acaricidal effect of extracts made from different plants on *T. urticae*, it was observed that the resulting effect varied depending on the plant species, extract concentration and application period (Tunc & Sahinkaya 1998; Topakcı et al. 2005; Sertkaya et al. 2010; Topuz & Madanlar, 2011; Chen & Dai 2017). In our study, it was observed that the lupine plant extract resulted death rate of 50% at above concentration starting from 3%. Based on these results, it appears that lupine plant extract has a high acaricidal effect on *T. urticae*. The seeds of lupine contain lupinine, sparteine, anagyrine and angustifoline as alkaloids; they also contain lupine and vernine as glycosides (Tüzün 2013). One might conclude that the insecticidal effect of the lupine extract may be resulting from these alkaloids and secondary compounds such as glycoside. Previous studies have already demonstrated the nematicidal, molluscicidal and insecticidal effects of lupinine and sparteine (Duke 1992; Bermudez-Torres et al. 2009; Yildiz 2011).

3.3. Insecticidal effect of lupin seed extract on callosobruchus maculatus F.

Lupin seed extract resulted a mortality rate of 100% against *C. maculatus* adult at 10% concentration observed after 72 hours. Excluding the lowest application concentration over 90% mortality rate was observed using lupin extract as the data was recorded after 72 hours (Table 4).

Table 4- Mortality percent of lupin seed extract against *Callosobruchus maculatus* F. adults at different concentrations and time of exposure

Mortality (% ± SE)			
Concentrations (% w/w)	*Time of exposure (h)*		
	24	*48*	*72*
10	68.42±3.75	91.10±2.35	100±0.00
5	43.89±2.25	75.03±2.21	96.89±3.37
2.500	38.63±1.79	76.74±3.01	96.33±2.98
1.250	8.74±0.82	75.03±1.73	88.89±1.89
0.625	8.74±0.95	16.08±0.32	44.44±0.94

Lupine seed extract was the most effective with LD_{50} values of 0.55 % after 72 hours and 1.21% after 48 hours of treatment (Table 5).

Table 5- LD_{50} and LD_{90} values and fiducial limits of lupin seed extract against *Callosobruchus maculatus* at different times

Time of exposure (h)	*n*[a]	*Slope±SE*	*LC_{50} (Fiducial limit)*[b]	*LC_{90} (Fiducial limit)*[b]	*Heterogeneity*	χ^2
24	360	0.166±0.026	7.26 (5.70-9.48)	14.98 (12.00-21.18)	1.22	15.98
48	360	0.151±0.031	1.21 (0.48-1.80)	8.06 (5.38- 20.08)	2.32	30.27
72	360	0.193±0.33	0.55 (0.24-0.81)	4.98 (2.78-22.75)	1.65	21.33

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Based on these results, it was observed that the lupin extract demonstrated a high level of insecticidal effect on *C. maculatus.* No previous study on the insecticidal effect of lupin seed extract on *C. maculatus* has been found in the literature. Previously some researchers have indicated that certain plant extracts had insecticidal effects on *C. maculatus* (Mahdian & Rahman 2008; Adedire et al. 2011; Cetin & Elma 2017; Louise et al. 2018).

3.4. Insecticidal effect of lupin seed extract on Plodia interpunctella (Hubner)

Lupin seed extract resulted in a low toxic effect on *P. interpunctella* larvae in general. Looking at Table 6, it can see that the death rate was observed just 50% after 24 hours at a concentration level of 25% and the highest was recorded 64% after 72 hours. And with other concentrations, the mortality remained below 40% for all exposure times.

Table 6- Mortality percent of lupin seed extract against *Plodia interpunctella* (Hubner) larvae at different concentrations and time of exposure

Concentrations (% w/w)	*Mortality (% ± SE)*		
	Time of exposure (h)		
	24	*48*	*72*
25	50.84±8.42	59.32±7.19	64.40±9.59
12.50	28.80±4.15	42.37±3.66	42.37±1.38
6.25	20.33±7.71	20.33±3.66	20.33±7.71
3.12	6.77±2.77	8.46±4.15	10.16±1.38

The LD_{50} and LD_{90} values of lupin extract against the *P. interpunctella* are shown in Table 7. Lupin seed extract showed low toxic effect on *P. interpunctella* adults after 24, 48 and 72 hours with LC_{50} of 23.08%, 19.45% and 18.36%, respectively.

Table 7- LD_{50} and LD_{90} values and fiducial limits of lupin seed extract against *Plodia interpunctella* at different times

Time of exposure (h)	*n*[a]	*Slope±SE*	*LC_{50} (Fiducial limit)*[b]	*LC_{90} (Fiducial limit)*[b]	*Heterogeneity*	χ^2
24	360	0.067±0.010	23.08 (18.14 -33.18)	42.12 (32.36-67.07)	1.94	25.21
48	360	0.076±0.010	19.45 (15.83-25.25)	36.33 (29.28-50.82)	1.66	21.57
72	360	0.079±0.010	18.36 (15.53-22.32)	34.58 (29.03-44.24)	1.10	14.41

a: Total number of tested adults; b: 95 % lower and upper fiducial limits

Bouayad et al. (2013), studies the impact of methanol extracts of ten plants on the growth of the *P. interpunctella* larvae. They indicated that the growth rate was below 2.5% for larvae, which were exposed to *Ajuga iva, Rosmarinus officinalis*, *Centaurium erythraea* extracts.

There is the restricted number of studies on the insecticidal activity of lupin extract on pests. Isaev (1939), reported that 0.05% concentration of *Lupinus angustifolius* was sprayed as a spray on the adults of *Nematus ribesii* (gooseberry sawfly) (Tenthredinidae- Hymenoptera) and 100% mortality was observed after 48 hours. In another study, the insecticidal effect of 3 lupin species extracts (*L. montanus*, *L. stipulatus* and *L. aschenbornii*) and the spartein alkaloid obtained from them, was researched on *Spodoptera frugiperda* larvae. As a result, *L. stipulatus* extract was found to be the most toxic against the larvae. And also, the extracts of the other two lupin species as well was found to be at least as effective as sparteine (Bermu´dez-Torres et al. 2009).

4. Conclusions

Local lupin seed has high phenol, flavonoid and antioxidant activity. Lupin seed extract showed a toxic effect on *Callosobruchus maculatus* and *Tetranychus urticae*. Synthetic pesticides, which are widely used today, are known for causing adverse effects on human beings, the environment, and other creatures. Therefore, especially in the last 15-20 years many studies have been conducted on plants which are known for their biological activities towards pests. From this perspective, further studies that will determine the active ingredients or substances contained in lupin extract that have the insecticidal effect and research the effects of these ingredients on these pests would provide significant findings. It was also concluded that further studies on their acaricidal efficacy under field and greenhouse conditions would provide new findings resulted the potential of lupin seed extract having the potential to be used to manage *Tetranychus urticae* especially sustainable agriculture and integrated pest management.

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The Use of Landscape Character Analysis to Reveal Differences Between Protected and Nonprotected Landscapes in Kapısuyu Basin

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ABSTRACT
The European Landscape Convention (ELC) has directed the landscape classification towards landscape character analysis. Landscape character analysis provides a character-based classification that can combine different values or variables and be applied at different scales to define the landscapes of each country and define the forces on the landscape. In this study, the Kapısuyu Basin of Küre Mountains National Park, which is one of the hot spots in the world in terms of different landscape character and natural quality, was classified by landscape character analysis.

In this study, Kapısuyu basin was analyzed on an analytical ground according to the landscape variables and the basin landscape types, and the landscape character area map were obtained based on the dominant features of the area and the cultural landscape pattern. Throughout the basin, 345 landscape character types and 21 landscape character area were identified. Despite having similar values, the surface area of the protected area in the national park and the rural area had significant differences in landscape character ratios and patchiness ratio. Patchiness was seen to be higher in rural areas. When looked at Shannon Diversity Index (SDI) values, it is seen that a high diversity of Landscape Character Types (LCT) exist in the rural areas. Within the scope of this study, the fact that the landscape character analysis performed at the basin scale in the protected area can be evaluated together with different variables and interpreted from the perspective of holistic landscape planning shows that the technique is a positive approach in the evaluation of protected areas.

Keywords: Landscape character analysis, Landscape variables, Landscape metrics, Küre mountains national park, Kapısuyu basin, Turkey

1. Introduction

When the landscape is defined as a whole of natural, cultural, ecological, archaeological, historical, aesthetic, social and perceptual attributes, various approaches related to landscape classification have been developed as it becomes difficult to classify the landscape. Since the landscape classification is critical in monitoring the landscape change and taking precautions, landscape researches has directed the landscape classification towards landscape character analysis with the European Landscape Convention (ELC). ELC refers to landscape characterization by evaluating Landscape as "an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors" (ELC Article No. 1) (Council of Europe 2000). Landscape characterization includes two different approaches and classification techniques: landscape character analysis (biophysical values-biophysical approach) and landscape character assessment. Using these techniques, member countries of the ELC developed a typology to classify landscapes of each country and evaluated the landscape at different scales with different variables (Görmüş et al. 2013; Erikstad et al. 2015).

Landscape characterization provides a character-based classification that can combine different values or variables and be applied at different scales in order for each country to describe its landscapes and determine the forces on the landscapes. Depending on the natural or cultural values of the landscape, landscape characterization approach and the variables used may differ. In the classification of natural landscape, landscape character analysis is made, and biophysical variables are taken into account. In the classification of cultural landscape, landscape character evaluation is taken into account and anthropogenic variables and perceptual variables are used.

Landscape Character Analysis (LCA) is performed to define the character of the areas representing the relationship between human and place, to determine the main factors of character change and to evaluate biodiversity values in natural resources (Kim and Pauleit 2007). LCA is considered as a biophysical approach based on natural sciences (Bastian 2008; Sarlöv Herlin 2016) and is adopted by physical geographers and landscape ecologists (Bastian 2008). In the European Landscape Atlas (LANMAP2)

(Pedroli et al. 2007), which is one of the first projects of landscape character analysis studies in Europe, landscape was examined in five groups: geographical landscape, landscape habitats, visual and perceptual landscape, historical landscape and cultural landscape. The variables of these groups vary according to the landscape features of the country. Just as there are countries that use biophysical, natural and cultural variables together or both, some countries focus on historical features (Görmüş et al. 2013). LCA studies were conducted at different scales and approaches ever since Turkey became one of the contracting parties of ELC contract in 2003 (Görmüş & Oğuz 2010; Atik & Ortaceşme 2010; Uzun et al. 2010; Şahin et al. 2011; Görmüş 2012). ELC entered into force in Turkey since 2004 (Atik et al. 2015) and it continues out within the scope of LCA studies of institutional pilot projects and academic research projects (Görmüş et al. 2013).

Despite these studies, the landscape character analysis has not been reflected in the planning legislation and pilot projects are still performed in Turkey. According to Atik et al. (2015), implementing a landscape-scale approach in land use planning and policy with the use of LCA will be an opportunity for the effective management and protection of Turkey's landscape. In order to have this opportunity, a framework model for different scales in the planning of the landscape character analysis in Turkey was developed by Görmüş et al. (2013) and the main basis of the model is the association of approaches and methods with national legislation. Because although planning legislation in Turkey has a structure that can be integrated into landscape character analysis, highlighting only ELC cannot ensure that landscape character analysis is included in the planning legislation. For this reason, it is necessary to relate planning legislation and practices to landscape character analysis approach and to demonstrate the efficiency of LCA in planning.

In Turkey, pilot LCA projects conducted and supported by different institutions were not developed at the national scale. Some projects have been developed on the scale of local administrative boundaries (provincial boundaries), some on NTUS (Nomenclature of Territorial Units for Statistics) regional administrative boundaries and some on the upper basin boundaries. However, in Europe, landscape character analysis studies have been started on a national scale to integrate the LCA approach to national planning legislation. Conversely, in Turkey, a holistic objective in this direction is not yet adopted, thus, conceptual debate about LCA studies are ongoing. The basis of these discussions is based on the fact that the LCA methodology, scale and data set are variable and cannot be standardized. The main argument of this study is the necessity of carrying out landscape character analysis at the basin level in countries like Turkey where there are landscapes with high topological variability and natural quality. It is anticipated that applying LCA in basin scale will provide a base data in macro and micro basins, data loss will not occur due to the detailing or roughening of the data, and data sets will provide continuity in the field. On the other hand, ELC brings the possibility of making landscape character a part of a political decision mechanism with the subjective quality that it brings to the definition of landscape. The working unit of landscape character analysis should be the basin to prevent the landscape from being integrated into a political decision through the character. Because the concept of basin defines an area and scale where everything is interrelated and interconnected just like the concept of landscape. This also applies to protected areas. Because, protected areas are formally and permanently provided with protection in order to prevent from conversion of their natural land cover and it is aimed at partial and total conservation. Such areas also serve ecosystem and habitat invaluably (MEP 2003). While buffer zone encircling the protected areas are mainly able to maintain the natural processes in these areas (Wiens et al. 2002); the unprotected areas, on the other hand, are at high stakes because of human induced disturbances by downsizing the protecting area and ruining ecological cycle and habitat outside it (Parks & Harcourt 2002; Hansen & DeFries 2007). Also, the landscape that surrounds the protected area may restrict the conservation alternatives (USGAO 1994; Cole & Landres 1996; McDonald et al. 2008).

Küre Mountains National Park is an important hotspot in terms of its biological diversity. The Kapısuyu basin, which contains a part of the national park, was chosen as a study area because it is one of the basins with different landscapes, high topographic variability, and biophysical, natural and cultural variables. In addition, a consideration of landscape character analysis both on the protected area and its' perimeter, and on the basin scale will provide a basis for discussing the contribution of landscape character analysis technique to protected area planning and basin planning.

In this study, Kapısuyu basin was analyzed on an analytical approach according to landscape variables and basin landscape types, and landscape character area map were obtained based on dominant features of the natural and cultural landscape pattern. The Kapısuyu basin has been evaluated in three zones: protected area, buffer zone and unprotected rural zone. The protected area consists of two different protected status areas in the Kapisuyu basin: KMNP protected area and Saraytepe archaeological area. The buffer zone is the interaction area determined to provide the protection specified in the KMNP management plan. In the study, it was named as KMNP buffer zone. Rural areas outside the protected area and buffer zone are rural areas without conservation status and are defined as unprotected rural zone in this study. Therefore, in the study, the interaction between the three regions (protected area, buffer zone and unprotected rural zone) is aimed to be resolved with LCA.

The distribution and pattern of landscape character types among the protected zone (KMNP Protected area and Saraytepe Archeological area), KMNP buffer zone, and unprotected rural zone were identified in the basin. This study draws the attention to the interaction between the areas having protection status and areas with no protection status. Moreover, this study aims to emphasize holistic landscape planning using landscape character in basin.

In this regard, the main objectives in this study are as follows: (1) In basin scale, identification of the landscape character types using biophysical, natural and cultural variables, (2) evaluation of patchiness and correlativity of landscape character types,

(3) the identification of the composition and the configuration of the landscape character types in the protected, buffer and unprotected zones of the Kapısuyu Basin, (4) discussion on its meaning in the nature conservation planning of these transitions and the evaluation of necessity of the landscape character analysis in the basin scale.

2. Material and Methods

2.1. Study area

Kapısuyu Basin (study area) starts at sea level and includes a part of Kastamonu-Bartın Küre Mountains National Park (KMNP) Protected area, Buffer Zone (Figure 1) and rural area. The basin is 8 km long to the east-west, 10-km long on north-south direction and 19.8 km wide on the southwest-northeast. Kapısuyu Basin is 10,600 ha and its elevation has ranged from + 0.00 (Sea Level) to 1384 meters (Figure 1). There are five areas of different status in the Kapisuyu Basin. These are the KMNP protected area, KMNP Buffer Zone, Archaeological Area, rural zone (villages and agricultural lands), and Kapısuyu beach. However, in this study, the Kapısuyu basin was evaluated in three zones considering the areal sizes, the protected zone, the buffer zone and the unprotected rural zone. The protected area includes KMNP protected area (2,117 ha) and Saraytepe archaeological area (146 ha), the interaction area determined to provide protection in the KMNP management plan covers the KMNP buffer zone (5,503 ha), and rural areas outside the protected area and buffer zone include the unprotected rural zone (2,826 ha). Different protection statuses and height variability nourish the habitat diversity of the basin and the distinctness of ecosystem layers.

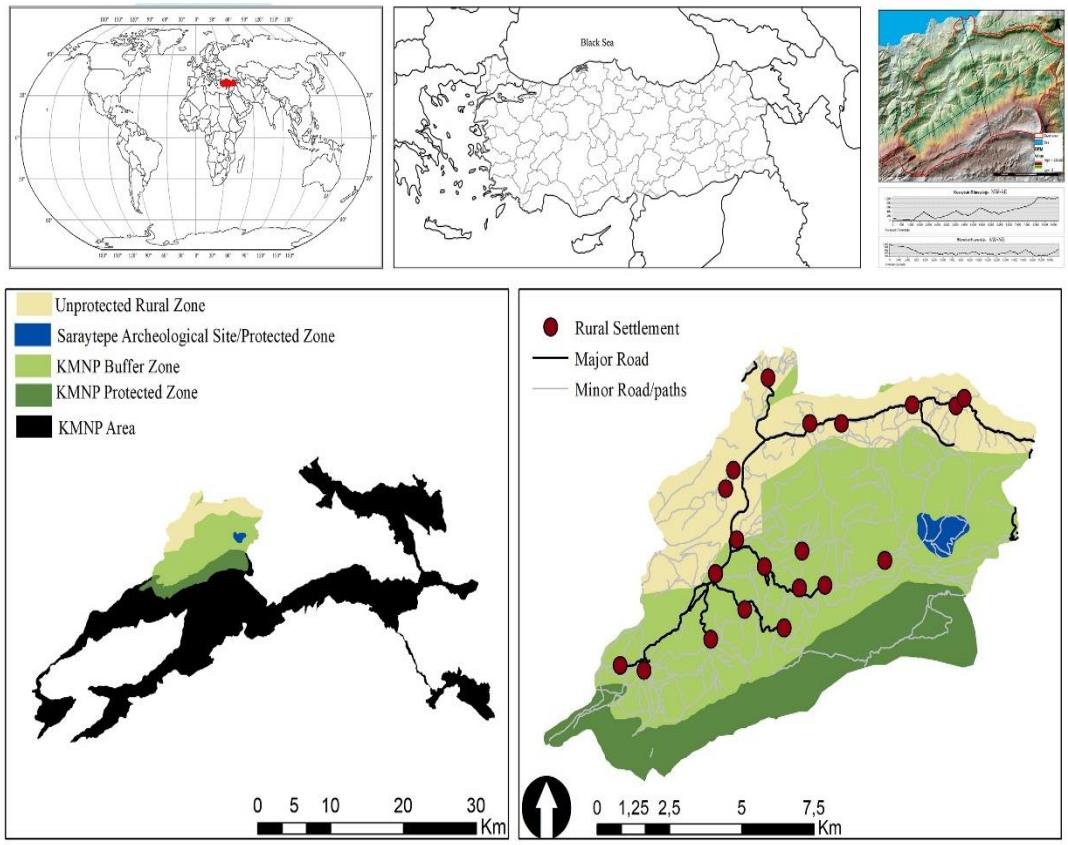

Figure 1- Location of study area

In order to carry out the inventory analysis of the study area, natural and cultural data were got via official correspondences from public and private institutions and organizations. In addition, the land use / cover map for the study area was obtained as a result of the classification of the RapidEye satellite image of 2011. For the accuracy assessment of the classification, total 235 ground control points were taken for each land use/cover category with GPS in the field studies.

2.2. Method

In this study, landscape character analysis was made at basin scale. The stages followed in the basin landscape character analysis are given below (Figure 2).

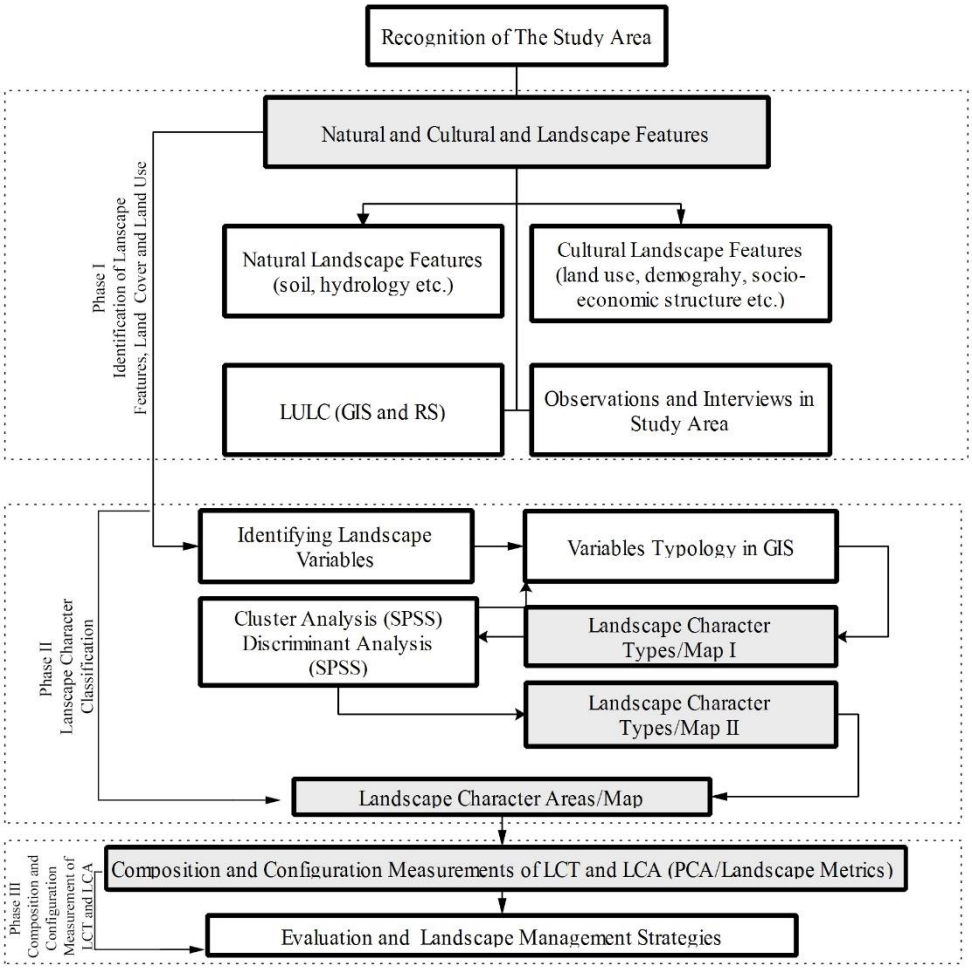

Figure 2- Flow chart of the study method

2.2.1 Phase I: Identification of Landscape features, land cover and land use

The natural and cultural landscape features of the basin are defined by the data obtained from the classification of the satellite image and the data obtained from the institutions.

In this study, 4 frames of RapidEye satellite images with a cloudiness rate of less than 10%, dated 06/2011, were used. The process of classifying satellite images consists of 3 stages. These are respectively image preprocessing, threshold-based object-based classification of image and accuracy assessment of classification.

Image preprocessing; this sub-stage consists of two processes in itself. These are respectively geometric correction and radiometric correction processes. ***Geometric correction;*** Since the RapidEye Ortho Level 3A satellite image used in the research was purchased with the orthorectification process completed, no additional geometric correction was required. ***Radiometric correction;*** Object radiations measured by the sensor system are affected by atmospheric conditions such as change in solar radiation, atmospheric dissipation and scattering, causing different pixel reflection values in different time zones and images obtained from different sensors. Atmospheric correction was applied to the multi-time satellite images used in the analysis under the radiometric correction title. In the study, FLAASH model is used in the atmospheric correction process, which allows the surface reflectance values to be obtained by deriving the atmospheric parameters such as surface reflectivity, surface altitude, water vapor content, aerosol and cloud optic thickness, surface and atmospheric temperatures (Görmüş et al. 2018). 4 frames of satellite images of 2011, whose radiometric and geometric corrections were made, mosaicking of satellite images were done and extracted according to the study area.

Threshold-based object-based classification of image; this technique was used to determine the meaningful pattern groups on the image or in other words, to separate each pixel in the image into different groups according to their spectral properties and to assign the pixel to the corresponding cluster according to the reflection values. Taking into account the purpose of the research and the spatial resolution of the satellite images, land use/land cover categories were determined for classification and classification was carried out. Determined land use/land cover categories for the research are settlement area, road, agricultural area, hazelnut garden, broad-leaved forest, coniferous forest, mixed forest, river, and barren land.

Threshold-based object-based classification process; In itself, it was carried out in 2 steps as segmentation phase and threshold determination phase.

Segmentation; According to the detail (class) inference targeted in the research, scale parameters, shape density values were produced, segmentation parameters were produced at different levels and with different algorithms. *Chessboard segmentation* algorithm is used to divide the image into identical segments in line with the value determined by the user. In this research, the scale parameter was specifically set to "1" to extract the linear classes (transportation networks and streams) at the pixel level on the image. "1" was used as the scale parameter that corresponds to 5x5 meters. *Multiresolution segmentation* algorithm, which clusters the objects on the image according to their similarities at different scales, was used by defining the scale parameter as 0.28, the shape parameter as 0 and the concentration parameter as 0.81.

Threshold determination; Threshold values were determined by establishing mathematical relationships between the bands for each land use/land cover category. In the research, the indices determined according to the land use/land cover categories and the mathematical operations performed to create the indices are as follows; Normalized Difference Vegetation Index (NDVI), Soil Adjusted Vegetation Index (SAVI), The Red-Edge Triangulated Vegetation Index (RTVI Core), and Normalized Difference Water Index (NDWI).

Accuracy analysis; The minimum accuracy for the study was determined as 80%. In the accuracy analysis, error matrices were created by examining the relation between KAPPA index class-based reference data (true ground control points 2011) and classification results. Ground control points used in the study were taken with GPS during the field studies carried out in June-August 2012 period and supported by photographs.

2.2.2. Phase 2: Landscape character classification

Identifying landscape character variables: To create landscape character analysis, it is necessary to identify the variables representing the landscape of the area. In the current study, the landscape elements representing the area are known as "landscape character variables" (Table 1). Biophysical and natural data were used to determine landscape character types at basin scale. Land form and location names were used in addition to landscape character types in determining landscape character areas.
The landscape character variables and sub-categories belonging to these variables in the landscape character analysis carried out on a basin scale are in Table 1.

Table 1- Basin scale landscape character variables

Landscape feature	*Type of variable*	*Variable Name*	*Sub-category*
Physical	Distinctive	Topography (T)	Altitude groups
			Slope steps
Natural	Distinctive	Hydrogeology (H)	Permeability State Porousness
Biological	Distinctive	Soil (S)	Large soil groups
Biological and human effect	Distinctive	Land cover/ land use (LU/LC)	Forest types
			Agricultural lands
			Settlement areas
			Rocky areas
Biological and human	Descriptor	Geomorphography	Definition of landform and land structure
Human effect	Descriptor	Cultural structure	Place names
Landscape	Indicative	Landscape indexes	Patch, class and landscape scale indexes

Variables Typology in GIS: Ordering of variables in the symbolic expression of landscape character types is in Figure 3. To ensure reliability and accuracy in the statistical classification of the variables in the basin scale, the ratio of variables in the basin were considered in scoring the sub-categories. Accordingly, the variable with the highest spatial ratio in the basin had the highest score. For example, in the topography variable hilly (100-499 m) areas were the most dominant ones in altitude groups. Therefore, they are represented with the number 3 (Table 2).

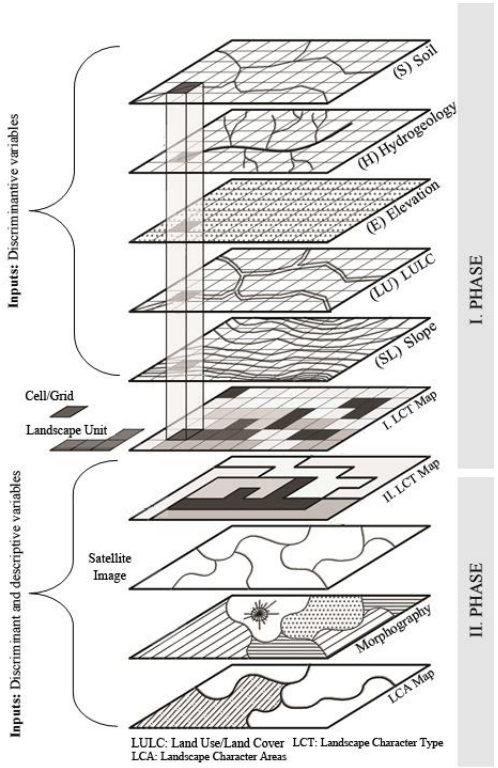

Figure 3- Phases of landscape characterization

Table 2- Variables and symbols used in determining landscape character types

Variable name	Sub-category	Criteria	Symbol
Elevation **(E)**	Elevation groups **(E)**	Plane (0-100m)	**1**
		Hilly (100-499m)	**3**
		Mountainous (500-1381m)	**2**
Slope **(SL)**	Slope steps **(SL)**	1-5: slightly sloping surfaces	**3**
		5-10: sloping surfaces	**2**
		10-20: moderately sloping	**4**
		20-50: remarkably sloping	**5**
		50-100: extremely sloping	**1**
Hydrology and hydrogeology **(H)**	Permeability state and porousness	Porous aquifer environment	**4**
		Karst aquifer environment	**3**
		Moderately permeable	**5**
		Low- permeable	**2**
		Impermeable	**1**
Soil **(S)**	Soil groups	Areas without a ground cover	**0**
		Alluvial soil.	**1**
		Grey-brown podzolic soil	**2**
		Red-yellow podzolic soil	**3**
Land cover/land use **(LU)**	Forest types	Mixed forest	**3**
		Leafy forest	**5**
		Coniferous forest	**2**
	Agricultural and settlement areas	Agriculture and settlement	**4**
	Rocky areas	Stony-open	**1**

Cluster analysis and Discriminant analysis: Clustering and discriminant analyses were utilized in order to categorize landscape characters. Landscape features for each unit square of the area and sub-sections are represented in the grid system as a hierarchical diagram and placed on the map, as well. So, it provides with polygonal combination of the unit squares with the same features. Afterwards, spatial maps of the area are superimposed and landscape units are these overlapping polygons.
Landscape types are formed by combining the landscape units defined. Units and polygons are classified via the Clustering Analysis and Discriminant Analysis. The overall aim of Clustering Analysis is to divide the data to be grouped by their

similarities and explain them. Based on the similarities between individuals or objects among all the variables in the study, grouping or clustering similar individuals into same groups and estimating to which group a new individual belongs is the basis of Clustering Analysis. (Hair et al. 1992; Tatlıdil 1996; Doğan 2002; Görmüş, 2012; Atik et al. 2015). Discriminant Analysis was used to measure the reliability of the classification carried out via Clustering Analysis (Figure 3).

Landscape character types/Map I: Grid cells were used as spatial units to identify the types (identifying the pattern of the classification). Each grid cell represents a polygon in GIS and integrates easier with other data. After the data sources were chosen and variables determined, variables were integrated into the grids by superimposing of data sets in GIS (Parametric method) and transferred into feature tables (Figure 3). In order to identify landscape types, the study area was divided into 30x30 m cells by a grid system. The variables were transferred into cells. Thus, each grid cell was characterized by the distinctive variables used for determining the groups in grid cells. 188,000 cells were obtained in the study. In the case that all types were spatially independent, 14,525 landscape units were obtained.

Landscape character types/Map II: As a result of clustering (unifying) cells of the same type in 14,525 landscape units, 345 landscape character types were determined. Landscape units belonging to the landscape character types were visualized on ArcMap 10.1 by color and name (1st Landscape character type map). By forming a landscape character type key, the code for each type was shown in the legend (Figure 4)

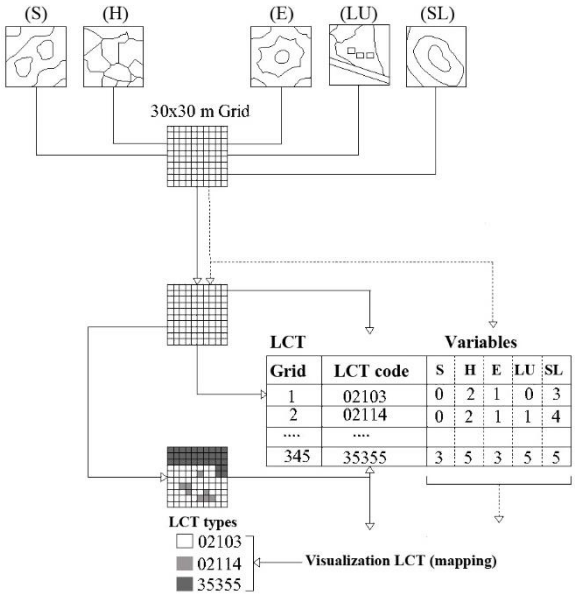

Figure 4- Typology of landscape types and landscape character type key

Landscape Character Areas Map: Character fields were defined by combining II. Map of Landscape character types with the land form and place names in Arc Map. 10.1 software.

2.2.3. Phase III: Composition and Configuration Measurements of LCT and LCA

The pattern of the landscape character types determined in the 2nd step was measured via landscape metrics (Figure 3). Of the configuration metrics defined in the Patch Analysis (Elkie et al. 1999; McGarigal 2002) module, patch density measured at class level, patch size metrics and patch shape metrics were used. Within the scope of the values obtained from the metrics, the composition and configuration of the landscape types around the basin and the distribution of landscape character types by area status were determined and identified. Abiding by the aims and objectives of the study, the landscape ecology principles (Turner 1989; Forman 1995; McGarigal & Cushman 2002; Mas et al. 2010) and metric set suggested by Botequilha-Leitão et al. (2006), a total number of nine composition and configuration metrics were used in the study. Patch Number: PN, Mean Patch Size: MPS, Mean Shape Index: MSI, Class Area: CA, Total Edge: TE, Edge Density: ED, Shannon's Diversity Index: SDI, SEI: Shannon Evenness Index and Shannon Index: SI.

The distribution of landscape character areas and landscape character types within the study area and their interaction with each other is determined. Composition and configuration of the landscape character types and landscape character areas identified are calculated via landscape metrics. This numeric data provides significant contributions in understanding direction of the change in landscape and the elements effecting the landscape character pattern. Based on landscape character type maps, landscape character area maps and numeric data of landscape metrics, problems that could arise from possible political decisions or change in land use and landscape planning and management strategies can be produced.

3. Results

3.1. Landscape character types and Landscape character areas

345 landscape character types were identified throughout the basin (Figure 5). At the end of the cluster analysis of the determined 345 LCTs, 21 landscape character areas (Figure 5) were obtained. The composition and configuration analysis of the identified landscape character types were measured using landscape metrics.

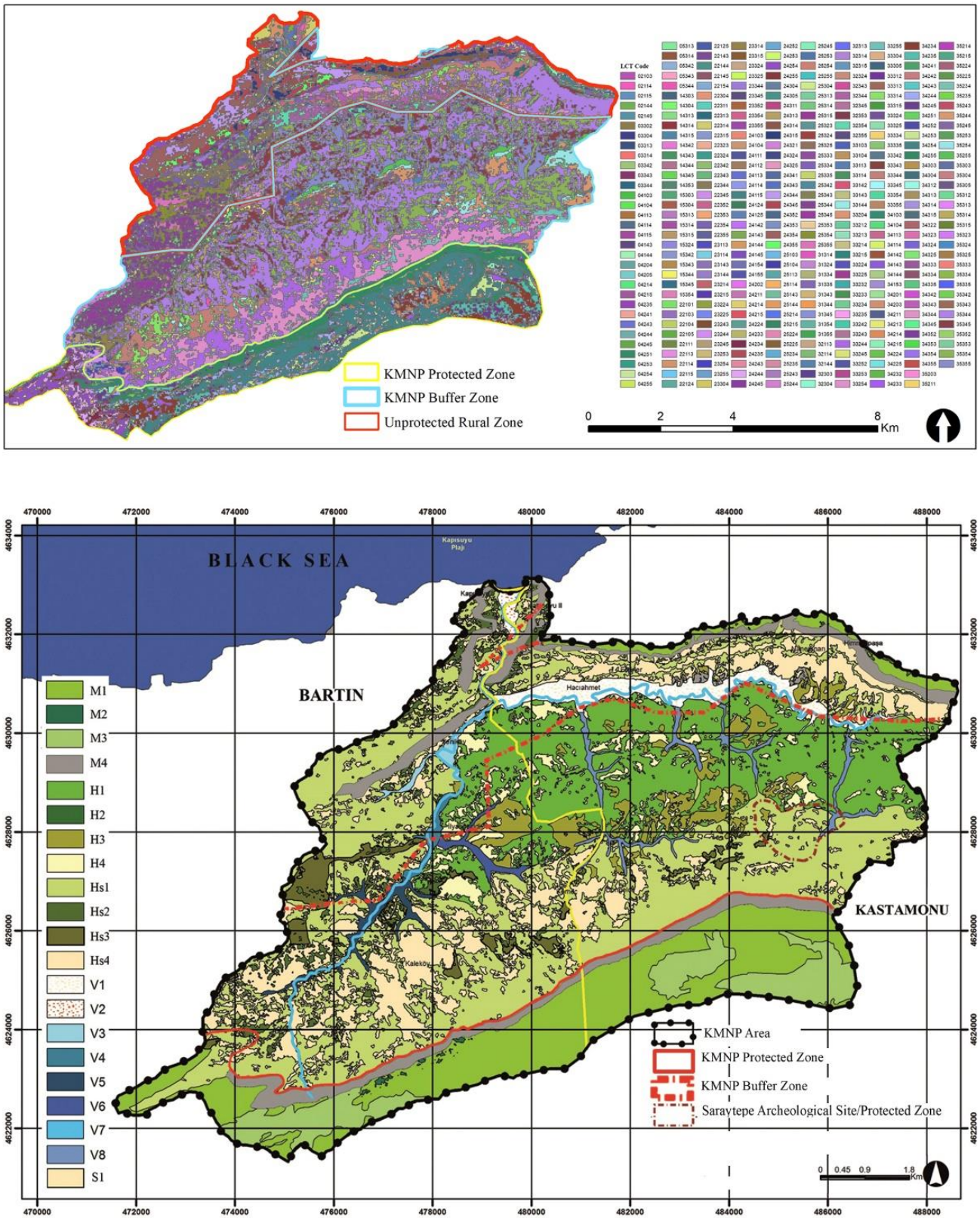

Figure 5- The codes and units of the landscape character types and landscape character areas throughout the basin

3.2 Composition and Configuration of LCT and LCA in the basin

Based on the metric values, disruption and patchiness of the landscape character types were interpreted for the whole basin and according to the protection status. Obtained metric values provide information on the structure and function of each character type in the basin. The distribution of the landscape character types in the basin were identified by their area status. The distribution of the landscape character types by area status (and their fragmentation states) were identified by considering ED, SI, PN, TE, MPS, MSI and CA (Figure 6) metrics together. Landscape diversity and patch condition in places with and unprotected rural zone status were identified by comparing SDI, SEI, PN and type number (Table 3 and Table 4). Although the surface area of KMNP Protected zone and unprotected rural zone have similar values, there are significant differences in landscape character type ratios and patchiness ratios. Patchiness in unprotected rural zone is higher. When SDI values are taken into consideration, the LCT diversity in unprotected rural zone is high. The areal distribution of landscape character types according to SEI values in KMNP Protected zone shows a more irregular structure compared to unprotected rural zone.

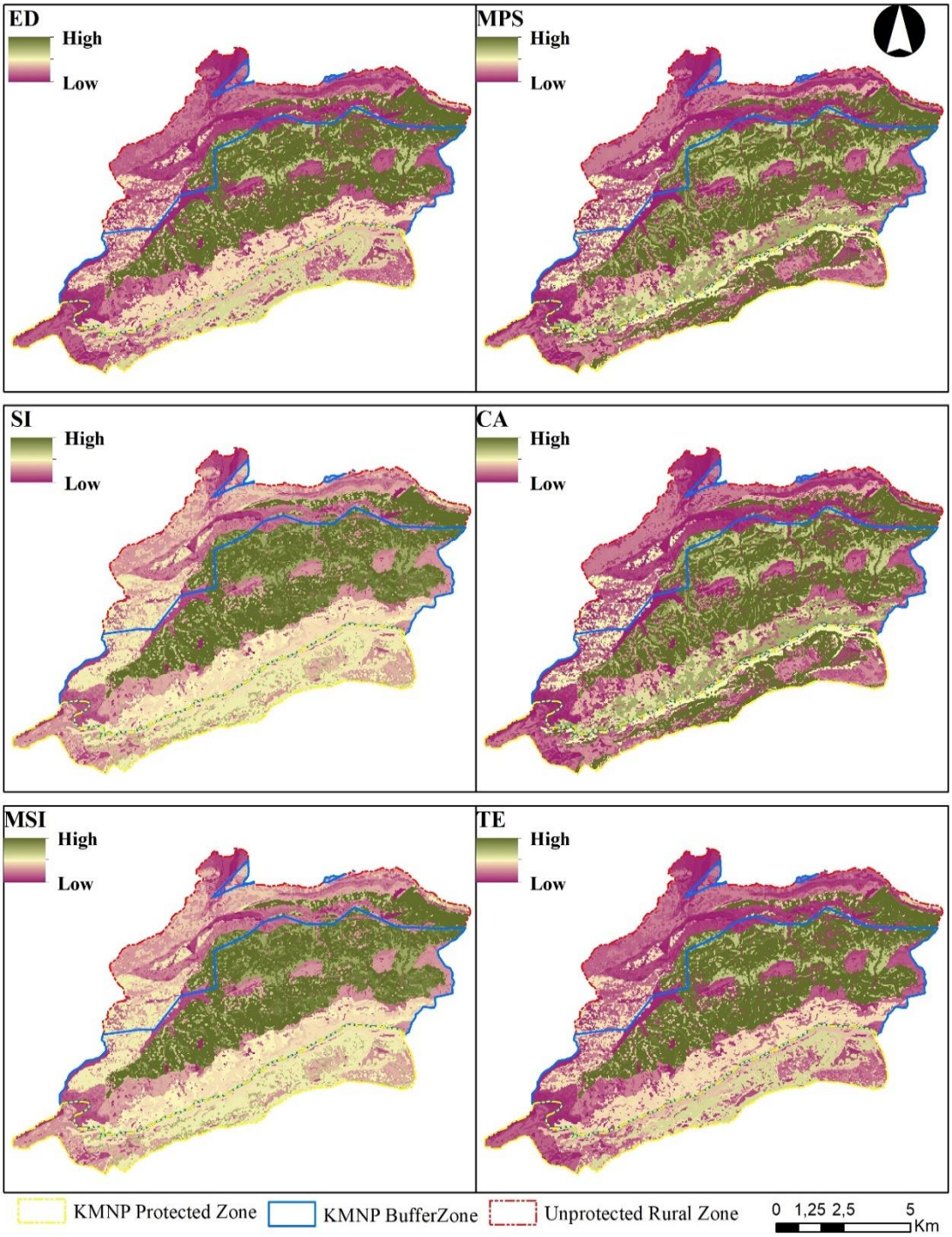

Figure 6- Landscape metrics value of LCT

Table 3- Landscape character type patchiness ratio and index values by area status

Areas (Surface/ha)	*LCT number*	*PN*	*SDI*	*SEI*	*LCT ratio in the basin (%)*	*Patchiness ratio in the basin (%)*
KMNP Protected zone (2.117)	61	2793	2.462685	0.599066	17.68	19.2
KMNP Buffer zone (5.650)	205	7598	3.125414	0.587152	59.4	52.3
Unprotected Rural Zone (2.827) (off Buffer zone)	252	4592	3.887638	0.703081	73	31.6
Archeologic Area (0.146)	24	199	1.770577	0.557126	6.9	1.1
Basin Area (10.600)	345	14525	3.86	0.66		

LCT: Landscape Character Type; PN: Patch Number; SDI: Shannon's Diversity Index; SEI: Shannon Evenness Index

Table 4- LCA measurements

Symbol	*Landscape Character Areas (LCA)*	*Patch density and patch size*			*Landscape diversity*		
		MPS	*PN*	*CA*	*SDI*	*SEI*	*PN (LCT cell)*
M1	Küre Mountains Broad-Leaved Forest	136.9	1	136.9	2.1	0.5	156
M2	Küre Mountains Coniferous Forest	0.5	20	0.9	1.9	0.8	54
M3	Küre Mountains Mixed Forest	17.5	3	52.7	2.1	0.6	48
M4	Küre Mountains Rocky and Carstic Landscape Character Area		5		2.9	0.6	179
H1	Kapısuyu Basin Hills Area Broad-Leaved Forest	1.2	134	165.8	2.3	0.5	464
H2	Kapısuyu Basin Hills Area Coniferous Forest	0.9	412	36.8	2.6	0.6	1089
H3	Kapısuyu Basin Hills Area Mixed Forest	0.9	43	42.8	2.3	0.5	395
H4	Kapısuyu Basin Hills Area Foothill Agriculture	1	469	46.1	1.9	0.4	129
Hs1	Kapısuyu Basin Broad-leaved forest on the slope	0.4	618	307.1	3.1	0.6	2826
Hs2	Kapısuyu Basin Coniferous forest on the slope	0.6	461	29.5	3.8	0.7	1296
Hs3	Kapısuyu Basin mixed forest on the slope	0. 9	24	223.9	3.1	0.7	77
Hs4	Kapısuyu Basin Foothill Agriculture	0.19	959	189	3.1	0.6	2826
V1	Emirler Valley Bottom Agriculture	26.2	1	26.2	2.9	0.6	79
V2	Kapısuyu Valley Bottom Agriculture	2.9	1	2.9	1.9	0.6	22
V3	İdare Stream Valley		1		3.2	0.8	57
V4	İlyas Stream Valley		1		2.9	0.8	34
V5	Başköy Stream Valley		1		2.7	0.7	45
V6	Atak Stream Valley		1		2.1	0.6	28
V7	Kapısuyu Stream Valley		1		1.9	0.6	18
V8	Hills Area Furrow Valleys		5		1.6	0.5	52
S1	Kapısuyu Sand Dune and Outlet		1		-	-	-

MPS: Mean Shape Index**; PN:** Number of Patch**; CA:** Class Area**; SDI:** Shannon's Diversity Index**,** Shannon's **Evenness Index LCT:** Landscape Character Type

Landscape diversity is higher in unprotected rural zone compared to the KMNP Protected zone and the areal distribution of landscape character types is more regular. According to the KMNP Protected zone and KMNP Buffer zone values, the patchiness ratio in the KMNP buffer zone as well as the landscape diversity is high and the distribution of landscape character types is more irregular. Although the Saraytepe archeological area is small area, the number of LCT it includes is high. The landscape character types' diversity in this area is small and the areal distribution of these types is irregular. The patchiness of the landscape character types in KMNP Protected zone, KMNP Buffer zone and unprotected rural zone is high in KMNP Buffer Zone, low in rural areas that off buffer zone and lowest in KMNP Buffer zone. The class area and patch number of these types have the highest value in national park protection area. The patchiness level of the types in KMNP Buffer zone and unprotected rural zone that is out of the buffer zone is highest in KMNP Buffer zone. (Table 3, Table 4).

4. Conclusions and Discussion

345 landscape character types were determined in the basin scale. The fact that the landscape character types are high indicates that physical, natural, biological and cultural factors are intense in basin. It is clear that topographic change has an impact on the formation of landscape character types. However, when considering the variables taken into account in determining LCT, it is clear that the most important factor providing the LCT diversity is human impact. When LCT correlation is examined in all three regions and between regions, it is concluded that the number of LCTs, patchiness in landscape and landscape regularity increases

with the human effect. It is seen that human intervention causes an increase in landscape character types and patchiness in landscape, and forms regularity in landscape (spatial order of land uses). The unprotected rural area is an area where the intervention is settled, however, the intervention shifts to the KMNP buffer zone, causing serious damage to the habitats due to the increase in the rates of patchiness in this zone. It is very clear that the buffer zone, which is designated to protect the protected area from cultural interventions of its surrounding, could not provide the protection duty. It is understood from the number of LCTs that the Saraytepe archaeological site in the buffer zone was also exposed to intervention due to the search for treasures. Inclusion of this area in the KMNP protection zone would be a more accurate approach in terms of protection. When the LCT number, irregularity and patchiness transitions between all three regions are taken into account, it is seen that LCA is useful in monitoring the transitions of areas with different conservation statuses and, if applied at the basin scale, can provide a more meaningful, accurate and rapid contribution to nature protection planning.

One of the most important problems of protected areas in Turkey is the landscape fragmentation caused by the usage pressure. It has been observed that efficient outputs will be obtained with landscape character analysis approach on basin scale about determining the interaction of the protected area with its environment in the planning of protected areas in Turkey.

Using LCA at the basin scale, where everything is interrelated and interconnected, enables planning decisions to be made in accordance with the scale. Determining the interaction between the pattern and the process at the appropriate scale is the most important situation in making the right decisions in landscape planning. Lack of basin management on a local and regional scale and the fact that the concept of "the bigger the better" of the scale is being the basic rule of decision makers may cause the ecological land use planning to be largely ignored. Habitats and ecosystems become more sensitive as a result of not using the appropriate scale. The fact that national, regional and local planning objectives are in conflict with each other in the management of KMNP and its environment (buffer zone), which is one of the areas with sensitive ecosystems, causes landscape fragmentation on the area. National park management interacts with national NGOs instead of local NGOs, developing conservation and use policies in the national park. The fact that these policies, which are implemented in specific areas in the national park, regardless of national and regional plans (The policies developed by the national park management with national NGOs can be in conflict with the national-regional plans and targets), do not find any response in the local population, causes an increase in usage pressures on the area. Because in these implementations, scale, landscape pattern, landscape process was applied specifically regardless of the process of the economic patterns developed by the local people with the protected area. Even if the implementations developed with this understanding are the correct uses, they attract the reaction of local communities, especially small groups that do not have economic investment power, and causes more damage to the habitats.

Landscape character analysis and landscape metrics were assessed together in the basin scale and thus, a new approach in determining the pattern-process interaction in protected area and its environment has been developed. In this approach the most dominant features of the basin were evaluated by raster and vector. Studying the area by dividing it into grid vectors made map production faster. No problems were encountered in calculating the units, obtained by combining similar grid cells, through landscape metrics. Assessing the pattern-process interaction with the environment of protected areas on a basin scale is necessary and important for ecology-based landscape planning. In this assessment, realizing the landscape classification with the landscape characterization technique allows for faster assessment of risk factors and monitoring of land use change. In addition, the applied technique and produced data can be used in landscape protection, protection of biological diversity and landscape management studies.

Landscape character analysis carried out within the scope of this study is carried out on grid base (at 30x 30 m cell level). Because this approach will save time in processing any map changes, it will make decision making process faster and more objective. Planning is established on decisions.

This study shows that the multivariate and multi-scale structure of landscape character analysis is a positive approach in the assessment of the protected areas. In addition, associating landscape character classification with landscape ecology through landscape metrics provides the opportunity to interpret landscape pattern and landscape process. Assessment of biophysical and anthropogenic variables by landscape character analysis technique and estimating the ecological structure of the area by measuring the obtained character types and character areas through landscape metrics gives a positive acceleration especially in protected area management.

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Spectroscopic Characterisation and Elemental Composition of Biochars Obtained from Different Agricultural Wastes

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ABSTRACT
The use of biochar (BC) has an environmental importance in terms of climate change, soil fertility, waste management and energy generation. The purpose of this study was to reveal some of the structural characteristics of BC produced from agricultural wastes by employing spectroscopic techniques within a short time frame. The BCs were produced via slow pyrolysis at 300 °C from four feedstocks: tea waste (TW), hazelnut husk (HH), rice husk (RH) and poultry litter (PL). The pH of plant-derived BC was alkaline (pH: 7-9), and the pH of manure-derived BC was strongly alkaline (pH: 10.1). PLBC has the highest (4.67 dS m^{-1}) electrical conductivity (EC) when compared to other BC materials. According to the X-ray fluorescence (XRF) analysis method, organic compound contents of TWBC and HSBC were found to be higher than the other two BCs, while the other two BCs (RHBC and PLBC) were richer in mineral content. TWBC and HHBC were composed of more mineral elements when compared to RHBC and PLBC, but the latter two were still rich in minerals. The surface area of RHBC was found higher (12.9 m^2 g^{-1}) than other BC materials. According to the X-ray fluorescence (XRF) analysis method, the total element content of PLBC was found higher than the other BCs. In addition, the silicon (Si) content of RHBC was considerably higher (16.4%). In PLBC's XRD diagram: quartz (SiO_2) at 3.41 (Å); calcite ($CaCO_3$) at 3.96, 2.94 and 1.91 (Å); sylvine (KCl) at 3.06 and 1.85 (Å); and whitlockite ($[Ca, Mg]_3 [PO_4]_2$) at 2.78 and 2.17 (Å) were found. In HHBC and RHBC diagrams, partially crystallized carbon (CryC) peaks were mainly observed between 1.20 and 2.34 (Å), and cristobalite peaks (i.e., amorphous SiO_2) were observed at 3.91 and 3.40 (Å).

Keywords: Biochar, Plant nutrients, XRD and XRF Spectroscopy, Spectroscopic characterisation

1. Introduction

Biochar (BC) is a material that is produced as a result of the conversion of various wastes from agricultural, environmental or industrial sources into coal by heating them in an oxygen-free environment (i.e., by pyrolysis). Because the waste is burned in an oxygen-free environment, it becomes a solid material that contains a large amount of organic carbon (OC). Moreover, its pH can be either acidic or alkaline, it is porous, and it has a high adsorption capacity (Lehman et al. 2006; Chan et al. 2007; Verheijen et al. 2010). In addition to its improving effects on soil properties, BCs can reduce the negative effects of pesticides, heavy metals and hydrocarbons in soil (Ogawa et al. 2006; Glaser et al. 2009; Beesley et al. 2011; Cabrera et al. 2011; Tang et al. 2013).

Although there are many studies on various feedstocks and the BCs derived from these studies on different types of feedstocks, different pyrolysis conditions and different use areas of BCs are still ongoing in different countries (Enders et al. 2012; Ahmad et al. 2013; Zhao & Nartey 2014; Prakongkep et al. 2015). Animal and plant wastes are often used as feedstocks in BC production. However, main chemical and structural properties of the biomass material can affect the composition of the obtained BC and, hence, its behaviour in soil (Downie et al. 2009). Lehmann & Joseph (2015) suggested that BC was the best material for soil improvement when compared to other organic soil conditioners and the effects of organic soil conditioners were directly linked to their properties, such as their pH, surface area, ash content and plant nutrient content.

The type and amount of materials that are used as feedstocks for BC production may vary depending on the country. Scott et al. (2014) indicated that since each feedstock contained different amount of nutrients, plants could have used them at different levels. Many studies have investigated the use of BC in different areas, other than for agricultural purposes, such as for water treatment, briquette making and energy production (Nakka 2015). Recent studies have shown that chemical methods for analysing BC are not preferred by researchers because they are both time consuming and costly; instead, because of recent technological developments, physical methods are increasingly preferred, as they are less costly and give quick results. For inert materials such as BC, more detailed and precise results are obtained by spectroscopic analyses methods. Recently, advanced

spectroscopic techniques have been used to characterise BC (Igalavithana et al. 2017), such as the X-ray fluorescence (XRF) and X-ray diffractometer (XRD) techniques, which had the lowest cost and user friendly. Hammes et al. (2007) listed several methods for BC characterisation, some of which are not only time consuming (about 24 h) but also quite expensive. In addition, some of these methods consume a large amount of chemicals. Bellon-Maurel & McBratney (2011) reviewed the pioneering studies on the adoption of spectroscopic techniques, which were found to be simpler and more cost-effective ways in which to measure BC characteristics.

The structure of BC derived from various feedstocks and similar feedstocks under different temperatures and duration may be different. For this reason, it is very important to determine the BC structures derived from different feedstocks that uses spectroscopic characterisation processes to obtain fast, practical and more accurate results. XRD and XRF spectroscopic techniques were used in this study. XRD analysis is advantageous for BC that has a high crystallinity because this analysis method provides both three-dimensional information (e.g., crystallite size and interlayer spacing) and information related to other characteristics of carbonaceous materials such as BC (Lehmann & Joseph 2015). Minerals in feedstock mostly determine the ash fraction composition of BC; however, most inorganic components do not volatilize at the temperature typically used in the pyrolysis process (105 °C). As such, one way of determining the elements that are present in BC and determining their quality is to use XRF spectroscopy. XRF analysis data is usually presented as the weight percentage of the most widely recognized elemental oxide. The structure of BC derived from the same feedstocks but in different countries may be different. For this reason, studying the BC characterisation data from different countries would be useful for researchers, as it would allow them to form a library of BC characteristics. The purpose of this study was to reveal some of the structural characteristics of BC produced from different types of agricultural wastes in a single country, using appropriate spectroscopic techniques to determine their general chemical composition in a short timeframe.

2. Material and Methods

2.1. Materials

This study was carried out to transform different agricultural wastes into BC and to determine the roles of these obtained BCs in agricultural sustainability using spectroscopic methods. The feedstocks were obtained from various regions in Turkey: tea waste, TW (*Camellia sinensis* L.) from the General Directorate of Tea Enterprises (CAYKUR); hazelnut husk, HH *(Corylus avellana* L.) from the Hazelnut Producers Union (PANKOBIRLIK); rice husks, RH (*Oryza sativa* L.) from a commercial enterprise in the Çorum Osmancık region; and poultry litter, PL from the Ankara University Research and Application Farm. After the feedstocks were sieved through a 0.5 mm sieve, they were dried in a 105 °C oven for 24 h before pyrolysis and underwent slow pyrolysis in an electric oven at 300 °C, in an oxygen-free environment. The temperature of the oven gradually changed from 10 °C d^{-1} to 300 °C d^{-1} within 2 h, at which time BC production was completed. The BCs were prepared for analysis 10 h after pyrolysis was completed. The degree of mineral composition and crystallinity of the BCs were determined by XRD at the laboratories of the Earth Sciences Application and Research Centre (YEBIM) of Ankara University.

2.2. Methods

According to Rajkovich et al. (2012), the pH of the BCs was measured (1:20 water suspension) with a pH meter (Mettler Toledo, FP20), and the electrical conductivity (EC) was measured (1:20 water suspension) with an EC meter (Consort, C 3010). According to ASTM (2007), the ash and organic matter (OM) content of the BCs were determined by incineration at 550 °C. The total nitrogen (N) content of BCs was determined using the Kjeldahl method (Bremner 1965), the total potassium (K) content was determined using flame photometry (Rayment & Higginson 1992) and the total phosphorus (P) content was determined using the yellow vanadomolybdophosphoric acid method (Kuo 1996). The surface area was measured on a surface analyser (Nova, Quantachrome Instruments) at 77 K, while the N sorption was determined using the Brunauer-Emmett-Teller (BET) equation (Brunauer et al. 1938).

Multi-element concentration was determined using polarised energy-dispersive XRF (PED-XRF; Spectro XLAB 2000). A total of 4 g of powdered BC samples were mixed with 0.9 g of wachs, which was used as a blinder, and the mixture was pressed at 20 N in an automatic press machine to get a 32 mm diameter pressed-disc pellet (Koralay 2010). Wachs is a type of alkane paraffin that contains hydrocarbons (CH), meaning that the wachs has an organic chemical composition and does not contain any inorganic content. The author who used the PED-XRF spectrometer also separately analysed the wachs alone and the sample combined with the wachs. In this way, it was possible to see that the wachs did not change the chemical composition of the samples.

The spectrometer was equipped with a 400 W rhodium (Rh) anode X-ray tube and a 0.5 mm beryllium (Be) side window. The detector in the spectrometer was silicon (Si; lithium [Li]), with cooled liquid N_2 and a resolution of < 150 eV at Mn Ka, 5000 cps (Kadıoğlu et al. 2009; Güllü & Kadıoğlu 2017). Diffraction patterns were collected with an Inel Equinox 1000 instrument that was equipped with a cobalt (Co) tube at a wavelength of λ= 1.788970 Å and a 120° curved position-sensitive detector (CPS-120). The data was converted to numeric values relevant to a copper (Cu) cathode (λ= 1.541874 Å) for an easy evaluation. The raw data was smoothed, and the baseline was corrected.

3. Results and Discussion

3.1. Properties of the materials

General properties of the feedstocks and BC materials are shown in Table 1 and Table 2. In terms of the general properties of the feedstocks, it was seen that the highest pH occurred in rice husk (RH) and the lowest in tea waste (TW), while the highest EC value was found in poultry litter (PL) and the lowest in rice husk (RH). As for the organic matter (OM) content, the highest value was seen in TW and the lowest in RH, while the highest value for N content was in PL and the lowest in RH. It was further found that the highest P value was in PL and the lowest in HH, while the highest K value was in PL and the lowest in TW.

Table 1- General properties of the feedstocks

Raw materials	*pH (w v^{-1})*	*EC (dS m^{-1})*	*OM (%)*	*Org C (%)*	*N (%)*	*P (%)*	*K (%)*
TW	5.22	1.17	96.3	55.9	3.19	0.33	0.82
HH	5.75	1.90	94.6	54.9	2.94	0.16	0.93
RH	7.24	0.48	56.4	32.8	0.92	0.36	1.35
PL	7.31	3.88	78.0	45.2	4.33	0.84	4.83

TW: Tea waste; HH: Hazelnut husk; RH: Rice husk; PL: Poultry litter

BC derived from plant wastes (i.e., [TWBC], [HHBC] and [RHBC]) showed neutral or low alkalinity pH, with no salinity problem, while the pH value of BC derived from animal waste (i.e., [PLBC]) was found to have strong alkalinity (pH: 10.1) and to had salinity (Table 2). The EC value of PLBC (4.67 dS m^{-1}) was 6 to 11 times higher than that of the other BCs. Past studies have reported that the EC value of PLBC is generally higher than those of other plant-derived BCs, with values varied from 5.66 dS m^{-1} to 33.7 dS m^{-1} (Evans et al. 2017; Clemente et al. 2018). The high EC value indicates that PLBC is more readily soluble, to some extent, than are other BCs.

The high pH, EC and organic C values of PLBC are compatible with the results of a study by Sikder & Joardar (2019). It was also stated in previous studies that the pH, salinity and N values of animal-derived BCs are higher than those of plant-derived BCs (Scott 2014 et al; Jassal et al. 2015). It is possible to get an idea of the mineral matter contained in BC by reviewing its ash content. Higher ash content was detected in the samples of plant-derived RHBC (33.81%) and animal-derived PLBC (33.83%) than was detected in the other samples, indicating that RHBC and PLBC had a higher mineral content. The ash content of TWBC (7.96%) and HHBC (5.51%) were quite low. A comparison of the raw and BC materials showed that the pH of all materials increased after the BC creation process, while the N, pH and K values decreased. The high mineral content in all the BCs indicated that these materials were rich in various elements including plant nutrients. However, when the XRD results were analysed, it was seen that these BCs contained different mineral substances. For example, PLBC contained minerals such as sylvine (KCl) and calcite ($CaCO_3$), while RHBC contained cristobalite (SiO_2), which could be a result of Si rather than plant nutrients. The mineral content levels were much lower (mostly below 20%) in BCs derived from plant residues than the animal-derived BC (Zhao et al. 2013). Some research revealed that, according to data through various processes (including slow pyrolysis), BC derived from PL had the highest ash content (Field et al. 2013; Qambrani et al. 2017). Conversely, the ash content of BCs derived from wood was low (< 10%). Products with ash content is greater than 50% are included under the category of pyrogenic carbonaceous material and are not considered BCs (European Biochar Certificate [EBC] 2015).

Table 2- Physicochemical properties of the BCs

BC materials	*pH (w v^{-1})*	*EC (dS m^{-1})*	*Ash (%)*	*OM (%)*	*Org C (%)*	*C:N*	*N (%)*	*P (%)*	*K (%)*	*Specific surface area (m^2 g^{-1})*
TWBC	7.42	0.78	7.96	92.1	53.5	22.2	2.40	0.04	0.30	8.26
HHBC	7.33	0.42	5.51	94.5	54.9	77.3	0.71	0.03	0.10	3.68
RHBC	7.93	0.74	33.8	66.2	38.5	47.6	0.81	0.08	0.22	12.9
PLBC	10.1	4.67	33.8	66.2	38.5	9.08	4.24	0.09	0.40	7.36

TWBC: Tea waste biochar; HHBC: Hazelnut husk biochar, RHBC: Rice husk biochar; PLBC: Poultry litter biochar

Black color was dominant in the BC samples obtained in this study. Armynah et al. (2018) pointed out that the colour of samples is related to the ash content. There was also a high ash content in grey BC samples. It was thought that the ash contents of PLBC and RHBC BCs were higher than those of the others because of the presence of calcite ($CaCO_3$) and quartz (SiO_2). These results are consistent with the XRD results. PLBC was similar to RHBC in terms of its organic matter (OM) and ash content levels (%). TWBC and HHBC had high levels of organic compounds, while RHBC and PLBC were found to be rich in terms of mineral content (Table 2). This may be due to the volatility of the OM in the plant-based feedstocks, such as the easily decomposable organic C found in TW and HH. This is followed by TWBC, RHBC and HHBC, respectively. The total N value of TWBC was higher than that of the other plant-derived BCs; this can be attributed to the higher content of green plant parts

found in the feedstock, as the tea is grown in humid regions and is a shrub plant. The N values of the other two plant-derived BCs were quite low.

PLBC and TWBC had the highest N contents of the BC materials studied (Table 2). However, when comparing these to the N contents of the feedstocks, their N contents before being processed into BCs were greater. In other words, the N contents of the materials decreased through the BC process. A transition in the chemical structure of the remaining N in the BC also occurred following the loss of N at higher temperatures. The volatilisation of most N and sulphur (S) compounds occurs above 200 °C and 375 °C, respectively, whereas the volatilisation of K and P occurs between 700 °C and 800 °C, respectively (DeLuca 2009). The amount of K in TWBC was found higher than the other plant-derived BCs. Scott et al. (2014) stated that plant-derived BC contains lower levels of N and P than does animal-derived BC and that the nutrition content of plant-derived BC is not high enough to allow it to be used for fertilisation.

In the current study, the highest surface area among the samples was found to occur in RHBC (12.9 $m^2 g^{-1}$). Wang et al. (2018) stated that Si content-rich BCs have a porous structure after Si release. In the present study, the surface area values of TWBC and PLBC were similar (8.26 and 7.36 $m^2 g^{-1}$), while the lowest surface area was found in HHBC (3.68 $m^2 g^{-1}$). Fang and Xu (2014) reported that 26.7% of BC samples derived from hazelnut shells are made of cellulose, 30.29% of hemicellulose and 43.01% of lignin. Thus, it was assumed that the low surface area of HHBC was due to the fact that it had more lignin in it than did the other BCs. Among other feedstocks, a more C-rich BC can be derived from woody biomass, which can have different amounts of lignin, cellulose and hemicellulose but which has small amounts of other inorganic compounds and organic extractives (Suliman et al. 2016). Xu & Chen (2013) found that the higher the content of lignin and minerals a woody biomass has, the more BC it can produce. Hence, woody biomass is one of the most important sources of BC. The dynamic degradation of organic materials and vascular bundles, and the formation of channel structures that occurs during pyrolysis, may cause a rise in the outer area and pore volumes of BC (Kim et al. 2013; Li et al. 2013). BC derived from rice straw is different from that derived from other feedstocks, as it has a low amount of lignin and a high amount of Si and K (Bourke et al. 2007; Keiluweit et al. 2010). The most important contrasts between a biomass and the BC derived from it are the surface area, pore structure (i.e., micropores, mesopores and macropores) and physicochemical properties such as composition, elemental makeup and ash content. In all cases, however, BCs contain more ash and carbon than do raw samples (Apaydın-Varol & Pütün 2012).

3.2. XRF spectroscopy

In terms of the total element content of the BC samples, as determined by XRF, the PLBC was the richest in terms of mineral contents (Table 4). In general, the plant-derived BCs had a much less favourable nutrient content than the animal-derived BC. The ratios of C/P and C/N of most wood-based and nut-based BCs are remarkably high. On the contrary, these ratios were much lower in BCs derived from manure, crop and food waste. BCs derived from manure were the most nutrient-rich, particularly in terms of C and P. Condensed wood-derived BCs were often the most nutrient- and ash-rich, as this material was C-rich (Singh et al. 2010).

The nutrient content of a BC is highly dependent on the type of material used as a feedstock. For example, the N and P content is usually highest in manure-derived BCs, followed by those derived from grass and wood; however, the C content is usually higher in wood-derived BCs, than that of grass- and manure-derived BCs (El-Naggar et al. 2019). The total element content of a BC does not always reflect the amount of plant nutrients available for plant growth, as this also depends on the pyrolysis temperature (Gunarathne et al. 2017). Elements such as calcium (Ca) and strontium (Sr) sometimes occur in PLBC as a result of some of the minerals included in feed. For example, various minerals, such as limestone and zeolite, are added to feeds to meet the nutritional needs of the chickens (Bintaş 2014); because the digestive system of chickens is weak, toxic elements (e.g., tin [Sn], lead [Pb], Cu, zinc [Zn], chromium [Cr] and iron [Fe]) that are difficult to digest are thrown out directly in the faeces.

Si is the most abundant element in soil, so all plants have different amounts of Si in their tissues, depending on the species. Rice is known to absorb more Si from the soil, for metabolic activities, than does any other plant, which explains the high Si content of RHBC (16.4%) found in this study. The Si content of the plants varies from 0.1% to 10%, depending on the species (Tamai & Feng Ma 2003). While some plants contain 1% to 2% Si (dry matter), rice (including the stem and leaves) contains 10% to 20% Si. RHs are composed of ash, Si and C, with Si protecting the plant's C from degrading via encapsulation (Wilding et al. 1967). The sulphur (S) content of all the BCs were found to be low, as C, N and S evaporate with increasing temperatures; however, micronutrients have been reported to increase their availability due to low evaporation (Chan & Xu 2009).

In their study on BCs produced from 14 waste materials, Prakongkep et al. (2015) stated that plant nutrients mostly form in crystalline minerals on the surface or cell structures of BCs. In the present study, the most significant difference in the microelements of the four BCs was observed in relation to Zn and Cu (Table 4); although the Zn and Cu contents of the other BCs were not very low, the Zn (1098 mg kg^{-1}) and Cu (206.9 mg kg^{-1}) contents of PLBC were 14-28 times and 8-17 times higher than those of the other BCs, respectively.

During the XRF measurements, each sample was measured three times, and the average of the measurements was given as the result. The spectrometer was calibrated using two standard rocks, G01-GSD-09 and K04-NIST-2704, based on the certificated concentrations of the elements under investigation (Table 3). The standard samples were run through the spectrometer on a different date for a full analysis, and the concentrations of the elements measured this time are shown in Table 3. XRF data is more reliable than is experimental data because of the use of a standard reference material for the measurements and integration of the calculations.

Table 3- The concentration of certificated elements in the standard rocks used in the PED-XRF analysis for calibration of the spectrometer, and the concentration of standard sediments measured by PED-XRF in the main analysis

Elements	*Unit*	*Certificated concentration G01-GSD-09 (Sediment)*	*Measured concentration G01-GSD-09 (Sediment)*	*Certificated concentration K04-NIST-2704 (River Sediment)*	*Measured concentration K04-NIST-2704 (River Sediment)*
Na	%	0.755 ± 0.084	0.79	0.201 ± 0.076	0.36
Mg	%	1.824 ± 0.024	1.76	1.369 ± 0.022	1.98
Al	%	5.972 ± 0.018	5.10	7.006 ± 0.02	8.26
Si	%	28.17 ± 0.03	27.98	25.78 ± 0.03	26.28
P	%	0.0853 ± 0.0013	0.091	0.1274 ± 0.0014	0.23
S	µg g^{-1}	365 ± 4.4	371	4116 ± 16	4287
Cl	µg g^{-1}	95.6 ± 1.2	92.87	186.6 ± 2	191
K	%	1.744 ± 0.004	1.65	2.152 ± 0.004	2.95
Ca	%	4.252 ± 0.006	4.65	2.739 ± 0.004	3.10
Ti	%	0.5349 ± 0.0013	0.43	0.4629 ± 0.0012	0.52
V	µg g^{-1}	93.7 ± 4.3	92.76	104.6 ± 4	106.20
Cr	µg g^{-1}	162.7 ± 1.5	160.72	177.1 ± 1.4	171.26
Mn	%	0.06287 ± 0.00024	0.072	0.06069 ± 0.00022	0.0762
Fe	%	3.528 ± 0.005	3.80	4.277 ± 0.006	5.72
Co	µg g^{-1}	13.9 ± 1.9	14.82	27.1 ± 2.7	28.22
Ni	µg g^{-1}	33.4 ± 1.1	34.27	44.5 ± 1.2	45.29
Cu	µg g^{-1}	36 ± 0.9	35.72	100 ± 1.4	102.21
Zn	µg g^{-1}	71.8 ± 1	70.65	422.5 ± v	430
Ge	µg g^{-1}	1.4 ± 0.3	1.22	2 ± 0.3	2.77
As	µg g^{-1}	10.3 ± 0.5	9.82	23.5 ± 1.1	25.76
Se	µg g^{-1}	0.3 ± 02	0.1	0.5 ± 0.2	0.9
Br	µg g^{-1}	0.8 ± 0.2	0.92	6.1 ± 0.2	8.62
Rb	µg g^{-1}	80.6 ± 0.4	78.92	100.8 ± 0.5	103.71
Sr	µg g^{-1}	166.1 ± 0.6	168.21	128.6 ± 0.5	131.23
Y	µg g^{-1}	26.3 ± 0.4	27.87	30.5 ± 0.4	32.82
Zr	µg g^{-1}	381 ± 0.9	376.23	281.9 ± 0.7	287.5
Nb	µg g^{-1}	15.5 ± 0.2	14.22	12.8 ± 0.2	13.77
Mo	µg g^{-1}	1.2 ± 0.2	1.67	4.7 ± 0.2	5.90
Cd	µg g^{-1}	0.5 ± 0	0.4	2.9 ± 0.2	3.98
In	µg g^{-1}	0.7 ± -0.4	0.62	0.7 ± 0	0.21
Sn	µg g^{-1}	3.1 ± 0.1	3.98	9.6 ± 0.2	11.62
Sb	µg g^{-1}	1 ± 0.1	1.77	3.7 ± 0.2	4.66
Te	µg g^{-1}	1.2 ± 0	1.90	1.2 ± 0	1.87
I	µg g^{-1}	1.4 ± 0.4	1.0	1.3 ± 0.4	1.90
Cs	µg g^{-1}	7.1 ± 0.5	6.12	5.2 ± 0.5	7.72
Ba	µg g^{-1}	430.1 ± 1.5	439	421.6 ± 1.5	430.23
La	µg g^{-1}	42.3 ± 1.3	45.43	29.5 ± 1.3	31.43
Ce	µg g^{-1}	81.4 ± 1.7	83.87	58.6 ± 1.7	60.9
Nd	µg g^{-1}	43.9 ± 2.9	44.93	27.6 ± 2.7	28.40
Hf	µg g^{-1}	7 ± 1.8	6.73	9.5 ± 2.3	11.04
Ta	µg g^{-1}	5.8 ± -5	5.44	8.0 ± -5.2	9.10
W	µg g^{-1}	2.7 ± 0	2.1	4.2 ± -2.4	5.62
Hg	µg g^{-1}	0.8 ± 0	0.98	0.7 ± 0.4	0.4
Pb	µg g^{-1}	22.9 ± 0.6	22.83	157.8 ± 1.2	167
Bi	µg g^{-1}	0.7 ± 0	0.63	0.9 ± 0	0.54
Th	µg g^{-1}	13.3 ± 0.5	14.76	11.4 ± 0.6	9.72
U	µg g^{-1}	2.5 ± 0.8	13.11	2.8 ± 0.9	3.30

In Table 4, it can be seen that, according to XRF analysis, the cobalt (Co) and molybdenum (Mo) contents of the BCs were also relatively high, and that Sr and Sn were more commonly observed than were other elements. These elements are essential microelements in terms of plant nutrition; however, soil contamination can occur when large amounts of them are incorporated into soil.

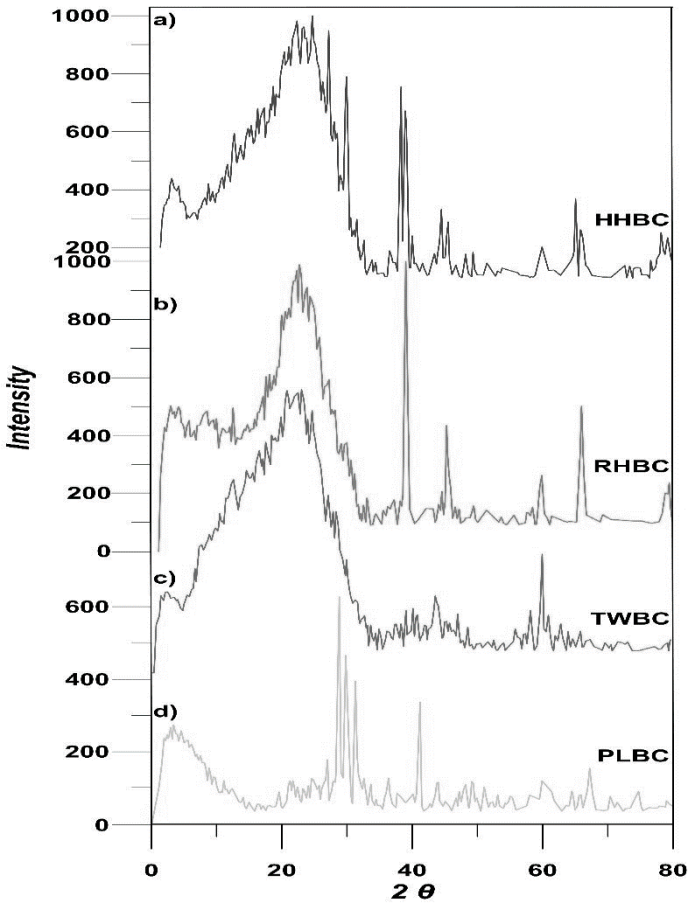
Table 4- Total element contents of BCs determined by XRF

Compounds	*TWBC*	*HHBC*	*RHBC*	*PLBC*
		(%)		
Na	0.02	0.03	0.03	0.70
Mg	0.22	0.23	0.33	1.39
Al	0.25	0.22	0.00	0.27
Si	0.56	0.73	16.4	1.24
P	0.30	0.11	0.24	2.44
S	0.26	0.16	0.30	1.35
Cl	0.06	0.17	0.01	0.39
K	2.93	1.38	2.13	8.66
Ca	0.65	2.42	0.57	5.29
Fe	0.05	0.21	0.43	0.52
Ti	0.005	0.02	0.01	0.02
V	0.0008	0.0008	0.0003	0.005
Cr	0.0006	0.001	0.001	0.003
Mn	0.16	0.03	0.03	0.12
Elements		*(mg kg^{-1})*		
Co	4.10	4.00	8.10	14.0
Ni	10.4	12.1	6.50	37.7
Cu	25,4	25.9	12.4	206.9
Zn	37.1	46.3	78.3	1098
Ge	0.10	0.20	0.20	0.40
As	0.20	2.80	2.10	1.50
Se	1.00	1.00	0.10	1.10
Sr	43.0	71.0	27.7	124.8
Mo	1.00	1.60	1.50	9.70
Cd	0.50	0.60	0.60	1.80
Sn	53.0	55.8	4.7	148.2
I	1.60	2.20	1.80	11.7
Ba	48.2	68.8	21.7	89,5
Hg	0.30	0.30	0.40	0.50
Pb	1.30	4.40	2.20	5.90
U	2.40	4.00	1.40	1.90
Ga	0.5	0.8	0.3	0.6
Br	6.3	1.3	8.8	25
Rb	40.7	15.6	8.8	52.6
Y	0.3	0.3	0.3	0.4
Zr	1.7	11.1	4.1	4.5
Nb	1	2.1	1.6	3.9
Ag	<0.1	<0.1	<0.1	0.9
In	0.5	0.7	0.6	0.8
Sb	0.6	0.8	0.7	0.8
Te	0.8	1.4	1	1
Cs	4	3.8	3.2	3.2
La	5.4	21.5	7	6.7
Ce	8.3	15.7	9.7	13.1
Hf	1.6	1.8	1.2	6.7
Ta	1.8	2	1.8	12.7
W	1.3	1.5	1.9	5.7
Tl	2	0.3	0.3	0.5
Bi	0.3	2	0.3	0.4
Th	0.3	0.1	0.4	0.4

TWBC: Tea waste biochar; HHBC: Hazelnut husk biochar, RHBC: Rice husk biochar; PLBC: Poultry litter biochar

3.3. XRD analysis

The diagrams obtained from an XRD analysis of the BCs revealed amorphous crystalline structures (Figure 1).



a) HHBC: Hazelnut husk biochar; b) RHBC: Rice husk biochar; c) TWBC: Tea waste biochar; d) PLBC: Poultry litter biochar

Figure 1- XRD diagrams of the BCs

The minerals seen in the PLBC diagrams were as follows: quartz (SiO_2) in 3.41 (Å); calcite ($CaCO_3$) in 3.96, 2.94 and 1.91 (Å); sylvine (KCl) in 3.06 and 1.85 (Å); and whitlockite ($[Ca, Mg]_3 [PO_4]_2$) in 2.78 and 2.17 (Å) (Table 5). Table 5 shows the overall mineralogical composition of the BCs. Besides existing minerals, the presence of amorphous C and amorphous Si was also noteworthy. According to the results of the XRD analysis of the BCs, amorphous C content was determined elementally with minerals such as quartz, cristobalite, calcite, sylvine and whitlockite. The chemical compositions in the detected minerals also improve the chemical and physical structure of the soil in terms of fertility. In addition, the presence of C in amorphous element oxides creates a similar positive effect in the soil.

Table 5- The minerals observed in the BCs

Characteristic Peaks (Å)	*Minerals*	*Abbreviations*	*BCs*
3.41	Quartz	Q	PLBC
3.91; 3.40;	Cristobalite	Crb	RHBC HHBC
3.96; 2.94;1.91	Calcite	Ca	PLBC
3.06; 1.85	Sylvine	Syl	PLBC
278;2.17	Whitlockite	Wh	PLBC
-	Amorphous Carbon	AC	TWBC
4.02;2.97	Amorphous silica	AS	HHBC
2.34;2.33;1.94;1.92;1.42;1.41; 2.30;1.94;1.41;1.20	Partially crystallized carbon	CryC	HHBC RHBC

TWBC: Tea waste biochar; HHBC: Hazelnut husk biochar; RHBC: Rice husk biochar; PLBC: Poultry litter biochar

The XRD diagrams of the plant-derived BCs (i.e., HHBC and RHBC) were found to be similar (see Table 5 and Figure 1). In HHBC and RHBC, partially crystallized carbon (CryC) peaks were mainly observed between 1.20 and 2.34 (Å), and cristobalite peaks (i.e., amorphous SiO_2) in 3.91 and 3.40 (Å). In addition, small amounts of calcite and sylvine, as well as minerals such as CryC and cristobalite, were detected.

Qadeer et al. (1994) showed that the diffractogram obtained from an XRD analysis of a RH was amorphous but confirmed that it had some local crystal structure, with highly conjugated aromatic compounds. Other researchers have stated that the gaps in the range of 5.8 Å and 3.89 Å-indicating the crystalline structure of cellulose in the rice-can be defined at pitch intervals of 6.0 Å, 5.8 Å, 4.44 Å, 4.04 Å, 3.89 Å, 2.52 Å and 3.89 Å (Keiluweit et al. 2010; Al-Wabel et al. 2013). In the XRD diffractogram in the present study, TWBC, which did not show a crystalline peak, can be said to be an amorphous C structure, possibly consisting of randomly ordered aromatic C layers. HHBC had a different structure than did the other materials, with an amorphous structure as well as Si and C content.

The chemical and spectroscopic properties of the BCs differed depending on the feedstock used to create them. Compared to the characteristics derived by the pyrolysis of different materials at the same temperature, PLBC, which is derived from manure, has better properties in terms of its elemental content and specific surface area when compared to those of plant-derived BCs. However, it was not superior in terms of pH. This is because this material contains a high mineral fraction, the most basic indicator of which is its high ash content and low C content. The arsenic (As) content of PLBC was found to be quite low. Because N is lost during pyrolysis, the N content is also quite low. Furthermore, it is not rich in Ca or P. This is similar to what was found in BCs obtained from plant base materials. However, the high salt content of PLCB should be taken into consideration when using it. It was seen that an amorphous structure is formed as a result of the burning of C in plant-derived BCs. While partly CryC was observed in RHBC and HHBC, amorphous C was found to be prominent in TWBC; this C was recalcitrant. It was also seen that RHBC, a plant-derived BC, is richer in terms of microelements—and especially of Co and Zn—when compared to other BCs.

4. Conclusions

Among the BCs reviewed in this study, PLBC was found to be preferable for use in agriculture as a source of plant nutrients. RHBC can be used as a source of Si to obtain silica for various purposes. BCs with a high pH value can be used to regulate the pH level in acidic soils. However, whether it is necessary to convert all plant waste into BC should be discussed further, as the high energy cost required for pyrolysis must be compared with the benefits and economic returns of producing BC. Therefore, researchers should study BC made with the same or different materials in different regions and should classify them into groups according to their suitability in various fields, such as those of agronomics, industry and energy. The overall benefit of BC could be better understood if a database was established that clearly shows which material can be used for which purpose. In this way, researchers working in multidisciplinary fields could select the appropriate BC from the database and use it for its ideal purposes. Feedstocks can be transformed into suitable BC material, according to its intended use, by designing the chemical structure of the BC before pyrolysis. Identifying and cataloguing the characteristics of various BCs will allow researchers to gain easy access to information about the BCs that are ideal for certain intended uses. As such, it is thought that studies such as this one will contribute to the establishment of a database of BCs and their potential uses. It has been seen that spectroscopic methods (e.g., XRF and XRD spectroscopy) can be used to determine the mineral content and structure of BCs quickly and at low cost. Studies on BC reveal the necessity of using spectroscopic methods due not only to their speed and low cost but also to their analytical reliability and more accurate results.

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Searching of Pregnancy Rate in Repeat Breeder Cows by Embryo Transfer Practices

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ABSTRACT

The aim of this study is to compare the pregnancy rates by applying embryo transfer to the cows which are not pregnant and should be removed from herd as repeat breeder. In this study, 87 randomly selected Holstein cows utilized. The repeat breeder cows (n=45) were selected from at least one giving birth, having regular sexual cycle, missing clinical worsening into genital organ and not displaying an abnormal discharge. On the other hand, it was selected from non-pregnant cows which inseminated artificially at least 3 times or more. Besides, cows that used as the control group (n=42) were selected from the cows without any artificial insemination postnatally. The PGF2α application was performed to all recipient cows which are considered to benefit from as a recipient in control and testing groups just 24 days before the flushing day. After this application, the cows showing estrous symptoms were recorded and determined as candidate recipients. The pregnancy rates were 35.6% and 50% for testing and control groups, respectively and the difference between the groups was significant (P<0,05). As a result, even this difference between the groups it has been concluded that embryo transfer can be used to conceive especially for high-yielding cows as a treatment method for repeat breeder cows. Thereby, embryo transfer from the cows with high superior characteristics to the high milk yielding cows which have infertility problems can economically be beneficial by utilizing the high milk yield in the later lactation without any replacement cost.

Keywords: Embryo transfer, Holstein, Infertility, Repeat breeder

1. Introduction

Many problems are faced affecting of cows milk and reproduction yield in countries where modern dairy farming is carried out. One of these is repeat breeder cow (RBC) problem. RBC is defined as cows that do not become pregnant even though they are inseminated at least 3 or more times. In a wide definition about this problem; the animals which is younger than 10 years old and had at least one birth, having regular sexual cycles, have not a clinical disorder in the genital organs and not showing an abnormal discharge, however, do not conceive of mating with a fertile bull or artificial inseminating 3 or more times are called repeat breeder cows (Alaçam 2010; Kaymaz 2012).

The ethiology of RBC syndrome is not fully know and has multifactoria causes. The cow, the bull and several environmental/handling factors are incriminated. All of them are often overlapped and it is difficult to determine the primary origin. While influence of the age of the mother, genetic factors, uterine infection and repeat estrous cycles, anatomical defects of the genital tract, hormonal dysfunctions, early embryonic deaths, inadequate follicular growth and effect of nutrition (the importance of nutrition in all vital processes is indisputable, and the qualitative and quantitative differences in the ration in dairy cattle may cause reproductive dysfunctions) can be considered as maternal reasons; influence of bull fertility and semen quality, site of semen deposition and estrus return, time of semen deposition are bull related factors. Except those, environmental factors can also be included in the ethiology of repeat breeder syndrome. These are season, stress, estrus detection, hygiene at artificial insemination and parturition (Perez-Marin et al. 2012).

Embryo transfer applications are one of the most important ways to assure genetic improvement rapidly in dairy cattle and increase the number of elite female and male animals in the herd (Seidel & Seidel 1991; Akyol 2001; Bilby 2010; Tekeli 2010). Besides, embryo transfer is one of the modern techniques used to increase success in animal breeding in the most effective way (Bülbül & Dursun 2005). The most important goal of embryo transfer applications consisting of a series of biological processes is to provide healthy and having good genetic capacity offsprings by obtained high quality oocytes and embryos (Santos et al. 2004).

Embryo transfer into lactating dairy cattle has shown beneficial effects in improving fertility in dairy cattle, especially during summer heat stress. The transfer of an embryo could bypass certain causes of infertility (i.e., fertilization failure and

early embryonic loss). Therefore, embryo transfer can be utilized to improve pregnancy rates in repeat breeder dairy cattle (Bilby 2010).

The aim of this study is to investigate whether it is possible to conceive by applying embryo transfer to the cows which do not conceive and be required to be selected from the herd despite being inseminated at least 3 times. Thus, it is tried that cows with RBC syndrome are conceived by embryo transfer and it will be shown that economic gain can be achieved by keeping them in herd for a while. Various studies have been worked in Turkey with repeat breeder cows. Most of these studies are based on artificial insemination with hormonal treatments. But, in this study, Repeat breeder cows are tried to be treated by fresh embryo transfer.

2. Material and Methods

2.1. Animals

In this study, embryos obtained from lactating cows selected as donors at the Eastern Mediterranean Agricultural Research Institute were transferred to the cows that were trial group that was repeat breeder cows and the control group that did not receive any postpartum treatment. The cows in this enterprise are Holstein and their ages are between 3 and 8 years old. In the study, 87 head cows randomly selected. Repeat breeder cows (n= 45) determined from the cows which have given at least one birth, have regular sexual cycles, have no clinical problem in their genital organs and show no abnormal discharge, but are not pregnant even if artificial insemination has been performed three or more times. The cows to form the control group (n= 42) were selected from animals that were not inseminated after birth.

In the farm, cows are housed in open barns and fans are used with water spraying as cooling systems to minimize the heat stress, which is an important effect of the Mediterranean climate. Wheat straw, alfalfa, corn silage and vetch as roughage and concentrated feed with 18% protein produced in the enterprise was used in animals ration. Water was provided with automatic waterers as adlibitum.

2.2. Superovulation protocol and evaluation of embryos

Progesterone release intravaginal device (PRID) (PRID-Delta, Ceva, Turkey) that releases controlled hormone (progesterone), the most preferred method to synchronize follicular development, were administered before superovulation protocol. Following this application, 7 days later, FSH (Folltropın-V, Bioniche Animal Health Europe Ltd., Ireland) injections were started to administere in decreasing doses intramuscularly (i.m.), 4 days-12 hours apart-twice a day-8 times in total. 2 cc PGF2α (Lutelen, Topkim, Turkey) injected on the mornings 3rd and 4th days of FSH application in order to regression of the existing corpus luteum and providing ovulation. PRIDs were taken out on the 3rd day evening of FSH injections. The donor cows were inseminated 3 times at the 12th, 24th and 48th hours following the last FSH application. 2.5 cc GnRH (Receptal, MSD Animal Health, Germany) was injected with 2nd and 3rd artificial inseminations. Uterine flushing was made on the 7th day following the first insemination. Uterine flushing was performed by non-surgical method. Embryos were recovered with flushing medium in a sterile bottle. After the uterine flushing process is completed, 2 cc PGF2α was made to be lysed corpus luteum and 500 mg benzathine cefapirin (Metricure, MSD Animal Health, The Netherlands) was administered intrauterinely to prevent uterine infections. Embryo scanning were carried out in petri dishes using by heated stereo microscopes (Leica, S8APO, Japan) after flushing medium in sterile bottle was filtered and portioned out in smaller amounts. The recovered embryos were put in to culture solution (same as holding solution) and kept in an incubator (Binder, USA) providing 38.5 °C, 5% CO2 and high humidity environment until transfer.

Classification of recovered embryos for quality and development stages were made according to Kanagawa et al. (1995), Silva et al. (2009), and Kaymaz (2012)'s evaluation criterias.

2.3. Selection and preparation of recipient cows

Repeat breeder cows selected as Trial Group (n=45) were determined from non-pregnant cows that they showed regular sexual cycles, no disorder was detected in their genital organs by clinical examinations although they were inseminated at least 3 times. The animals in the Control Group (n=42) were selected from the between cows that had at least 90 postpartum days, were showing a regular sexual cycle, had no clinical problems on their genital organs and had no artificial insemination.

A double dose of PGF2α injection, one of the methods of synchronization, was made to recipient cows in order to perform embryo transfer on the 7th day of oestrus. For this, the first PGF2α application was injected 24 days before the uterus flushing day. After 14 days, the second injection of PGF2α was applied and cows in oestrus were followed between 48th and 96th hours. Animals showing signs of oestrus were recorded and identified as candidates as recipients. Candidate cows were evaluated in both groups after ultrasound (Ultrasonic scanner, HS-101V, Honda, JAPAN) examination. Their uterus and ovarian structures were evaluated. Cows suitable to be recipient were selected for study. The fresh embryos were transferred to the recipient cows that had the appropriate corpus luteum (CL) on the day of the transfer and showed no pathological symptoms.

2.4. Transfer of embryos

The animals in the trial and control groups to be used as recipients should show oestrus 6-8 days ago. For this purpose, PGF2α injected intramuscularly twice with an interval of 14 days to cows that can be included in the study. These cows were monitored and those that showed oestrus symptoms were determined and recorded. The presence of quality CL in these determined cows recorded by using transrectal ultrasound technique; fresh embryo transfers were performed to the recipients which have good quality CL. Embryos of 7-8 days old obtained from donor cows were transferred by embryo transfer catheter to recipients' cornu uteri on the right or left where CL is detected by ultrasound. Pregnancy examinations in recipient animals were performed 28 days after transfer (35 days after oestrus) with ultrasound.

2.5. Statistical analyses

SPSS (Version 23) package program was utilized for statistical analysis of the study. Pregnancy of the groups was compared statistically using Chi-Square test.

3. Results and Discussion

Sixteen head repeat breeder cows were pregnant from 45 recipient cows in the trial group. In the control group, twenty one of 42 recipient cows which embryo were transferred, were diagnosed in pregnancy. As a result of the statistical analysis, pregnancy rates in the trial and control groups were 35.6% and 50%, respectively. In statistical comparison of both groups, there was a significant statistical difference between the rates of pregnancy at $P<0.05$ level ($p= 0.003$). Nevertheless, Considering the difficulties of getting pregnant with artificial insemination in repeat breeder cows, it can be said that the rate of pregnancy rate is high by performing embryo transfer. Pregnancy rates of the groups were found as shown in Table 1.

Table 1- Pregnancy rates of the groups

Groups	*n*	*Pregnant*	*Non-Pregnant*	*Pregnancy %*	*Non-Pregnancy %*
Trial Group	45	16	29	35.6	64.4
Control Group	42	21	21	50	50

Embryo transfer studies with repeat breeder cows are few. While other studies mostly were performed with frozen-thawed embryos, fresh transfer process was carried out in this study.

The pregnancy rates following either a controlled internal drug release (CIDR)-based timed artificial insemination (TAI) or an embryo transfer (TET) protocol were compared in lactating repeat breeder dairy cows. In the pregnancy examination performed on the 60th day, the rate of conception by embryo transfer was higher in repeat breeder cows compared to artificial insemination. It has been reported that embryo transfer is effectively increase the pregnancy rate in lactating repeat breeder dairy cows (Santos et al. 2004).

Dochi et al. (2008), thawed the embryos they obtained with in vitro fertilization application after freezing and transferred them to repeat breeder cows. Embryos were transferred 7 or 8 days after a natural estrus into one of two groups of animals that were either inseminated with frozen-thawed semen or were not inseminated (without-AI group) in the same estrous cycle as the embryo transfer. Both heifers and cows had higher pregnancy rates following embryo transfer and AI (with-AI group) than following embryo transfer only (without-AI group) (49.2% vs 29.5%; 41.5% vs 20.4%). Compared to this study and Dochi et al.'s study (2008), we had a higher rate of pregnancy rate in the group of directly embryo transferred. This may have been caused by Dochi et al. (2008) using frozen thawed embryos. Because pregnancy rate is lower in frozen embryo transfer compared to fresh transfer (Riha et al. 2002; Kızıl et al. 2012). In a study of non-pregnant cows despite artificial insemination at least 3 times, PGF2α treatment was performed to show the cows oestrus and it was found that the rate of conception was high as a result of embryo transfer in recipient cows (Rodrigues et al. 2010).

Stradaioli et al. (2015) have worked with 44 referred as clinically normal repeat breeder cows of 6 different dairy herds. Pregnancy rate after embryo transfer performed to repeat breeder cows was found 37.14%. In 6 dairy farms they have chosen for the study, it has been reported that pregnancy rates after artificial insemination vary between 20-35%. In both studies revealed that, further studies need to be made and embryo transfer may be a good treatment alternative in repeat breeder cows.

Karasahin et al. (2016) transferred to repeat breeder cows after the embryos are frozen by vitrification method. On the 60th day after the transfer, pregnancy examination was performed by rectal palpation and the pregnancy rate was found 28.13%. Comparing the study presented with this study, it is seen that the pregnancy rate is low in this study, although the results are close. The fact that frozen embryos were used in the study of Karasahin et al. (2016) and pregnancy examinations were performed on the 60th day may be the reasons for the low pregnancy rate compared to our study. It should also be considered that late embryonic deaths may have reduced the pregnancy rate. Late embryonic death-fetal death rates have been reported as

14%, 7.2% and 6.9% in different studies (Aslan & Wesenauer 1999; Silke et al. 2002; Kızıl 2011). Santos et al. (2004) reported the rate of embryonic death at the age of 30 to 45 days as 0.23% to 2.67% (average 0.85%) and overall 3.2% to 42.7% (average 12.8%).

In the study, the pregnancy rate in the repeat breeder cows group was found to be 35.6%. Whereas, it is very difficult to conceive these animals, which are defined as repeat breeder cows, by artificial insemination. There are studies reporting pregnancy rates as 20% and 18.75% in repeat breeder cows when artificial insemination is performed without applying any treatment (Selvaraju & Veerapandian 2010; Ergene 2012). In this case, it shows us that embryo transfer application is a useful and suitable alternative for the treatment of this syndrome by getting pregnant repeat breeder cows.

Three individuals may be involved in reduce pregnancy rates in natural service or even AI. These are sire, dam and embryo. Pregnancy rate generally depends upon three individuals who interact in various ways, often in a common environment. Heat stress at day 1 or days 1 - 3 reduced embryo survival and pregnancy rates. However, heat stress at days 3, 5 or 7 did not affect pregnancy rates indicating that thermal tolerance is acquired as the embryo ages. Considering that there is no sperm utilizing for recipient cows due to embryo transfer and the embryo to be transferred to the recipient is good quality and has passed a specific stage (age), by eliminating the other two factors, only the factor that may arise from the recipient cow remain. To put it more clearly, this allows us to eliminate two important disadvantages that reduce the rate of conception and also, Since the embryo to be transferred will be at the age of 7 days, high results can be obtained compared to artificial insemination in the rate of conception under heat stress. It is stated that it is possible to achieve high pregnancy rates with embryo transfer by using frozen-thawed embryos in seasons when hot stress is effective and causes infertility (Rutledge 2001).

4. Conclusions

Consequently, according to the data obtained, ıt has been concluded that embryo transfer applications can be recommended as a successful treatment alternative for repeat breeder cows and the determination of the presence of corpus luteum on the transfer day with synchronous heat detection in the recipient cows to be transferred is of great importance in terms of pregnancy rates to be achieved. So, it can be helped to the breeding of the herd or animal with the transfer of the embryos of the cows with superior characteristics to the cows with superior characteristics but having fertility problems and also more efficient use of repeat breeder recipients in production. Embryo transfer is one of the most important and effective methods used in animal breeding with new methods and technologies developed in the last 50 years. It is also evaluated that with the introduction of widespread use in our country, it will make important contributions to meet our quality breeding pregnant heifer needs with more affordable costs.

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Proximate Composition, Fatty Acid and Amino Acid Profiles of Narrow-Barred Spanish Mackerel (*Scomberomorus commerson*) Fillets from İskenderun Bay in The North-Eastern Mediterranean Sea

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ABSTRACT
The present study aims to determine the proximate, fatty acid and amino acid profiling of consumed fresh narrow-barred Spanish mackerel (*Scomberomorus commerson*). Landed fish were freshly sampled (total length 33.7-48.7 cm and weight 617-1260 g) from the Yumurtalık Bay (north-eastern Mediterranean Sea) in Turkey during January, February and March. The protein values were highest in January (22.89%) while the and lowest in February (21.38%) and March (21.73%). Lipid and ash values were not significantly differencing among sampling time. The fatty acid data revealed that the saturated fatty acid values were found higher than the polyunsaturated and monounsaturated fatty acid values. In general, the fillets were abundant in palmitic acid (C16:0), stearic acid (C18:0), oleic acid (18:1n-9) and docosapentaenoic acid (C22:6n-3; DHA) values, regardless of the sampled months. DHA value was recorded as 315.08 mg $100g^{-1}$ in January, while it increased to 327.55 mg $100g^{-1}$ in the March samples. A total of 16 amino acids were determined from the fresh fillets. Compared with the other essential amino acids, the concentration of lysine and leucine were found to be higher in the fillet. At the same time, the lower rates of tryptophan were detected in examined samples for all months. Consequently, this study shows that the narrow-barred Spanish mackerel as a finfish (commercially valuable) from the north-eastern Mediterranean Sea has precious nutritional values that of the protein, fatty acids and amino acids during the sampling period. This fish can be recommended in terms of detected essential fatty acid and amino acid profile that completely nutritious for the human as well as other organisms' dietary requirements.

Keywords: Essential amino acids, Fatty acids, Proximate composition, *Scomberomorus commerson*

1. Introduction

Narrow-barred Spanish mackerel is a member of the Scombridae family, and it is usually found in a large area centered in world especially Southeast Asia and the fish migrate to the eastern Mediterranean Sea via the Suez Canal and move westward toward Tunisia (Froese & Pauly 2019). In the Mediterranean Sea, the presence of narrow-barred Spanish mackerel was first recorded in Palestine (Hornell 1935), whereas its presence has been known in the Mersin and Iskenderun Bays, Turkey, since 1981 (Gücü et al. 1994). Moreover, the presence of this fish was last recorded in the northern Aegean Sea in Izmir Bay in 2018 (Akyol and Tosunoğlu 2019). This immigrant fish species is consumed by hunting. This pelagic fish is increasingly being caught by commercial fisheries, and it has often been reported to be caught by the local fisheries of Güllük, Gökova and Mersin Gulfs (Gücü et al. 1994; Torcu et al. 1997).

Fish are an important source of protein, play an important role in human nutrition and possess high digestibility, biological and growth-promoting values. Fish are also the source of essential elements, particularly n-3 polyunsaturated fatty acids (n-3 PUFAs). Such fatty acids present in fish, especially docosahexaenoic acid, are beneficial for the development of the brain and visual system in infants as well as for reducing the incidence of various disorders in adults, including high cholesterol levels, stroke and heart diseases (Von Schacky et al. 1999; Connor 2000; Arts et al. 2001; Lauritzen et al. 2001; Nordov et al. 2001; Silvers & Scott 2002).

During the last two decades, PUFAs have attracted a great interest among scientists for their medicinal and nutritional properties. The abundance of unsaturated fatty acids is the most valuable characteristic of the fish (Nordov et al. 2001; Türkmen et al. 2008). Polyunsaturated fatty acids of the ω-6 and ω-3 families in particular are recognized as essential biochemical components of the human diet (Aktaş & Halperin 2004). Therefore, the approximate biochemical composition of one species helps evaluate its nutritional and edibility in terms of energy units in comparison with those of other species. Information about the biochemical composition of *Scomberomorus commerson* is of great importance in assessing its nutritional value, but it also

facilitates quality assessment and optimum use of this natural resource. However, no information is available regarding the biochemical composition of *S. commerson*. This study was aimed at determining the changes in the proximate fatty acid (FA) and amino acid composition in fillets of fish whose prevalence continues to rapidly increase in the Yumurtalık Bay, Adana, Turkey. To the best of our knowledge, this is the first report to investigate the proximate composition, fatty acid and amino acids profile of these fish species from İskenderun Bay in The North-Eastern Mediterranean Sea caught during the consumption months.

2. Material and Methods

The research was conducted from January to March 2019. Sampling of mackerel fish was performed in Yumurtalık Bay, Adana, Turkey. Narrow-barred Spanish mackerel were caught by professional fishermen at the coast of the Mediterranean of Turkey (Figure 1). The months when the fish were caught at the Mediterranean shores were January, February and March (İsmail Kamburlu (fishermen), pers comn.). Six samples of the fish species caught during each of these three months in 2019 (18 samples) were placed in styrofoam in box with ice and brought to the laboratory within 2 hours and stored at -20 °C until analysis. The min-max length and weight of the narrow-barred Spanish mackerel were 33.7-48.7 cm (40.83±1.15) and 617-1260 g (935.16±62.52), respectively. The muscle tissues of fish were homogenized, manually separated and analyses in triplicate with regard to nutritional value, fatty acid composition and amino acid composition.

Figure 1- Map of the fish catching area

2.1. Chemical analyses

The samples were thawed at +4 °C and 6 fish fillet samples from each month were homogenized using a blender. Proximate composition analysis (moisture, ash, lipid, and crude protein) of the homogenized samples was determined using the standard procedures of AOAC (1995).

Moisture content was measured by drying samples to constant weight at 103 °C for 24 h. Ash content was determined by burning the samples at 600 °C for 5 h. Protein (N × 6.25) content was determined using an automated Kjeldahl Kjeltec 2200 (Foss Tecator, Högans, Sweden).

2.2. Analytical methods

Lipids were extracted according to the procedure described by Folch et al. (1957). Following lipid extraction, fatty acid methyl esters (FAME) were prepared according to the method described by Metcalfe and Schmitz (1961) and analysed as previously described (Czesny and Dabrowski 1998) with some modifications. Briefly, FAME obtained were separated in a gas chromatographic column (Agilent 6820 A), which was equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 µm). The injector temperature program involved maintenance of 190 ºC for 35 min, followed by temperature increase at a rate of 30 ºC min-1 to 220 ºC where it was maintained for 5 min. Carrier gas was hydrogen (2 mL min-1), with a split ratio of 30:1. The individual fatty acids were identified by comparing their retention times with that of a standard mix of fatty acids (Supelco 37 component FAME mix). Amino acid composition analysis of the fish fillet samples was conducted using the Ultra-Fast Liquid Chromatography system equipped with a UV detector (Gheshlaghi et al. 2008).

Fatty acids per 100 g of total lipid (TL) require to be the derivation of a reasonable factor (F) correlating the total quantity of fatty acids to a given quantity of total lipid (Weihrauch et al. 1977). Fatty acids in fish muscle levels (mg $100g^{-1}$) were converted with them following formula:

$$FA\ (mg\ 100g^{-1}) = (F\ X\ FA \cdot TL) \cdot 10^{3}$$

In these conversions, F indicates fatty acid conversion factor (0.90 for fish with 5% fat; for fish with <5% fat, it is calculated from the equation F = 0.933 - (0.143 - TL)). FA represents fatty acids. TL represents the total lipids.

The atherogenicity index (AI), thrombogenicity index (TI) and peroxidisability index (PI) were calculated by following the equation suggested by Ulbricht and Southgate (1991) and making slight modifications to it as described by Canto et al. (2015). The hypocholesterolaemic to hypercholesterolaemic ratio (H/H) was calculated using the formula developed by Santos-Silva et al. (2002).

2.3. Statistical analysis

The proximate compositions of amino acids and fatty acids (n=6) have been reported as mean ± standard deviation values. Data were analyzed using the one-way analysis of variance (ANOVA) test at a significance level of 0.05% after confirmation of normality and homogeneity of variance. When significant differences were detected, data were subjected to Student-Newman-Keuls post hoc test for identifying homogeneous subsets. All computations were performed using SPSS16.0 (SPSS Inc. Chicago, IL, USA).

3. Results and Discussion

3.1. Proximate composition of narrow-barred Spanish mackerel

Caught during the consumption months of the narrow-barred Spanish mackerel are given in Table 1. Landed fish were during the months of January, February and March, and statistical differences (P<0.05) were noted for moisture and protein value. The value of moisture in fish fillet usually determines its nutritional taste and value (Gökoğlu et al. 2004). Comparisons made among the groups of narrow-barred Spanish mackerel caught on each of the months revealed that the highest moisture value was found in the February and March samples (73.36 and 73.97%, respectively), and the lowest value was found in the January sample (72.64%). Moisture value in fish effects of the textural properties are very high, the fish will have a soft and mushy texture (Lazo et al. 2017). Moreover, measurement of ash value is mostly dependent on the mineral values in each fish sample as well as the feeding patterns, growth phase, seasons and habitat or environment of the fish species (Suryaningrum et al. 2010). Moisture value in the samples will also have a large impact on the protein value measured in the fish and the higher protein value in the samples will result in a lower moisture value than in fresh samples (Gökoğlu et al. 2004; Sebranek 2009). Protein value was the highest in the January sample (22.89%) compared with that in the other 2 months, followed by the February (21.39%) and March (21.73%) samples. Bandarra et al. (2001) and Çelik (2008) found similar results and reported the protein content of horse mackerel. Reduction of muscle protein in adult fish has been mentioned in cases of mobilization under prolonged fasting (Love, 1992). However, there have been some cases where seasonal protein changes have been reported for wild fish populations (Gökçe et al. 2004; Patrick et al. 2008).

Table 1- Proximate composition (%) of narrow-barred Spanish Mackerel during the sampling periods

Proximate Composition	*Sampling Time*		
	19- January	*19- February*	*19- March*
Moisture	72.64± 1.09^{b}	73.36± 0.42^{a}	73.97± 0.56^{a}
Ash	3.01± 0.21	2.97± 0.10	2.99± 0.13
Protein	22.89± 0.63^{a}	21.39± 0.38^{b}	21.73± 0.91^{b}
Lipid	2.06± 0.10	2.11± 0.20	2.06± 0.01

For the data relative to fish fillet, values are mean ± SD. (n= 6; number of fishes per sampling time), and values in the same row with different superscript letters indicate statistically significant difference (P<0.05)

Monthly sampling did not significantly (P>0.05) affect the lipid value in the flesh. All the other species exhibited fillet fat of < 1%, which would categorize them into low-fat species (Huynh and Kitts 2009). The lipid value of fish is also attributed to environmental factors like nutritional supply and food sources and directly affects odor and flavor density (Puwastien et al. 1999; Lazo et al. 2017; Vijayan et al. 2016).

3.2. Fatty Acid Composition of narrow-barred Spanish mackerel

The fatty acid composition of narrow-barred Spanish mackerel caught during the consumption months is shown in Table 2. At all sampling time, narrow-barred Spanish mackerel showed the highest levels of total saturated fatty acids (SFAs), followed by PUFAs and monounsaturated fatty acids (MUFAs). In agreement with our results, Rajaram et al. (2018) and Osman et al. (2001) reported similar predominant FAs as well as equivalent SFA–PUFA–MUFA patterns in Spanish mackerel fillets. The fatty acid

compositions varied during the sampling time. The fatty acid composition of fish flesh in March samples had the highest ($P<0.05$) levels of total saturates. The SFA value of narrow-barred Spanish mackerel in the March sample was 286.95 mg $100g^{-1}$ followed by 261.53 and 255.79 mg $100g^{-1}$ in the January and February samples, respectively; moreover, the MUFA value was 317.16, 309.34 and 288.32 mg $100g^{-1}$ in February, January and March. In this study, the highest PUFA level among those caught in consumption months was found in February and March (548.06 and 550.96 mg $100g^{-1}$). The results of this study were consistent with the results of various studies in the northeastern Mediterranean (Ozogul et al. 2009; Durmuş 2018; Köşker 2020) There is a common view of the positive effects of PUFAs on human health (Lunn and Theobald 2006; Fung et al. 2009; Hellberg et al. 2012). The accumulation of fatty acids in fish muscle is affected by various factors such as diet and genetics, as well as sexual maturity, geographical location and hunting season (Horn et al. 2018).

In general, viewed individually fatty acids, including palmitic acid, stearic acid, oleic acid and DHA were abundantly found in narrow-barred Spanish mackerel flesh, independent of the sampling time. Prato and Biandolino (2012) also reported oleic acid as the most abundant of the MUFAs in most marine fishes. MUFA and SFA are used as metabolic energy sources for these species, and long chain n-3 fatty acids are essentially essential for structural purposes, namely as components of membrane phospholipids. Moreover, MUFA are more efficiently transformed into energy via the process of β-oxidation than n-6 PUFA (Turchini et al. 2007). This observation can be explained by the fact that SFA and MUFA are largely represented in neutral lipids and are more prone to migration (Turchini et al. 2007).

Table 2- Fatty acids (mg/100g) composition of narrow-barred Spanish Mackerel

Fatty Acids	*Sampling Time*		
	19- January	*19- February*	*19- March*
C14:0	11.35 ± 1.65	11.81 ± 1.56	11.23 ± 0.41
C15:0	7.44 ± 1.73	7.39 ± 1.08	8.98 ± 0.14
C16:0	373.08 ± 5.89^{b}	429.50 ± 5.41^{a}	398.32 ± 5.52^{ab}
C18:0	261.53 ± 5.78^{b}	255.79 ± 4.84^{b}	$286,95^{a} \pm 4.16^{a}$
C20:0	10.81 ± 0.07^{b}	15.14 ± 0.80^{a}	10.58 ± 0.17^{b}
C22:0	11.88 ± 0.43^{a}	10.54 ± 0.37^{b}	10.98 ± 0.17^{b}
C24:0	1.68 ± 0.01^{ab}	2.25 ± 0.19^{a}	1.10 ± 0.20^{b}
SFA	685.68 ± 10.37^{b}	741.00 ± 9.06^{a}	735.97 ± 10.04^{a}
C14:1	1.95 ± 0.17	2.00 ± 0.37	2.01 ± 0.01
C15:1	2.89 ± 0.17^{b}	3.49 ± 0.48^{a}	2.78 ± 0.01^{b}
C16:1	58.90 ± 2.30^{b}	64.88 ± 0.62^{a}	61.46 ± 0.45^{ab}
C17:1	6.44 ± 0.65^{b}	7.99 ± 0.07^{a}	5.85 ± 0.09^{b}
C18:1n9	158.85 ± 3.85^{a}	147.53 ± 0.07^{b}	131.16 ± 5.85^{c}
C18:1n7	58.33 ± 3.92	61.52 ± 3.39	61.66 ± 3.14
C20:1n9	9.45 ± 0.57^{c}	16.23 ± 1.04^{a}	10.75 ± 0.17^{b}
C22:1n9	4.37 ± 0.13	4.93 ± 0.47	4.40 ± 0.07
C24:1n9	7.03 ± 0.48	7.63 ± 0.07	7.16 ± 0.43
MUFA	309.34 ± 3.72	317.166 ± 1.76	288.32 ± 8.90
C18:2n6	17.19 ± 0.01^{c}	17.22 ± 0.01^{b}	17.48 ± 0.02^{a}
C18:3n6	$7.47^{a} \pm 1.33^{ab}$	$7.67^{a} \pm 1.47$	5.94 ± 0.09^{b}
C18:3n3	75.55 ± 4.71	77.39 ± 3.87	77.37 ± 2.04
C20:2n6	3.49 ± 0.26	3.50 ± 0.26	3.61 ± 0.02
C20:3n6	11.43 ± 0.07	11.73 ± 0.10	10.41± 1.10
C20:4n6	3.01 ± 0.00^{a}	3.10 ± 0.03^{a}	2.91 ± 1.11^{b}
C20:5n3	67.32 ± 4.66	67.62 ± 3.79	68.44 ± 4.74
C22:5n3	26.48 ± 1.49	27.40 ± 1.25	25.11 ± 0.38
C22:6n3	315.08 ± 9.38^{b}	322.76 ± 2.78^{ab}	327.55 ± 3.94^{a}
PUFA	535.04 ± 1.35^{b}	548.06 ± 4.97^{a}	550.96 ± 7.66^{a}
EPA+DHA	382.40 ± 8.22^{b}	391.37 ± 5.11^{a}	392.42 ± 3.15^{a}
PUFA/SFA	0.78 ± 0.04	0.74 ± 0.01	0.74 ± 0.04
n6	42.60 ± 1.18^{a}	43.19 ± 1.82^{a}	39.83 ± 0.54^{b}
n3	484.43 ± 11.41^{b}	496.26 ± 1.87^{a}	497.94 ± 4.14^{a}
n6/n3	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.00
n3/n6	11.39 ± 0.95	11.51 ± 0.53	12.36 ± 0.04
DHA/EPA	4.68 ± 0.12	4.76 ± 0.17	4.82 ± 0.29
IA	0.81 ± 0.04	0.86 ± 0.01	0.87 ± 0.05
IT	0.29 ± 0.02	0.31 ± 0.00	0.31 ± 0.02
PI	1.02 ± 0.06^{a}	0.91 ± 0.04^{b}	0.99 ± 0.04^{a}
HH	1.62 ± 0.11^{a}	1.41 ± 0.02^{b}	1.49 ± 0.07^{b}

For the data relative to fish fillet, values are mean ± SD. (n= 6; number of fishes per sampling time); and values in the same row with different superscript letters indicate statistically significant difference ($P<0.05$)

In the fatty acid analyses of narrow-barred Spanish mackerel, the predominant SFAs were palmitic acids and stearic acid. Generally, the palmitic acid value in fish was considerably higher than the stearic acid value at all sampling time. Oleic acid was the most abundant fatty acid of the MUFAs in flesh of fish and statistically different in those caught in consumption months, the highest amount was found in January (158.85 mg $100g^{-1}$). No significant differences in vaccenic acid (C18:1n-7) were found among the fish samples of each month. Linoleic acid (C18:2n-6) value in fish was significantly higher in the March sample (17.48 mg $100g^{-1}$) compared with its value at all other sampling times (February, 17.22 mg $100g^{-1}$ and January, 17.19 mg $100g^{-1}$). Osman et al. (2001) also reported high linoleic acid values in comparison with other n-6 fatty acids for fish species with low fat contents from tropical marine waters. In addition, linoleic acid and α-linolenic acid (18:3n-3) PUFAs are essential nutrients that must be obtained from food (Das 2006).

There were no significant differences in either the α-linolenic acid or EPA value among all sampling times (Table 2). The EPA value of narrow-barred Spanish mackerel was almost the same at all sampling times and EPA ratios in the range of 67.32 and 68.44 (mg $100g^{-1}$) were determined. Contrastingly, the DHA value, which significantly affected the change in the total PUFA value, were influenced by the month in which the fish were caught. DHA value decreased to 315.08 mg $100g^{-1}$ in January, while it increased to 327.55 mg $100g^{-1}$ in March. The difference between months is thought to accumulate DHA found to increase gradually in the body by getting used to the ambient conditions during the stay in the same bay in this fish, which is a migratory species. Seafood contains high and balanced amounts of polyunsaturated fatty acids, especially EPA and DHA (Lunn and Theobald 2006; Ozogul et al. 2009, 2018). Both EPA and DHA are known to be important for human health (Cooner 2000). It was found that this species meets 250-500 mg daily EPA and DHA intake against the risk of cardiovascular disease recommended by EFSA (2012) during the caught in consumption months, Briefly, PUFA value of flesh fish in the March samples (550.96 mg $100g^{-1}$) were significantly higher than that in other sample months. Furthermore, fish need PUFA to adapt to lower water temperatures, and cold-sea fish are rich in n-3 fatty acids (Chanmugam et al. 1986).

The n3/n6 ratio in narrow-barred Spanish mackerel fluctuated within the range of 11.39-12.36 and PI, AI and TI values were calculated to determine lipid quality based on fatty acid data. No significant differences in PI, AI and TI values were found among the monthly samples. The AI and TI ratios across the months ranged from 0.81 to 0.87 and from 0.28 to 0.31, respectively (Table 2). PI values fluctuated within the range of 1.02-0.91 in narrow-barred Spanish mackerel. The n-3/n-6 ratio is a good index to compare the relative nutritional value of fish oil. A higher rate is essential to reduce coronary heart disease, plasma lipid levels and cancer risks (Kinsella et al. 1990). SFAs, MUFAs, and n-6 PUFAs, are TI and AI that these lipids index show potential effects on dietary quality and coronary artery diseases. (Jankowska et al. 2010; Görgün & Zengin 2015). The findings of many other researchers also have seasonal changes, species, gender, size, food availability, geographic location; breeding status, water temperature and salinity rate affect the amount of fatty acids in other fish species (Vlieg & Body 1988; Saoud et al. 2008).

3.3. Amino acids composition of narrow-barred Spanish mackerel

The amino acid profile of the fresh sample was examined. In the fresh sample, 16 amino acids, including 8 essential amino acids (EAAs) and 9 nonessential amino acids (n-EAA) were detected (Table 3). The amino acids in fish have been very important in terms of nutrition and flavor (Antoine et al. 2001).

When the first sampling results were evaluated, among all the detected amino acids, glutamic acid value was the highest (3.81%) and tryptophan value was the lowest (0.22%) in January. In the January sample, EAA concentrations from the highest to lowest were as follows: lysine (2.23%), leucine (1.56%), valine (1.31%), histidine (1.22%), isoleucine (1.21%), threonine (1.05%), phenylalanine (1.02), methionine (0.58%) and tryptophan (0.19%). Among all the EAAs in the fish fillet samples, the concentrations of lysine and leucine were the highest at 2.23% and 1.56%, respectively, while the concentration of tryptophan was the lowest. Similar results on amino acid content were reported by Paratama et al. 2017. These results are Peng et al. (2013) shows that the highest leucine and lysine in the yellow fin tuna from the Serranidae family. Amino acid composition determines the quality of a protein, which is among the most important macronutrients in human diet. Leucine, valine, isoleucine and lysine are categorized as EAAs because a human body cannot produce them s on its own; these EAAs are derived from various external food sources and it is well known that each of the amino acids contributes to the basic taste of a product. Among the n-EAAs in the fish fillet samples, the concentrations of glutamic acid and aspartic acid were the highest at 3.81% and 2.23%, respectively (Table 3). In February, during the second sampling, the concentrations of only some amino acids were changed. The total value of methionine (0.64%), leucine (1.86%) and lysine (2.67%) were increased, and that of histidine (1.12%) and isoleucine (1.01%) was decreased in the fresh fillet; these values were significantly different across the sampling times. Among the n-EAAs in fish fillet samples from February, there was a significant difference in the concentrations of glutamic acid and aspartic acid, which decreased and increased at 3.43% and 2.63%, respectively. Data analysis revealed that the concentrations of histidine (1.25%), methionine (0.74%), isoleucine (1.38%) and leucine (1.93%) increased, whereas that of lysine (2.02%) decreased significantly ($P>0.05$) in the March samples. On the other hand, the n-EAAs, glutamic acid (2.01%) and aspartic acid (2.10%), significantly decreased in the March samples (Table 3). The presence of glycine, alanine, valine, leucine, tyrosine and phenylalanine in the peptides also gives a bitter taste, since proline mostly gives the bitter taste of peptides. The taste of glycine and alanine are active ingredients, and it is well known to have sweetness in various seafood (Pratama et al. 2017). Aspartic acid and glutamic acid have an important role in enzyme active cores, and maintain the solubility properties of proteins (Sikorski et al. 1990; Belitz et al. 2001). Glutamate gives umami taste when its concentration in the foodstuff rises above the taste threshold, and this may be

an indicator of protein intake (Kawai et al. 2009; Zhao et al. 2016). Also, the less glutamic acid contained in fish meat it would result in less savory taste of the fish meat (Suryaningrum et al. 2010). Non-essential amino acids can be synthesized by transferring an amino group to α-keto acids that can be derived from non-protein sources such as glucose (Webster and Lim 2002; Litwack 2017). This may be the cause of non-essential amino acids that increase at sampling times.

Table 3- Amino Acids Composition of narrow-barred Spanish Mackerel

Amino Acids	*Amino Acids profile of mackerel (%)*		
	19-January	*19 -February*	*19- March*
Histidine	1.23±0.21^{a}	1.12±0.29^{b}	1.25±0.10^{a}
Threonine	1.05±0.01	1.04±0.08	1.04±0.14
Methionine	0.58±0.03^{b}	0.64±0.11^{ab}	0.74±0.01^{a}
Valine	1.30±0.18	1.30±0.18	1.35±0.18
Phenylalanine	1.02±0.04	1.02±0.01	0.99±0.10
Isoleucine	1.31±0.32^{a}	1.01±0.02^{b}	1.38±0.32^{a}
Leucine	1.56±0.26^{b}	1.86±0.06^{a}	1.93±0.17^{a}
Lysine	2.23±0.08^{ab}	2.67±0.06^{a}	2.02±0.16^{b}
Tryptophan	0.22±0.01	0.24±0.01	0.24±0.01
Serine	1.88±0.03	1.86±0.03	1.81±0.93
Glycine	1.13±0.02	1.16±0.0	1.13±0.12
Aspartic Acid	2.23±0.00^{ab}	2.63±0.00^{a}	2.10±0.44^{b}
Glutamic Acid	3.81±0.02^{a}	3.43±0.02^{b}	2.01±0.75^{c}
Arginine	1.29±0.20	1.26±0.14	1.26±0.90
Alanine	1.42±0.10	1.40±0.10	1.40±0.10
Tyrosine	0.78±0.12	0.76±0.12	0.77±0.12

For the data relative to fish fillet, values are mean ± SD. (n= 6; number of fishes per sampling time); and values in the same row with different superscript letters indicate statistically significant difference (P<0.05)

In conclusion, the present study demonstrates that narrow-barred Spanish mackerel, which are caught and landed in the north-eastern Mediterranean Sea, have a commercial value and are rich in proteins, fatty acid and amino acids during the sampling time. Moreover, the amounts of EPA and n3/n6 were not significantly different at all sampling times. This species has been found to meet the daily intake of 250-500 mg of EPA and DHA against the risk of cardiovascular disease recommended by EFSA (2012) caught during the months of consumption. This fish can be recommended in terms of detected essential fatty acid and amino acid profile that completely nutritious for the human as well as other organisms' dietary requirements.

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Determination of Melatonin Differences between Day and Night Milk in Dairy Cattle

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ABSTRACT
The aim of this study was to determine the difference between melatonin hormone in night milk and melatonin concentration in day milk of Holstein cows. In this study, daytime milk obtained from 40 heads Holstein cows in the first lactation raised in a private dairy cattle farm in Karapınar district of Konya state province in Turkey and night milk samples taken from the same cows that were kept in darkness for one week were used. The milk samples were collected from day and night milking in order to determine the melatonin concentration with the help of Bovine Melatonin (MLT) Elisa Kit. In this study, melatonin concentration in day time and night time milk were determined as respectively 103.70 ± 6.61 pg mL^{-1} and 163.13 ± 8.96 pg mL^{-1}. The difference between melatonin levels of day and night milk was statistically significant ($P<0.01$). Since significant difference occurred between melatonin concentration between day and night milk at the end of the study, it can be stated that night milk can be used for medical purposes that is used cure of some illnesses and producers can be provided with a new production source.

Keywords: Holstein, Melatonin, Night milk, Day-time milk, Dairy cattle

1. Introduction

Melatonin is a hormone secreted from the gland called pineal or epiphysis located at the base of the brain, which weighs 100-180 mg in mature humans and a tiny pea-sized gland. Melatonin in the blood is found between 60-70% depending on the albumin (Ölmez et al. 2000; Atasoy & Erbaş 2017). Melatonin secretion is not restricted to mammals but is also produced in non-mammalian vertebrates, in some invertebrates, and in many plants, with the same molecular structure. The synthesis of melatonin is strictly controlled by lighting conditions and shows a clear circadian rhythm with low values during the daytime and significant increase at night (Karasek & Winczyk 2006; Srinivasan et al. 2011). This hormone secret during sleep at night, is intensely secreted, especially between 23 and 05 hours. For this intense secretion to occur, the environment must be dark (Jainudeen 2000; Ölmez et al. 2000; Karasek & Winczyk 2006; Balch 2010; Atasoy & Erbaş 2017). Pineal gland is present in all vertebrates. Mammalian pineal is derived from photo-receptor cells capable of synthesizing melatonin (Gupta & Spessert 2007). This gland consists of specialized epithelial cells called pinealocytes. In mammals, the pineal gland loses its ability to synthesize melatonin when exposed to direct light. Melatonin synthesis is at the lowest level during the day (light phase) and at the highest level at night (dark phase) (Boztepe 2019).

Melatonin is synthesized from tryptophan amino acid in all vertebrates. It is first converted to 5-hydroxy tryptophan by the tryptophan hydroxylase enzyme and then to 5-hydroxytryptamine, that is, serotonin, by the aromatic L-amino acid decarboxylase enzyme. Serotonin is converted to the N-acetylserotonin form by the AA-NAT (Arylalkylamine-N-acetyltransferase) enzyme. Finally, N-acetylserotonin is converted to N-acetylmethoxideriptamine, melatonin, via the HIOMT (hydroxyindole-O-methyltransferase) enzyme. While AA-NAT limits melatonin secretion with very low activity during the day, it reaches high activity at night. As a result, the rate of conversion of serotonin to melatonin is minimal as it leads to the accumulation of serotonin in pinealocytes during daytime. AA-NAT activity increases at the onset of darkness, which regulates the rate of melatonin synthesis. It causes norepinephrine (NE) secretion to increase from the end of the postganglionic sympathetic nerve fibers present in the pineal gland. NE plays a role as a second means on pinealocytes via membrane-bound adrenergic receptors and cAMP, leading to an increase in melatonin synthesis and secretion (Gupta & Spessert 2007). According to Özçelik et al. (2013), in human the secretion of melatonin hormone depends on the sensitivity of pinealocyte cells to light. With this sensitivity, light inhibition is eliminated in the dark, and melatonin secretion is increased again. Especially between 23:00 and 05:00 at night,

melatonin secretion peaks and its concentration in the blood increases 3-10 times. In humans, the release of melatonin starts to increase in the evening at 21.00-22.00 hours and reaches the highest level at 02.00-04.00 hours. It starts to decrease at 05.00-07.00 in the morning and drops to basal levels after 07.00. While the blood concentration of melatonin is approximately 0-20 v during the day, it increases to 50-200 pg mL^{-1} during the night. An average of 30 mg melatonin is synthesized overnight. Singh and Rao (2016) reported that the melatonin exchange interval in low-density in human, cow and goat milk was 5-25 pg mL^{-1}. Romanini et al. (2019) found the highest concentration of melatonin in individual cow's milk as 41.94 pg mL^{-1}. They reported that there were seasonal changes and that the highest melatonin concentration was reached at night hours in winter.

Melatonin is a very powerful antioxidant, it is stronger than vitamin C, E or beta carotene, and it prevents harmful oxidation. In this way, it can reduce the risk of hypertension, heart attack and some types of cancer. It has also been stated that it regulates the immune system, prevents memory loss, vascular congestion and paralysis, and can be useful in the treatment of Alzheimer's and cancer (Yazıcı & Köse 2004; Hardeland et al. 2011; Özçelik et al. 2013; Salt et al. 2017). In addition, there is no toxic level of melatonin consumption (Balch 2010). In case of covering long distances in a short time, biological dysfunctions occur in the body, and the biological balance is disrupted due to the changes of the biological clock. According to some sources, melatonin plays a role in the regulation of biological clock (Liu & Borjigin 2006). In fact, the arrangement of the biological clock provides regular secretion of melatonin in the body and provides protective and therapeutic functions. Jimenez et al. (2009) have reported that goat somatic cell count (SCC) is a very important quality indicator, and it is related to the function of the immune cells in the breast and oxidative metabolism. Researchers have stated that melatonin plays a positive role in oxidative metabolism. The same study group divided 60 Verata goats into two groups in order to demonstrate the effects of melatonin implant reduction on SCC in dairy goats. The first group was the melatonin implanted and the other was the non-implanted control group. All animals were followed bacteriologically during lactation and evaluated for clinical mastitis. Blood and milk samples were taken three days after birth and four times at monthly intervals. At the end of the experiment, the milk composition did not change and SCC decreased significantly in the middle of lactation in the implant group compared to the control group.

Because of these benefits of melatonin, determination of natural resources is very important. In animal products milk etc, melatonin is more abundant in night milk than in day-time milk. There have been some studies on this subject abroad (Valtonen et al. 2005; Asher et al. 2015; Romanini et al. 2019) but the studies on this issue are quite insufficient in our country. This study was conducted to determine the level of melatonin in milk obtained from day and night milking of Holstein cows, and the production status of a natural source of melatonin.

2. Material and Methods

2.1. Material

The animal material of the study consisted of the milk samples obtained from the first lactating Holstein cows which were raised farm in Karapınar district of Konya province in Turkey. Milk samples were collected from the same cows as day milk (evening milking) at the beginning of the study and night milk after one week darkening application at nights. Cows are milked three times a day at 07.00 in the morning, at 16.00 in the evening and at 01.00 at night.

2.2. Method

The samples to be analysed were obtained through milking the milk produced during the day by 40 heads of Holstein cows in the first lactation.

The cows were kept in a completely darkened barn for a week. Because of milking program differenced the cows is being probably mastitis, since the management of the enterprise could not be interfered, the animals were exposed to light in the milking house for their night milking.

Milk samples were homogenously taken (from onset to end of milking) carefully taken into 50 mL tubes to represent the total milk with the help of milk sampling apparatus which can be mounted to the milking system. The milk samples were transferred to the laboratory in cold chain, and dark environment being homogenized and filled into 8 mL polypropylene tubes.

In the laboratory, the samples were centrifuged twice at 4500 rpm for 15 minutes. After each centrifugation, the fat layer accumulated in the upper part was removed and the milk samples completely separated from the fat were kept at -80 ^{o}C until study day. On the study day, the samples were first thawed to -20 ^{o}C, then to +4 ^{o}C and finally at room temperature. After thawing, homogenization was achieved through vortexing. Homogenized samples were prepared using the MYBIOSOURCE brand Bovine Melatonin (MLT) ELISA Kit (Competitive ELISA) (MBS743340, Mybiosource, California, USA) and in accordance with the kit procedure, using the Rayto RT-2600 Microplate Washer (India) washer and BMG LABTECH (Enzyme-Linked Immuno Sorbent Assay (ELISA) scanner Melatonin levels were determined in pg mL^{-1}. The quantitation limits of the melatonin kit used were between 0-1000 pg mL^{-1} and no dilution was applied to the samples.

Differences between melatonin levels in day and night milk were determined by pairing t-test in order to prevent dependence since the samples were taken from the same animals before and after darkening (Kesici & Kocabaş 2007). Statistical analyses were performed with the help of MINITAB statistical package program (Minitab 2010).

3. Results and Discussion

Melatonin levels were determined with ELISA method from the samples of the daytime milk taken at the beginning of the study and the milk samples obtained only in the evening without any additional application (feeding, lighting, etc.) from the same cows in the barn prevented from receiving light for a week.

At the end of the study, the rate of melatonin in day milk was 103.70 pg mL^{-1} and in night milk it was 163.13 pg mL^{-1} (Table 1). The difference between melatonin levels of day and night milk was statistically significant ($P<0.01$).

Table 1- Melatonin levels and standard errors in day and night milk

Samples	*n*	$\bar{X} \pm S_{\bar{X}}$	*Min*	*Max*
Day Milk (pg mL^{-1})	40	103.70±6.61	28.76	213.68
Night Milk (pg mL^{-1})	40	163.13±8.96	64.58	322.08
Difference (pg mL^{-1})	40	59.43±12.81	1.26	293.32

The difference of between day and night milk was 59.43 pg mL^{-1}. (Table 1, Figure 1). This difference is important only in determining the difference between day and night milk without any application. This difference is also above the highest melatonin value of 41.49 pg mL^{-1} in the milk produced overnight. Asher et al. (2015) detected melatonin levels in milk in darkness at night and in limited lighting conditions at night. Melatonin level of the night milk in the dark is around 30 pg mL^{-1}, while in the group with limited illumination it is between 15-20 pg mL^{-1}. The difference between melatonin levels of daytime milk and animals kept in total darkness and cows with limited illumination at night was also significant. Melatonin detected in daytime milk of animals kept in the dark at night decreased with limited illumination. That is, any lighting or light leads to a decrease in the level of melatonin.

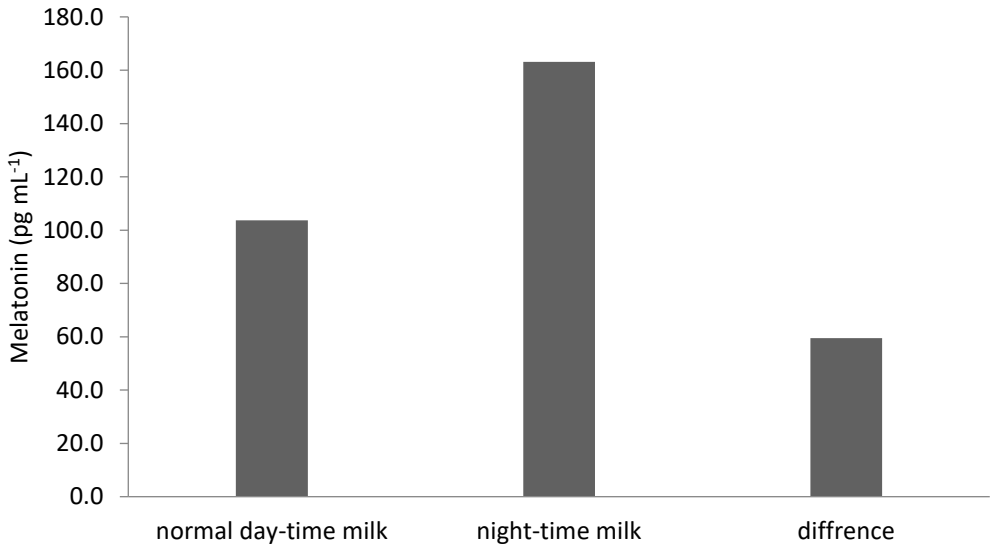

Figure 1- Melatonin levels in day and night milk

Asher et al. (2015) also studied the effects of the seasons on melatonin levels. Differences in melatonin levels of daytime milk and night milk in dark or limited lighting conditions between winter and summer months were found statistically significant. While melatonin levels under darkened conditions were around 22 pg mL^{-1} in low yield animals, it was 16 pg mL^{-1} in high yield animals under the same conditions in winter. The same researchers investigated differences between melatonin levels in cow milk, UHT (Ultra High Temperature) milk and tank milk. While there was no statistically significant difference between cow milk and UHT milk in winter, the difference between tank milk was significant. At the same time, the difference between UHT milk and tank milk is statistically insignificant.

In this study, melatonin levels for both day and night milk were found higher than those reported in the literature (Asher et al. 2015; Romanini et al. 2019). The possible cause of this difference may be attributed to the different kits used to determine melatonin level and the differences in seasons. That is, the reference range for melatonin levels in the Elisa kit (MYBIOSOURCE brand Bovine Melatonin (MLT) ELISA Kit (Competitive ELISA) (Catalog Number: MBS743340)) catalog used in this study is between 0 and 1000 while this reference ranges from 0 to 50 in the catalogs of different companies.

4. Conclusions

Valtonen et al. (2005) reported that even the lowest dose of melatonin, 0.1 mg, was 10 times greater than total melatonin secreted at night. So, it is impossible to meet this amount by consuming melatonin-rich milk. Accordingly, at least half a liter of ‘’night milk’’ produced from cows should be consumed by adults, especially children and the elderly as a supplement to patients' melatonin secretion basing on the reports of Valtonen et al. (2005). When this is realized, it is likely that complaints about many diseases or disorders on which melatonin is known to be effective will be reduced. As demonstrated in some studies, melatonin has a protective effect. Therefore, consuming this milk, at least for protection, will have important health-related contributions. Although the level of melatonin in milk is far from meeting the needs of people, meeting the milk needs from night milk can be helpful in reducing many melatonin-induced diseases or symptoms. In the study on this issue, Konturek et al. (2007) reported that plasma melatonin levels decreased with age. It is a known fact that the complaints about illness and health increase at the age of 50s-55s normally. It is also known that these complaints increase in later ages. That is, plasma melatonin level decreases with age and when these known facts are evaluated together, it can be concluded that the event is related to melatonin. Thus, plasma melatonin levels in the 21-25 age range reach up to 80 pg mL^{-1}, while this value is almost halved in the 51-55 age range. According to the results of the present study, an average of 163 pg mL^{-1} melatonin was detected in the milk obtained from cows overnight. Considering that a glass is 200 mL, 163x200 = 32600 pg of melatonin is consumed. At night, one glass of milk at night can contribute to increase the decreased level of melatonin in the 51-55 age range to reach the 21-25 age range and decrease the possible complaints.

Melatonin has a vital role in humans, animals and plants and is essential for a healthy life, and even the resources rich in melatonin should be evaluated for a healthy and happy life. For example, in Finland (Valtonen et al. 2005) and Germany (Mullins 2010), melatonin-rich milk produced from cattle under the name of ‘’night time milk’’ has been commercially produced. In this regard, Valtonen et al. (2005) reported that melatonin secretion decreased with age and that they used the night milk called “night time milk’’ as the material for the elimination of sleep disorders in elderly people, and they accomplished positive results when the patients were given approximately 0.61 liters day^{-1}. Bae et al. (2016) compared the effects of consuming normal milk containing 100 pg melatonin and 47.5 mg tryptophan amino acid with a glass of night milk containing 1000 pg melatonin and 58.24 mg tryptophan amino acid. As a result, a glass of overnight milk containing 1000 pg of melatonin increased sleep comfort and decreased insomnia throughout the day.

Regarding this melatonin-rich (approximately 67% richer), which can be called “night time milk” or night milk; (1) new studies related to production should be conducted, (2) the time when the levels of melatonin are highest in milk at night should be determined, (3) which light sources are more suitable for the production of night milk with melatonin should be ascertained, (4) how tryptophan contribution in feed will contribute to melatonin production in “night milk’’ should be established as tryptophan amino acid is the source of melatonin, and (5) the effects of “night milk’’ on patients should be investigated, and also (6) this melatonin-rich milk is marketed as “night time milk’’ in some countries. In Turkey, these studies should be carried out and melatonin-rich milk should be more produced in dairy cattle farms and it is important that this product is delivered to the consumers.

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Efficacy of Various Entomopathogenic Fungi Strains as Biocontrol Agents for Control of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae)

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ABSTRACT
Cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is considered an important bruchid pest in cowpea seed storages. The used pesticides against this pest have caused the occurrence of resistant populations and direct toxicity to the users. The objective of this study was to evaluate the mortality effects of six entomopathogenic fungi isolates obtained from ARSEF (USA) collection [*Paecilomyces farinosus* (2538), *Isaria fumosorosea* (4501), *Isaria farinosa* (3580), *Beauveria bassiana* (4984), *Lecanicillium muscarium* (972) and *Lecanicillium muscarium* (5128)] against *C. maculatus* adults under laboratory conditions (26±2 °C, 70±5% RH and 16h light: 8h dark). The isolates were cultivated in Potato Dextrose Agar (PDA, Oxoid, CM0139) medium at 26±2 ºC in dark conditions for two weeks before using them as control agents. Spore suspensions of the isolates were prepared at two different concentrations ($1x10^5$ and $1x10^7$) and mixed with Tween 20 (0.04%). Each concentration was replicated three times and the mortality rates were observed on the 2nd, 4th, 6th, 8th and 10th day of incubations. As a commercial control, a Mycotal extraction of *L. muscarium* and as a negative control, Tween 20+sterile water was used. Six entomopathogenic fungal isolates at both conidial concentrations yielded high mortalities (from 62.6% to 100%) of *C. maculatus* adults. These results illustrated that tested fungi strains led to significant mortalities on *C. maculatus* adults in all the treatments as compared to the controls. Consequently, these fungi strains were regarded as an encouraging alternative method to control the population of *C. maculatus* adults in the stored cowpea grains.

Keywords: Entomopathogenic fungus, Biological control, Cowpea seed beetle, Bruchid

1. Introduction

Legume plants are very important source of protein for human feeding in undeveloped countries. Among them, cowpea (*Vigna unguiculata* (L.) Walp.: Fabaceae) is grown for its green beans and seeds by farmers especially in tropical and subtropical regions of the world. It is also cultivated all over the world for animal feed (Ofuya & Akhidue 2005). Due to their prosperous source of nutritious elements, the fresh and green crusts of cowpea are consumed as vegetable. In addition, the leaf, branch and stem parts of the plant are used as fresh animal food for livestocks (Remya 2007).

Cowpea seed beetle (*Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)) is one of the most destructive pests on cowpea and other legumes growing in tropical and sub-tropical countries, both in fresh green crusts in fields and in stored seeds (Singh & Van Emden 1979). The adults are not harmful. But, the larvae of this pest feed on cowpea (*V. unguiculata* (L.)), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), soybean (*Glycine max* Mer.) and haricot beans (*Phaseolus vulgaris* L.) (Mahfuz & Khalequzzaman 2007). The female adults of *C. maculatus* lay their eggs in the fresh cowpeas before the reaping in the field. The larvae from these eggs bore into the cowpea seeds, develop by feeding the embryo of the seeds and mature just about in a month in storage conditions (Fox & Tatar 1994). Therefore, the larvae can lead to both quantitative (due to grain weight loss caused by larvae feeding) and qualitative (due to product alterations such as loss of nutritious and aesthetic values, resulting increased levels of losses in the grain mass) damages on cowpea grains (Moina et al. 1998). This pest requires great care due to the potentials of severe damages mentioned above.

In the past, many commercial and persistent insecticides such as Phosphine, Methyl bromide, Deltamethrin and Malathion were largely used to control this pest in stored cowpea. But, these chemicals pose risks such as food and environmental

pollution, toxicities to non-target organisms, pest resistance, pesticide residues, direct toxicities to users and ozone depletion (Arthur 1996; Isman 2006; Khashaveh et al. 2011). Therefore, health authorities are reluctant to allow chemical insecticides due to their residues on stored grains. New alternative control strategies are required, because the commercial insecticides have quite toxic impacts on environment and human health. The growing research on biological protectant alternatives has revealed the positive role of microbial insecticides (Sheeba et al. 2001). One of the most promising and environment-friendly alternatives for pest control is entomopathogenic fungi. They are among the first entomopathogenic organisms used against harmful arthropods. Entomopathogenic fungi cause disease by infecting to insects or other arthropods and subsequently provoke rapid declines in large populations of their arthropod hosts. They have garnered the most interest to research for utilizing as microbial insecticides. For these reasons, their roles are crucial on biological control of hazardous insects because of environmentally safe and very low toxicity to mammalians (Cox & Wilkin 1996). The use of entomopathogenic fungi for biological control of the pests is an attractive alternative to classical pesticides, because these beneficial fungi are very friendly control agents against a wide range of organisms, and have no detrimental effects on environment (Khetan 2001; Sevim et al. 2013). Therefore, entomopathogenic fungi could be a viable alternative method to control this pest. Up to now, there are many studies with the use of entomopathogenic fungi to control insect pests, but very little focused on using fungi as control agents against stored product pests (Ferroni 1977; Serale & Doberski 1984; Moina et al. 1998; Sheeba et al. 2001; Gökçe & Er 2005; Sevim et al. 2010; Shifa Vanmathi et al. 2011; Muştu et al. 2014; Reddy et al. 2014; Erler & Ateş 2015; Komaki et al. 2017; Usanmaz Bozhüyük et al. 2018; Bjornson & Elkabir 2019).

The main objective of the present study was to investigate the efficacies of various entomopathogenic fungi isolates [(*Paecilomyces farinosus* (2538), *Isaria fumosorosea* (4501), *I. farinosa* (3580), *Beauveria bassiana* (4984), *Lecanicillium muscarium* (972) and *L. muscarium* (5128))] for the control of the adults of important and destructive storage pest; *C. maculatus*, under laboratory conditions.

2. Material and Methods

2.1. Storage pest insect

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) adults were collected from private store houses in Muğla, Turkey and kept on cowpea (*Vigna unguiculata* (L.) seeds. After, a certain amount of cowpea seeds was purchased from a local market and maintained at a freezer in two days at -15 ˚C in order to prevent any arthropod pests prior to in the bioassay. Then, *C. maculatus* adults were reared in 1 L jars containing cowpea seeds. The cultures were maintained in the dark conditions in a growth chamber set at 26±2 ˚C and 70±5% RH without exposure to any insecticide for several generations. Newly emerged adults (three days-old, mixed males and females) were used for subsequent experiments. All experimental procedures were carried out under the same conditions as the cultures. Experiments were carried out in three replicates with 25 adults of this pest in each Petri dish. Sufficient amount (1 seed/1 insect) of cowpea seeds were placed in each Petri dish for the adult insects during entomopathogenic tests.

2. 2. Entomopathogenic fungi isolates and preparation

The entomopathogenic fungi strains [(*Paecilomyces farinosus* (2538), *Isaria fumosorosea* (4501), *I. farinosa* (3580), *Beauveria bassiana* (4984), *Lecanicillium muscarium* (972) and *L. muscarium* (5128)] were obtained from an entomopathogenic fungi collection (ARSEF, USA). Another *L. muscarium* isolate, used as commercial control, was obtained from a commercial product (Mycotal, Koppert, NL). Fungal isolates were cultivated in the Potato Dextrose Agar (PDA, Oxoid, CM0139) medium at 26±2 ºC in dark for two weeks and used as spray source on the storage pests. Harvested conidia from 14-day-old cultures grown on PDA plates were thoroughly mixed with the carrier in screw capped bottles in 3 mL distilled sterile water. Spore solutions of entomopathogenic fungi isolates were prepared at $1x10^5$ and $1x10^7$ concentrations and mixed with Tween 20 (0.04%). The suspension was sieved and 1 mL of prepared suspension was sprayed on each replicate of 25 beetles in each Petri dishes. The sprayed Petri dishes were then incubated in an incubator at 26±2 ºC and the dead beetles were counted in each 48 h. For evaluating the conidial viability, the spores of different isolates were saved in the suspension of distilled sterile water and Tween 20, checked by light microscopy (Olympus BH2) after 2, 4, 6, 8 and 10 days.

2. 3. Bioassays

In order to test the toxicity of six entomopathogenic fungi, the applications were carried by adding 1×10^5 and 1×10^7 conidia to 1 mL in 9 cm diameter sterile Petri dishes with two layers of drying paper. Then, 25 newly emerged adults of *C. maculatus* were collected with an aspirator and placed in each Petri dish. Separately, sufficient amount of cowpea seeds (1 seed/1 insect) were added to each Petri dish. The prepared entomopathogenic fungal suspensions were sprayed on the adults and seeds contained in Petri dishes and incubated at 26±2 ºC. Each assay was repeated three times for each dose and exposure time combination. Mycotal extraction of *L. lecanii* was utilized as commercial control and distilled sterile water with Tween 20 as negative control in the study. After these treatments, the alive and dead *C. maculatus* adult individuals were counted in every 48 h for 10 days (Table 1).

Table 1- Percent mortalities of *Callosobruchus maculatus* (Fab.) adults inoculated with two different conidial concentrations ($1x10^5$ and $1x10^7$) of six entomopathogenic fungi isolates

Treatment Entomopathogenic fungi	*Dose*	*Callobruchus maculatus (Fabricius)*				
		Mortality (%)[a]				
		Days After Treatment[b]				
		2	*4*	*6*	*8*	*10*
Paecilomyces farinosus (2538)	$1x10^5$	65.3±3.52 bc	73.3±4.80 bc	84.0±8.0 b	94.6±2.66 cd	97.3±1.33 bc
	$1x10^7$	90.6±7.42e	92.0 ± 6.11cd	98.6 ± 1.33de	100 ± 0.0e	100 ± 0.0c
Isaria fumosorosea (4501)	$1x10^5$	77.3 ± 11.3bcde	84.0 ± 8.0bcd	90.6 ± 4.80bcde	97.3 ± 1.33cde	98.6 ±1.33bc
	$1x10^7$	76.0 ± 0.0bcde	80.0 ± 2.30bcd	86.6 ± 5.33bc	97.3 ± 1.33cde	100 ± 0.0c
Beauveria bassiana (4984)	$1x10^5$	78.6 ± 13.3bcde	78.6 ± 13.3bcd	89.3 ± 4.80bcde	98.6 ±1.33de	100 ± 0.0c
	$1x10^7$	86.6 ± 6.66de	86.6 ± 6.66bcd	100 ± 0.0e	100 ± 0.0e	100 ± 0.0c
Lecanicillium muscarium (972)	$1x10^5$	62.6 ± 4.80b	70.6 ± 4.80b	90.6 ± 4.80bcde	98.6 ± 1.33de	98.6 ± 1.33bc
	$1x10^7$	84.0 ± 12.0cde	92.0 ± 4.0cd	97.3 ± 2.66cde	100 ± 0.0e	100 ± 0.0c
Isaria farinosa (3580)	$1x10^5$	72.0 ± 12.0bcde	72.0 ± 12.0b	85.3 ± 8.11b	98.6 ± 1.33de	100 ± 0.0c
	$1x10^7$	78.6 ± 2.30de	88.0 ± 9.33bcd	97.3 ± 1.33cde	100 ± 0.0e	100 ± 0.0c
Lecanicillium muscarium (5128)	$1x10^5$	69.3 ± 7.05bcd	74.6 ± 9.33bc	88.0 ± 6.11bcd	93.3 ± 3.52c	97.3 ± 2.66bc
	$1x10^7$	89.3 ± 5.33 e	96.0 ± 2.30d	98.6 ± 1.33de	100 ± 0.0e	100 ± 0.0c
Commercial Control (*L. muscarium* (Mycotal))	$1x10^5$	78.6 ±1.33bcde	84.0 ± 2.30bcd	89.3 ± 1.33bcde	89.3 ± 1.33b	96.0 ± 2.30bc
	$1x10^7$	82.6 ± 7.42 cde	82.6 ± 7.42bcd	88.0 ± 2.30bcd	89.3 ± 1.33b	94.6 ± 3.52b
Negative Control (Tween20+steril water)	-	0.0 ± 0.0a	1.33 ± 1.11a	1.33 ± 1.11a	2.66 ± 1.11a	2.66 ± 1.11a

[a]: Numbers in each column are Mean ± SE of three replicates, each set-up with 25 adults; [b]: Exposure duration (day)
[*]: Values followed by different letters in the same column differ significantly at P<0.05

2. 4. Statistical analysis

Percent mortalities of *C. maculatus* adults were subjected to ANOVA with SPSS 17.0 software package and means were separated by Duncan's multiple range test at P<0.05.

3. Results

Mortality rates of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) adults treated with six entomopathogenic fungi isolates are provided in Table 1. The results showed that all tested entomopathogenic fungi isolates had different mortality effects on *C. maculatus* adults as compared to control treatments. In all experiments, the mortality rates generally increased with increasing exposure durations. The strain used as commercial control (*Lecanicillium muscarium*, Mycotal) was failed to achieve 100% mortality and the greatest rate of mortality with this *L. muscarium* strain was 94.6%. According to the results, the earliest 100% mortality on *C. maculatus* adults were obtained at 6 days after treatment (DAT) with *Beauveria bassiana* (4984) sprayed at $1x10^7$ spore concentration, under 26±2 °C and 70±5% RH. On the other hand, mortality rates were considerable higher even in the first day counts (2 DAT), $1x10^7$ concentration of *Paecilomyces farinosus* (2538) provided over 90% average mortality, where *B. bassiana* isolate (4501) killed 78.6% of the adults at lower ($1x10^5$) dosage, which was on par with the same dosage mortality results of commercial control (*L. muscarium*). In the second count day (4 DAT), one of the *L. muscarium* isolates (5128) were able to kill about 96% of the adults at $1x10^7$ dosage while *Isaria fumosorosea* (4501) treatments at lower dose have the highest mortality (84.0%) among the others. All of the isolates, except for *I. fumosorosea*, displayed over 95% mortality at 6^{th} day after treatments. The second greatest mortality at that day was achieved in *L. muscarium* (5128) treated Petri dishes, 98.6% of adults were recorded dead at high-dose treatments, which was closely followed by *I. farinosa* (98.6%), again at $1x10^7$ spore concentration. In the 8^{th} day after treatments, 100% of the adults were dead in Petri dishes treated with the higher dose of entomopathogenic isolates, again, with the exception of *I. fumosorosea*. And finally, at 10^{th} day, over 97% mortality was recorded in all fungi, tested. As seen in Table 1, the first two days (2^{nd} and 3^{rd}) and second two days (6^{th} and 8^{th}) had different efficiency levels. It is also worth to mention that all isolates yielded significantly different results at lower and higher dosages, but *I. fumosorosea* treatment yielded similar outcomes throughout the experimental period, except for 4^{th} day.

4. Discussion

In this study, six entomopathogenic fungi isolates [(*Paecilomyces farinosus* (2538), *Isaria fumosorosea* (4501), *I. farinosa* (3580), *Beauveria bassiana* (4984), *Lecanicillium muscarium* (972) and *L. muscarium* (5128)] were found as pathogenic on *C. maculatus* adults. Mortality rates of all isolates on *C. maculatus* adults, at both doses (1×10^5 and 1×10^7), increased gradually with longer exposure durations. Mortality of *C. maculatus* adults varied between 97.3 and 100%. Specifically, the adult mortalities were 97.3% with *L. muscarium* (5128) and *P. farinosus* (2538) at $1x10^5$ dose on 10^{th} day of treatment and 100% with all tested entomopathogenic fungi isolates at $1x10^7$ dose (Table 1).

Many studies were carried out by different researchers about the mortality effects of various entomopathogenic fungi against *C. maculatus*. Currently, *B. bassiana* has been identified as one of the most successful entomopathogenic fungi. The use of *B. bassiana* to control *C. maculatus* was studied by various researchers worldwide. Cherry et al. (2005) reported that the evolution of cumulative mortality among *C. maculatus* adults following immersion in aqueous conidial suspensions of *B. bassiana* 0362 yielded greater efficacy for *B. bassiana* 0362. The same authors pointed out that this was the most noticeable at $1x10^8$ conidia mL^{-1} where 100% mortality was achieved with *B. bassiana* 0362 after 6 days of treatment. Draganova et al. (2007) found that the isolates 417, 412, 414 and 426 of *B. bassiana* caused mycoses on *C. maculatus* adults, and the highest lethal effect to *C. maculatus* adults was expressed as 100% mortality on the 6^{th}, 7^{th} and 8^{th} day, respectively. Shifa Vanmathi et al. (2011) recorded the mortality rates at 1×10^5 (40%, 80%, 85.70%, 91.52%, 97.90%) and 1×10^7 doses (13.33%, 33.33%, 71.42%, 71.42% and 97.78%) after 24, 48, 72, 92 and 120 h on *C. maculatus* adults, respectively. Kılıç et al. (2019) determined that nine different isolates of *B. bassiana* led to different mortality rates at 1×10^7 dose and after 12 h of treatment on *Helicoverpa armigera* (Hübner) (51.9-68.1%), *Spodoptera littoralis* (Hübner) (45.5-54.4%), *Tenebrio molitor* (L.) (66.7-81.5%) and *Blattella germanica* (L.) (3.33-6.70%). In present study, *B. bassiana* (4984) isolates caused the mortality rates at 1×10^5 (86.60%, 86.60%, 100%, 100%, 100%) and 1×10^7 doses (78.60%, 78.60%, 89.30%, 98.60%, 100%) after 2, 4, 6, 8 and 10 days of the treatments on *C. maculatus* adults, respectively (Table 1). *I. fumosorosea* has been known as a common entomopathogenic fungus all over the world for more than 30 years (Zimmermann 2008). Sevim et al. (2013) determined that *I. fumosorosea* KTU–42 caused different mortality rates on *C. ciliata* adults (63%) and nymphs (50%). In the present study, we found that *I. fumosorosea* (4501) isolate caused different mortality rates in the 1×10^5 (76 %, 80%, 86.6%, 97.3%, 100%) and 1×10^7 (77.3%, 84%, 90.6%, 97.3%, 98.6%) doses after 2, 4, 6, 8 and 10 days of the treatments on *C. maculatus* adults, respectively (Table 1). *I. farinosa* is one of the most the commercially produced entomopathogenic fungi, which have an estimated 700 species of entomopathogenic fungi in approximately 90 genera. Yang et al. (2009) stated that *I. farinosa* had mortality effect on the larvae, pupae and adults of *Pissodes punctatus* (Coleoptera: Curculionidae) up to 88%. In another study, Muştu et al. (2011) recorded that on the 9^{th} and 12^{nd} days of the incubation, *I. farinosa* caused different mortality rates (22.5 and 45%) at $1x10^6$ and $1x10^8$ conidial concentrations (mL^{-1}) (52.5 and 70%) on *A. rostrata* adults. Correlatively, Demirci et al. (2011) found that *I. farinosa* had different mortality rates at 1×10^8, 1×10^7, 1×10^6, 1×10^5 doses and 95% RH on the adults of *Planococcus citri* (Risso) (84.53%, 32.29%, 19.24%, 20.54%), respectively. Muştu et al. (2014) determined that *I. farinosa* isolate had significant mortality on the sun pest adults (*Eurygaster austriaca* (Schrk.)). They found that *I. farinosa* caused different mortality rates (0.00%, 5.00%, 10.00% and 33.75%, 63.75%, 86.25%) at 1×10^6 and 1×10^8 conidia concentration doses mL^{-1} after 6, 9 and 12 days of treatments on *E. austriaca* adults, respectively. In present study, *I. farinosa* (3580) isolate caused different mortality rates (72%, 72%, 85.3%, 98.6%, 100%; and 78.6%, 88%, 97.3%, 100%, 100%, respectively) at 1×10^5 and 1×10^7 doses on the 2^{nd}, 4^{th}, 6^{th} and 10^{th} days of the treatment on *C. maculatus* adults. These percentages were significantly higher than the control ($P<0.05$) (Table 1). *P. farinosus* has been known as an effective entomopathogenic fungus for more than 30 years. Today, because it is one of the most important biocontrol agents, its various strains are successfully used for biocontrol of different pestiferous insects, such as whiteflies (Zimmermann 2008). Vidal et al. (1997) demonstrated that 29 isolates of *P. farinosus* had highly significant mortality (from 68 to 94%) on the silverleaf whitefly, *Bemicia argentifolii* Bellows & Perring. Simova & Draganova (2003) found that *P. farinosus* had a mortality effect on two spotted spidermite, *Tetranychus urticae* K.. In another study, it was stated that *P. farinosus* isolates (290 and 290re) showed lethal effect on *Ips sexdentatus* Boer. and *I. acuminatus* Gyll. adults (45.00 and 66.67%, respectively) at 1×10^8 conidia/mL (Draganova et al. 2007). In this study, it was found that *P. farinosus* isolate (2538) caused different mortality rates on *C. maculatus* adults in the 1×10^5 and 1×10^7 doses after 2, 4, 6 and 10 days of the treatments, as 90.6%, 92.0%, 98.6%, 100%, 100%; and 65.3%, 73.3%, 84.0%, 94.6% and 97.3%, respectively (Table 1). *L. muscarium* is also among the important entomopathogenic fungi. It is known as one of the important natural enemies of *Scolypopa australis* (Walker) in kiwi orchards (Marshall et al. 2003). This fungus is a commercially produced entomopathogenic fungi and has been commercialized worldwide as the biopesticides Mycotal (a commercial formulation of *L. muscarium* produced) against whiteflies and thrips; and Verticillin against whiteflies, aphids and mites (Faria & Wraight 2007). In a previous study, it was stated that *L. muscarium* isolates had mortality effects on the adults and nymphs of *Bactericera cockerelli* (Sulc) (up to 100% for adults and 70% for nymphs) at the 1×10^7 dose after seven days of the treatments (Mauchline & Stannard 2013). In present study, *L. muscarium* isolate (972) caused greater mortality effect (84.0%, 92.0%, 97.3%, 100%, 100%) at 1×10^7 dose than at 1×10^5 dose (62.6%, 70.6%, 90.6%, 98.6%, 98.6%) within all exposure times of the treatments on *C. maculatus* adults. However, *L. muscarium* isolate (5128) caused 89.3%, 96.0%, 98.6%, 100% and 100% mortality rates at 1×10^7 dose and after 2, 4, 6, 8 and 10 days on *C. maculatus* adults in this study, respectively. But, the same fungus isolate had lower mortality effect (69.3%, 74.6%, 88.0%, 93.3% and 97.3%) at 1×10^5 dose and within the same exposure times. These percentages were significantly higher than the control ($P<0.05$) (Table 1). Komaki et al. (2017) tested six entomopathogenic fungi isolates ((*P. farinosus* (2538), *I. fumosorosea* (4501), *I. farinosa* (3580), *B. bassiana* (4984), *L. muscarium* (972) and *L. muscarium* (5128)) against *Tribolium confusum* du Val., 1863 adults under laboratory conditions. In their study, these entomopathogenic fungi isolates led to the mortalities between 37.3 (for *I. farinosa* (3580) at 1×10^5 dose) and 100% (for *P. farinosus* (2538) and *B. bassiana* (4984) at 1×10^7 dose) on *T. confusum* adults after 10 days of treatment. In present study, the mortality rates were recorded as between 62.6 (for *L. muscarium* (972) at 1×10^5 dose) and 100% (for all fungi isolates at different doses) on *C. maculatus* adults after 10 days of treatment. According to these results, six fungi isolates showed greater effect on *C. maculatus* adults than *T. confusum* adults (Table 1).

5. Conclusions

In the present study, six entomopathogenic fungi isolates [(*P. farinosus* (2538), *I. fumosorosea* (4501), *I. farinosa* (3580), *B. bassiana* (4984) and *L. muscarium* (2 isolates)] were tested against *C. maculatus* adults under laboratory conditions. Among the tested fungi isolates, *L. muscarium* (972) caused the greatest mortality rates (between 84 and 100%) on *C. maculatus* adults at 26±2 °C, 70±5% RH and with $1x10^7$ conidial concentrations (mL^{-1}) on the 2nd, 4th, 6th, 8th and 10th days. The lowest mortality was recorded at $1x10^5$ conidial concentration of *L. muscarium* (972) isolate as 62.60% on the 2nd day of the treatment. Present findings showed that the fungal isolates used in this study could be used as possible biocontrol agents against *C. maculatus* adults. Further studies should be carried out to evaluate the effectiveness of these isolates in the field. It was concluded that use of biological control agent of *L. muscarium* (972) isolate as a part of integrated pest management strategy may reduce the future dependence on chemical control.

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Effect of Methyl Jasmonate Treatments on Fruit Quality and Antioxidant Enzyme Activities of Sour Cherry (*Prunus cerasus* L.) During Cold Storage

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ABSTRACT
The study was carried out to investigate the effect of methyl jasmonate (MeJA) treatments (0.5 and 1.0 mM MeJA) on quality characteristics such as weight loss, respiration rate, ethylene production, color, total phenolic content (TPC), total antioxidant capacity (TAC) and antioxidant enzyme activities of sour cherry fruit (*Prunus cerasus* L. cv. 'Kütahya') during cold storage. Fruit were stored at 0±1 °C and 90±5% RH for 36 days. The results indicated that MeJA treatments showed higher levels of total phenolic content, total antioxidant capacity and quality and were also effective on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), malondialdehyde (MDA), ethylene production and respiration rate. In conclusion, 0.5 mM MeJA treatment showed the best maintaining of fruit quality among the concentrations of MeJA. It can be suggested that sour cherry could be stored successfully for 36 days at 0 °C following treatment of MeJA.

Keywords: Antioxidant enzymes, Ethylene production, MeJA, Respiration rate, Sour cherry

1. Introduction

Sour cherry (*Prunus cerasus* L.), a stone fruit species and originated from northeastern Anatolia, belongs to the *Rosaceae* family (Önal 2002; Ferretti et al. 2010). Total annual production of sour cherry was 1.52 million tones worldwide in 2018 (FAO 2020). Turkey is one of important countries in terms of sour cherry production and the fruit has been widely consumed as fruit juice, jam, vine or dried fruit (Eksi & Akdag 2007; Lončarić et al. 2016). The fruit is rich in anthocyanins which known as an antioxidant due to ability to counteract oxygen free radicals. Sour cherry has an important nutritional value because of its high level of vitamins, fibers, and polyphenolids (anthocyanins and other flavonoids), in addition to alkaloids and melatonin (Jia et al. 2012).

Although fresh consumption of this fruit has not been widely common (Lončarić et al. 2016), recent studies showed that there has been an increasing demand for fresh consumption due to its health benefits resulted from regular intake of anthocyanins and polyphenolics (Beattie et al. 2005; Kim et al. 2005; Piccolella et al. 2008).

Methyl Jasmonate (MeJA) is a natural compound used both preharvest (Saracoglu et al. 2017) and postharvest (Öztürk et al. 2019) to extend shelf life, as well as maintain the quality of products. The treatment of MeJA increases antioxidant activity during postharvest period since stimulates the activities of several antioxidant enzymes such as superoxide dismutase (SOD) (Cao et al. 2009a), catalase (CAT) (Asghari & Hasanlooe 2015), ascorbate peroxidase (APX) (Cao et al. 2009b), polyphenol oxidase (PPO) (Asghari & Hasanlooe 2015). Moreover, the treatments of MeJA poise membrane structure and bring down lipid peroxidation (Ziosi et al. 2008). So, the postharvest treatment of MeJA in horticultural crops is remarkable in order to maintaining storage quality, delaying senescence and improving resistant responses.

Unfortunately, there have been no published studies about the effect of MeJA on sour cherry quality and postharvest life. So, the aim of this study was to investigate the effect of MeJA treatments with stretch film on sour cherry quality, ripening and senescence at 0 °C storage temperature and during 36 days.

2. Material and Methods

2.1. Plant materials

The sour cherry fruit *(Prunus cerasus* L. cv. 'Kütahya') were manually harvested at optimal harvest date (when the entire surface of fruit had light red color) in Gevaş (38° 32′ 28″ N, 43° 26′ 03″ E, 1730 m in elevation) district of Van Province, Turkey and used in this study. The samples were pre-cooled for 12 hours at +4 °C temperature.

2.2. Methods

The fruit suitable for experiment, as in the same size and free from defects were selected and divided into three groups. Samples in the first group was immersed in distilled water as a control for 10 minutes. The second and third group fruit were immersed in 0.5 and 1.0 mM MeJA (PubChem CID: 5281929, 95% Sigma Aldrich, cat no.392707) solutions for 10 minutes, respectively. After treatments, all fruit were dried on papers at room condition (25 °C). Later, the fruit were placed in foam plates (each package per 400 g) and covered with stretch film having 8 µ thickness, then stored for 36 days at 0 °C temperature and 90-95% relative humidity (RH). During storage period, changes in fruit quality were determined some physical, chemical and physiological analysis mentioned below.

2.3. Weight loss

Weight loss during the storage period was measured daily and calculated as percentage of initial weight.

2.4. Titratable acidity (TA), fruit juice pH, soluble solids content (SSC), color

Titratable acidity (TA) was measured by titrating of fruit juice by 0.1 N NaOH till pH= 8.1 and the results were assessed in % citric acid (Cemeroğlu 2007). The pH values were measured by a pH meter (AZ 8601, Hengxin Company, China). Soluble solids content (SSC) was detected with a digital hand refractometer (Atago, Tokyo, Japan) and results were presented as percent. Fruit color was measured by a chromameter (Minolta CR-400; Osaka, Japan) in *L*, a*, C*° and *h* °color space system and 10 fruits were measured randomly for each replication.

2.5. Total phenolic content (TPC) and total antioxidant capacity (TAC)

Total phenolic content was determined with a spectrophotometer (Thermo Scientific Genesys 10S UV-VIS) at 725 nm as described by the Swain & Hillis (1959) and was assessed in gallic acid equivalent (GAE) mg100 g^{-1} FW.

Ferric Reducing Antioxidant Power (FRAP) method was utilized to evaluate the total antioxidant capacity at 593 nm and was assessed in µmol trolox equivalent (TE) g^{-1} FW (Benzie & Strain 1996).

2.6. Antioxidative enzyme analyzes

The activity of superoxide dismutase (SOD) and catalase (CAT) enzymes was spectrophotometrically measured according to the methods of Jebara et al. (2005), Bagcı (2010), Alp & Kabay (2019) at 560 nm and 240 nm, respectively. Ascorbate peroxidase (APX) activity was also measured at 290 nm as in Nakano et al. (1981). The levels of lipid peroxidation were assessed with regard to malondialdehyde (MDA) content according to Bagcı (2010)s' method.

2.7. Respiration rate and ethylene production

For respiration rate determinations, fruit (each replication an average of 250 g) were kept at room temperature in closed jars for 2 hours and the carbon dioxide (CO_2) emission of fruit was detected in headspace gas sample with the Headspace Gas Analyzer GS3/L analyzer. The respiration rate values presented as mL CO_2 kg^{-1} h^{-1} (Cavusoglu et al. 2020).

The ethylene production of samples was assessed in aforementioned headspace gas samples with a gas-tight syringe and a GC-FID (GC-2010 Plus) as described by Guillén et al. (2013). The ethylene production was presented is assessed as µL C_2H_4 kg^{-1} h^{-1}.

2.8. Oxygen and Carbon dioxide concentrations in the packages

The oxygen (O_2) and CO_2 concentrations in the packages was detected by Headspace Gas Analyzer GS3 / L analyzer.

2.9. Statistical analysis

This study was carried out as completely randomized experimental design with three replications and each package was

considered one replication. Descriptive statistics for the studied variables were presented as Mean and Standard Error of Mean (SEM). Two-way Factorial ANOVA was performed to data. Treatments at different MeJA concentrations and storage period were considered as factors. Duncans' Multiple Range Test comparisons were also used to identify different levels of treatment and storage factors. Statistical significance level was considered as 5% and SPSS (Ver. 21) statistical program was used for all statistical computations.

3. Results

3.1. Weight loss

The weight loss was 7.33% in control fruit at the end of storage period and, respectively, higher than in sour cherry fruit treated with 1 mM and 0.5 mM MeJA (Table 1). Among the storage periods, weight losses were significant in all the sampling.

Table 1- The changes in weight loss during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
Weight loss	0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000± 0.000 F[1]
	5	1.001 ± 0.101	0.922 ± 0.044	0.928 ± 0.009	0.950 ± 0.032 E
	10	2.523 ± 0.332	2.089 ± 0.020	2.127 ± 0.405	2.246 ± 0.161 D
	15	3.577 ± 0.463	2.949 ± 0.028	2.986 ± 0.409	3.170 ± 0.205 C
	25	5.685 ± 0.724	4.666 ± 0.042	4.272 ± 0.014	4.874 ± 0.325 B
	36	7.331 ± 1.449	6.384 ± 0.057	6.847 ± 0.003	6.853 ± 0.412 A
	Means	3.352 ± 0.795	2.834 ± 0.653	2.859 ± 0.681	
	Significant effects; Ptreatment = 0.846 Pstorage = 0.001 Ptreatment x Pstorage = 0.890				

[1]: Letters show differences among storage periods at P<0.05 error level

3.2. Titratable acidity (TA), fruit juice pH, soluble solids content (SSC), Color

The results in table 2 showed that the fruit treated with MeJA resulted in higher levels of TA compared with untreated fruit. The highest level of TA was detected in fruit treated with 1 mM MeJA followed by fruit treated with 0.5 mM MeJA after 36 days of storage period. Among storage periods, TA values were significantly changed in all treatments. Significant differences were observed between fruit treated with MeJA and control fruit in terms of TA values.

Table 2- The changes in titratable acidity (TA), pH and soluble solids content (SSC) during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
TA	0	2.320 ± 0.144 A a	2.320 ± 0.144 A a	2.320 ± 0.144 A a	2.320 ± 0.064
	5	1.277 ± 0.054 CD c	1.677 ± 0.205 BC b	1.725 ± 0.131 BC a	1.559 ± 0.110
	10	1.568 ± 0.038 BC b	1.975 ± 0.029 AB a	1.991 ± 0.134 AB a	1.844 ± 0.094
	15	1.824 ± 0.288 B a	1.120 ± 0.032 E c	1.456 ± 0.029 C b	1.466 ± 0.148
	25	1.002 ± 0.029 E b	1.200 ± 0.003 CD a	1.037 ± 0.006 D b	1.079 ± 0.039
	36	1.479 ± 0.116 BCD b	1.527 ± 0.041 CD a	1.555 ± 0.154 C a	1.520 ± 0.052
	Means	1.578 ± 0.132	1.636 ± 0.130	1.680 ± 0.127	
	Significant effects; Ptreatment = 0.856 Pstorage = 0.001 Ptreatment x Pstorage = 0.015				
pH	0	3.195 ± 0.015	3.195 ± 0.015	3.195 ± 0.015	3,195 ± 0,006 B[1]
	5	3.400 ± 0.040	3.325 ± 0.015	3.405 ± 0.035	3,376 ± 0,021 A
	10	3.380 ± 0.030	3.405 ± 0.005	3.365 ± 0.055	3,383 ± 0,017 A
	15	3.345 ± 0.015	3.385 ± 0.015	3.310 ± 0.020	3,346 ± 0,015 A
	25	3.395 ± 0.025	3.390 ± 0.020	3.365 ± 0.025	3,383 ± 0,012 A
	36	3.440 ± 0.000	3.405 ± 0.005	3.410 ± 0.010	3,418 ± 0,007 A
	Means	3.359 ±0.024	3.350 ±0.022	3.341 ±0.023	
	Significant effects; Ptreatment = 0.875 Pstorage = 0.001 Ptreatment x Pstorage = 0.260				
SSC	0	15.900 ± 0.100 A a[1]	15.900 ± 0.100 BC a	15.900 ± 0.100 B a	15.900 ± 0.044
	5	13.000 ± 0.300 C b c	14.000 ± 0.800 CD b	19.050 ± 0.650 A a	15.350 ± 1.216
	10	13.650 ± 1.050 BC b	16.750 ± 0.050 A a	16.000 ± 0.100 B a	15.466 ± 0.650
	15	13.750 ± 0.050 BC a	13.250 ± 0.050 D a	12.600 ± 0.200 D b	13.200 ± 0.217
	25	10.050 ± 0.350 D c	13.700 ± 0.400 D a	12.150 ± 0.050 D b	11.966 ± 0.217
	36	15.100 ± 0.100 AB a	15.250 ± 0.150 BC a	14.500 ± 0.000 C b	14.950 ± 0.152
	Means	13.575 ± 0.575	14.808 ± 0.395	15.033 ± 0.705	
	Significant effects; Ptreatment = 0.169 Pstorage = 0.001 Ptreatment x Pstorage = 0.001				

[1]: Differences among storage periods was shown with capital letters for the same treatment (P<0.05), differences among treatments was shown with small letters for the same storage period (P<0.05)

The level of pH increased in all fruit, regardless of treatment, during the storage period. The least level of pH (3.40) was found in fruit treated with 0.5 mM MeJA at the end of storage period (Table 2). The difference of pH values among the storage periods was significant ($P<0.05$) in all the sampling times. It was determined that SSC levels decreased at the end compared with the beginning of storage period. The highest value was 15.25% in fruit treated with 0.5 mM MeJA after 36 days of storage (Table 2). Among storage periods, SSC contents were significantly in both fruit treated with MeJA and control fruit. Significant differences were observed between fruit treated with MeJA and control fruit (Table 2).

Although there were fluctuations in all treatments during the storage period, a decrease trend in L^*, a^*, C° and h° values were observed during storage period. The highest values of L^* observed in fruit treated with 1 mM MeJA. Also, the least values of a^* was found in fruit treated with MeJA at the end of storage period (Table 3). Among storage periods, L^*, a^*, C^* and hue angle values were significant in in all the sampling.

Table 3- The changes in L^*, a^*, C° and h° during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
L^* (Lightness)	0	27.215 ± 0.895	27.215 ± 0.895	27.215 ± 0.895	27.215 ± 0.400 C[1]
	5	30.475 ± 0.065	30.520 ± 0.790	30.305 ± 0.435	30.433 ± 0.237 A
	10	30.010 ± 0.220	30.545 ± 0.895	30.545 ± 0.565	30.366 ± 0.301 A
	15	28.940 ± 0.430	28.065 ± 0.165	25.945 ± 0.495	27.650 ± 0.588 C
	25	27.910 ± 0.030	28.915 ± 0.045	28.490 ± 0.450	28.438 ± 0.218 B
	36	24.365 ± 1.655	24.295 ± 1.495	26.125 ± 1.655	24.928 ± 0.810 D
	Means	28.152 ± 0.657	28.259 ± 0.697	28.104 ±0.613	
	Significant effects; Ptreatment = 0.986 Pstorage = 0.001 Ptreatment x Pstorage = 0.435				
a^*	0	23.150 ± 0.810	23.150 ± 0.810	23.150 ± 0.810	27.215 ± 0.400 C
	5	25.050 ± 0.630	23.640 ± 1.060	23.210 ± 0.470	30.433 ± 0.237 A
	10	21.525 ± 1.375	23.705 ± 1.245	22.195 ± 0.295	30.366 ± 0.301 A
	15	21.595 ± 0.485	17.410 ± 1.200	18.185 ± 0.325	27.650 ± 0.588 C
	25	23.190 ± 0.990	22.795 ± 0.225	19.765 ± 1.405	28.438 ± 0.218 B
	36	21.625 ± 1.715	19.105 ± 1.875	18.880 ±0.700	24.928 ± 0.810 D
	Means	22.689 ± 0.504	21.634 ± 0.821	20.897 ± 0.655	
	Significant effects; Ptreatment = 0.183 Pstorage = 0.001 Ptreatment x Pstorage = 0.205				
C°	0	24.680 ± 0.970	24.680 ± 0.970	24.680 ± 0.970	24.680 ± 0.433 B
	5	26.740 ± 0.570	25.215 ± 1.445	24.960 ± 0.570	25.638 ± 0.553 A
	10	22.745 ±1.405	25.050 ± 1.510	22.050 ± 0.950	23.281 ± 0.820 C
	15	22.785 ± 0.735	18.150 ± 1.320	19.330 ± 0.040	20.088 ± 0.962 D
	25	24.255 ± 1.065	23.885 ± 0.215	20.620 ± 1.540	22.920 ± 0.877 C
	36	23.085 ± 1.755	20.050 ± 1.990	19.810 ± 0.700	20.981 ± 0.972 D
	Means	24.048 ± 0.549	22.838 ± 0.919	21.908 ± 0.725	
	Significant effects; Ptreatment = 0.143 Pstorage = 0.001 Ptreatment x Pstorage = 0.241				
h°	0	19.180 ± 0.720	19.180 ± 0.720	19.180 ± 0.720	19.180 ± 0.321 A
	5	19.220 ± 0.690	18.500 ± 0.910	18.950 ± 0.680	18.890 ± 0.367 B
	10	17.870 ± 0.630	17.870 ± 1.190	19.415 ± 1.685	18.385 ± 0.645 B
	15	17.250 ± 0.860	16.440 ± 0.060	18.740 ± 2.060	17.476 ± 0.716 C
	25	16.235 ± 0.445	16.425 ± 0.315	16.835 ± 0.425	16.498 ± 0.210 D
	36	16.155 ± 0.045	17.160 ± 0.240	16.600 ± 0.440	16.638 ± 0.225 D
	Means	17.651 ± 0.418	17.595 ± 0.373	18.286 ± 0.494	
	Significant effects; Ptreatment = 0.462 Pstorage = 0.001 Ptreatment x Pstorage = 0.894				

[1]: Differences among storage periods was shown with capital letters for the same treatment ($P<0.05$)

3.3. Total phenolic content (TPC) and total antioxidant capacity (TAC)

The level of total phenolics decreased regularly in all fruit, during storage period. The highest value (22.702 mg100 g^{-1}) was determined in fruit treated with 0.5 mM MeJA at the end of storage period (Table 4). Furthermore, similar patterns were found in antioxidant capacity and the antioxidant capacity in fruit treated with MeJA was higher than in the control fruit (Table 4). Among storage periods, total antioxidant capacity (TAC) and total phenolic content (TPC) were significant in all the sampling.

Table 4- The changes in total antioxidant capacity (TAC) and total phenolic content (TPC) during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
TPC	0	28.851 ± 1.984	28.851 ± 1.984	28.851 ± 1.984	28.851 ± 0.887 A[1]
	5	25.326 ± 0.395	26.185 ± 0.079	26.309 ± 0.559	25.939 ± 0.264 AB
	10	23.502 ± 0.214	24.766 ± 0.784	24.243 ± 0.603	24.170 ± 0.349 BC
	15	22.872 ± 0.840	23.766 ± 0.095	23.830 ± 3.131	23.489 ± 0.859 BC
	25	20.728 ± 2.022	23.125 ± 3.634	22.484 ± 1.116	22.111 ± 1.200 C
	36	19.409 ± 0.262	22.703 ±0.284	21.413 ± 0.001	21.174 ± 0.614 C
	Means	23.448 ± 0.998	24.898 ± 0.820	24.521 ± 0.887	
	Significant effects; Ptreatment = 0.508 Pstorage = 0.001 Ptreatment x Pstorage = 0.997				
TAC	0	0.749 ± 0.033	0.749 ± 0.033	0.749 ± 0.033	0.749 ± 0.014 A
	5	0 .682 ± 0.006	0.711 ± 0.113	0.663 ± 0.091	0.685 ± 0.038 B
	10	0.638 ± 0.003	0.679 ± 0.003	0.654 ± 0.011	0.656 ± 0.008 C
	15	0.565 ± 0.005	0.660 ± 0.022	0.619 ± 0.017	0.614 ± 0.018 D
	25	0.541 ± 0.005	0.629 ± 0.074	0.598 ± 0.008	0.589 ± 0.025 E
	36	0 .546 ± 0.073	0.606 ± 0.041	0.575 ± 0.024	0.575 ± 0.025 E
	Means	0.619 ± 0.025	0.672 ± 0.023	0.642 ± 0.021	
	Significant effects; Ptreatment = 0.294 Pstorage = 0.001 Ptreatment x Pstorage = 0.994				

[1]:Differences among storage periods was shown with capital letters for the same treatment (P<0.05)

3.4. Antioxidative enzyme analyzes

During the storage period, the enzyme activity of CAT and SOD reached a peak at 15th day in 0.5 mM MeJA treated fruit. In addition, the highest levels of SOD, APX and CAT were found in fruit treated with 0.5 mM MeJA followed by fruit treated with 1 mM MeJA at the end of storage period (Table 5). In addition, the level of MDA increased in all treatments during the storage period. However, the lowest level of MDA was found in fruit treated with MeJA (Table 5).

Table 5- The changes in CAT (mmol g^{-1} FW), APX (mmol g^{-1} FW), SOD (unit g^{-1} FW) and MDA (nmol g^{-1} FW) enzyme activities during storage of 'Kütahya' sour cherry (*Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
CAT	0	0.021 ± 0.001	0.021 ± 0.001	0.021 ± 0.001	0.020 ± 0.000 B[1]
	5	0.042 ± 0.003	0.098 ± 0.058	0.069 ± 0.002	0.069 ± 0.018 A
	10	0.044 ± 0.005	0.126 ± 0.000	0.075 ± 0.004	0.081 ± 0.015 A
	15	0.075 ± 0.011	0.148 ± 0.002	0.089 ± 0.016	0.103 ± 0.015 A
	25	0.067 ± 0.008	0.140 ± 0.001	0.072 ± 0.003	0.092 ± 0.015 A
	36	0.066 ± 0.058	0.134 ± 0.001	0.074 ± 0.001	0.091 ± 0.020A
	Means	0.052 ± 0.009 b[1]	0.111 ± 0.014 a	0.066 ± 0.006 b	
	Significant effects; Ptreatment = 0.002 Pstorage = 0.010 Ptreatment x Pstorage = 0.709				
SOD	0	150.013 ± 6.652	150.013 ± 6.652	150.013 ± 6.652	150.012 ± 20.975 D
	5	171.022 ± 11.733	240.633 ± 21.189	285.671 ± 26.298	232.441 ± 23.023 C
	10	213.161 ± 15.863	294.347 ± 12.174	295.218 ± 21.268	267.575 ± 18.786 B
	15	268.846 ± 20.070	337.808 ± 31.940	302.535 ± 6.280	303.062 ± 16.001 A
	25	195.592 ± 65.981	264.959 ± 56.428	229.757 ± 21.595	230.102 ± 26.344 C
	36	137.772 ± 0.814	210.237 ± 83.514	174.075 ± 0.473	174.028 ± 25.299 D
	Means	189.400 ±15.887	249.665 ± 22.491	239.544 ± 18.783	
	Significant effects; Ptreatment = 0.075 Pstorage = 0.001 Ptreatment x Pstorage = 0.810				
APX	0	0.326 ± 0.014	0.326 ± 0.014	0.326 ± 0.014	0.326 ± 0.006 D
	5	0.356 ± 0.007	1.243 ± 0.218	1.576 ± 0.093	1.058 ± 0.238 C
	10	0 .804 ± 0.006	1.168 ± 0.088	1.483 ± 0.716	1.151 ± 0.223 C
	15	0.881 ± 0.017	1.531 ± 0.882	1.558 ± 0.185	1.323 ± 0.271 C
	25	1.678 ± 0.031	1.932 ± 0.020	1.724 ± 1.384	1.777 ± 0.360 B
	36	1.958 ± 0.020	2.460 ± 0.239	2.287 ± 0.134	2.234 ± 0.117 A
	Means	1.000 ±0.186	1.443 ± 0.231	1.492 ± 0.262	
	Significant effects; Ptreatment = 0.261 Pstorage = 0.001 Ptreatment x Pstorage = 0.964				
MDA	0	38.469 ± 2.035	38.469 ± 2.035	38.469 ± 2.035	38.468 ± 0.910 E
	5	49.243 ± 2.604	47.509 ± 3.573	49.490 ± 0.005	48.747 ± 1.207 D
	10	56.926 ± 1.535	50.446 ± 2.419	51.772 ± 0.138	53.047 ± 1.452 C
	15	59.712 ± 2.022	53.765 ± 1.839	56.590 ± 0.671	56.689 ± 1.306 BC
	25	63.545 ± 1.778	56.847 ± 1.982	59.010 ± 6.201	59.800 ± 2.143 B
	36	69.717 ± 4.927	67.400 ± 3.159	65.095 ± 7.898	67.404 ± 2.674 A
	Means	56.268 ± 3.155	52.405 ± 2.775	53.404 ± 2.819	
	Significant effects; Ptreatment = 0.629 Pstorage = 0.001 Ptreatment x Pstorage = 0.979				

[1]: Differences among storage periods was shown with capital letters for the same treatment (P <0.05), differences among treatments was shown with small letters for the same storage period (P<0.05)

Among storage periods, CAT, APX, SOD and MDA enzyme activities were significant. On the other hand, significant differences were observed among treatments in CAT enzyme activity.

3.5. Respiration rate and ethylene production

Ethylene production decreased in all treatments during the storage period, whereas respiration rates increased. MeJA treatments were effective on respiration rates especially suppression of ethylene production compared with control fruit (Table 6). Among storage periods, respiration rate and ethylene production were significant in all the sampling.

Table 6- The changes in respiration rate and ethylene production during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
Respiration rate	0	127.769 ± 8.605	127.769 ± 8.605	127.769 ± 8.605	127.769 ± 3.848 A[1]
	5	77.448 ± 0.238	59.917 ± 18.573	103.619 ± 7.263	80.328 ± 9.539 CD
	10	106.608 ± 9.376	101.918 ± 5.384	97.414 ± 0.321	101.980 ± 3.258 B
	15	103.121 ± 1.488	100.857 ± 7.738	97.242 ± 1.906	100.407 ± 2.356 B
	25	89.793 ± 1.481	95.261 ± 1.412	90.426 ± 0.822	91.827 ± 1.231 BC
	36	80.206 ± 9.769	74.474 ± 2.260	73.163 ± 0.302	75.947 ± 2.929 D
	Means	97.491 ± 5.577	93.366 ± 7.078	98.272 ± 5.110	
	Significant effects; Ptreatment = 0.806 Pstorage = 0.001 Ptreatment x Pstorage = 0.684				
Ethylene production	0	0.166 ± 0.020	0.166 ± 0.020	0.166 ± 0.020	0.166 ± 0.008 D
	5	0.282 ± 0.056	0.179 ± 0.084	0.235 ± 0.024	0.231 ± 0.032 A
	10	0.197 ± 0.039	0.215 ± 0.066	0.227 ± 0.040	0.212 ± 0.022 A
	15	0 .174 ± 0.004	0.123 ± 0.005	0.133 ± 0.003	0.143 ± 0.010 B
	25	0.159 ± 0.018	0.113 ± 0.005	0 .142 ± 0.017	0.137 ± 0.010 B
	36	0 .158 ± 0.004	0.112 ± 0.004	0.107 ± 0.005	0.125 ± 0.010 C
	Means	0.189 ± 0.015	0.151 ± 0.017	0.168 ± 0.015	
	Significant effects; Ptreatment = 0.276 Pstorage = 0.001 Ptreatment x Pstorage = 0.872				

[1]: Differences among storage periods was shown with capital letters for the same treatment (P <0.05)

3.6. Oxygen and Carbon dioxide concentrations in the packages

During the storage period, the concentration of O_2 decreased inside the packages, while CO_2 levels increased, as expected. CO_2 levels increased significantly inside the package for the first five days of storage, while, the O_2 levels were reduced. At the end of the storage, the lower CO_2 levels were reported in fruit treated with MeJA compared with untreated fruit, and also the higher O_2 levels were detected in fruit treated with MeJA (Table 7). Among storage periods, concentration of O_2 and CO_2 inside the packages were significant in all the sampling.

Table 7- The changes in concentration of O_2 and CO_2 in the packages during storage of 'Kütahya' sour cherry *(Prunus cerasus* L.) fruit during 36 d at 0 °C. Data was presented as means ± SEM

Parameters	*Storage period*	*Control*	*0.5 mM MeJA*	*1 mM MeJA*	*Means*
O_2	0	20.900 ± 0.000	20.900 ± 0.000	20.900 ± 0.000	20.900 ± 0.000 A[1]
	5	16.150 ± 0.250	16.400 ± 1.200	13.400 ± 0.400	15.316 ± 0.693 B
	10	15.350 ± 0.950	16.350 ± 0.150	15.800 ± 0.800	15.833 ± 0.371 B
	15	16.500 ± 1.500	15.050 ± 1.550	15.700 ± 2.100	15.750 ± 0.821 B
	25	13.800 ± 0.500	13.500 ± 1.600	13.550 ± 0.850	13.616 ± 0.488 B
	36	13.350 ± 1.550	15.350 ± 1.550	16.350 ± 2.550	15.016 ± 1.031 B
	Means	16.008 ± 0.801	16.258 ± 0.781	15.950 ± 0.865	
	Significant effects; Ptreatment = 0.961 Pstorage = 0.001 Ptreatment x Pstorage = 0.662				
CO_2	0	0.300 ± 0.000	0.300 ± 0.000	0.300 ± 0.000	0.300 ± 0.000 B
	5	1.900 ± 0.200	1.950 ± 0.650	2.600 ± 0.100	2.150 ± 0.227 A
	10	2.000 ± 0.300	2.050 ± 0.550	2.250 ± 0.550	2.100 ± 0.220 A
	15	2.200 ± 0.000	2.650 ± 0.250	2.550 ± 0.350	2.466 ± 0.140 A
	25	2.700 ± 0.200	2.600 ± 0.400	2.950 ± 0.350	2.750 ± 0.160 A
	36	2.450 ± 0.250	2.300 ± 0.100	2.150 ± 0.350	2.300 ± 0.126 A
	Means	1.925 ± 0.240	1.975 ± 0.267	2.133 ± 0.278	
	Significant effects; Ptreatment = 0.843 Pstorage = 0.001 Ptreatment x Pstorage = 0.927				

[1]: Differences among storage periods was shown with capital letters for the same treatment (P <0.05)

4. Discussion

Most commodities come in possession of unmarketable as fresh products with a weight loss more than 10%. Fruit treated with MeJA after harvest has been suggested to have a positive effect on quality parameters such as color, firmness and weight loss (Fan et al. 2016a; Fan et al. 2016b). In our study, fruit treated with MeJA and especially 0.5 mM MeJA had a positive impact in delaying weight loss comparing to control.

It has been suggested that the amount of acidity decreases since fruit use up organic acids through respiration process during the storage period (Jin et al. 2012). Zhang et al. (2009) found that pears treated with MeJA after harvest showed delayed rotting and did not adversely affect quality parameters such as titratable acidity (TA), firmness and soluble solids content (SSC). Researchers suggested that MeJA treatment resulted in higher level of TA compared with untreated fruit and had positive effects on fruit quality (Wang & Zheng 2005; Casado et al. 2014; Akan et al. 2019). In the current study, it was found that the fruit treated with 0.5 mM MeJA have a positive influence in terms of SSC. On the other hand, the fruit treated with 1 mM MeJA t had higher levels of TA. The higher values of pH observed in untreated fruit.

It has been reported that the treatment of MeJA after harvest prevents color changes in the fruit skin (Martínez-Espláa et al. 2014; Öztürk et al. 2014). In addition, many researchers have mentioned that the change of hue angle value is an indicator of the color changes in the fruit skin (Rudell et al. 2005; Greer 2005; Rudell & Mattheis 2008). In the current study, it was observed that MeJA treatments had a positive effect on hue angle values. Moreover, the higher values of L^* was found in fruit treated with 1 mM MeJA.

The antioxidants associated with phenolic compounds are effective against degenerative diseases (Aviram et al. 2008; Mertens-Talcott et al. 2006). Many researchers believed that MeJA treatments generally increased antioxidant capacity and total phenolic content (Wang and Zheng 2005; Chanjirakul et al. 2006; Wang et al. 2008). We also found that antioxidant capacity and total phenolic content enhanced in fruit treated with MeJA comparing to untreated fruit.

Antioxidant enzymes provide an essential role, defending plants from injury caused by the accumulation of reactive oxygen species (ROS) (Groppa & Benavides 2008; Duan et al. 2008; Kıpçak et al. 2019). Ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) are important in performing mostly related to antioxidant enzymatic systems actuated in plants to scavenge the detrimental effects of oxidative stress (Gill & Tuteja 2010; Karuppanapandian et al. 2011). Accumulation of ROS could possibly lead to an increase in lipid peroxidation, leading to devastation to membranes, and therefore enhance deposit of MDA (Xie et al. 2008).

Previous studies showed that activities of superoxide dismutase and catalase are effectively impressed by MeJA in postharvest treatments (Chanjirakul et al. 2006; 2007; Meng et al. 2017). In the current study, it was approved that the activities of superoxide dismutase and catalase increased in fruit treated with MeJA comparing to untreated (control) fruit after 36 days of storage. Fan et al. (2016a) suggested that exogenous treatment of MeJA significantly decreased MDA content in cowpea fruit. Our findings revealed similar results. Fruit treated with MeJA had lower level of MDA content than untreated (control) fruit. Moreover, evidence shows that postharvest treatment of MeJA results in higher activities of APX, SOD and CAT during the storage period than control fruit in peach and loquat fruit (Jin et al. 2009; Cao et al. 2009; Zapata et al. 2014). In the present study, we obtained similar findings. That is the fruit treated with MeJA showed the higher level of APX content compared with control fruit.

Previous studies showed that the Modified Atmosphere Packaging (MAP) treatment reduces respiration rate and ethylene production in cherry fruit (Yarılgac et al. 2019). It was reported that MeJA increases the respiration rate, when treated in the beginning of ripening stage and also claimed it has no effect on respiration rate in mango fruit (Lalel et al. 2003). In addition, Öztürk et al. (2019) suggested that MeJA treatment led to a lower respiration rate compared to control fruit. The effect of MeJA on respiration rate (Öztürk et al. 2019) and ethylene production (Zapata et al. 2014) may diverse depending on the maturity period, fruit type or MeJA concentration. In our study, we found that the respiration rate and ethylene production was lower in fruit treated with MeJA comparing to control fruit.

In conclusion, MeJA treatments showed higher levels of total phenolic content, total antioxidant capacity and quality and were also effective on ethylene production and respiration rate. This positive effect of MeJA may be due to stimulation of antioxidant enzyme activity. 0.5 mM MeJA treatment showed the best results among the all of MeJA concentrations. It can be suggested that sour cherry could be stored successfully for 36 days at 0 °C following treatment by MeJA.

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The Estimation of Live Weight from Body Measurements in Different Meat-Type Lambs

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ABSTRACT
The present study aimed to analyze the live weight (LW) and some body measurements to estimate the LW of lambs in five different meat-type sheep breeds reared in the same flock under intensive conditions. A total of 202 head of lambs, including both genders of Kivircik (K), Bandirma (B), Karacabey Merino (KM), Hampshire Down x Merino crossbreed (HM), and Ramlic (R), were conducted in this study. Birth weights ranged between 3.94-5.07 kg in those breeds and were affected significantly by breed and birth types (P<0.001). The effect of sex was clearly seen with the weaning period; males lambs were 2.61 kg heavier than female lambs (P<0.001). The importance of breed differences on chest circumference (CC) increased as the lambs grew older. On the other hand, sex had a significant effect on all body measurements at the pre- and post-weaning period (P<0.01 and P<0.001); however, did not on body length (BL) at the pre-weaning period (P>0.05). In contrast, the importance of birth type on BL and CC decreased as the lambs grew older (P>0.05). The results of regressing LW and body measurements show that LW was very highly (P<0.001) correlated with body measurements (*r*=0.682–0.892). The highest correlations were observed between LW and CC (*r*=0.802-0.892) in B, KM, and R lambs. These results suggest that using body measurements as a correction factor has a great benefit in those breeds, such as withers height and CC are used to estimate LW in KM and R lambs, effectively.

Keywords: Estimation, Body measurements, Live weight, Sheep breeds

1. Introduction

Sheep breeding plays a crucial role in enhancing the rural economy, and it is known by one of the most adaptive small ruminants to adverse environmental conditions in Turkey. Despite its great impact on the rural economy, the extensive lamb production system is still practiced in most of the country with traditional methods of management and natural grazing (Kaymakçı & Sönmez 1996; Akçapınar 2000). There are more than 37 million sheep with various native breeds in different geographical regions of Turkey (TurkStat 2019). Approximately 80-90% of Turkey's sheep populations are fat-tailed breeds, such as Akkaraman in the Central Anatolia, Morkaraman in the Eastern Anatolia, Awassi in the Southeastern Anatolia. There have also been thin-tailed breeds like Chios and Kivircik crossbreeds in the Aegean, Pirlak in the Mediterranean, and Karayaka in the Black Sea region. These native breeds are generally resistant to disease, tolerate extreme temperatures, have a strong flocking instinct, and can able to adapt poor nursing and feeding conditions. Nowadays, sheep meat plays an important role in demanding red meat production. On the other hand, the latest statistic shows that it is far from meeting the increasing demand of the growing human population because only 9.1% of total red meat production is covered by sheep meat in Turkey (TurkStat 2019). This situation is in contradiction with Turkey's well-known property as a country of small ruminants. Crossbreeding studies of native breeds with Merino, fast-growing and early developing breeds, and Kivircik, known as having good quality carcass and flavor, have increased with consideration of consumer preferences in the last few decades (Akçapınar et al. 2001; Yılmaz et al. 2002; Ekiz & Altınel 2006). Although it is necessary to define the factors associated with age, live weight (LW), and body measurements not only to select better animals to achieve more genetic improvement and reproductive efficiency but also high carcass yield in domesticated sheep breeding (Aksoy et al. 2019). Various studies showed that body measurements could be used to study the interaction between heredity and environment (Wolf et al. 1981; Vatankhah & Talebi 2008), and in assessing growth rate, feed utilization, and carcass characteristics in farm animals (Kirton et al. 1995; Sarı et al. 2014; Da Silva 2019) and LW can be predicted from body measurements (Ali et al. 2014; Sari et al. 2014; Şahin et al. 2018). Also, estimating the LW using body measurements is more practical than weighting in rural areas because of requiring less labor. The aim of the current study was to evaluate the effects of breed type, as well as birth type, sex, and age of dam, on early growth performance; and to get a simple linear regression equation of LW, especially for small-scale farm owners, in Kivircik (**K**), Bandirma (**B**, *German Black Head Mutton x Kivircik crossbreed)*, Karacabey Merino (**KM**), Hampshire Down x Merino crossbreed (**HM**), and Ramlic (**R**) lambs under intensive conditions.

2. Material and Methods

2.1. Animals and measurements

The data in this study originated from the flocks of Sheep Breeding Research Institute, Balikesir, Turkey. A total of 202 head of lambs, K (n=51; 36 Female (F), 15 Male (M)), B (n=49; 34 F, 15 M), KM (n=47; 33F, 14 M), HM (n=27; 16 F, 11 M), and R (n=28; 14 F, 14 M), were used in this study. The reproduction system was based on a once-a-year mating; the general breeding season was started in June, leading to lambing ewes between November and January. During pre-weaning time, lambs have suckled their mothers, and from 15 days of age, they had access to commercial starter feed and alfalfa hay *ad libitum*, and they weaned at an average age of 90±6 days. At the beginning of May 2018, a single flock was created from later-born lambs to investigate the variation in genes affecting growth, meat quality, and yield in these meat-type sheep breeds. Lambs were housed in cross-ventilated pens and had free access to water and mineral licking stones. An average 600 g/lamb of concentrate feed, 100 g/lamb of alfalfa hay, and 300 g/lamb of vetches-wheat mixtures hay per day were given up to post-weaning period (135 days of age).

Body measurements considered in this study were lamb LW, body length (BL), withers height (WH), back height (BH), and chest circumference (CC) measurements.

LW of lambs was recorded at birth, weaning (90 days of age), and 135 days of age for calculation of the average daily gain (ADG) and standardized for pre- and post-weaning LW. Individual LW of lambs was determined prior to before morning feeding to avoid error due to stomach fill. Body linear measurements were taken on animals in a standing position with a raised head by the same technician in order to avoid intra-individual variations, according to Yilmaz et al. (2013). Circumference was measured with a flexible calibrated tape, whereas calipers were used for length and width.

All animal care and handling procedures of the present study were reviewed and approved by the Ethical Committee of the Sheep Breeding Research Institute (Approval number:13360037). All efforts were made to minimize any discomfort during body measurements.

2.2. Statistical analysis

The effect of independent factors (breed type, sex of lamb, birth type, and age of dam) on considered traits were analyzed using the GLM procedure of Minitab (2014) statistical package programs, and least-squares means were compared using the Tukey's multiple comparison tests. The model used for the least-squares (LS) analysis was as follows:

$$Y_{ijklm} = \mu + B_i + S_j + T_k + A_l + E_{ijklm} \quad (1)$$

Where: Y_{ijklm} = the observation of the m^{th} animal within the l^{th} age group of the k^{th} type of birth and j^{th} sex category of the i^{th} breed; μ: overall mean; B_i: effect of the i^{th} breed (i: K, B, KM, HM, R); S_j : effect of the j^{th} sex (j: male, female); T_k: effect of the k^{th} type of birth (k: single, twin); A_l: effect of the dam l^{th} age (l: 2, 3, 4, 5, 6, 7+); E_{ijklm} : residual error.

Stepwise multiple regressions were fitted to get a prediction equation of LW from body measurements considering raw correlations between all studied body measurements. The REG procedure of Minitab (2014) was used to determine the relative importance of body measurements.

3. Results and Discussion

In the present study, LW and some body measurements of K, B, KM, HM, and R lambs were determined, which are essential to evaluate some production characteristics of lambs from meat-type breeds. Results of LS means and standard errors (SE) of LW and average daily gain (ADG) at different periods are given in Table 1. Single lambs were 0.68 kg heavier (P<0.001) at birth than those born as twins. With the exception of birth weight, all LW was significantly greater in males than females (P<0.001). Birth weights ranged between 3.94-5.07 kg in K, B, KM, HM, and R (P<0.001). Also, the average birth weight of KM and HM lambs were significantly different from K, B, and R lambs. Age of dam or sex did not, however, breed and birth type affect birth weight. Such differences have been well documented by Bingöl & Bingöl (2015) and Öztürk et al. (2012).

At weaning age (90 days) and up to the post-weaning (135 days) period, a significant difference in LW was observed between those breeds (Table 1). HM and KM lambs were 20.59% and 24.26% heavier than B lambs at birth (5.07 and 4.92 versus 4.08 kg, P<0.001) but at weaning period bodyweight of B lambs became the highest one (34.44 kg, P<0.001). This difference between those three breed types tended to decrease with age, and the advantage of B lambs in LW did not persist in the post-weaning period. Conversely, there were no significant differences observed between K and R lambs in all periods. The results obtained from the present study on birth weight, especially for K, KM, HM breeds were higher than several estimates in the literature, for example, Koyuncu et al. (1999), Cemal et al. (2005), Koyuncu & Uzun (2009).

Table 1- LS means (±SE) of LW (kg) and ADG (g/day) different periods

Factors		*LW*	*ADG*				
	No. of lambs	*Birth (0 days)*	*Weaning (90 days)*	*135 days*	*Pre-weaning (0–90 days)*	*Post-weaning (90–135 days)*	*Overall (0–135 days)*
Breed type		***	***	***	***	***	***
K	51	3.94±0.11^{b}	28.92±0.53^{c}	35.73±0.68^{b}	277.63±5.36^{cd}	133.21±7.97^{b}	235.52±4.76^{b}
B	49	4.08±0.11^{b}	34.44±0.53^{a}	40.84±0.68^{a}	337.39±5.38^{a}	144.64±7.99^{b}	272.32±4.78^{a}
KM	47	4.92±0.10^{a}	31.60±0.48^{b}	40.77±0.62^{a}	296.39±4.92^{bc}	200.94±7.30^{a}	265.53±4.37^{a}
HM	27	5.07±0.14^{a}	32.77±0.67^{ab}	41.98±0.86^{a}	307.71±6.82^{b}	203.00±10.10^{a}	273.37±6.05^{a}
R	28	4.17±0.14^{b}	27.90±0.67^{c}	35.03±0.86^{b}	263.62±6.81^{d}	165.40±10.10^{b}	228.54±6.05^{b}
Sex of lamb		NS	***	***	***	***	***
Male	69	4.49±0.09	32.43±0.43^{a}	41.77±0.55^{a}	310.39±4.38^{a}	204.55±6.51^{a}	276.17±3.89^{a}
Female	133	4.38±0.07	29.83±0.36^{b}	35.97±0.46^{b}	282.71±3.66^{b}	134.30±5.44^{b}	233.95±3.25^{b}
Birth type		***	***	***	***	NS	**
Single	97	4.78±0.08^{a}	32.43±0.38^{a}	40.08±0.49^{a}	307.28±3.84^{a}	169.72±5.70	261.50±3.41^{a}
Twin	105	4.10±0.08^{b}	29.82±0.41^{b}	37.66±0.53^{b}	285.82±4.15^{b}	169.13±6.17	248.62±3.69^{b}
Age of dam		NS	NS	NS	NS	NS	NS
2	7	3.87±0.27	29.64±1.34	37.90±1.71	286.30±13.60	172.70±20.20	252.10±12.00
3	26	4.40±0.13	32.08±0.66	39.92±0.85	307.57±6.69	171.09±9.94	263.16±5.94
4	30	4.59±0.13	30.86±0.62	38.84±0.80	291.88±6.34	174.22±9.41	253.70±5.63
5	40	4.47±0.11	31.01±0.54	39.01±0.69	294.90±5.47	174.74±8.13	255.83±4.86
6	48	4.65±0.10	31.76±0.49	38.97±0.62	301.17±4.93	162.65±7.32	254.18±4.38
7+	51	4.64±0.09	31.41±0.46	38.58±0.59	297.49±4.67	161.14±6.94	251.42±4.15

LW: Live weight; *ADG:* Average daily gain; *K:* Kivircik; *B:* Bandirma; *KM:* Karacabey Merino; *HM;* Hampshire Down x Merino crossbreed*; R:* Ramlic. Different letters (a, b, c) in the same column are statistically different (P<0.05). NS: Not Significant; **: P<0.01; ***: P<0.001

ADG is one of the most important indicators of the profitability of lamb production in both pre- and post-weaning periods. ADG of lambs was significantly affected by sex and breed type (P<0.001). With the exception of the post-weaning period, birth type affected ADG while the age of the dam did not. From birth to 90 days of age, B lambs had higher ADG than the other breeds (337.39 g, P<0.001). On the other hand, HM and KM lambs had higher ADG from 90 to 135 days of age than the other breeds (Table 1). Also, single-born lambs had higher ADG at the pre-weaning period (21.46 g/day; P<0.001) and higher overall ADG (12.88 g/day; P<0.01) compared with twins. However, the effect of birth type was not significant at post-weaning (P>0.05). The present findings are in agreement with the results reported by other authors (Cemal et al. 2005; Koyuncu & Uzun 2009; Norouzian 2015).

To estimate genetic inheritance, the morphological differences among breeds could be used as an indicator for each sheep breed (Marković et al. 2019). Also, estimating the environmental effects on these traits is important for manipulating management practices to get more income. In the current study, the results of body measurements at weaning (90 days of age) and 135 days of age are presented in Table 2. CC has been suggested to be the most satisfactory single variable in estimating LW by Ibiwoye et al. (1993). In the current study, B and KM lambs had higher CC at the weaning period (P<0.01). Moreover, the differences between CC of lambs became more significant, and HM lambs had higher CC at the post-weaning period (P<0.001). The results of CC obtained from the conducted study are higher than reports of Isik & Aksoy (2015) for CC traits in Bafra sheep breed at 6 months age.

Another trait that could be used to estimate body weight is WH. Topal and Macit (2004) reported that the combination of WH and CC could be used to estimate body weight in Morkaraman sheep. WH was varied between 56.06-58.16 cm at weaning and 61.56-64.12 cm at 135 days of age within those breeds. WH of male lambs were 1.95 and 2.65 cm higher than female lambs at weaning and 135 days of age-old, respectively (P<0.001). Similar results were obtained by Silva Souza et al. (2019) for Piata, Xaraes, and Paiaguas sheep breeds at 270 days of age in Brazil. Also, considering the weaning and post-weaning periods obtained results for BL and BH were consistent with the literature (Akçapınar et al. 2001; Ambarcioglu et al. 2017; Sahin et al. 2018).

The results from this study indicate that the importance of breed differences on CC increased as the lambs grew older. On the other hand, sex had a significant effect on all body measurements at the pre- and post-weaning period (significance varied between P<0.01 and P<0.001); however, did not on BL pre-weaning period (P>0.05). In contrast, the importance of birth type on WH and CC decreased as the lambs grew older. This may be explained by differences in the characteristics of those breeds (Yılmaz et al. 2013).

Table 2- LS means (±SE) of body measurements (cm) at weaning (90 days of age) and post-weaning time (135 days of age)

		Weaning (90 days)					*Post-weaning (135 days)*			
Factors	*N*	*BL*	*WH*	*BH*	*CC*	*N*	*BL*	*WH*	*BH*	*CC*
Breed type		***	***	***	**		***	***	***	***
K	51	60.32± 0.52^{b}	57.61± 0.38^{a-c}	58.13± 0.40^{ab}	74.22± 0.67^{ab}	51	63.29± 0.51^{b}	63.40± 0.36a	63.50± 0.36^{ab}	78.02± 0.67^{b}
B	49	62.18± 0.52^{a}	58.16± 0.39^{ab}	58.00± 0.40^{ab}	76.08± 0.67^{a}	49	66.65± 0.51^{a}	63.32± 0.36^{a}	63.19± 0.36^{b}	81.55± 0.67^{a}
KM	47	60.41± 0.47^{ab}	58.88± 0.35^{a}	59.43± 0.36^{a}	75.01± 0.61^{a}	47	65.19± 0.47^{a}	64.12± 0.33^{a}	64.68± 0.33^{a}	81.23± 0.61^{a}
HM	27	59.71± 0.66^{b}	56.66± 0.49^{bc}	57.11± 0.50^{bc}	74.27± 0.85^{ab}	27	66.01± 0.65^{a}	63.27± 0.46^{ab}	63.20± 0.46^{ab}	82.16± 0.85^{a}
R	28	56.77± 0.66^{c}	56.06± 0.49^{c}	56.01± 0.50^{c}	71.75± 0.85^{b}	28	61.79± 0.65^{b}	61.56± 0.46^{b}	60.64± 0.46^{c}	77.45± 0.85^{b}
Sex of lamb		NS	***	***	**		***	***	***	***
Male	69	60.29± 0.42	58.45± 0.31^{a}	58.75± 0,32^{a}	75.24± 0.55^{a}	69	65.98± 0.42^{a}	64.46± 0.30^{a}	64.39± 0.30^{a}	81.50± 0.55^{a}
Female	133	59.47± 0.35	56.50± 0.26^{b}	56.72± 0.27^{b}	73.29± 0.46^{b}	133	63.19± 0.35^{b}	61.81± 0.25^{b}	61.69± 0.25^{b}	78.67± 0.46^{b}
Birth type		NS	***	***	**		NS	**	***	*
Single	97	60.30± 0.37	58.25± 0.28^{a}	58.65± 0.28^{a}	75.21± 0.48^{a}	97	64.90± 0.37	63.71± 0.26^{a}	63.64± 0.26^{a}	80.82± 0.48^{a}
Twin	105	59.46± 0.40	56.70± 0.30^{b}	56.82± 0.31^{b}	73.32± 0.52^{b}	105	64.27± 0.40	62.56± 0.28^{b}	62.44± 0.28^{b}	79.35± 0.52^{b}

K: Kivircik; *B*: Bandirma; *KM*: Karacabey Merino; *HM*:Hampshire Down x Merino crossbreed; *R*: Ramlic. *BL*: Body length; *WH*: Withers height; *BH*: Back height; *CC*: Chest circumference; Different letters (a, b, c, d) in the same column are statistically different (P<0.05). N: Number of lambs; NS: Not Significant;*: P<0.05; **: P<0.01; ***: P<0.001

Except for some fluctuations, B and KM lambs were highest for all physical measurements during the pre-weaning period. This might be attributed to the superiority of mothering ability and the higher birth weight of these breeds. These results are consistent with a previous study conducted for Hamari and Kabashi breeds (Ali et al. 2014).

The results of regressing LW and body measurements are presented in Table 3. In general terms, LW was very highly (P<0.001) correlated with body measurements (r=0.682–0.892). In B, KM, and R lambs, the highest correlations were observed between LW and CC (0.802-0.892), whereas BL (0.864) and WH (0.784) was the most correlated trait with LW in HM and K lambs, respectively. On the other hand, the least correlation between LW and other traits was observed in K, KM, and R lambs for BL (0.764-0.837); HM lambs for WH (0.854), and B lambs for BH values (0.682). The obtained results of phenotypic correlation coefficients between LW and body measurements were lower than the results obtained from four different Iranian sheep (Mehrebani, Zandi, Shaal, and Macoei) breeds (Shirzeyli et al. 2013). The difference between those breeds is natural due to having different morphology.

Table 3- Phenotypic correlations between body measurements in different meat-type lambs

Trait	*LW*					*BL*					*WH*					*BH*				
Breed	*K*	*B*	*KM*	*HM*	*R*	*K*	*B*	*KM*	*HM*	*R*	*K*	*B*	*KM*	*HM*	*R*	*K*	*B*	*KM*	*HM*	*R*
BL	0.764***	0.693***	0.726***	**0.864*** **	0.837***															
WH	0.784***	0.733***	0.825***	**0.854*** **	0.852***	0.619***	0.698***	0.723***	0.779***	**0.836*** **										
BH	0.774***	0.682***	0.804***	**0.862*** **	0.855***	0.615***	0.645***	0.713***	0.774***	**0.797*** **	0.911***	0.906***	0.933***	**0.953*** **	0.915***					
CC	0.764***	0.802***	0.827***	0.858***	**0.892*** **	0.618***	0.589***	0.750***	0.809***	**0.817*** **	0.598***	0.654***	0.732***	0.675***	**0.770*** **	0.563***	0.570***	0.750***	0.681***	**0.821*** **

K: Kivircik; *B*: Bandirma; *KM:* Karacabey Merino; *HM*: Hampshire Down x Merino crossbreed; *R*: Ramlic.; *LW:* Live weight; *BL:* Body length; *WH:* Withers height; *BH*: Back height; *CC*: Chest circumference; ***: P<0.001

As understood from Table 3, it was seen that body measurements such as CC and WH had a high relationship with LW of lambs in those sheep breeds. Also, there was a moderate to a strong relationship between the other studied body measurements (0.589-0.953). Inconsistent with this study, Afolayan et al. (2006) noted that various body measurements related to animal size and weight display moderate to a high correlation between one another. The obtained correlation between LW and BL (0.693-0864), LW, and WH (0.733-0.854), LW, and CC (0.764-0.892) were higher than obtained results from Mahmut et al. (2014) except BL (0.693) for B lambs and CC (0.764) for K lambs.

Multiple regression analysis was also performed to get simple linear regression and partial regression equations for those breeds. According to obtained results, as shown in Tablo 4, there was no significant effect of sex on regression analysis for R lambs. The lowest and the highest determination coefficient ($R^{2)}$ value was found in B and H lambs as 0.75 and 0.92, respectively. Eck et al. (2019) noted that although the high correlation between WH and BH, using WH as a correction factor has a great benefit instead of BH because the lambs stood better on their forelegs than on their hind legs. The results obtained from our study are in accordance with this study, but they differ due to the significant effect of BH on the regression equation. The differences between morphological characters of breeds, weaning time, location, season, or breeding systems may be the reason among these two studies.

Table 4- Simple linear regression equations for estimating LW in different meat-type lambs

Breed	***Sex***	***Equation***	***R^2***	***MSE***
K	M	*LW= -53.82 + 0.383(BL) + 0.286(WH) + 0.293(BH) + 0.378(CC)*	0.84	2.12
	F	*LW= -63.93 + 0.482(BL) + 0.283 (WH)+ 0.329(BH) + 0.385(CC)*	0.81	2.29
	-			
B	M	*LW= -48.68 + 0.326(BL) + 0.317(WH) + 0.593(CC)*	0.75	2.46
	F	*LW= -51.95 + 0.326(BL) + 0.360(WH) + 0,592(CC)*	0.74	2.48
	-			
KM	M	*LW= -53.51 + 0.795(WH) + 0.527(CC)*	0.79	2.93
	F	*LW= -55.55 + 0.782(WH) + 0.556(CC)*	0.79	2.94
	-			
HM	M	*LW= -47.53 + 0.296(BL) + 0.385(WH) + 0.140(BH) + 0.460(CC)*	0.92	1.91
	F	*LW= -55.01 + 0.315(BL) + 0.663(BH) + 0.416(CC)*	0.89	2.16
	-			
R	M	*LW= -50.10 + 0.594(WH) + 0.630(CC)*	0.86	2.28
	F			
	-			

K: Kivircik; *B*: Bandirma; *KM*: Karacabey Merino; *HM*: Hampshire Down x Merino crossbreed; *R:* Ramlic.; *M:* Male; *F:* Female; *LW*: Live weight; *BL:* Body length; *WH:* Withers height; *BH:* Back height; *CC*: Chest circumference; R^2, Determination coefficient; *MSE:* Mean square error

4. Conclusions

LW and body measurements are important indicators to predict the growth of both indigenous and crossbred sheep breeds. They can be used to select animals with better production characteristics that result in increased production, productivity, and profitability of the farm. The results from the present study indicated that the relationships between LW and body measurements are affected by such factors of breed and birth type with sex differences in those meat-type sheep breeds. Obtained results from this study also show that B, KM, and HM lambs were notably heavier and grew faster than K and R lambs, and had better growth characteristics in the same rearing system. Also, some useful information was collected that could be used in selection programs and obtained some regression equations to estimate LW from body measurements. Moreover, the high correlations between LW and such body measurements, e.g., WH and CC, would imply these two traits could be used to determine LW effectively in KM and R lambs rather than BL and BH.

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Comparison of Ethylene Sensitivity of Three Tomato Cultivars From Different Tomato Types and Effects of Ethylene on Postharvest Performance

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ABSTRACT
The aim of study was to investigate ethylene sensitivity of different types of tomatoes and the effects of ethylene on their postharvest performance. For that purpose, beefsteak, heirloom and cluster types of tomato fruit were harvested at the breaker maturity stage and divided into two groups one of which was treated with 150 µL L^{-1} ethylene and the other was untreated for comparison. Ethylene treated and untreated fruit were stored at 12 °C and 90±5% relative humidity for 35 days and subsamples removed every 7 days for postharvest quality analysis. After each removal time, fruit were kept at 20 °C for 3 days in order to determine shelf life performance. Ethylene treatment lead to increase respiration rate, ethylene production, weight loss but decreased fruit firmness in all tested tomato cultivars. Minimum ethylene production and respiration rate occurred in untreated beefsteak tomatoes. At the end of cold storage and shelf life period, the highest *L** values and fruit firmness were recorded for control beefsteak tomatoes. The conclusion drawn from this experiment was that the cluster type of tomatoes was more sensitive, while beefsteak type of tomatoes was found to be less sensitive to ethylene treatment as they had the highest and lowest amount of ethylene productions respectively. Untreated beefsteak tomatoes exhibited maximal postharvest quality compared to other treatments after 35 days cold storage and shelf life.

Keywords: Cold storage, Ethylene, Quality, Respiration rate, Shelf life, Tomatoes

1. Introduction

Internationally tomato is the leading vegetable with an annual production of 177 million tons (MT). China ranked 1st with the production of 61.6 MT whereas Turkey ranked 4th with 12.1 MT production (FAO 2018). Approximately 70% of tomatoes are freshly consumed while the remaining 30% of tomatoes are processed for making tomato sauce and a range of tomato-based products including ketchup and juice (Erturk & Cirka 2015). Tomato, being a climacteric fruit, is highly sensitive to the ripening hormone ethylene. Ethylene induces the ripening of climacteric fruit and is highly effective in modulating biochemical reactions in fruit. Ethylene affects not only biochemical composition but also increases respiration rate and senescence of fruit and vegetables (Prasanna et al. 2007). Additionally, chlorophyll degradation and softening of tomato fruit are caused by ethylene (Akbudak et al. 2007). Effects of ethylene in horticultural produces are mainly dependent upon the cultivar, maturity stage, application dose and temperature (Nagata et al. 1995; Wills et al. 1998; De Wild et al. 2005). Endogenous or exogenous treatment of ethylene is widely used to stimulate and initiate ripening in climacteric fruits. Ethylene is applied to fruit for ripening and improvement in quality of color (Dhall & Singh 2013). Similar to other fruits and vegetables, maturation causes changes in color, texture, flavor and chemical structure of tomatoes.

The attainment of consumer satisfaction is a challenging task for marketing and therefore breeders are introducing different tomato types and cultivars every year. Respiration rate, ethylene sensitivity, sugars, acids and other biochemical properties vary according to type of tomatoes. In general, tomatoes with higher sugar and acid content have a better taste than those with lower acid and sugar content (Cantwell 2010).

Ethylene production by tomato fruit varies according to type and maturity stage of fruit (Baldwin 2004). Different tomato types show different ripening behavior. Therefore, it is important to determine the response of ethylene in these different types of tomato to benefit commercial growers, breeders, wholesalers and retailers. Therefore, the aim of study was to investigate ethylene sensitivity of different types of tomatoes and the effects of ethylene on their postharvest performance.

2. Material and Methods

Beefsteak (cv. Tybif), heirloom (cv. Yuksel Koy) and cluster (cv. Merkur) types of tomato were harvested at 'breaker stage'. All fruits were obtained from a commercial greenhouse in Antalya, Turkey (36°59'57.3" N 30°51'20.4" E). During the entire vegetation period, uniform irrigation and fertigation management procedures were applied to the tested tomato types. All fruits were harvested on the same day and fruit were immediately transported to the postharvest physiology laboratory at Akdeniz University, Antalya, Turkey. Fruit with any defects i.e. decayed, bruised and non-uniform, were discarded and the remainder were split into two groups. The first group was treated with 150 µl L^{-1} of ethylene at 20 °C in a 20 m^3 room for 40 min and the second group was left untreated (control). Both groups of fruit samples were stored at 12 °C and 90±5% relative humidity for 35 days. Fruit samples for quality analysis were removed from storage at 7 days intervals and kept at 20 ºC and 60±5% relative humidity for additional 3 days to simulate shelf life performance.

For ethylene production, 10 fruits from each treatment were enclosed in 5 L airtight jars for 1 h at 20 °C, then a 1 mL gas sample was withdrawn using a gastight syringe and injected into a gas chromatography (GC; Finnigan Trace Ultra, Thermo Electron S.p.A. Strada Rivoltana 20900 Radano, Milan-Italy) equipped with GS-GASPRO, 113-4362 Capillary column, 60 m x 0.322 mm calibrated with standard ethylene. The temperature of detector, oven and injection were 170 °C, 90 °C and 100 °C, respectively. Flow rates of carrier gas helium, air and hydrogen were 25 mL min^{-1}, 350 mL min^{-1} and 35 mL min^{-1}, respectively. Ethylene production was reported as µL C_2H_4 kg^{-1} h^{-1} (Dogan et al. 2017).

Respiration rates of fruits were measured as CO_2 production. For that purpose, 10 fruits from each treatment were enclosed in 5 L airtight jars for 1 h at 20 °C, then a 1 mL gas sample was taken from the headspace and injected into GC equipped with 80/100 Porapak N, 182.88 cm x 0.635 cm column calibrated with standard CO_2. The temperatures of detector, oven and injection temperature were 100 °C, 65 °C and 100 °C, respectively. Flow rates of carrier gas helium, air and hydrogen were 10 mL min^{-1}, 400 mL min^{-1} and 45 mL min^{-1}, respectively. Respiration rates were reported as mL CO_2 kg^{-1} h^{-1} (Dogan et al. 2017). The ethylene production and respiration rate analysis were carried out with the same tomatoes for 35 days of storage.

Weight loss was determined by weighing tomatoes at the beginning of the experiment (day 0) and at 7 days intervals. Cumulative weight loss was expressed as percentage loss of the initial total weight.

Color changes of tomatoes were recorded with a color meter (CR-400, Minolta, Ramsey, NJ, USA), which directly gave CIE *L**, hue angle (*h*°) and chroma (*C**) values. Color measurements were made from 3 different points on the equatorial region of the fruit surface to represent the entire fruit sample. (Mcguire 1992). Total soluble solids (TSS) content was measured with a digital refractometer (Hanna HI 96801) and the TSS was expressed as percent (%). For titratable acidity (TA), the juice of tomato fruit was obtained using a blender. Determination of TA was done by titrating a juice sample of 2 mL with 38 mL of distilled water along with 0.1 N NaOH to an end point of 8.1. Each sample was titrated three times and means calculated. The TA was determined as g citric acid kg^{-1}. Fruit firmness of tomato was measured using a penetrometer (FT 011) with 3 mm plunger. Measurements were carried out on three different points of each fruit and firmness was determined in Newton (N). The amount of unmarketable fruit was expressed in percent. The calculation was done according to the following equation (1) used by Jan & Rab (2012).

Amount of unmarketable fruit (%) = number of deteriorated fruit/ total number of fruit x100 (1)

A completely randomized design with three replications was used for the experiment. Each replication contained ten fruits. Means calculated were subjected to Duncan's multiple range test to determine significant differences. Mean values obtained were analyzed with SAS program.

3. Results and Discussion

3.1. Ethylene production and respiration rate

3.1.1. Ethylene production

Ethylene treated heirloom and beefsteak types of tomato had maximum ethylene production after 21 days storage compared with 28 days for the same types without ethylene treatment. Both control and ethylene treated cluster type tomatoes reached peak ethylene production after 21 days. Maximum ethylene production (3.527 µL C_2H_4 kg^{-1} h^{-1}) occurred in the ethylene treated cluster type with the least ethylene (1.225 µL C_2H_4 kg^{-1} h^{-1}) in control beefsteak tomatoes (Figure 1).

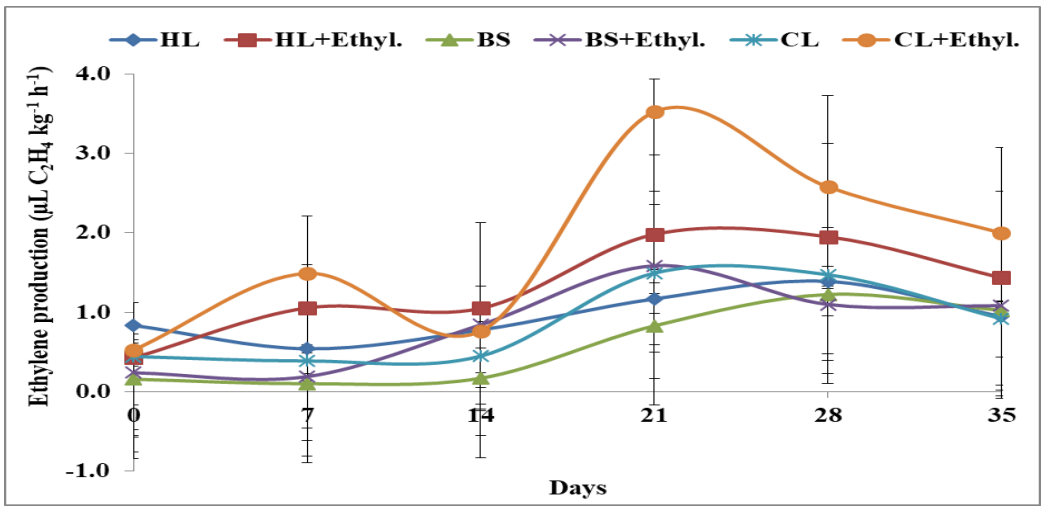

Figure 1- Effect of 150 µl L^{-1} ethylene treatment on ethylene production in different types of tomatoes at 12 °C. Vertical lines represent standard deviations of the means (n=3). †BS = Beefsteak, BS+Ethyl. = Beefsteak+Ethylene, HL= Heirloom, HL+Ethyl. = Heirloom+Ethylene, CL= Cluster, CL+Ethyl.= Cluster+Ethylene

Extension in storage resulted in increase of ethylene in this study with higher ethylene production in ethylene treated fruit which agreed with the result of Chomchalow et al. (2002) who reported an increase in ethylene production with advanced ripening in tomatoes treated by ethylene. Maximum ethylene production was obtained in ethylene treated fruit during our study as compared to untreated fruit which agreed to the outcome of Dong et al. (2001) who reported that ethylene treated 'Flavortop' nectarines had higher ethylene production.

3.1.2. Respiration rate

Control heirloom type had a climacteric maximum after 35 days of storage compared with 14 days for ethylene treated heirloom tomatoes. Control beefsteak type reached a climacteric maximum in 14 days while ethylene treated beefsteak type of tomatoes had climacteric maximum on 28th day of storage. Control cluster type of tomatoes reached climacteric maximum on 35th day with ethylene treated cluster type had climacteric maximum on day 0. Maximum respiration rate of 2.171 mL CO_2 kg^{-1} h^{-1} occurred in control cluster type after 35 days storage with minimum respiration rate of 1.072 mL CO_2 kg^{-1} h^{-1} occurred in control beefsteak type of tomatoes 14 days after storage (Figure 2).

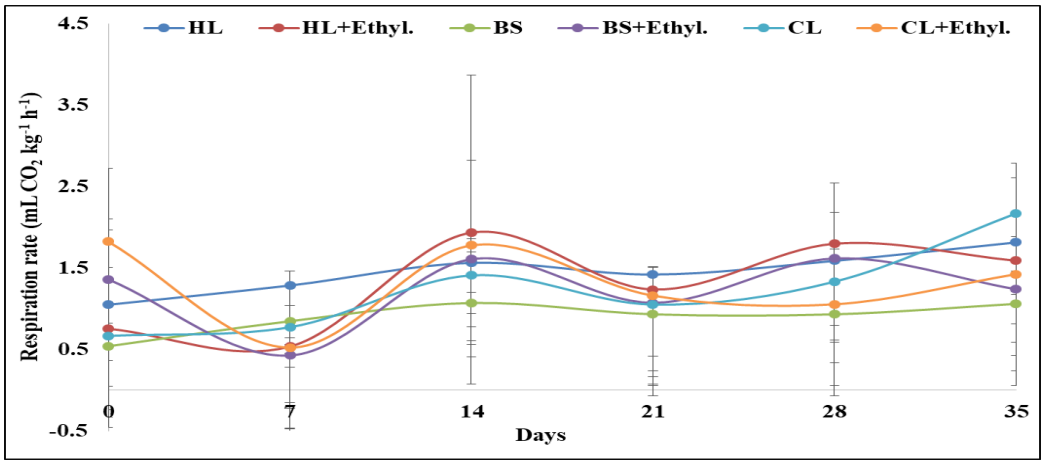

Figure 2- Effect of 150 µl L^{-1} ethylene treatment on respiration rate in different types of tomatoes at 12 °C. Vertical lines represent standard deviations of the means (n=3). †BS = Beefsteak, BS+Ethyl.= Beefsteak+Ethylene, HL = Heirloom, HL+Ethyl.= Heirloom+Ethylene, CL= Cluster, CL+Ethyl. = Cluster+Ethylene

Rise in respiration rate of tomatoes was observed by Karacali (1990) as noticed in our study. Boe & Salunkhe (1967) in tomatoes and Elmi et al. (2017) in strawberries reported that the ethylene treatment increased the rate of CO_2 production. However, their outcome contradicted with cluster type tomatoes where control treatment had higher CO_2 production than ethylene treated tomatoes. Respiration rate of tomato fruit is one of the vital indicators of senescence climacteric fruit (Maharaj et al. 1999). Similarly, according to Gonzalez-Aguilar et al. (2010) respiration rate and ethylene productions are main components to determine decay incidence of fruit and vegetables.

3.2. Weight loss

Ethylene treatment in all tomato types resulted in higher amount of weight loss in the fruit. Weight loss of tomatoes increased both in cold storage and shelf life during storage period. After cold storage, greatest weight loss (5.90%) was from ethylene treated cluster fruit whereas lowest weight loss (2.37%) was in control beefsteak tomatoes (Table 1). At the end of 35+3 days storage and shelf life period, maximum weight loss (8.48%) occurred in ethylene treated cluster tomatoes whereas minimum weight loss (3.84%) occurred in control beefsteak tomatoes (Table 2). The interactions between storage duration and treatments were significant in both cold storage and shelf life conditions at P≤0.05.

Table 1- Effect of ethylene on weight loss, color (*L, *C**, *h°*), total soluble solids, titratable acidity, fruit firmness and amount of unmarketable fruit of different types of tomatoes during storage at 12 °C**

Parameters	*Treatments*	*Storage duration (Days)*					
		0	**7**	**14**	**21**	**28**	**35**
Weight loss (%)	**BS**†	-	0.68jk*	0.87jk	1.09jk	1.60fk	2.37df
	BS+Ethyl.	-	0.53k	0.89jk	1.16hk	2.21dh	3.25bd
	HL	-	0.49k	0.80jk	1.00jk	1.60fk	2.40df
	HL+Ethyl.	-	0.65jk	0.94jk	1.20gk	2.10ei	2.85ce
	CL	-	0.64jk	1.72fj	2.08ei	3.68bc	5.11a
	CL+Ethyl.	-	0.63jk	1.20gk	2.27dg	4.13b	5.90a
	$LSD_{5\%}$: St. Dur.: 0.4131 **St. Dur. × Trt.:** 0.9237 **Trt.:** 0.3771						
Brightness (*L)**	**BS**	54.69a	54.36ab	54.03ab	52.85ae	53.33ad	50.18ah
	BS+Ethyl.	53.67ad	53.84ac	53.37ad	49.89bi	48.46ei	46.23hl
	HL	52.30af	50.94ag	49.13di	46.61gk	45.98hl	43.57jm
	HL+Ethyl.	49.37ci	48.64ei	46.86gj	43.71jl	42.29kn	40.33mn
	CL	50.08bi	49.81bi	46.10gk	42.02ln	41.00mn	40.08mn
	CL+Ethyl.	50.57ah	48.23fi	45.64il	43.34jm	40.06mn	37.96n
	$LSD_{5\%}$: St. Dur.: 1.5526 **St. Dur. × Trt.:** 3.8031 **Trt.:** 1.5526						
Chroma (*C)**	**BS**	26.81kl	26.68kl	29.87hk	36.05ae	37.83ad	39.20a
	BS+Ethyl.	26.55kl	27.13kl	27.69kl	32.43fi	35.36bg	38.39a
	HL	27.50kl	28.26jl	29.33ik	33.81eg	34.61dg	36.93ae
	HL+Ethyl.	26.62kl	28.36jl	28.45jl	33.84eg	34.67cg	35.86af
	CL	25.54l	27.46kl	31.92gj	35.33bg	38.32ad	38.48ab
	CL+Ethyl.	26.18kl	29.61ik	31.86gj	37.06ae	35.91af	33.56eh
	$LSD_{5\%}$: St. Dur.: 1.535 **St. Dur. × Trt.:** 3.7599 **Trt.:** 1.535						
Hue angle (*h°*)	**BS**	115.61a	111.11a	96.77be	84.02eh	67.35hn	60.64jq
	BS+Ethyl.	115.28a	101.44ad	84.24eh	74.64gj	67.74hm	65.48io
	HL	113.56ab	104.70ac	87.19dg	75.28gj	63.31ip	53.56lq
	HL+Ethyl.	112.31ab	92.51cf	70.73gl	57.98jq	50.42mq	48.17oq
	CL	78.95fi	72.44gk	56.81kq	49.73nq	47.61oq	45.27q
	CL+Ethyl.	79.11fi	63.98ip	52.83mq	49.58nq	46.32pq	45.09q
	$LSD_{5\%}$: St. Dur.: 6.0791 **St. Dur. × Trt.:** 14.891 **Trt.:** 6.0791						
Total soluble solids (TSS) (%)	**BS**	4.10ae	4.10ae	3.90be	3.97ae	4.10ae	3.87ce
	BS+Ethyl.	4.00ae	3.90be	3.87ce	3.93ae	4.00ae	3.87ce
	HL	4.23ab	3.97ae	4.13ad	4.03ae	4.00ae	3.83de
	HL+Ethyl.	4.13ad	4.27a	3.93ae	3.77e	3.90be	4.03ae
	CL	3.93ae	3.97ae	4.03ae	4.07ae	4.10ae	4.20ac
	CL+Ethyl.	3.90be	3.97ae	3.97ae	4.07ae	4.13ad	4.20ac
	$LSD_{5\%}$: St. Dur.: 0.11 **St. Dur. × Trt.:** 0.2695 **Trt.:** 0.11						
Titratable acidity (g citric acid kg^{-1})	**BS**	5.50cd	4.13fj	3.50ik	3.30jk	3.20jk	3.17jk
	BS+Ethyl.	3.87fk	3.37ik	3.30jk	3.27jk	3.17jk	3.03jk
	HL	8.13a	6.93b	4.80cf	4.73dg	4.30ei	3.53ik
	HL+Ethyl.	7.93a	5.13ce	5.10ce	3.97fk	3.80gk	3.27jk
	CL	5.70c	5.17ce	4.33ei	3.80gk	3.80gk	3.63hk
	CL+Ethyl.	4.73dg	4.57dh	3.80gk	3.47ik	3.40ik	3.40ik
	$LSD_{5\%}$: St. Dur.: 0.3308 St. Dur. × Trt.: 0.8102 **Trt.:** 0.3308						
Fruit firmness (N)	**BS**	13.20a	12.37ae	11.54bf	10.54fh	7.45jl	6.83km
	BS+Ethyl.	12.47ad	11.89bf	11.42bg	9.92gi	7.50jl	6.11ln
	HL	12.63ac	11.37bg	8.63ij	6.87km	5.38mo	4.43op
	HL+Ethyl.	12.36ae	10.89eg	9.30hi	6.83km	4.85np	3.52p
	CL	12.66ab	10.86eg	10.61fh	7.58jl	6.72km	4.16op
	CL+Ethyl.	12.62ac	11.07cg	10.98dg	7.76jk	4.51op	3.82p
	$LSD_{5\%}$: 0.544 **St. Dur.: St. Dur. × Trt.:** 1.3324 **Trt.:** 0.544						
Amount of unmarketable fruit (%)	**BS**	0h	0h	0h	8.06gh	19.25df	30.40bc
	BS+Ethyl.	0h	0h	0h	13.94fg	23.40ce	34.78b
	HL	0h	0h	0h	13.40fg	20.78df	30.40bc
	HL+Ethyl.	0h	0h	0h	4.84h	15.01fg	51.96a
	CL	0h	0h	0h	4.73h	15.98ef	25.50cd
	CL+Ethyl.	0h	0h	1.23h	3.09h	15.51fg	45.48a
	$LSD_{5\%}$: St. Dur.: St. Dur. × Trt.: Trt.:						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS: Beefsteak; BS+Ethyl.: Beefsteak+Ethylene; HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene; LSD: Least significant difference; St. Dur.: Storage duration; St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments

Table 2- Effect of ethylene on weight loss, color (*L, *C**, *h*°) and total soluble solids contents of different types of tomatoes under shelf life at 20 °C**

Parameters	*Treatments*	*Storage duration (Days)*					
		0	**7+3**	**14+3**	**21+3**	**28+3**	**35+3**
Weight loss (%)	**BS**†	-	1.89ik*	1.98hk	2.34gk	2.40gk	3.84df
	BS+Ethyl.	-	1.30k	2.00gk	2.23gk	3.18eh	5.73bc
	HL	-	1.80jk	2.17gk	2.11gk	2.93gj	4.72cd
	HL+Ethyl.	-	1.64k	2.52gk	2.18gk	3.10ei	4.52d
	CL	-	2.97ej	3.76df	4.03df	7.60a	7.76a
	CL+Ethyl.	-	2.47gk	3.27eg	4.18de	6.43b	8.48a
	$LSD_{5\%}$: St. Dur.: 0.4315 **St. Dur. × Trt.:** 1.0569 **Trt.:** 0.4726						
Brightness (*L)**	**BS**	54.69a	52.23ac	50.95be	50.80be	47.13gn	45.30kr
	BS+Ethyl.	53.67ab	51.29bd	49.97ch	48.49dk	48.80dj	44.46mr
	HL	52.30ac	47.37fm	47.00gn	44.05nr	42.97qs	42.30rs
	HL+Ethyl.	49.37ci	48.34dk	45.86jp	44.72lr	44.07nr	42.76qs
	CL	50.08cg	47.76el	46.41ip	44.74lr	43.34ps	42.93qs
	CL+Ethyl.	50.57bf	48.11dk	46.81ho	43.61os	43.27ps	40.80s
	$LSD_{5\%}$: St. Dur.: 1.106 **St. Dur. × Trt.:** 2.7091 **Trt.:** 1.106						
Chroma (*C)**	**BS**	26.81l	31.63ik	33.70ei	34.00ei	33.99ei	37.62ac
	BS+Ethyl.	26.55l	30.25k	32.80gk	33.00gk	35.04ch	36.15bf
	HL	27.50l	31.55ik	32.35hk	33.34fj	33.67fj	40.02a
	HL+Ethyl.	26.62l	30.84jk	32.61hk	33.11fk	33.41fj	34.22di
	CL	25.54l	35.09ch	36.76de	35.82cf	37.23bd	38.94ab
	CL+Ethyl.	26.18l	34.77ch	35.76cg	37.11bd	37.23bd	34.21di
	$LSD_{5\%}$: St. Dur.: 1.028 **St. Dur. × Trt.:** 2.518 **Trt.:** 1.028						
Hue angle (*h*°)	**BS**	115.61a	78.95b	63.67ce	66.41ce	60.84df	47.60gi
	BS+Ethyl.	115.28a	83.13b	65.99ce	57.24eg	51.16fi	47.45gi
	HL	112.31a	82.95b	65.57ce	47.57gi	46.72gi	43.79hi
	HL+Ethyl.	113.56a	73.56bc	57.57dg	52.96fh	50.68fi	43.11hi
	CL	79.11b	60.72df	59.33df	51.00fi	44.06hi	41.93hi
	CL+Ethyl.	78.95b	68.29cd	57.60dg	44.10hi	43.07hi	41.22i
	$LSD_{5\%}$: St. Dur.: 3.7691 **St. Dur. × Trt.:** 9.2323 **Trt.:** 3.7691						
Total soluble solids (TSS) (%)	**BS**	4.10af	4.30ab	3.80gh	4.07bf	3.97ch	3.83fh
	BS+Ethyl.	4.00ch	4.03bh	4.00ch	4.13ae	3.90eh	3.87eh
	HL	4.23ac	4.10af	4.00ch	4.00ch	3.87eh	3.77h
	HL+Ethyl.	4.13ae	4.37a	4.10af	4.00ch	3.90eh	3.80gh
	CL	3.93dh	4.03bh	4.13ae	4.13ae	4.13ae	4.20ad
	CL+Ethyl.	3.90eh	3.77h	4.03bh	4.07bg	4.10af	4.13ae
	$LSD_{5\%}$: St. Dur.: 0.1149 **St. Dur. × Trt.:** 0.2815 **Trt.:** 0.1149						
Titratable acidity (g citric acid kg^{-1})	**BS**	5.50bc*	3.63fl	3.33hl	3.27il	3.07jl	2.90kl
	BS+Ethyl.	3.87ek	3.37hl	3.30il	3.27il	3.30il	2.80l
	HL	8.13a	5.37bd	4.33eh	4.23ei	4.10ei	3.80el
	HL+Ethyl.	7.93a	4.40eg	4.03ej	3.87ek	3.80el	3.37hl
	CL	5.70b	3.83ek	3.67fl	3.37hl	3.33hl	3.23il
	CL+Ethyl.	4.73ce	4.57df	3.80el	3.47gl	3.43gl	3.40hl
	$LSD_{5\%}$: St. Dur.: 0.3337 **St. Dur. × Trt.:** 0.8174 **Trt.:** 0.3337						
Fruit firmness (N)	**BS**	13.20a	9.35de	9.07de	8.93de	7.28gf	6.45gi
	BS+Ethyl.	12.47a	10.98b	8.50de	8.05ef	6.34gi	5.70hk
	HL	12.63a	6.07hj	5.93hj	4.61kn	4.59kn	3.29op
	HL+Ethyl.	12.36a	5.20jm	4.96jn	4.78kn	4.18mp	3.28op
	CL	12.66a	10.44bc	6.81gh	4.34lo	4.54ln	3.37op
	CL+Ethyl.	12.62a	8.50de	6.40gi	5.33il	3.92np	3.15op
	$LSD_{5\%}$: 0.459 **St. Dur.: St. Dur. × Trt.:** 1.1243 **Trt.:** 0.459						
Amount of unmarketable fruit (%)	**BS**	-	0e	0e	0e	33.33d	66.67b
	BS+Ethyl.	-	0e	0e	33.33d	33.33d	66.67b
	HL	-	0e	0e	0e	33.33d	33.33d
	HL+Ethyl.	-	0e	0e	0e	44.44c	66.67b
	CL	-	0e	0e	0e	33.33d	83.33a
	CL+Ethyl.	-	0e	0e	33.33d	44.44c	72.22b
	$LSD_{5\%}$: St. Dur.: 4.0581 **St. Dur. × Trt.:** 9.9403 **Trt.:** 4.4454						

*: Means with different letters are statistically significant at P≤0.05 according to Duncan's multiple range test; †BS: Beefsteak; BS+Ethyl.: Beefsteak+Ethylene; HL: Heirloom; HL+Ethyl.: Heirloom+Ethylene; CL: Cluster; CL+Ethyl.: Cluster+Ethylene, LSD: Least significant difference; St. Dur.: Storage duration; St. Dur. × Trt.: Storage duration × Treatments; Trt: Treatments.

Increases in weight losses with tomato ripening was reported by Sammi & Masud (2007) which was similar outcome obtained in this study. In this experiment ethylene treated fruit had higher weight losses as compared to control which agreed with the outcome of Dhall & Singh (2013) who expressed that ethylene treated tomatoes had more weight loss than control treatment. They mentioned that this increases in weight loss may be due to the rise in respiration rate during ripening.

3.3. Fruit color (L, C*, h°)*

The *L** values tended to decline with time in cold storage and shelf life. In general, ethylene treatment in tomato fruit resulted in lower *L** values than the untreated ones. The interactions between storage duration and treatments were significant under cold

storage and shelf life at P≤0.05. After cold storage, the highest *L** value (50.18) was in control beefsteak whereas the lowest *L** value (37.96) was in ethylene treated cluster tomatoes after 35 days storage (Table 1). At the end of shelf life, maximum *L** value (45.30) was in untreated beefsteak tomatoes and minimum (40.80) *L** value was in ethylene treated cluster tomatoes treated after 35+3 days storage (Table 2).

Decrease in *L** values with storage extension of tomatoes were reported by Fagundes et al. (2015). According to these researchers the decrease in *L** values may be due to increase in the red color of tomatoes during storage. Camelo & Gomez (2004) mentioned that as the red pigmentation of tomatoes started to synthesize the *L** values showed decrease and had attained the dark red color.

Interactions between storage duration and treatments were statistically significant at P≤0.05. After cold storage, the highest *C** value (39.20) were in control beefsteak tomatoes and ethylene treated cluster tomatoes had the lowest *C** value (33.56) (Table 1). After shelf life, maximum *C** value (40.02) was in untreated heirloom tomatoes while minimum *C** value (34.21) was in ethylene treated cluster tomatoes (Table 2).

In the current study, different types of tomato exhibit increase in *C** values which was supported by the findings of Davila-Avina et al. (2011) who reported rise in *C** value throughout storage of tomatoes. Camelo & Gomez (2004) revealed that *C** had not been a good indicator to signify the ripening of tomatoes. However, it can be used as a suitable parameter for acceptance of consumers regarding tomatoes that are fully ripe.

In general, ethylene treated tomatoes had lower *h*° values than untreated ones and prolonging storage duration decreased *h*° values. After cold storage maximum *h*° value (65.48°) occurred in ethylene treated beefsteak type with minimum *h*° value (45.09°) in ethylene treated cluster tomatoes (Tables 1). After shelf life, highest *h*° value (47.60°) occurred in control beefsteak type, while the lowest *h*° value (41.22°) was in ethylene treated cluster tomatoes (Table 2).

Decreases in *h*° values of tomatoes with extending storage duration was found by Chomchalow et al. (2002) as obtained from this study. Cantwell (2010) reported that the lower the *h*° values the redder will be the fruit. Tomatoes attained red color with increase in storage and cluster type tomatoes was redder as compared to beefsteak and heirloom types in our study.

3.4. Total soluble solids (TSS)

TSS content in beefsteak and heirloom types decreased during storage but increased in cluster type fruit. After cold storage, highest TSS content (4.20%) occurred in both control and ethylene treated cluster type with lowest TSS content (3.83%) in control heirloom fruit (Table 1). At the end of shelf life period, maximum TSS content (4.20%) was in control cluster fruit while minimum TSS content (3.77%) was in control heirloom type tomatoes (Table 2).

Davila-Avina et al. (2011) expressed that tomato fruit harvested at pink maturity stage showed a decrease in TSS content during storage that agreed with our results regarding beefsteak and heirloom type of tomatoes however it contradicted with cluster type of tomatoes which exhibited increase in TSS content. Similar findings regarding cluster type of tomatoes were reported by Dhall & Singh (2013). They stated that this rise could be because of water loss, hydrolyzation of starch and other polysaccharides to soluble forms of sugar. However, their results contrasted with our findings for heirloom and beefsteak type of tomatoes which had a slight rise in TSS content at first and then decreased by the end of both cold storage and shelf life period. Increase in TSS content of cluster type of tomatoes with extending storage duration was reported by Mohammed et al. (1999).

3.5. Titratable acidity (TA)

The extension in storage duration considerably decreased the TA in cold storage and shelf life conditions. Ethylene treated tomatoes had lower TA than untreated ones. At the end of cold storage, the highest TA (3.63 g citric acid kg^{-1}) was exhibited by control cluster type whereas the lowest TA (3.03 g citric acid kg^{-1}) was recorded in ethylene treated beefsteak type of tomatoes (Table 1). At the end of shelf life period, the maximum TA (3.80 g citric acid kg^{-1}) was found in control heirloom type while minimum TA (2.80 g citric acid kg^{-1}) was found in ethylene treated beefsteak type of tomatoes (Table 2).

Decrease in TA with extension in storage duration was exhibited by different types of tomato in this study which agreed with the findings of Tigist et al. (2013) who stated that TA decreased with extension in storage. The reasons for decline in TA during our experiment can be due to the loss of citric and malic acid during ripening as reported by Sammi & Masud (2007) or it may be because of triggering of ethylene production that influence the organic acids and total soluble solids in tomatoes and other climacteric fruit as mentioned by Guilen et al. (2007).

3.6. Fruit firmness

Different types of tomatoes had a decline in fruit firmness with prolonging storage period. In general ethylene treated tomatoes had lower fruit firmness than non-treated tomatoes. Significant interaction between the storage duration and treatments existed

at P≤0.05. At the end of cold storage, the untreated beefsteak type of tomatoes had maximum fruit firmness (6.83 N) while minimum fruit firmness (3.52 N) was determined in ethylene treated heirloom type of tomatoes (Table 1). At the end of shelf life period, the highest fruit firmness (6.45 N) was exhibited by control beefsteak type whereas lowest fruit firmness (3.15 N) was displayed by ethylene treated cluster type of tomatoes (Table 2).

Dhall & Singh (2013) revealed that ethylene treated tomatoes had less fruit firmness than control fruit as obtained in our study. Nyalala & Wainright (1998) expressed that storage of tomatoes at high temperatures result in lower fruit firmness than those stored at low temperatures which can be because of increased activity of polygalacturonase at 20 °C as mentioned by Kapotis et al. (2004). These findings agreed with more decrease in fruit firmness of tomatoes in the shelf life period than cold storage in this study.

3.7. Amount of unmarketable fruit

Quantity of unmarketable fruit increased with time in storage and shelf life. Ethylene treatment resulted in more unmarketable fruit than in controls apart from cluster tomatoes after shelf life. Significant interaction (P≤0.05) between storage duration and treatments occurred. At the end of cold storage, maximum 51.96% of unmarketable fruit occurred in ethylene treated heirloom tomatoes whereas minimum unmarketable fruit (25.50%) was in control cluster type of tomatoes (Table 1). At the end of shelf life, the most unmarketable fruit (83.33%) was in control cluster fruit with the least (33.33%) was in control heirloom fruit (Table 2).

Our results regarding higher unmarketable fruit in ethylene treated tomatoes during cold storage was supported by Geeson et al. (1986). They reported that ethylene treatment had enhanced decay development in tomato however this outcome contradicted with results of shelf life where control cluster type of tomatoes had highest unmarketable fruit. According to Cheng & Shewfelt (1988) storage of tomatoes at 4 °C for 15 days and then ripening at ambient temperature increased ethylene production and vulnerability to decay which support our findings of higher amount of unmarketable fruit during shelf life as compared to cold storage. According to Gonzalez-Aguilar et al. (2010) CO_2 and ethylene productions are vital components which determine the level of decay development in fruit and vegetables.

4. Conclusions

The conclusion drawn from the results obtained is that cluster type tomatoes were recorded to be more sensitive to ethylene treatment than beefsteak and heirloom types as they had produced the highest ethylene during cold storage. Beefsteak type tomatoes retained better postharvest quality than heirloom and cluster types of tomatoes at the end of cold storage and shelf life. Ethylene treatment resulted in higher ethylene production, weight loss with lower fruit firmness. At the end of cold storage, minimum ethylene production, respiration rate and maximum L^*, C^*, fruit firmness were found in control beefsteak type. Ethylene application in beefsteak type resulted in maximum $h°$ value. The highest titratable acidity and lowest amount of unmarketable fruit were noticed in control cluster type of tomatoes. At the end of shelf-life period; minimum weight loss and maximum L^*, $h°$, fruit firmness was noted in control beefsteak type. The lowest amount of unmarketable fruit and highest titratable acidity was obtained in control heirloom type. The highest total soluble solids contents were observed in control cluster type of tomatoes. Variations among the different types of tomato existed which can be taken into consideration prior to storage by the commercial growers, storage operators and wholesalers.

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Potential Aphid (Hemiptera: Aphididae) Vectors of Plum-pox Virus (Virus:Potyviridae) and Status of Sharka Disease in Stone Fruit Orchards in the East Mediterranean Region of Turkey

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ABSTRACT
This study was conducted in stone fruit orchards in five provinces of the East Mediterranean Region of Turkey between the years of 2009-2011. The aim of the study was to determine the potential aphid vectors of the quarantine pathogen PPV (plum pox virus, family *Potyviridae*) that causes serious yield losses. During the surveys, 542 orchard/nurseries were sampled and 6 aphid species (Hemiptera: Aphididae) [*Brachycaudus persicae* (Passerini,1860), *B. helichrysi* (Kaltenbach, 1843), *B. cardui* (Linnaeus, 1758), *Hyalopterus pruni* (Geoffroy, 1762), *Myzus persicae* (Sulzer, 1776) and *M.cerasi* (Fabricius, 1775)] from stone fruit trees and 4 aphid species [*Aphis craccivora* (Koch, 1854), *A. fabae* Scopoli 1763, *A. gossypii* Glover 1877, *A. nasturtii* Kaltenbach 1843)] from weeds that were known as the efficient vectors of PPV were detected in the stone fruit orchards. Stone fruit samples (flower, leaf, fruit), weed samples and aphid samples were tested by DAS-ELISA to determine the presence of PPV. PPV infected samples that resulted uncertain from DAS-ELISA were processed to conventional RT-PCR (Reverse Transcription Polymerase Chain Reaction) to finalize the decision of PPV presence. A total of 8 orchards were resulted as PPV-infected with both analyzing methods in the region. Three of these orchards were infected with aphids as well. *B.cardui* collected from the PPV-infected orchard in Hatay-Samandağ were also run to analyse for the presence of PPV and resulted virus positive.

Keywords: Aphid vectors, Plum-pox virus, Sharka

1. Introduction

The majority of plant viruses rely on insect vectors for transmission. Acquisition and inoculation of viruses occurs during feeding activity of specific vectors (Stafford et al. 2012). Among insect vectors, aphids are the most common and efficient vectors owing to piercing-sucking mouthparts that provide transmission of the virus to healthy plants. Viruses were classified as stylet- borne (carried at the tips of the stylets, non-persistent) or circulative (ingested by aphids and circulated through the hemolymph, persistent) (Pirone & Harris 1977). Unlike some other viruses, PPV (plum pox virus, family *Potyviridae*) is not persistent in the aphid and is transferred from the mouthparts of the aphid between plants non-persistently. PPV, the causal agent of sharka virus disease, is one of the devastating organisms that threatens fruit production of stone fruits. Severe fruit deformation and premature fruit dropping is seen in the infected orchards that cause serious economic losses (Figure 1).

Sharka disease is listed in the domestic and external quarantine list of Turkey. The disease was first detected on plums in Edirne in 1969 in Turkey (Sahtiyancı 1969) but had a slow dispersion since that time. Following the first report, Elibuyuk (2003) detected PPV-M strain in 10 home gardens in Ankara region in apricot, plum and peach trees between 1995 and 2002. Afterwards, Koc & Baloglu (2006); Candresse (2007); Ulubas Serce et al. (2009); Akbas et al. (2011); Caglayan et al. (2013); Ceylan et al. (2014); Çağlayan & Yurdakul (2017) and Yurtmen et al. (2017) conducted studies related to the disease.

The foremost inoculum source of the virus is infected prunus trees. Wild woody and herbaceous hosts are also widespread and are potential reservoirs of the disease. Virus is transmitted from infected trees to healthy ones by means of grafting, infected propagation material and aphids (Celetti et al. 2008). Natural transmission of the virus is mediated by Aphids (Aphididae) in non-persistent manner. Aphids can acquire the virus in probes as short as 30 seconds, and can transmit for up to 1 hour (Labonne et al. 1995). *Myzus persicae*, *Aphis gossypii*, *A. spiraecola*, *A. fabae*, *Brachycaudus persicae* and *Hyalopterus pruni* transmitted PPV under laboratory conditions (Avinent et al. 1994; Gildow et al. 2004). Currently, up to 20 aphid species are known to transmit PPV (Levy et al. 2000; Labonne & Dallot 2006). Apart from cultivated Prunus, several weed species have been

identified as PPV herbaceous hosts under experimental and under field conditions (Milusheva & Rankova 2002; Viršček 2004; Celetti et al. 2008). As well as being a possible reservoir of PPV, weeds play an important role as being host plant for aphid vectors.

Figure 1- Sharka disease symptoms observed on apricot fruits (A), apricot leaves (B), peach fruits (C) and peach leaves (D)

In this study, the actual status of the disease in stone fruit orchards of eastern Mediterranean region were revealed. Aphid species were detected on Prunus trees and on weed species. Besides, weed species as possible reservoir of PPV both in the infected and non-infected orchards were detected. In this manner the risk of new introductions by aphid vectors were evaluated.

2. Material and Methods

Extensive surveys to detect PPV infection, aphid species composition in the region, potential vector aphids and weeds that host to aphids and the virus were performed yearly in spring and autumn between the years of 2009 and 2011.

2.1. Field surveys and samples

The study was carried out on stone fruit orchards (apricot, peach, nectarine, plum, cherry) in the largest *Prunus* cultivating areas in Adana (Ceyhan, Seyhan, Yüreğir, Karataş), Mersin (Arslanköy, Değirmendere, Çiviçukuru, Dikilitaş, Tarsus, Çamtepe, Yeşilovacık, Mut), Hatay (Samandağ, İskenderun), Kahramanmaraş (Andırın, Çokaklı, Merkez) and Osmaniye provinces in the Eastern Mediterranean Region of Turkey. Number of survey orchards was determined according to Bora and Karaca (1970). During the surveys, the minimum amount (0.01%) of total tree entity of the province were considered but was expanded as much as possible. As much flower, leaf and fruit as possible were sampled in the randomly selected orchards (Barnett 1986).

2.2. Method

2.2.1. Detection of PPV

During a 3 year period, stone fruit orchards were visually inspected for PPV symptoms. Samples of flowers, leaves and fruits from the symptomatic and asymptomatic trees were collected in Spring and Autumn periods. Each symptomatic plant, weeds and aphids were collected individually. If no symptom expression of sharka was seen, samples were taken from ten trees; one flower, one leaf and one fruit sample from four direction of the tree. The number of samples in the orchards were varied according to the size of the orchards, number of the symptomatic plants and the presence of the aphid vector and weeds hosting aphid species. In case of the presence of all these parameters, number of the subsampling were increased in the subjected orchard.

These samples were tested for the presence of PPV**.** Double Antibody Sandwich Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) method was used for the serological identification of the virus by using commercial PPV kit (Bioreba-PPV). The results were evaluated both visually and by spectrophotometer at 405 nm wavelength. Two-fold or more of negative control value was accepted as positive for PPV. The samples resulted serologically as being PPV-positive or PPV-suspected were run to conventional RT-PCR (Reverse Transcriptase Polymerase Chain Reaction), molecularly in order to clarify the results. As well

as plant samples of Prunus, the aphid species collected from suspected orchards were subjected to serological and molecular screening against PPV. The weeds collected from orchards were also tested for the presence of PPV.

2.2.2. Detection of aphids

Twigs and leaves of Prunus trees infested with aphids were cut and wrapped with paper, then were placed in a plastic bag and brought into laboratory in cold chain once a week. Weeds were uprooted for accurate identification. The aphid samples were taken not only from symptomatic trees but also from healthy ones and were stored in 70% alcohol for future identification. For peach trunk aphid, *Pterochloroides persicae*; samples were collected from the trunk and thick branches of the trees directly by a fine brush and placed in 70% ethanol in glass vials in the orchard. Nymphs were reared into adults in cages in the controlled rearing rooms adjusted 25±2 °C temperature and 70±10% relative humidity.

2.2.3. Identification

Aphid adults were identified into species by Dr. Işıl Özdemir (Ankara Plant Protection Central Research Institute) and weeds by Dr. Eda Aksoy (Directorate of Plant Biodiversity, Geofit Research and Training Center). Coccinellidae species were identified by Prof. Dr. Nedim Uygun (Çukurova University, Agriculture Faculty, Plant Protection Department-Retired). Identification of Neuroptera species were made by Prof. Dr. Faruk Özgür (Çukurova University, Agriculture Faculty, Plant Protection Department-Retired). Masaru Nishikawa (Ehime University; Entomological Laboratory, Matsuyama, Japan) identified the Forficulidae species.

3. Results and Discussion

3.1. Detection of PPV in stone fruit orchards

In the first year of the study (2009), five hectare of nectarine/peach mixed orchard located in Adana were found PPV-infected among 143 orchards sampled. In the second year, two PPV infected orchards were detected among 202 orchards sampled. The 0.5 hectares nectarine orchard located in Samandağ/Hatay and 8 hectares nectarine orchard in İskenderun/Hatay were eradicated. In 2011, two apricot and one peach orchards located in Hatay were also found infected with PPV. In the same year, 0.5 hectares of apricot orchard in Akdeniz district of Mersin and 1.5 hectares of plum orchard in Yeşilovacık districts of Mersin were found contaminated with sharka disease among 197 orchards sampled in 2011. The infection rates were shown in Table 1. All PPV infected orchards detected between 2009 and 2011 were eradicated by their owners.

Table 1- PPV infection rates of orchards sampled in 2009, 2010 and 2011 in East Mediterranean Region

Year	***Number of orchards sampled***	***Number of orchards PPV infected***	***Infection rate of sampled orchards (%)***
2009	143	1	0.70
2010	202	2	0.99
2011	197	5	2.54
TOTAL	542	8	1.47

3.2. Detection of aphid species

In order to detect the current status of aphid vectors in stone fruit orchards, surveys were conducted in the orchards of peach, apricot, nectarine, plum, cherry and almond for three years long. Aphid colonies were collected from both Prunus trees and from weeds in and around these orchards whether they were PPV-symptomatic or not. After that, the aphids were identified into species and listed in Table 2.

In the study, a total of 19 aphid species were collected between the years of 2009 and 2011. Of the total amount, seven species-*Brachycaudus persicae, B. cardui, B. helichrysi, Hyalopterus pruni, Pterochloroides persicae, Myzus persicae, M. cerasi* were collected from stone fruit trees. Eleven species including *Aphis pisum, A. craccivora, A. fabae, A. fabae solanella, A. gossypii, A. nasturtii, A. rumicis, Brevicoryne brassicae, Hayhurstia atriplicis, Lipaphis erysimi, Uroleucon (Uromelan) jaceae* were derived from weeds, and a species-*Macrosiphum rosae*- was on rose (Table 1). Among these species, *A. gossypii, A. craccivora, A. fabae, A. nasturtii, B. persicae, B. helichrysi, B. cardui, H.pruni, M. persicae,* and *M. cerasi* were ranked among the vectors of PPV with variable efficiency (Kunze & Krczal 1971; Avinent et al. 1994; Labonne et al. 1995; Levy et al. 2000; Pribek 2001; Gildow et al. 2004). Therefore, natural spread of Sharka disease by aphid vectors to the short distance exist potentially in the region.

Table 2- Aphid species detected in stone fruit orchards in 2009, 2010, 2011 and their vector status

Species	*Year*	*Location*	*Host Plant*	*Colonizes Prunus*	*Vector for PPV*
Acyrthosiphon pisum (Harris, 1776)	15.04.2010	Çiftlik district/Mersin	*Medicago* sp.	No	**No**
Aphis craccivora (Koch, 1854)	29.09.2009	Değirmendere/Mersin	*Chenopodium nigrum*	No	**Yes**
	25.05.2010	Karataş/Adana	Weed (not identified)		
	03.05.2011	Güzelyayla/Mersin	*Capsella bursa pastoris*		
	03.05.2011	Değirmendere/Mersin	*Vicia sativa*		
Aphis fabae Scopoli, 1763	20.04.2010	Seyhan/Adana	*Vicia sativa*	No	**Yes**
	03.05.2011	Güzelyayla/Mersin	*Vicia sativa*		
	03.05.2011	Değirmendere/Mersin	*Capsella bursa-pastoris*		
	03.05.2011	Güzelyayla/Mersin	*Neslia paniculata*		
	13.05.2011	Çiviçukuru/Mersin	*Vicia sativa*		
Aphis fabae solanella Theobald, 1914	06.10.2019	Tarsus/Mersin	*Solanum nigrum*	No	**No**
	04.06.2010	Samandağ/Hatay	*Solanum nigrum*		
	29.06.2010	Karataş/Adana	*Solanum nigrum*		
Aphis gossypii Glover, 1877	11.06.2009	Andırın/Kahramanmaraş	Weed (not identified)	No	**Yes**
	06.10.2009	Nacarlı/Sumaklı/Mersin	*Brassica rapa*		
	15.04.2010	Çiviçukuru/Mersin	*Chenopodium album*		
	02.06.2011	Dikilitaş/Mersin	*Chenopodium album*		
Aphis nasturtii Kaltenbach, 1843	03.05.2011	Değirmendere/Mersin	*Vicia sativa*	No	**Yes**
Aphis rumicis Linnaeus, 1758	06.10.2009	Tarsus/Mersin	Weed (not identified)	No	**No**
Brachycaudus(Acaudus) persicae (Passerini,1860)	03.05.2011	Değirmendere/Mersin	*Prunus persica*	Yes	**Yes**
Brachycaudus (Acaudus) cardui (Linnaeus, 1758)	21.05.2010	Yanpar/Mersin	*Prunus armeniaca*	Yes	**Yes**
	10.06.2010	İskenderun/Hatay	*Prunus persica* var *nucipersica*		
	30.09.2010	Arslanköy/Mersin	*Prunus persica*		
Brachycaudus helichrysi (Kaltenbach, 1843)	22.05.2009	Yüreğir/Adana	*Silybum marianum*	Yes	**Yes**
	26.04.2011	Merkez/Osmaniye	*Prunus armeniaca*		
	01.06.2011	Yeşilovacık/Mersin	*Prunus domestica*		
	02.06.2011	Bekirde mh./Mersin	*Prunus armeniaca*		
Brevicoryne brassicae (Linnaeus, 1758)	03.05.2011	Güzelyayla/Mersin	*Neslia paniculata*	No	**No**
Hayhurstia atriplicis (Linnaeus, 1761)	29.09.2009	Değirmendere/Mersin	*Chenopodium nigrum*	No	**No**
	30.09.2010	Arslanköy/Mersin	*Chenopodium album*		
Hyalopterus pruni (Geoffroy, 1762)	22.05.2009	Ceyhan/Adana	*Prunus armeniaca*	Yes	**Yes**
	22.05.2009	Yüreğir/Adana	*Prunus armeniaca*		
	22.05.2009	Sakırcalı/Osmaniye	*Prunus armeniaca*		
	22.05.2009	Ceyhan/Adana	*Prunus domestica*		
	01.06.2009	Mut/Mersin	*Prunus armeniaca-P. domestica*		
	10.06.2009	Samandağ/Hatay	*Prunus domestica*		
	15.04.2010	Çiviçukuru/Mersin	*Prunus armeniaca*		
	29.06.2010	Karataş/Adana	*Prunus persicae* var.*nucipersica*		
	26.04.2011	Merkez/Osmaniye	*Prunus armeniaca*		
	03.05.2011	Değirmendere/Mersin	*Prunus persica*		
	02.06.2011	Dikilitaş/Mersin	*Sonchus sp.*		
Lipaphis erysimi (Kaltenbach, 1843	03.05.2011	Güzelyayla/Mersin	*Neslia paniculata*	No	No
	13.05.2011	Çiviçukuru/Mersin	*Vicia sativa*		
Macrosiphum rosae (Linnaeus, 1758)	17.06.2009	Arslanköy/Mersin	Rosa L.	No	**Yes**
Myzus (Nectarosiphon) persicae (Sulzer, 1776)	29.09.2009	Değirmendere/Mersin	*Amaranthus retroflexus*	Yes	**Yes**
	29.09.2009	Değirmendere/Mersin	*Chenopodium album*		
	26.04.2011	Merkez/Osmaniye	*Prunus armeniaca*		
	02.06.2011	Dikilitaş/Mersin	*Chenopodium album*		
	02.06.2011	Dikilitaş/Mersin	*Sonchus* sp		
Myzus cerasi (Fabricius, 1775)	11.06.2009	Andırın/Kahramanmaraş	*Prunus avium*	Yes	**Yes**
	17.06.2009	Arslanköy/Mersin	*Prunus avium*		
Pterochloroides persicae (Cholodkovsky, 1899)	04.06.2010	Samandağ/Hatay	*Prunus persica* var. *nucipersica*	Yes	**No**
Uroleucon (Uromelan) jaceae (Linnaeus, 1758)	13.05.2011	Çiviçukuru/Mersin	*Prunus persica* *Sonchus oleracea*	No	**No**
	02.06.2011	Dikilitaş/Mersin	*Sonchus oleracea*		

3.2.1. Results of the first year (2009)

In the first year of the study, 143 samples were tested for PPV. One orchard located in Adana province were found infected with Sharka disease without any infestation of aphids. The orchards which were infested with aphid colonies were not resulted as PPV-positive. *Hyalopterus pruni* was the predominant species on Prunus trees in most of the orchards especially in plum and apricot. *Myzus persicae* was collected from weeds in autumn period. Most of the aphid species detected on the weeds were ranked among PPV vectors. The vector aphids obtained from the stone fruit trees and the weeds detected in 2009 posed a risk for transmission of PPV.

3.2.2. Results of the second year (2010)

In the second year, *H. pruni* kept on being the predominant species on trees followed by *Pterochloroides persicae* and *Brachycaudus cardui.* Among 202 samples, only two orchards were found Sharka infected in 2010. The PPV positive orchard in Samandağ/Hatay was infested with peach trunk aphid, *P. persicae.* Collected samples of *P. persicae* were analyzed by conventional RT-PCR and were resulted negative for PPV. Stoetzel & Miller (1998) recorded that *P. persicae* is not listed as transmitting a virus. Labonne & Dallot (2006) notified that the main sources of inoculum for the vectors are leaves and fruits of infected stone-fruit trees which empowers the thought of *P. persicae* is not a PPV-vector. *Brachycaudus cardui* colonies were collected from the second PPV-positive orchard that was located in İskenderun/Hatay. Aphids collected from this orchard were analyzed for the presence of PPV and resulted as positive. This species was cited as the efficient vector of PPV in the previous studies (Brunt et al. 1996; Isaac et al. 1998; Levy et al. 2000). Three aphid species apart from nine; *B. cardui*, *P. persicae* and *H.pruni,* detected in 2010, colonized on Prunus while rest of the aphids were weed colonizing species which most of them were listed in the PPV vectors. In cases where the weeds were infected with PPV, there is a further risk for transmission of the virus from weeds to Prunus spp. by aphid vectors.

3.2.3. Results of third year (2011)

In the third year, among a total of 197 samples, five PPV infected orchards were identified in the region. There was not aphid colonization in 3 of them in Hatay. The rest two PPV-infected orchards-a plum orchard in Yeşilovacık/Mersin and an apricot orchard in Dikilitaş/Mersin were colonized with *Brachycaudus helichrysi.* Although this species is one of the known effective vectors of PPV (Levy et al. 2000), serological and molecular analyzes of *B. helichrysi* collected from these two orchards resulted negative for PPV. According to this year's result, it is likely to state that the virus was either spread non-homogenously in the orchard or virus concentration was low in the plant part where the aphid fed. In other words, it is seen that the disease may appeared randomly in the orchard and/or spread of the virus may be very irregular in the tree. Besides, the effective spread of virus by aphids varies according to the aphid species, the host species, PPV strain, season and location (Levy et al. 2000). Wallis et al. (2005) reported that for effective PPV spread, timing of optimal host susceptibility, optimal virus titer and optimal vector population should coincide, all of which explains the negative result.

Excluding the two orchards in Mersin, all orchards infected with aphid colonies tested negative for PPV in 2011. Five of twelve aphid species (*B. persicae, B. helichrysi, H. pruni, M. persicae, P. persicae*) detected in 2011 were Prunus colonizing species and except *P. persicae* all were PPV vectors. Levy et al. (2000) listed *B. helichrysi* and *M. persicae* as the most efficient vector species. Rest of the aphids were non-prunus colonizing species. Among them *Aphis gossypii, Aphis craccivora* and *Aphis fabae* were listed as in the most efficient vector category (Levy et al. 2000; Gildow et al. 2004).

3.3. Detection of weed species hosting aphids and PPV

Weeds can provide inoculum sources for PPV and act as hosts to aphid vectors as well. In this study, the weed species growing in and around the stone fruit orchards were also collected and analyzed for the presence of PPV. All of them were given negative results against PPV. Both the weed species and the aphid colonies on the weeds were identified into species and listed in Table 3.

Table 3- The weed species existing in Prunus orchards and aphid species found on weeds

Weed Species	***Family***	***Aphid species present on weeds***	***Previous works***
*Capsella bursa-pastoris** (L.)	Brassicaceae (Syn.:Cruciferae)	***Aphis craccivora* ***Aphis fabae*	Milusheva & Rankova 2002, Brunt et al. 1996
Chenopodium album L.	Chenopodiaceae	***Aphis gossypii* ***Myzus persicae*	-
Chenopodium vulvaria L.			
Solanum nigrum L.*	Solanaceae	*Aphis fabae solanella*	Virščček et al. 2004
Medicago sp.	Leguminosae (Syn.:Fabaceae)	*Acyrthosiphon pisum*	-
Sonchus sp.*	Astaraceae	***Hyalopterus pruni* ***Myzus persicae* *Uroleucon****jaceae*	Virščček et al. 2004
Sonchus oleraceaus L.			-
Amaranthus retroflexus L.	Amaranthaceae	***Myzus persicae*	-
Neslia paniculata (L.)	Brassicaceae	***Aphis fabae* *Brevicoryne brassicae* *Lipaphis erysimi*	-
Silybium marianum (L.)	Astaraceae	***Brachycaudus helichrysi*	-
Vicia sativa L. *	Leguminosae (Syn.:Fabaceae)	***Aphis craccivora* ***Aphis fabae* *Aphis nasturtii*	Brunt et al. 1996

*: Herbaceous hosts of PPV; **: Vector aphids

Four of eleven weed species (*Capsella bursa-pastoris* L., *Solanum nigrum* L., *Sonchus* sp., *Vicia sativa)* were mentioned as natural hosts of PPV in previous works (Table 2). Families of Brassicaceae (Cruciferae), Chenopodiaceae, Amaranthaceae, Solanaceae and Leguminosae were reported as containing susceptible host plants to PPV (Brunt et al. 1996). Celetti et al. (2008) isolated PPV from several herbaceous hosts such as *Ranunculus repens, Medicago lupulina, Trifolium pratense, T. repens, Silene vulgaris* that were common weeds in stone fruit orchards in many regions of Ontario. Milusheva & Rankova (2002) reported that *Capsella bursa-pastoris* L. was the host of Sharka virus. Virščček (2004) conducted DAS-ELISA analyses to the samples collected from PPV infected peach orchards and found positive results for several common weed species such as *Taraxacum officinale, Convolvulus arvensis, Sonchus sp.* and *Solanum nigrum* L. in Slovenia. *Chenopodium foetidum* is shown as one of the several herbaceous plants which PPV can be artificially transmitted (OEPP/EPPO 2004).

Seven aphid species (*Aphis craccivora, A. fabae, A. gossypii, A. nasturtii, Brachycaudus helichrysi, Hyalopterus pruni, Myzus persicae)* that infest the weeds shown in Table 2 are PPV vectors. Three of twelve aphid species infesting weeds are also Prunus colonizing species (*B. helichrysi, H. pruni, M. persicae*) while the rest are not. Not only Prunus colonizing species but also the migratory ones have an important role in the spread of disease. According to the results, it can be concluded that PPV infested herbaceous hosts colonized by vector aphids pose a high risk for fruit trees as PPV inoculum reservoirs. The studies conducted in Prunus orchards and samplings of Prunus trees, herbaceous plants and aphids between 2009 and 2011 indicated that appropriate conditions existed for natural spread of PPV in stone fruit orchards in the region.

Besides, predator species of aphids were detected in the survey areas. Samples were taken from these orchards and were identified into species by experts. The list is shown in Table 4.

Table 4- Predator species obtained from aphid colonies

Family	*Species*	*Location*	*Date*	*Host Aphid species*
Syrphidae	*Episyrphus balteatus* (De Geer)	Hatay/Samandağ (1)	10.06.2009	-*Hyalopterus pruni* Geoffroy, 1762)
		Hatay/İskenderun (1)	10.06.2010	-*Brachycaudus cardui*
		Mersin/Güzelyayla(2)	07.10.2010	(Linnaeus, 1758)
	Metasyrphus corollae (Fabricius)	Mersin/Güzelyayla	07.10.2010	
	Paragus aegyptius Mac Quart	Mersin/Gözne	12.10.2010	
Coccinellidae	*Oenopia (Synharmonia) conglobata* (L.)	Hatay/İskenderun	10.06.2010	
	Hippodamia (Adonia) variegata (Goeze)	Osmaniye /Sakırcalı	22.05.2009	-*Brachycaudus helichrysi*
		Adana/Ceyhan	22.05.2009	(Kaltenbach, 1843)
		Hatay/Samandağ	10.06.2009	-*Hyalopterus pruni* Geoffroy, 1762)
		Adana/Seyhan	20.04.2010	
		Adana/Karataş	25.05.2010	
		Mersin/Merkez	07.10.2010	
	Coccinella semptempunctata (L.)	Adana/Seyhan	20.04.2010	*Aphis fabae*
	Adalia bipunctata (L.)	Mersin/Güzelyayla	07.10.2010	
Forficulidae	*Forficula lurida* Fischer 1853	Andırın/Çokaklı	11.06.2009	*Myzus cerasi* (Fabricius, 1775)
Chrysopidae	*Chrysoperla carnea* (Stephens)	Adana/Ceyhan	22.05.2009	*Hyalopterus pruni*
		Adana/Seyhan	20.04.2010	*Hyalopterus pruni*

4. Conclusions

In this study, the actual status of Sharka disease in stone fruit orchards in The Eastern Mediterranean Region of Turkey were revealed. The orchards tested positive for PPV were eradicated. Aphid species and weeds both in the infected and non-infected orchard areas were detected and the risk of new introductions by aphid vectors were evaluated.

A total of 542 Prunus samples were analyzed for PPV between 2009 and 2011. The survey orchards were located in intensive stone fruit plantings of 5 provinces of the region. Eight orchards tested positive and were eradicated by their owners. Sharka disease incidence was higher in Hatay and Mersin provinces. The PPV infected orchards detected in the region were 3 to 6 years old (the oldest was 10 years old) which set us thinking that the introduction of the disease agent to the region is occurred by infected propagation material imported from abroad or from the other regions of the country. Our results indicated that the prevalence of the disease was restricted in the region. Out of 542 sampled orchards 1.47% was found infected (Table 1). Another study conducted in Turkey in 56 provinces indicated that the incidence of the disease was 3.9% in 5.762 samples (Akbas et al. 2011).

According to findings of the study, of the total amount of 19 aphid species detected on Prunus trees and on weeds, six of seven aphid species on Prunus trees and four of twelve aphid species on weeds are PPV vectors. Not only Prunus colonizing aphids, but also the migratory ones have an important role in the spread of the disease. Accordingly, it can be concluded that vector aphids colonized on PPV infested Prunus trees or herbaceous hosts pose a high risk for healty fruit trees as PPV inoculum reservoirs. The studies conducted in Prunus orchards and samplings of Prunus trees, herbaceous plants and aphids between 2009 and 2011 indicated that appropirate conditions existed for natural spread of PPV in stone fruit orchards in the region.

It is a known fact that aphids transmit viruses non-persistently. This can be explained by feeding behaviour of aphids. Although aphids are host–specific in their choice of food sources and plants on which to colonize, they are not capable of identifying suitable hosts visually, and need to probe many plants through tactile and gustatory tests before reaching preferred hosts. When the aphid find the plant (ex.: PPV infected Prunus trees or weeds) distasteful or unsuitable to feed, it moves to a new host (ex.:healthy Prunus), and can in the process, acquire the virus from infected host and then spread it to the healthy ones (Celetti et al. 2008) in a few minutes. Insecticide applications may reduce the overall population of aphid vectors over a growing season but can not wipe off completely. Similarly, predator species detected during surveys (Table 4) and parasitoids can suppress the aphid population but can not wipe off completely. In another word, total control of aphid vectors is impossible to achieve (Anonymous 2008). As a single aphid can transmit PPV to a new host in a matter of seconds, presence of vector aphids in the region pose a high risk for the spread of disease.

Besides, the favorable conditions existed for the natural spread of PPV in stone fruit orchards in the region, the risk of PPV-infected propagation material should not be overlooked. Accordingly, excess responsibility should be undertaken related to quarantine and plant passport implementations. Preventation is the only practical management strategy to avoid sharka disease. Therefore, training of the farmers, officers and all units related with agriculture is as effective as removing symptomatic trees or eradication of the whole infected orchards. PPV infected trees/orchards should be uprooted immediately because natural

pathways (vector aphids) and the PPV host plants (Prunus and weeds) for the spread of the disease exists in the region. Otherwise aphids are able to transmit the disease to healthy trees in and around the orchard. Infected fruit should also be exterminated because aphids can acquire the virus not only from leaves but also from fruits. Recently, researchers in France and the United States demonstrated that aphids could acquire and transmit the virus from infected fruit to young peach seedlings. Doubtlessly, properly disposing the stone fruit cull piles away from susceptible stone fruit orchards is an important precaution (Celetti et al. 2008). Besides, strict control measures should be taken on the imported plant materials in order to avoid the entrance of PPV infected material to the country or a region.

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Prevalence of *Listeria* spp. in Seafood Samples and Control of *Listeria monocytogenes* with Using LISTEX™ P100 Bacteriophage Applications in Smoked Rainbow Trout

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ABSTRACT
This study was carried out to determine the presence of *Listeria* spp. in seafood and determine the effect of LISTEX™ P100 bacteriophage applications (incorporated into the sodium alginate based film and applied directly to the surface) on the smoked trout. In this study, *Listeria* spp. was isolated in 40 of the 100 products analyzed. Among the *Listeria* isolates, 8% correspond to *L. monocytogenes*, 15% to *L. innocua*, 6% to *L. seeligeri*, 10% to *L. welshimeri*, and 15 to *L. grayi*. *L. ivanovii* was not detected in any of the products analyzed. LISTEX™ P100 bacteriophage as antimicrobial compounds was incorporated into the sodium alginate based film for the first time. Bacteriophage in sodium alginate based film and direct bacteriophage applications in smoked trout were found to be effective in *L. monocytogenes* inactivation during storage. In addition, the preservation of phage stability of the two groups during storage indicates that in the smoked products can use in the control of *L. monocytogenes*.

Keywords: *Listeria monocytogenes*, Bacteriophage, LISTEX™ P100, Alginate film, Biopreservation, Seafood

1. Introduction

Listeria species are Gram-positive, non-spore-forming, facultative anaerobic bacteria widely distributed in the natural environment (Amajoud et al. 2018). Among *Listeria* genus, *Listeria monocytogenes* is most significant species because it causes listeriosis one of the most severe foodborne illness, whereas *Listeria innocua*, *Listeria welshimeri*, *Listeria ivanovii*, and *Listeria seeligeri* are associated with sporadic human infections (Amajoud et al. 2018).

In terms of food-borne illness, Ready-to-eat (RTE) foods are risky products for *L. monocytogenes* (Chibeu et al. 2013). Because, RTE are consumed directly, without a bacterial inactivation processing (Guenther et al. 2009). The salt content (2-8%), moisture contents (65-78%), pH (5.9-6.3) and water activity value (0.95-0.98) of the smoked seafood products, which are RTE products, facilitate the development of *L. monocytogenes* (Hwang et al. 2009). Therefore, smoked fish products are classified as risky products for listeriosis (Rørvik 2000).

The preservation methods and procedures applicable to minimally processed RTE foods are insufficient to ensure complete control of this microorganism (Guenther et al. 2009). The use of bacteriophage within these approaches is a good alternative (Oliveira et al. 2014), because the use of bacteriophage to prevent the development of *L. monocytogenes* in foods is very effective (Soni & Nannapaneni 2010; Rossi et al. 2011; Perera et al. 2015). Lytic bacteriophages are virus that infect bacterial cells and disrupt bacterial metabolism, causing bacterial lysis (Garcia et al. 2008). There are several commercially available phage products such as ListShield, EcoShield, Agriphage, SalmoFresh and LISTEX™ P100 (Hagens & Offerhaus 2008; Gálvez et al. 2014; Oliveira et al. 2014). The U.S. Food and Drug Administration (FDA) approved LISTEX™ P100 for use in foods to struggle with *L. monocytogenes* contamination (Soni and Nannapaneni 2010).

There are many studies in which bacteriophages are added directly to the surface of seafood products (Soni et al. 2009; Soni & Nannapaneni 2010; Galarce et al. 2014; Soni et al. 2014; Perera et al. 2015). As a result of these studies, it was reported that direct phage application to surface of products was effective in seafood. However, there are few studies in which a bacteriophage is incorporated into the film or within the absorbent pads (Gouvêa et al. 2015; Gouvêa et al. 2016). In these studies, the effects of phage containing films and pads on pathogen bacteria were investigated in vitro. There is only one study investigating the effect of bioactive packaging material containing bacteriophage (LISTEX™ P100) on *L. monocytogenes* in RTE turkey meat (Lone et al. 2016). However, in this study, we investigated the effect of sodium alginate-based film containing LISTEX™ P100 bacteriophage on *L. monocytogenes* in smoked trout fillets.

In this study was aimed to detect the presence of *Listeria* spp. in seafood sold in Turkey and to determine the effect of LISTEX™ P100 bacteriophage applications (incorporated to the film and applied directly to the surface) on *L. monocytogenes* in the smoked trout fillets.

2. Material and Methods

2.1. Investigation of Listeria spp. in seafood

In this study, a total of 100 raw and processed seafood products were purchased from supermarkets and restaurants in Turkey. These samples were investigated for the presence of *Listeria* spp. ISO (International Standards Organization 11290-1) method was used for the isolation of *Listeria* spp. (Anon 2000; ISO 2017). According to this method, the isolation process was carried out by pre-enrichment, selective enrichment, planting of solid medium and evaluation of colonies.

2.2. L. monocytogenes strains and *Bacteriophage source and plaque forming assay*

In this study, *Listeria monocytogenes* ATCC 7644 strain was used. The LISTEX™ P100 bacteriophage used in the study was obtained from Micreos (The Netherlands). Phage P100 stock solution in buffered saline had an approximate concentration of 10^{11} PFU (Plaque-forming units) mL^{-1}. To determine the phage titer of LISTEX^{TM} commercial suspension by double layer agar.

To determine the titre;

Phage titer (PFU mL^{-1}): N × 1/DF × 1/V

N: Number of plaques of lysis counted on a plate, DF: Dilution factor, V: Volume

2.3. Preparation of sodium alginate based film containing bacteriophage and evaluation of antimicrobial activity of film

Two types of film were prepared for evaluation of antimicrobial activity of sodium alginate based film containing bacteriophage. First one was sodium alginate film with phage P100 incorporated, other was control film not included phage P100.

1% sodium alginate based film solution was prepared; 1 g of sodium alginate is dissolved in 50 mL of sterile distilled water and mixed until dissolved in magnetic stirrer at 80 °C. The solution was cooled to 50 °C, it was added to 50 ml of 10^8 PFU mL^{-1} LISTEX™ P100 bacteriophage in film solution to achieve 1% concentration of solution. In this way, group of film containing bacteriophage was formed. For group of control film not containing bacteriophage; it was added to 50 mL of physiological salt water in film solution to achieve 1% concentration of solution. The films were poured into sterile petri dish to measure their antimicrobial activity. 3 mL of 0.05 M and 12.8 M $CaCl_2$ solutions used by Brachkova et al. (2012) were added to the films to strengthen the film (Han et al. 2018). To evaluate the antimicrobial activity of sodium alginate based films, a 1.75 cm diameter films were cut with a sterile lid.

The 24 hour cultures of *L. monocytogenes* (ATCC 7644) were diluted with PSW to standard concentration of 0.5 Mac Farland, and 100 µL of bacterial culture (10^8 CFU mL^{-1}) was spread to petri dishes containing Muller Hilton Agar (MHA, Merck). Films of 1.75 cm diameter have been placed on this plate. After plates' incubation, the diameter (mm) of the inhibition zones was recorded.

2.4. Effect of LISTEX™ P100 phage on L. monocytogenes (ATCC 7644) *in smoked trout*

2.4.1. Application of liquid smoked process to trout fillets

The fresh rainbow trout were obtained from a commercial company. The fish were filleted after harvest and immediately transported to the laboratory under a cold chain. Liquid smoke condensate (Red Arrow, SmokEz-C-10, mesguite water based) purchased from a food additive company (GMT Food Ingredients) was used to smoke the trout fillets. 100 mL liquid smoke condensate was mixed in 36% salt solution. Raw trout fillets were kept in this solution for 4 hours at 4 °C. At the end of this period, fillets were removed from this solution and dried for 25 min. Fillets were baked at 120-130 °C until their internal temperature reached 80 °C (Alçiçek 2010; Alcicek and Atar 2010). After cooking, the smoked trout were made into small fillets of 10 g.

2.4.2. Inoculation of smoked trout fillets with L. monocytogenes (ATCC 7644)

500 µL of *L. monocytogenes* culture (ATCC 7644) (6 log CFU g^{-1}) were poured on each side of raw trout sample (10 g). Total of 1000 µL of *L. monocytogenes* culture were used for contaminating both sides of fillets. After inoculation, the smoked fillets were allowed to air dry for 15 min in a Biosafety Level 2 laminar flow hood.

Experimental groups

Four different experimental groups were designed in this study.

Group 1: Group of smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644) coated with alginate film containing bacteriophage **(BF)**
Group 2: Group of smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644) coated with alginate film not containing bacteriophage **(CF)**
Group 3: Group of applied bacteriophage directly to the surface of smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644) **(B)**
Group 4: Group of smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644) **(C)**

2.4.3. Bacteriophage applications in smoked trout fillets inoculated with L. monocytogenes (ATCC 7644)

Smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644) were coated by dipping method with BF and CF film solutions that prepared as described in "Preparation of sodium alginate based film containing bacteriophage" section. And $CaCl_2$ solutions were added to the films to strengthen the film (Brachkova et al. 2012; Han et al. 2018).

For fillets applied bacteriophage directly to the surface (B), LISTEX™ P100 bacteriophage was inoculated 500 µL (8 log PFU g^{-1}) to two side of smoked trout fillets inoculated with *L. monocytogenes* (ATCC 7644).

In control group (C), smoked trout fillets were only inoculated with *L. monocytogenes* (ATCC 7644). Fillets (BF, CF, B and C) were stored in refrigerator at 10 °C for 7 days. The number of *L. monocytogenes* and bacteriophages were determined on the fillets at 0., 24., 48. and 96. hours and 7th day of storage.

2.4.4. Determination of L. monocytogenes and bacteriophage count in smoked trout fillets inoculated with L. monocytogenes during storage

For *L. monocytogenes* counting, each smoked trout fillet sample was aseptically homogenized and diluted. Serial dilutions of bacterial culture were spread on PALCAM Agar plates. They were incubated at 30 °C for 24-48 hours. At the end of the incubation, colonies were counted and the number of bacteria was determined as log CFU g^{-1}. For the determination of the number of bacteriophages, double layer agar method was used. They were incubated at 30 °C for 24 hours. At the end of the incubation, plaques of lysis were counted and the number of phages expressed as log PFU g^{-1}.

2.5. Statistical analysis

The obtained data were analyzed using Statistical Analyses Software Package (version 22, SPPS, Inc., Chicago, IL). The experimental data were subjected to One-way analysis of variance (ANOVA). The means comparison was performed by Duncan Multiple Comparison with the level of significant set at $P<0.05$.

3. Results and Discussion

3.1. The prevalences of Listeria spp. in seafood

Listeria spp. was isolated in 40 of the 100 products analyzed. Among the *Listeria* isolates, 8% correspond to *L. monocytogenes*, 15% to *L. innocua*, 6% to *L. seeligeri*, 10% to *L. welshimeri*, and 15 to *L. grayi*. *L. ivanovii* was not detected in any of the products analyzed (Table 1).

Table 1- Distribution of *Listeria* spp. in raw and processed seafood samples (%)

Listeria species	*Raw seafood*	*Processed seafood*	*Total*
L. monocytogenes	2	6	8
L. innocua	10	5	15
L. ivanovii	0	0	0
L. seeligeri	2	4	6
L. welshimeri	6	4	10
L. grayi	0	1	1

At the end of this study, *L. monocytogenes* was isolated in 8% of the seafood samples and this prevalence is 2% for raw seafood samples; 6% for processed seafood. Similarly, In the results of Fallah et al. (2013) and Yamazaki et al. (2000) it was found that *L. monocytogenes* number is higher in ready to eat and processed seafood products compared to raw seafood. The results show that preservation methods applied to processed seafood products and Ready-to-eat foods seem to be insufficient to prevent *Listeria monocytogenes* contamination and growth (Guenther et al. 2009).

Our results show that *L. monocytogenes*, *L. seeligeri* and *L. grayi* prevalence were higher in processed seafood products compared to raw seafood. The high prevalence detected in these products could be due to many factors such as cross contamination and insufficient cooking process (Jamali et al. 2013).

3.2. Antimicrobial activity of sodium alginate based film containing bacteriophage

Films incorporated with bacteriophage are promising for future application in food packaging (Gouvêa et al. 2015). It was been observed to not develop of bacterial cells around the sodium alginate based film containing LISTEX™ P100 bacteriophage. This condition was caused by the lysis caused by bacteriophage. No lysis was observed around the film in the control film group. The sodium alginate based film containing bacteriophage was determined to be 4.3±0.11 mm in inhibition zone against *L. monocytogenes* in MHA (Figure 1). Gouvêa et al. (2016) used absorbent food pads containing bacteriophage, similar to our results; the researchers reported that these pads were effective on the pathogen bacteria in the medium against *Salmonella Typhimurium* pathogen.

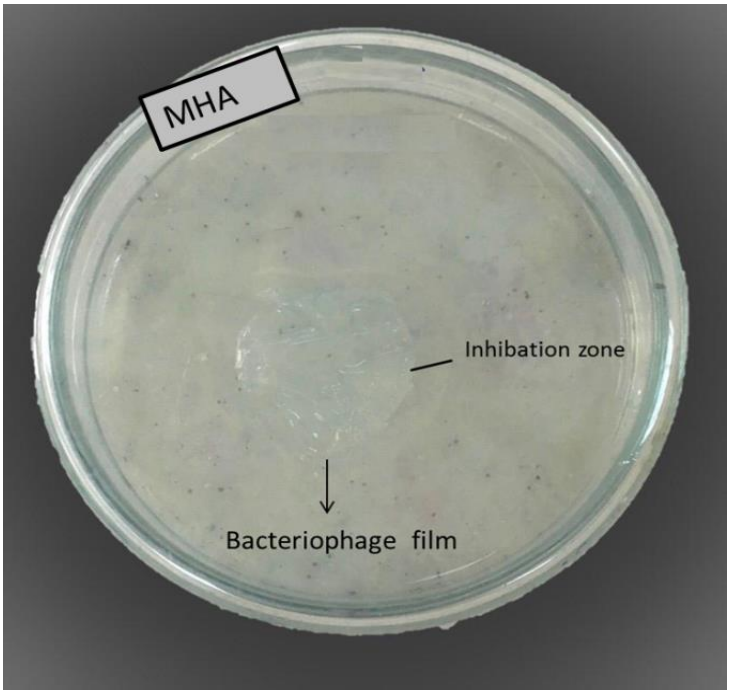

Figure 1- Antimicrobial activity of sodium alginate based film containing bacteriophage against *Listeria monocytogenes* on MHA plates

3.3. The effects of bacteriophage applications on L. monocytogenes in smoked trout fillets

The effect of LISTEX™ P100 on *L. monocytogenes* contaminating smoked trout fillets during storage at 10 °C was dependent on the way of application (Figure 2).

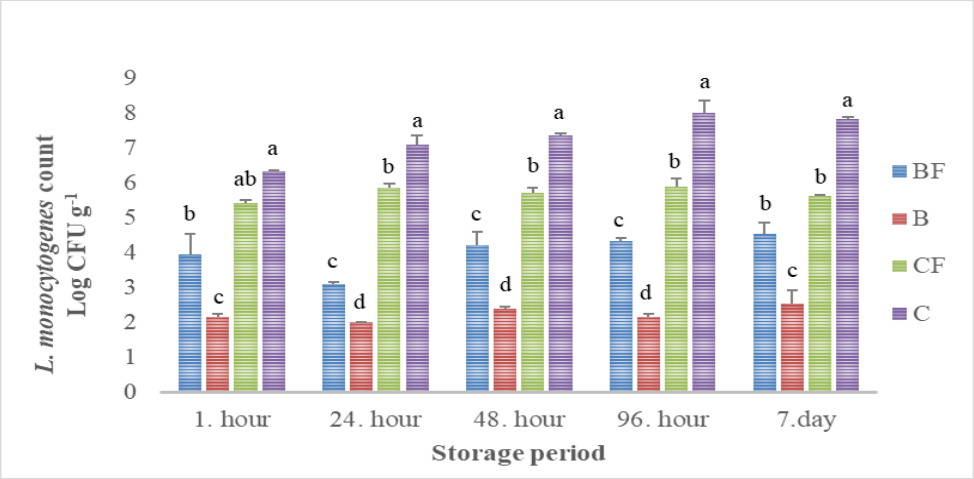

Figure 2- The effects of bacteriophage on *L. monocytogenes* in smoked trout fillets stored at 10 °C (log CFU g^{-1}) BF: Film containing bacteriophage, B: Applying of bacteriophage directly to the surface, CF: Film not containing bacteriophage, C: phage not added *Different letters (a, b) represent statistical differences among groups in same storage period (P<0.05)

The number of *L. monocytogenes* in BF Group provided 1.48 log CFU g^{-1} reduction compared to the CF Group in 1st hour of storage, however, this decrease was not found to be statistically significant (P>0.05). The number of *L. monocytogenes* in BF Group provided 2.37 log CFU g^{-1} reduction compared to C Group (P<0.05). During 7 days of storage, although the most effective bacteriophage application to inactivation of *L. monocytogenes* was the direct bacteriophage application (B) (P<0.05), application of film containing bacteriophage (BF) was found to be effective compare to the control group (C) (P<0.05).

Soni & Nannapaneni (2010) investigated the effect of 10^8 PFU g^{-1} bacteriophage on *L. monocytogenes* in raw salmon fillets during 10 days of storage. During storage, researchers determined similarly our results that the number of *L. monocytogenes* in fillet was lower in the group that were applied bacteriophage compared to the group that were not applied bacteriophage (P<0.05).

Gutiérrez et al. (2017) reported that the 10^9 PFU mL^{-1} LISTEX™ P100 bacteriophage application in dried hams was effective (below the detectable limit) in reducing the number of *L. monocytogenes* (10^3, 10^4, 10^5 CFU cm^{-2}) in storage at 4 ºC and 12 ºC at the end of 24 hours.

In this study performed by us, it determined that direct bacteriophage application at the end of the 24th hour resulted in a decrease of 5 log CFU g^{-1} in the number of *L. monocytogenes* in the fillets compared to the control group and it determined that the number of *L. monocytogenes* at 24 hours in the fillets was 2.15 log CFU g^{-1}. This difference between the two studies is thought to be caused by the difference between the numbers of *L. monocytogenes* and bacteriophages initially added. It was reported by Carlton et al. (2005) that the differences in the number of added phages were effective on the number of *L. monocytogenes.* The researchers stated that low dose phage (1.5 x10^8 PFU g^{-1}) application on the development of *L. monocytogenes* on cheese yielded 2-3 log reduction in bacterial count, whereas high dose phage application was effective in inactivating *L. monocytogenes* completely. Similarly, Perera et al. (2015) reported that cold smoked trout fillets that were inoculated with *L. monocytogenes* detected a 0.4 and 1.0 log reduction in the number of bacteria in the fillets at the 24th hour following low-dose (10^5 PFU g^{-1}) and high-dose (10^6 PFU g^{-1}) bacteriophage (ListShield) application.

Although the number of *L. monocytogenes* in bacteriophage application applied directly to the surface of smoked trout fillets resulted in an average reduction 1.78 log (P<0.05) compared to the number of *L. monocytogenes* in application bacteriophage in alginate films over 7 days of storage, application of bacteriophage in alginate films resulted in a 1.67 log reduction in storage compared to the control film without the addition of bacteriophage. Similarly, Lone et al. (2016) reported that the phage-based material they developed had a significant antimicrobial effect on contaminated foods.

In this study, *L. monocytogenes* could not be completely eliminated with bacteriophage applications. But the smoked trout fillets were contaminated with high levels of bacteria in this study, unlikely to be encountered in real life. However, LISTEX™ P100 applications (incorporated into the sodium alginate based film and applied directly to the surface) were generally found to be effective in inactivation of *L. monocytogenes* during 7 days of storage. For this reason, it is possible to say that it will be more effective to the phages both directly applied to the fillets and incorporated into the sodium alginate films in commercial food processing facilities.

3.4. Stability of phage LISTEX™ P100 in smoked trout fillets

The stabiliy of bacteriophages during storage at 10 °C for 7 days in smoked trout fillets contaminated with *L. monocytogenes* is shown in Figure 3.

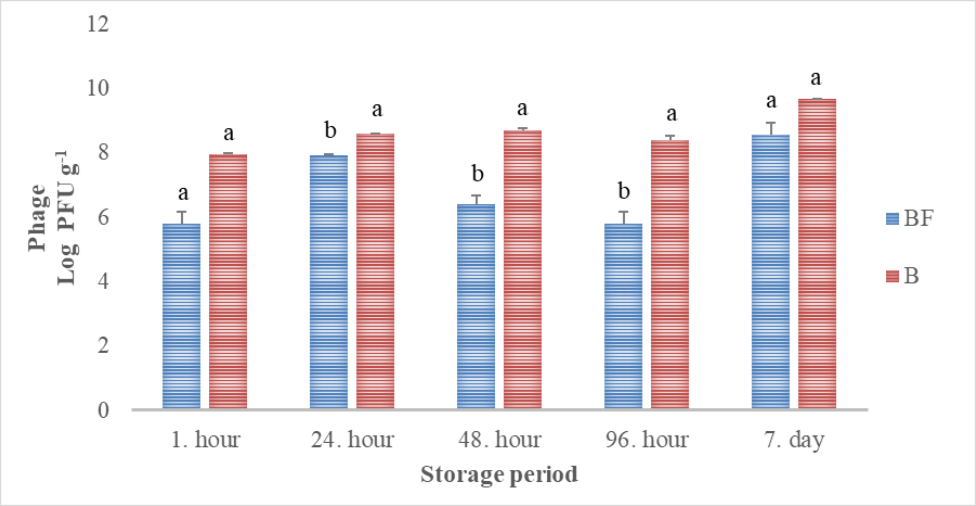

Figure 3- Stability of phage LISTEX™ P100 during the 7 days shelf life of smoked trout fillets stored at 10 °C (log PFU g^{-1})
BF: Film containing bacteriophage, B: Applying of bacteriophage directly to the surface *Different letters (a, b) represent statistical differences among groups in same storage period (P<0.05).

The incorporation of phages into the sodium alginate films resulted in a phage titer of 5.80 log PFU g^{-1} in trout fillets after 1 hour of storage at 10 °C, and the titer increased up to 8.54 log PFU g^{-1} at day 7 of storage (P<0.05). The phage titer was higher when the phage product was directed applied on the trout fillets. Indeed, 7.95 log PFU g^{-1} and 9.67 PFU g^{-1} were detected at the first hour and the day 7, respectively (P<0.05).

At the end of this study, the number of phages increased in the two groups in which bacteriophage was applied compared to the beginning of storage (P<0.05). This is the result of the natural phage cycle (which starts with one phage infecting one bacterial cell and resulting in 100–200 phages) (Moye et al. 2018). Unlike our findings, Soni & Nannapaneni (2010) reported that phage stability remained constant at 4 °C for 10 days and that the number of 10^8 log PFU g^{-1} bacteriophages initially added at the end of storage was about 8 log PFU g^{-1}. In this study, we have demonstrated that bacteriophage LISTEX™ P100 was able to reduce *L. monocytogenes* counts in a model broth system and on raw salmon fillet tissue.

4. Conclusions

Phage biocontrol is increasingly accepted as a natural and green technology, effective at specifically targeting bacterial pathogens in various foods, in order to safeguard the food chain. Because of the specificity of bacteriophages, phage biocontrol offers a unique opportunity to target pathogenic bacteria in foods without disturbing the normal microflora of foods (Moye et al. 2018). In this study, LISTEX™ P100 bacteriophage applications (incorporated into the sodium alginate based film and applied directly to the surface) were found to be effective in *L. monocytogenes* inactivation during 7 days storage. Also in this study, it was concluded that LISTEX™ P100 bacteriophage addition into sodium alginate film for the first time was be effective in control of *L. monocytogenes* in smoked trout fillets. Considering the results obtained, it may be concluded that the applications of LISTEX™ P100 bacteriophage could be very effective for the specific biocontrol of *L. monocytogenes* in smoked rainbow trout.

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Foliar Application of Humic Acid and Some Exo-and Endophytic Growth Hormones on Yield, Yield Components and Fatty Acid Composition in Safflower (*Carthamus tinctorius* L.) under Drought Stress

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ABSTRACT
A two-year field experiment was conducted in Varamin-Pishva Branch, Islamic Azad University, Iran to study the impact of drought stress and foliar application of some hormones on the safflower growth. The drought stress was induced at three levels and considered as main plots. Irrigating after 75% water depletion was considered to be normal irrigation (control), irrigating after 60 and 45% water depletion, defined as mild and severe drought stress, respectively. The foliar application of humic acid (HA), salicylic acid (SA), Gibberellic acid (GA_3), ascorbic acid (AA), water, and the non-foliar application was considered as sub-plots. The main effect of irrigation regimes was significant on seed yield, oil yield, palmitic acid, and water use efficiency (WUE). Also, the main effect of foliar applications was significant for seed yield, oil yield, stearic acid, and WUE. Normal irrigation produced the maximum oil yield (2270 kg ha^{-1}) that was decreased by 25.9% and 37.1% under mild and severe stress regimes, respectively. The maximum and minimum oil yields were produced by the application of SA and non-foliar treatment with average values 1970 and 1357 kg ha^{-1}, respectively. Although the palmitic acid content was enhanced under the drought stress conditions, oleic acid content was significantly decreased in such conditions. The current findings suggest that the foliar application of SA can be recommended when optimal water supply was applied to increase the quality and quantity of safflower oilseed.

Keywords: Ascorbic acid, Foliar spray, Gibberellic acid, Safflower (Carthamus tinctorius L.), Salicylic acid

1. Introduction

The Safflower (*Carthamus tinctorius* L.) is one of the oldest oilseed crops, which is native to the Middle East (Mohammadi et al. 2019), and it is usually grown for flowers, medicinal products, cooking and coloring purposes (Çamaş & Esendal 2006). Environmental factors and different types of stress conditions affect growth parameters, herbage yield, and essential oil composition of medicinal and aromatic plants. The scarcity of water has a direct effect on plant biomass, affecting also physiological and morphological traits, including canopy architecture and plant antioxidant system (Keshavarz & Khodabin 2019; Chavoushi et al. 2020). Decreases in chlorophyll content, yield and yield components were observed for the safflower under drought stress conditions (Mona et al. 2012). Drought stress severely affected safflower productivity and biological yield compared to normal watering. Plant hormones can play vital roles in plant growth and development processes response to various abiotic and biotic stress (Shaki et al. 2019). Plant hormones have been used to improve the growth and development of different crop species (Keshavarz et al. 2016; Dotto & Neumann Silva 2017; Shaki et al. 2020). The Gibberellic acid (GA_3) plays essential roles in seed germination, stem elongation, leaf expansion and reproductive development (Khan & Chaudhry 2006). Baydar (2002) reported that the oil synthesis of safflower increased significantly from 33.8% to 38.8% with the application of 300 ppm GA_3 at the budding stage. In another study, Bibi et al. (2003) indicated that increasing concentrations of GA_3 gradually improved the oil content of sunflower. The salicylic acid (SA) is a natural substance that can induce stem elongation, leaf epinasty and yield increase (Keshavarz et al. 2016). The ability of exogenous SA to enhance antioxidant protection, to increase the accumulation of osmolytes, and to maintain optimum chlorophyll pigments under stress conditions has been suggested as potential mechanisms of drought tolerance in safflower (Ghassemi-Golezani et al. 2020). SA regulates various aspects of plant responses to stress through extensive signaling cross-talk with other hormones such as GA_3 and indol-3-acetic acid (Jayakannan et al. 2013). Several studies have found that treatment with SA improved the tolerance of safflower to drought stress (Chavoushi et al. 2019, Chavoushi et al. 2020). Shaki et al. (2019) found that SA improved the biomass production and fatty acid composition of the safflower in drought conditions. The application of (humic acid) HA is a practice that can improve water use efficiency (WUE) and decrease the effect of drought stress on plants (Zhang et al. 2013). Tohidi Moghadam et al. (2014) and Yadollahi et

al. (2015) demonstrated a significant increase in the safflower and corn (*Zea mays* L.) yield by applying HA under drought conditions. Similarly, Khademian et al. (2019) found that the application of humic acid improved plant photosynthesis in drought conditions via increasing the rate of gas exchange and electron transport flux in safflower (*Carthamus tinctorius* L.). This study aims to compare the drought stress and foliar application of some exo–and endophytic growth hormones on leaf area index (LAI), water use efficiency and seed quality and quantity of safflower.

2. Material and Methods

This experiment was conducted in the research field of Agriculture Faculty of Tarbiat Modares University, Tehran, Iran during two cropping seasons (in 2017-2018 and 2018-2019) to investigate the effect of foliar application of HA, SA, GA_3 and ascorbic acid (AA) on quantitative and qualitative characteristics of the safflower under drought stress conditions. Weather conditions such as temperature and precipitation were obtained from Chitgar (51˚10'E, 33' 44' N, 1305.2 m asl) weather station which is 1 km from field condition. The average annual temperature is 14.2 °C and the average annual rainfall is 244 mm in Karaj. The soil of the experimental site was classified as clay loamy soil, organic matter of 1.28 and pH was 7.8. Available soil nitrogen and phosphorus at depth of 60 cm were 14.9 ppm and 41.8 ppm, respectively. The experiments were designed with a split-plot design in a randomized complete block design. The safflower (*Carthamus tinctorius* L.) seeds registered and provided by the National Seed and Plant Improvement Institute, Iran, Karaj. Each experiment included fifty-four experimental units including, three irrigation treatment and six foliar applications with three replications. A drip irrigation system was used to watered all treatments to ensure uniform seed germination. Irrigation regimes (drought stress) were started early spring (April 4th, both year, flowering stage) and continued throughout the season (ending mid-July, both year). The plots were irrigated at 75% of field capacity (no stress), 60% of field capacity (mild stress) and 45% of field capacity (severe stress). Irrigation water (V_w in m^3) was estimated based soil moisture at field capacity using by TDR method (Time Domain Reflectometry method, model 4593, soil moisture equipment, Santa Barbara), according to the following equation (1):

$$V_w: (\theta_{F.C} - \theta_i) \times D \times A \quad \text{(Equation 1)}$$

Where, $\theta_{F.C}$ is the volume of soil moisture at field capacity, *A* is the main plot area (m^2) and *D is* the root depth. Soil moisture was monitored throughout the whole growth period by taking soil samples at the depth of 60 cm, with a neutron probe (twice weekly). The amounts of water to plots were controlled by contour. WUE was calculated by dividing the marketable yields by the volume of applied water.

The foliar application [(F_1: HA 300 mg L^{-1}, F_2: SA 100 mg L^{-1}, F_3: GA_3 50 mg L^{-1}, F_4: AA 150 mg L^{-1}, F_5: Water and F_6: non-foliar application)] was considered as a sub-factor and were applied at rosette stage (Lewis & McFarlane 1986) with a pressurized backpack sprayer (12 L capacity) calibrated to deliver 1000 L ha^{-1} of spray solution. The experimental field was prepared by shallow plowing, followed by disking in September and October of each year (2017 and 2018). Each experimental plot had six rows (6 m) and 5 cm spacing between plants in rows with 50 cm distanced. In both years seeds were cultivated by hand on the 20th and 25th of November. After seed sowing, irrigation water was reapplied when 25% of the available soil water was used until the plants were established. The amounts of urea (350 kg ha^{-1} urea) were applied (based on local recommended) at three different stages; one-third before seed sowing and mixed into the soil, one-third at stem elongation stage and one-third at capitulum emergence stages stage by spreading the fertilizer onto the soil surface just before irrigation. All operations related to the harvesting except irrigation were carried out uniformly and according to the traditions of the area.

At the first of the Capitulum forming stage, three representative plants per plot were chosen for the leaf area index (using a leaf area meter, Delta-T Devices Ltd., Cambridge, UK). In both years the final harvest was performed on August 16th at the physiological maturity stage. To determine morphologically and 1000 seed weight, 10 plants from each plot and to measure seed yield, 2 m^2 of each plot from the middle of each plot were hand-harvested at the physiological maturity stage.

The percentage of seed oil and gas chromatography analysis was done according to the method described by Azadmard-Damirchi et al. (2005). Oil percentage was measured by Inframatic 8620 Percor, England. Oil yield was obtained by multiplying seed yield by oil percentage. The oil sample for each treatment was converted to methyl esters in the presence of Menthol BF_3^{-1} (14% w/w) reagent and using sodium methoxide as a catalyst. Samples of 200 mg oil were added to 7 ml sodium methylate (0.5 M) and heated to boiling for 10 min. After that 5 ml Boron Trifluoride in menthol was added to the mixture, heated again for 2 min and 6 ml n-hexane (GC grade) added to complex and heated for 2 min. In the end, 50 ml saturated saline water added and suspension strongly shaken for 1 min. The upper phase (0.5 µL) was taken and analyzed by GC (GC 8000, Carlo-Erba Instruments, Italy) equipped with the flame ionization detector (FID) on a DB23 fused silica capillary column (30m by 0.25mm i.d., d_r =0.25µm film; J and W Scientific, Folsom, CA). Nitrogen was utilized as carrier gas at a flow rate of 4.93 mL min^{-1} and the split ratio was 21.28 mL min^{-1}. The GC process was conducted as split mode injection at an oven temperature of 120 °C for 1 min, raised to 220 °C for 15 °C min^{-1} then kept at 220 °C for 15 min. The injector and detector temperature were set at 250 °C. Peak identification was performed by comparing with the retention time of valid commercial standards (Sigma Co., USA). The fatty acid content of stearic acid, oleic acid, palmitic acid, and linolenic acid was shown as a percentage of the oil.

The main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) in SAS (SAS Institute Inc. 2002). The significance of differences among treatment means was tested using LSD at a probability level of 1% and 5% and the significant interaction effects were separated by the slicing method.

3. Results

Significance for irrigation regimes, foliar application treatments, two-way interactions, and three-way interactions for the studied traits are presented in Table 1. The main effect of year was not statistically significant on all studied traits except for the LAI ($P<0.01$), which might be due to the same mean temperature in both years (Figure 1). The results of the combined analysis of variance showed that the irrigation regimes differed significantly in terms of seed yield, oil yield, palmitic acid, and WUE (Table 1). Also, foliar application treatments differed significantly for seed yield, oil yield, stearic acid, and WUE (Table 1). The two-way interaction of irrigation regimes × foliar application treatments was significant for oleic acid ($P\leq0.01$).

Table 1- Combined analysis of variance on agronomic and some physiological traits of the safflower as affected by foliar application treatments and irrigation regimes

S.O.V	*D.F*	*LAI*	*1000-seed weight (g)*	*Seed yield (kg h^{-1})*	*Oil yield (kg h^{-1})*	*Oil content (%)*
Year (Y)	1	0.35*	22.18^{ns}	25223.0^{ns}	3956.5 ^{ns}	1.12^{ns}
Y (Block)	4	0.02	6.10	12483841.3	1763257.5	5.51
Drought stress (S)	2	2.51**	15.19^{na}	62172702.5**	7122808.9 **	14.09^{ns}
Y×S	2	0.21*	1.74^{na}	42397.8^{ns}	1919.8 ^{ns}	0.64^{ns}
Block (Y×S)	8	0.02	6.48	1234367.5	179032.3	5.57
Foliar application (FA)	5	0.09^{ns}	32.96**	8408850.5**	957661.5 **	6.76^{ns}
Y×FA	5	0.01^{ns}	6.83^{na}	2310162.7^{ns}	195590.7 ^{ns}	39.76**
S×FA	10	0.03^{ns}	2.31^{na}	820422.5^{ns}	86084.4 ^{ns}	14.61**
Y×S×FA	10	0.09*	5.30^{na}	292093.6^{ns}	93744.8 ^{ns}	13.41**
Error	60	0.04	5.24	1345210.3	171839.9	4.68
CV (%)		18.27	6.72	23.32	23.46	6.06
S.O.V	*D.F*	*Palmitic acid (%)*	*Stearic acid (%)*	*Oleic acid (%)*	*Linoleic acid (%)*	*WUE (kg ha^{-1} mm^{-1})*
Year (Y)	1	0.168^{ns}	0.013^{ns}	0.19^{ns}	0.002^{ns}	0.001^{ns}
Y (Block)	4	0.047	0.276	27.74	3.66	0.599
Drought stress (S)	2	0.615**	0.167^{ns}	480.04**	5.33^{ns}	0.198*
Y×S	2	0.005^{ns}	0.009^{ns}	0.25^{ns}	2.11^{ns}	0.003^{ns}
Block (Y×S)	8	0.041	0.102	34.33	1.95	0.027
Foliar application (FA)	5	0.018^{ns}	0.164*	13.92**	3.34^{ns}	0.437**
Y×FA	5	0.007^{ns}	0.082^{ns}	1.39^{ns}	1.99^{ns}	0.130^{ns}
S×FA	10	0.051^{ns}	0.085^{ns}	9.35**	1.16^{ns}	0.0355^{ns}
Y×S×FA	10	0.034^{ns}	0.043^{ns}	0.80^{ns}	0.57^{ns}	0.0166^{ns}
Error	60	0.035	0.050	3.52	1.54	0.069
CV (%)		2.99	8.10	11.37	1.63	23.27

S.O.V.: the Source Of Variation; D.F: the Degree of Freedom; LAI: Leaf Area Index; WUE: Water Use Efficiency; CV: Coefficient of Variance; ns: not significant; * and ** Significant at the 5% and 1% levels of probability, respectively

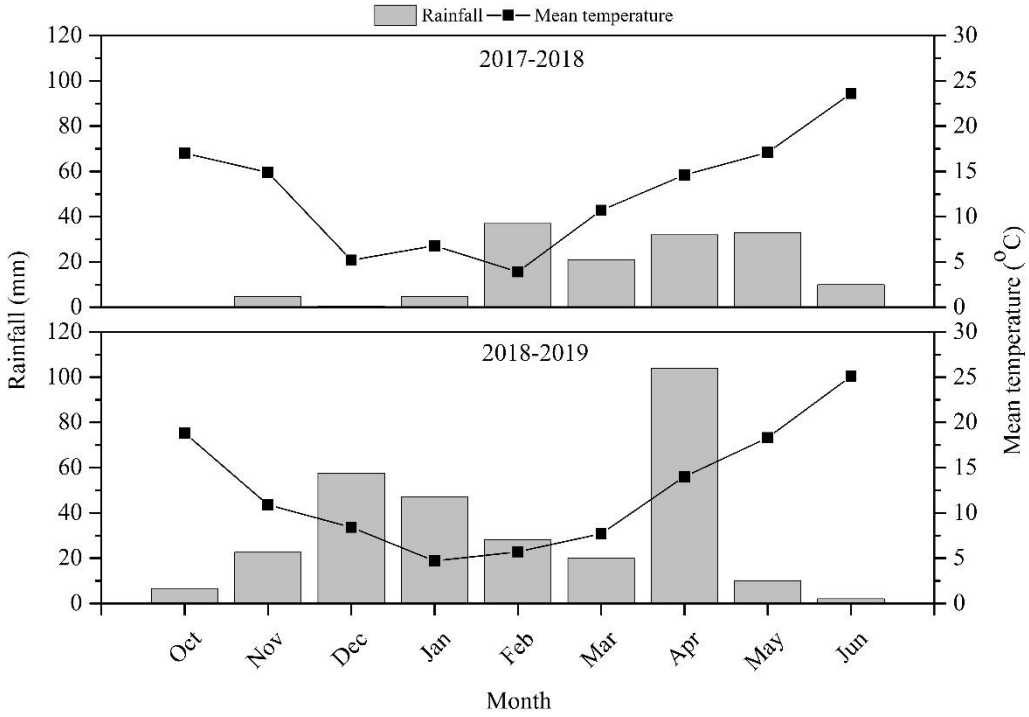

Figure 1- Rainfall and mean temperatures during the growing season

3.1. Agronomic traits

The LAI responded differently to irrigation regimes and the foliar application treatments each year, so means are presented separately for each year (Table 2). Averaged over foliar application treatments in the first year, the LAI decreased by 21.5% and 52.4% under mild and severe stress, respectively, compared with the well-watered regime (Table 2). In the second year, the no-stress regime produced the highest the LAI, with an average 2.53 that was reduced by 43.1% and 66.3% under the mild and severe stress regimes, respectively (Table 2).

Table 2- Three-way interaction year × irrigation regime × foliar application on LAI and oil content

		LAI			*Oil content (%)*		
Year	*Treatment*	*No stress*	*Mild stress*	*Severe stress*	*No stress*	*Mild stress*	*Severe stress*
	HA	1.07 a	1.80 a	1.08 a	34.46 b	36.95 ab	39.93 a
	SA	1.71 a	1.66 a	0.95 a	34.03 b	34.18 bc	34.64 c
First	GA_3	2.64 a	1.17 a	0.89 a	35.53 ab	35.36 abc	34.46 c
	AA	1.96 a	1.25 a	0.83 a	37.40 a	38.67 a	36.15 bc
	Water	1.61 a	1.23 a	0.76 a	35.40 ab	34.66 bc	38.38 ab
	No treatment	1.60 a	1.20 a	0.53 a	33.31 b	32.58 c	34.59 c
	HA	3.13 a	1.65 ab	0.53 b	35.67 abc	37.43 ab	34.28 a
	SA	3.01 a	1.28 abc	1.10 a	36.74 ab	30.86 c	38.76 a
Second	GA_3	2.90 a	1.71 a	0.78 ab	36.86 ab	33.67 bc	35.88 a
	AA	1.82 b	1.22 bc	0.91 a	31.84 c	36.81 ab	34.79 a
	Water	2.76 a	1.61 abc	0.83 a	32.22 bc	34.05 bc	37.56 a
	No treatment	1.60 b	1.17 c	0.97 a	39.85 a	39.57 a	37.56 a

L.A.I: Leaf Area Index; HA: Humic Acid; SA: Salicylic Acid; GA_3: Gibberellic Acid; AA: Ascorbic Acid. Means followed by similar letters in columns are not significantly different at 5% probability level by Least Significant Difference test

Although in the first year, there was no significant difference between the foliar application treatments in each irrigation regime (control, mild and severe stress) in terms of the LAI, a statistically significant difference was observed among foliar application treatments in the three irrigation regimes in the second year (Table 2). The HA, the GA_3, and the SA treatments showed the highest LAI, with average values 3.13, 1.71, and 1.10 under the control, mild and severe stress regimes, respectively (Table 2). The 1000-seed weight showed a various response under foliar application treatments, ranging from 35.12 g for the GA_3 treatment to 31.63 g for the non-foliar treatment (Table 3). The maximum seed yield was observed in the no stress regime, with an average 6449.1 kg ha^{-1} that was reduced by 29.6% and 39.1% under mild and severe stress, respectively (Table 4). The seed yield was significantly affected by the foliar application treatments so that the SA and non-foliar treatments, with average values 5634.8 and 3757.1 kg ha^{-1}, respectively (Table 3), produced the maximum and minimum seed yields.

Table 3- Main effect of foliar application treatments on different traits of safflower

Treatment	*1000-seed weight (g)*	*Seed yield (kg h^{-1})*	*Oil yield (kg h^{-1})*	*Stearic acid (%)*	*WUE (kg ha^{-1} mm^{-1})*
HA	34.74 ab	4817.5 b	1746.6 ab	2.95 a	1.08 b
SA	35.12 a	5634.8 a	1970.6 a	2.79 b	1.29 a
GA_3	34.83 a	5337.1 ab	1886.9 ab	2.68 b	1.21 ab
AA	34.72 ab	5446.3 ab	1954.7 ab	2.77 b	1.23 ab
Water	33.25 b	4827.5 b	1684.6 b	2.76 b	1.07 b
No treatment	31.63 c	3757.1 c	1357.4 c	2.70 b	0.86 c

HA: Humic Acid; SA: Salicylic Acid; GA_3: Gibberellic Acid; AA: Ascorbic Acid; WUE: Water Use Efficiency; Means followed by similar letters in columns are not significantly different at 5% probability level by Least Significant Difference test.

3.2. Oil content and its yield level

There were significant differences in oil yield of the safflower at the different irrigation regimes (Table 1), so that the control treatment produced the maximum oil yield, with an average 2270.57 kg ha^{-1} that was decreased by 25.9% and 37.1% under mild and severe stress, respectively (Table 4). The foliar application treatments had a significant effect on the oil yield of safflower (Table 1). The highest oil yield was observed in the SA treatment with an average 1970.6 kg ha^{-1} that was 11.36%, 4.24%, 0.80%, 14.51%, and 31.11% higher than the SA, the GA_3, the AA, water, and non-foliar treatments, respectively (Table 3). In terms of oil content, averaged across foliar application treatments in the first year, the no-stress regime had the lowest oil content (35.02%) which was increased by 1.1% and 2.7% under the mild and severe stress regimes, respectively (Table 2). Averaged by the foliar application treatments in the second year, the oil content of control, mild and severe stress regimes were 35.53%, 35.39%, and 36.74%, respectively (Table 2).

The oil content of safflower responded differently to irrigation regimes and the foliar application treatments in each year (Table 2). In the first year, the oil content varied from 37.40% for the AA treatment to 33.31% for the non-foliar treatment in the optimal water supply (control), from 38.67% for the AA treatment to 32.58% for the non-foliar treatment in mild drought stress, and from 39.93% for the HA treatment to 34.46% for the GA_3 treatment (Table 2). In 2017-2018 (first year), the control treatment (the non-foliar application) had the maximum oil content with an average 39.85%, 39.57%, and 37.56% in the control, mild and severe regimes, respectively (Table 2).

Table 4- Main effect of irrigation regimes on different traits of safflower

Drought Treatment	*Seed yield (Kg ha^{-1})*	*Oil yield (Kg ha^{-1})*	*Palmitic acid (%)*	*WUE (kg ha^{-1} mm^{-1})*
No stress	6449.1 a	2270.57 a	6.16 b	1.13 ab
Mild stress	4540.1 b	1601.55 b	6.23 b	1.05 b
Severe stress	3930.0 c	1428.24 b	6.41 a	1.20 a

WUE: Water Use Efficiency; Means followed by similar letters in columns are not significantly different at 5% probability level by Least Significant Difference test

3.3. Fatty acid composition

Although the linoleic acid was not affected by the irrigation regimes and foliar application treatments, the palmitic, stearic, and oleic acids showed a different response to these treatments (Table 1). When compared with the control irrigation (no stress), mild and severe stress increased palmitic acid by 1.13% and 4.05%, respectively, while the oleic acid content was reduced by 30.64% and 30.41% (averaged over the foliar application treatments) compared with the well-watered regime (Table 4).

The maximum stearic acid content belonged to the HA treatment with an average of 2.95% that was 5.42%, 9.15%, 6.10%, 6.44%, and 8.47% higher than the SA, the GA_3, the AA, the water, and non-foliar, respectively (Table 3). The oleic acid content responded differently to irrigation regimes and the foliar application treatments (Table 5). The AA treatments had the maximum oleic acid content with average values of 23.12%, 15.27%, and 15.19% under control, mild, and severe stress, respectively (Table 5). It is worth noting that there was no significant difference among the foliar application treatments under the severe stress condition in terms of the oleic acid content. Moreover, foliar application treatments did not show any significant difference in terms of the linoleic acid content.

3.4. Water Use Efficiency (WUE)

The average WUE was different in the three drought stress irrigation regimes (Table 4). The maximum WUE was observed when the severe drought stress regime imposed during the growing season with an average 1.20 kg ha^{-1} mm^{-1}, while the mild stress showed the minimum amount of WUE with an average 1.05 kg ha^{-1} mm^{-1} (Table 4). A significant difference was observed among the foliar application treatments in terms of the WUE (Table 3). The foliar application of SA showed the maximum WUE with an average 1.29 kg ha^{-1} mm^{-1} that was 16.27%, 6.20%, 4.65%, 17.05%, and 33.33% higher than the WUE with foliar application of the HA, the GA_3, AA, water, and non-foliar treatment, respectively (Table 3).

Table 5- Interaction between irrigation regime and foliar application on oleic acid

	Oleic acid (%)		
Treatment	*No stress*	*Mild stress*	*Severe stress*
HA	19.42 ab	15.27 a	14.23 a
SA	22.93 a	13.77 b	14.35 a
GA_3	20.72 ab	14.02 ab	14.08 a
AA	23.12 a	15.26 a	15.19 a
Water	20.84 ab	13.90 ab	14.35 a
No treatment	17.31 b	14.01 ab	14.32 a

HA: Humic Acid, SA: Salicylic Acid; GA_3: Gibberellic Acid; AA: Ascorbic Acid; Means followed by similar letters in columns are not significantly different at 5% probability level by Least Significant Difference test.

4. Discussion

Drought stress inhibits plant growth and productivity via limiting photosynthesis. Change in photosystem I and II activities can cause some modifications in the thylakoid membrane proteins, leading to a decline in electron transfer from the light-harvesting antenna to photosystem II which eventually may lead to photo-inhibitory destruction of photosystem II reaction centers (Ghassemi-Golezani et al. 2020). The results of this research indicated that the safflower LAI decreased when plants experienced drought stress conditions. Indeed, the reduction in LAI under drought stress is a typical symptom of oxidative stress (Mohammadi et al. 2018). Reduction in the LAI under drought stress led to the loss of plant biomass because of lower photosynthesis rates (Shaki et al. 2109). The plants usually decrease leaf areas to minimize water loss by transpiration under drought stress conditions (Keshavarz et al. 2018). The results in the current study are in agreement with Chavoushi et al. (2019) who found that the LAI was significantly reduced when water stress was imposed over the growing period. The drought stress irrigation regimes affected seed yield safflower. The number of seeds *per capitulum*, the 1000-seed weight, and the LAI decreased under drought stress, leading to a reduction in seed yield (Golparvar 2011). Some authors have reported a decrease in seed yield for safflower (Soheili-Movahhed et al. 2019; Chavoushi et al. 2020). The 1000-seed weight was different among the foliar application treatments and was enhanced by the application of the plant growth regulators. Foliar application of humic acid or salicylic acid increased 1000-seed weight, seed yield and oil yield safflower. Increasing these traits by application of plant hormones could be resulted from the rising photosystem II activity due to conformational modifications in some proteins (Ghassemi-Golezani et al. 2020), affecting changes in the properties of photosystem II electron acceptors. In addition, the increasing grain weight might be attributed to an increase in the period or rate of grain filling period (Mohtashami et al. 2016). The decreased in seed weight under

drought stress has been indicated in safflower (Movahhedy-Dehnavy et al. 2009) Also, the stress resistance increases through mechanisms of metabolic defence under the foliar application of the plant growth regulators, leading to better plant growth and seed yield (Ullah et al. 2012, Keshavarz & Sadegh Ghol Moghadam 2017). The earlier study confirmed that foliar application of safflower with some nutritions effectively prevented the plant from oxidative stress (Janmohammadi et al. 2016). In a previous study, Moradi et al. (2017) concluded that the seed yield of safflower increased by 6.02% when fulvic acid was added by the foliar application.

Although genotype is considered the most important factor to determine the oil content, the drought stress may affect the seed oil content (Amini et al. 2014). As previously explained, water stress conditions affected plant growth and development, leading to an effect on seed oil composition. The optimal water supply over the growing period could enhance oil content in safflower, while drought stress reduces it (Soheili-Movahhed et al. 2019). A reduction in oil content might be attributed to the oxidation of polyunsaturated fatty acids (Golparvar 2011). The reduction in oil content when drought stress occurred during the growing season is supported by some reports (Mona et al. 2012). The plant growth regulators can improve seed oil content due to their positive effect in seed during biosynthesis and storage of fatty acid and oil over the seed filling stage (Ali et al. 2013). Golkar et al. (2019) showed that salicylic acid enhances safflower tolerance to drought stress by increasing the antioxidative activities. Antioxidative activities have an important role in alleviating drought oxidative stress in plants (Chavoushi et al. 2020). Exogenous SA can protect photosynthetic pigments from oxidative damage that may help to maintain normal photosynthesis of safflower. The reduction of oil content under drought conditions is most likely caused by the inhibition of leaf area, Relative water content and fluorescence parameters of safflower plants (Amiri et al. 2017). In their previous study, Ullah et al. (2012) investigated the effects of plant growth regulators (the SA) on different traits of canola (*Brassica napus* L.) and concluded that the SA was as an ineffective treatment concerning the reduction of the adverse effects of drought stress, while significantly enhanced the oil content of canola.

Increased in total saturated fatty acids and decreased in the unsaturated fatty acids under drought conditions were reported by Mona et al. (2012). A decrease in the oleic and stearic acid contents and an increase in the linoleic and palmitic acid contents have been reported by Sabagh et al. (2019) in sunflower (*Helianthus annuus* L.) seed oil under drought conditions. The oil composition of safflower is mainly due to its ability to accumulate phenolic and flavonoid compounds (Alizadeh-Yeloojeh et al. 2020). Treating the safflower plants by the plant growth regulators can affect the fatty acid compositions (Mona et al. 2012). The exo-application of plant growth regulators significantly enhanced the quality of oil in the safflower by improving the processes of physiological and biochemical of stress-induced plants (Sabagh et al. 2019). There is a result in the use of salicylic acid affecting the oil composition of safflower (*Carthamus tinctorius* L.) under drought stress (Shaki et al. 2019). The increasing oleic acid content might be attributed to the effect of the plant growth regulators on enzymes involved in fatty acid unsaturation (Ullah et al. 2012).

The safflower WUE was affected by the drought stress irrigation regimes. The amount of irrigation during the growing season and seed yield affects the WUE. Although the control regime had the maximum seed yield, the highest WUE was not observed in this regime. Based on harvested seed yield and the amount of irrigation, the severe stress was detected as the best irrigation regime in terms of the WUE. As previously mentioned, the seed yield of safflower was improved through the application of plant growth regulators, so that the highest seed yield was observed in the salicylic acid treatment. In a recent study, Tayebi et al. (2018) found that SA can preserve water content of plant cells by stimulating biosynthesis of osmolytes such as glycine betaine, soluble sugars, proline, polyphenols and polyamines. Badpa et al. (2016) showed that SA treatment increased the endogenous content of plant growth regulators in safflower stress conditions. Since the average amount of irrigation water was constant among the foliar application treatments, higher seed yield led to the greater WUE.

The current study evaluated the effects of the foliar application of the plant growth regulators on different traits of safflower under the drought stress. The results showed that the seed and oil yields of safflower were reduced when drought stress regimes were applied during the growing season due to adverse effects of drought stress on agronomic traits such as the LAI and the 1000-seed weight. The oil quality of the safflower under drought stress conditions was decreased mainly due to the reduction in the oleic acid content. Generally, treating the safflower plant by the SA when the optimal water supply (control) was applied resulted in the best quality and quantity of the safflower oilseed.

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Effect of Ginger Essential Oil on *in Vitro* Gas Production, Rumen Fermentation and Methane Production

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ABSTRACT
In this study, control (0), 50, 100, 200, 400, 800 and 1000 mg L^{-1} ginger essential oil (GEO) (*Zingiber officinale* Roscoe*)* was added to rumen liquid (RL). Then, the effects of the GEO added to the RL *In vitro* gas production, organic matter digestibility (OMD), metabolisable energy (ME), rumen fermentation parameters and methane (CH_4) production were examined on these samples. It was determined that the addition of the GEO to RL decreased the *in vitro* gas production of *Trifolium pratense* hay (TPH), the OMD and ME contents, total volatile fatty acids (TVFA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and other volatile fatty acids (OVFA) ($P<0.05$). Moreover, it was determined that while the productions of carbon dioxide (CO_2), CH_4 and ammonia nitrogen (NH_3-N) decreased, the ratios of the rumen pH and AA/PA increased ($P<0.05$) depending on the increase in the dose of GEO. In conclusion, it was determined that the GEO dose which had the highest negative effect on the *in vitro* gas production, the rumen fermentation, the nutrient digestibility, the CH_4 and the CO_2 production was 1000 mg L^{-1}. It was concluded that since high doses of GEO affect rumen fermentation and digestion of feeds negatively, it would be appropriate to use 200 mg L^{-1}.

Keywords: Ruminant nutrition, Zingiber, Rumen parameters, Methane, Fatty acids

1. Introduction

In livestock sector, antibiotics have been commonly used with the aim of increasing the feed conversation and preventing diseases and metabolic disorders (Jouany & Morgavi 2007). However, the use of antibiotics in animal feeding was banned after January of 2006 as required by the decision made by the European Union in 2003 on the grounds that they pose a risk for human health (Chesson 2006). In order to solve this problem, the number of studies carried out with the aim of developing feed additives as an alternative to antibiotics has increased. As a result of these studies, it was put forward that aromatic plants and essential oils extracted from these plants would be an alternative to antibiotics (Chao et al. 2000; Meliani et al. 2014; Sharma et al. 2016; Mahboubi 2019).

There are many active metabolites such as zingiberene in the structure of the essential oils obtained from the ginger plant (Raina et al. 2005; Sharma et al. 2016). It is reported that these active compounds existing in the ginger essential oil (GEO) do not only have antiseptic, antimicrobial, antioxidant features, but they are also effective against common cold, vomiting control, heart diseases, stomach ulcers, tumor growth, rheumatism and migraine (Raina et al. 2005; Meliani et al. 2014; Mahboubi 2019). It is also stated that the GEO shows antibacterial feature against gram positive and gram negative bacteria (Chao et al. 2000; Meliani et al. 2014; Nanon et al. 2015; Faniyi et al. 2019).

It is reported that the GEO with above-mentioned features will be used to manipulate the rumen fermentation (Soroor & Moeini 2015; Faniyi et al. 2016; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Faniyi et al. 2019). Moreover, it was also determined that the GEO has an effect on the digestibility of feeds, metabolisable energy (ME) and methane (CH_4) production (Nanon et al. 2015; Soroor & Moeini 2015; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Faniyi et al. 2019). For this reason, it is important to reveal the effects of GEO on rumen fermentation, digestion of feeds and the CH_4 production in rumen.

This study was carried out with the aim of determining the effects of different doses of GEO (0, 50, 100, 200, 400, 800 and 1000 mg L^{-1} to rumen liquid (RL)) on *in vitro* gas production, the digestibility of feeds, the rumen fermentation and CH_4 production.

2. Material and Methods

2.1. Feed and animal material

The feed material of the study was *Trifolium pratense* hay (TPH) and it was used in the study after being ground in the grinder with a sieve-diameter of 1 mm. The GEO used in the study was obtained from the market in pure form (catalogue no: W252204-8007-08-7) (Sigma-Aldrich). The RL used in the study was taken from 3 rams, Kivircik breed, with a rumen canula. During the study, the animals were fed with complete ration (TMR) composed of 60% of alfalfa hay and 40% of concentrate feed mix (18% crude protein and 2750 kcal kg^{-1} ME Dry $Matter^{-1}$ (DM)) and water was continuously available in front of them.

2.2. Implementation of in vitro gas production technique

In the determination of the TPH's *in vitro* gas production and the levels of organic matter digestibility (OMD) and ME, the *in vitro* gas production technique developed by Menke & Steingass (1988) was used. In order to determine the *in vitro* gas production, special glass tubes with a volume of 100 mL (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschieβ, Germany) were used and about 200±10 mg of feed samples were put into the syringes for each dose of GEO (0, 50, 100, 200, 400, 800 and 1000 mg L^{-1} to RL) in triplicates. 30 mL of RL/buffer solution prepared in accordance with the method reported by Menke et al. (1979) was added into the syringes. Following this procedure, the syringes were taken into incubation in the water bath of 39°C and the *in vitro* gas productions were measured at the intervals of 3, 6, 12, 24, 48, 72 and 96 hours, respectively.

At the 96 hours of the incubation, the pH, the total volatile fatty acid (TVFA) and the ammonia nitrogen (NH_3-N) levels in the RL in the syringes were determined. Moreover, the carbon dioxide (CO_2) and the CH_4 gases produced were calculated using the concentration of the individual volatile fatty acids (VFA) via the following equations (Blümmel et al. 1999).

CO_2 = Acetic acid (AA)/ 2 + Propionic acid (PA)/ 4 + 1.5 x Butyric acid (BA)
CH_4 = (AA + 2 x BA) - CO_2
The concentration of VFA was taken as mmol.
The OMD of the feed raw materials and their ME were determined via the following equations reported by Menke & Steingass (1988).
OMD, % = 15.38 + 0.8453 x GP + 0.0595 x VP + 0.0675 x CA
ME, MJ/kg DM = 2.20 + 0.1357 x GP + 0.0057 x CP + 0.0002859 x EE^2
(GP: The net gas production at the end of the 24-hour of incubation duration of 200 mg of dry forage sample, CP: % Crude protein, EE: % Ether extract and CA: % Crude ash).

The true dry matter of NDF digestibility were determined by using the Ankom $Daisy^{II}$ incubator (ANKOM Technology Corp., Fairport, NY, USA, 2008).

2.3. Chemical analysis

The dry matter, CA, CP and EE analyses of TPH were determined with the methods reported by AOAC (2000); the analysis of the cell wall components was determined with the methods reported by Van Soest et al. (1991) using the ANKOM 200 Fiber Analyzer device (ANKOM Technology Corp., Fairport, NY, USA, 2008).

The pH of the RL was determined via a digital pH-meter (Sartorius PB-20, Goettingen, Germany). Ruminal ammonia nitrogen (NH_3-N) analysis was done in RL used in *in vitro* gas production at 96^{th} hour. RL was taken 10 mL and put into tubes (15 mL). Then, 0.1 mL of 1 M hydrochloric acid (HCI) was added to stop the microorganism activity. Ruminal NH_3-N analysis was distilled by Kjeldahl method. For this purpose, 10 mL of RL was placed in the sample setting unit of the Kjeldahl device and 3 mL of 1 N sodium hydroxide (NaOH) solution was added. For distillate 50 mL of 2% boric acid was placed and 3-4 drops of indicator were placed on it. Subsequently, 175 mL of distillate were collected. The distillate collected was titrated with 0.1 N sulfuric acid (H_2SO_4). The amount of sulfuric acid spent in titration (mL) was determined. Then NH_3-N was calculated in mg (Blümmel et al. 1997).

RL volatile fatty acids (acetic, butyric, propionic, valaric, isovaleric and isobutyric acid) analysis was done in RL used in *in vitro* gas production at 96^{th} hour. RL was taken 10 mL and put into tubes (15 mL). Then, 1.0 mL of 25% phosphoric acid was added to RL (Wiedmeier et al. 1987). RL was centrifuged at 14000 rpm and determined by gas chromatography (Agilent Technologies 6890N gas chromatography, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 um df. Max. Temp: 260 °C. Cat. 11023) RL volatile fatty acids.

2.4. Statistical analyses

The research was conducted by a completely randomized design with three replications. The data obtained from the research was subjected to analysis of variance (Snedecor & Cochran 1967) and the differences between means was determined with

Duncan multiple comparison test using SAS programme (2004).

3. Results and Discussion

3.1. Chemical composition of TPH

The organic matter, CA, CP, EE, NDF, ADF, ADL, cellulose and hemicellulose contents of TPH were calculated as 93.82%, 6.18, 17.38, 3.81, 51.08, 36.66, 8.56, 14.43 and 28.10 respectively. The chemical composition of TPH was found similar to that reported by NRC (2007).

Table 1- Chemical composition of TPH, %

Ingredients	*%*
Organic matters	93.82
Crude ash	6.18
Crude protein	17.38
Ether extract	3.81
Neutral detergent fiber, (NDF)	51.08
Acid detergent fiber, (ADF)	36.66
Acid detergent lignin, (ADL)	8.56
Cellulose	28.10
Hemicellulose	14.43

3.2. Effect of GEO on in vitro gas production

The GEO addition decreased the *in vitro* gas production of TPH in all the incubation periods (P<0.05). The lowest *in vitro* gas production was found in the group with 64.89 mL and 1000 mg L^{-1} addition and the highest *in vitro* gas production was found in the control group with 74.66 mL and without GEO addition. The decrease in the *in vitro* gas production occurring depending on the increase in the GEO dose added to RL can be explained by the antimicrobial features of active components existing in the structure of GEO and, as a result of this, their limiting rumen microorganisms (Chao et al. 2000; Meliani et al. 2014; Nanon et al. 2015; Soroor & Moeini 2015; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Faniyi et al. 2019). In many previous studies, it was reported that GEO decreased the *in vitro* gas production (Tag El-Din et al. 2012; Meliani et al. 2014; Nanon et al. 2015; Soroor & Moeini 2015; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Faniyi et al. 2019). The findings of this study support the results reported by Tag El-Din et al. (2012), Kurniawati et al. (2018), Mekuiko Watsop et al. (2018). However, Nanon et al. (2015) showed that GEO addition (1600 mg/kg DM) affected the *in vitro* gas production negatively.

Table 2- Effects of GEO and its different doses on the *in vitro* gas production of TPH, mL

Incubation duration, hour	*GEO, mg L^{-1}*							
	Control (0)	*50*	*100*	*200*	*400*	*800*	*1000*	*SEM**
3	18.80^{a}	18.41^{ab}	17.83^{ab}	17.35^{b}	16.17^{c}	14.95^{d}	14.48^{d}	0.625
6	29.76^{a}	28.65^{b}	27.77^{bc}	27.65^{c}	25.48^{d}	23.90^{e}	22.71^{f}	0.549
12	45.43^{a}	44.47^{ab}	43.14^{bc}	41.29^{cd}	40.29^{d}	39.21^{d}	36.71^{e}	1.268
24	57.77^{a}	56.02^{b}	55.34^{b}	63.60^{c}	51.76^{d}	49.93^{e}	48.67^{e}	0.880
48	67.70^{a}	64.61^{b}	61.36^{c}	59.18^{cd}	57.65^{de}	55.67^{e}	53.11^{f}	1.401
72	71.62^{a}	70.06^{b}	69.62^{bc}	68.42^{cd}	67.41^{d}	65.86^{e}	63.33^{f}	0.746
96	74.66^{a}	72.22^{b}	71.10^{c}	69.55^{d}	69.16^{de}	68.25^{e}	64.89^{f}	0.608

*: Standard error mean. Differences between the means shown with different letters on the same line are significant (P<0.05)

The positive effect of the GEO added to RL on the digestibility of feeds can be explained by the GEO's showing antimicrobial activity against microorganisms (Tag El-Din et al. 2012; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018). The organic components existing in GEO are reported to show an antibacterial effect by breaking the cell wall structure of microorganisms as it is in other essential oils (Sharma et al. 2016; Mahboubi 2019). It can be stated that the development of rumen microorganisms is limited via a similar mechanism (Tag El-Din et al. 2012; Soroor & Moeini 2015; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018) and, depending on this, *in vitro* gas production decreases.

3.3. Effect of GEO on the OMD and ME

Supplementation of GEO significantly affected OMD and ME content of TPH. The OMD and ME content ranged from %78.72 to 71.03 and 11.44 to 10.21 MJ/kg DM respectively (P<0.05). These parameters significantly decreased with increasing level of GEO supplementation.

Table 3- Effects of GEO and its different doses on the OMD of TPH under *in vitro* conditions and ME

Parameters	*GEO, mg L^{-1}*							
	Control (0)	*50*	*100*	*200*	*400*	*800*	*1000*	*SEM**
OMD, %	78.72^{a}	77.25^{b}	76.67^{b}	75.20^{c}	73.65^{e}	72.10^{e}	71.03^{e}	0.743
ME, MJ/kg DM	11.44^{a}	11.21^{b}	11.11^{b}	10.88^{c}	10.63^{d}	10.38^{e}	10.21^{e}	0.118

OMD: Organic matter digestion; ME: Metabolisable energy; *: Standard error mean. Differences between the means shown with different letters on the same line are significant (P<0.05).

It can be explained by the finding that the GEO added to RL in increasing doses caused a low level of *in vitro* gas production by showing antimicrobial effect (Tag El-Din et al. 2012; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Mahboubi 2019). However, Soroor & Moeini (2015) found that the GEO increased the *in vitro* gas production. The finding that the *in vitro* gas production was found high in the mentioned study can be explained by the use of a different ration and the low dose of GEO (60 mg L^{-1}).

The decrease in the OMD determined in the study was also found in the studies made by Tag El-Din et al. (2012) and Mekuiko Watsop et al. (2018), Mahboubi (2019) working with different feeds and GEO doses. However, in the studies carried out by Soroor & Moeini (2015), Medjekal et al. (2017) and Kurniawati et al (2018), the GEO affected the digestibility of feeds positively. This can be explained by the fact that these researchers worked with low doses (60 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1}, respectively). Nanon et al (2015) reported in their study that the GEO addition (1600 mg/kg DM) to the ration did not affect the digestion of dry matter negatively.

With the GEO addition in different doses to RL, the ME content of TPH changed between 11.44 and 10.21 MJ/kg DM. As the GEO dose increased, the ME level decreased. Similarly, Tag El-Din et al. (2012) determined in their study that the addition of ginger decreased the ME level.

3.4. Effect of GEO on rumen fermentation

The addition of GEO to RL decreased the TVFA and the AA, PA and BA significantly (P<0.05). Depending on the GEO doses, the TVFA ranged from 90.05 to 68.88 mmol/L. The lowest TVFA was determined in the experimental group into which the GEO was added in the dose of 1000 mg L^{-1}. Moreover, the AA, PA and BA levels of RL varied between 47.92-35.69 mmol/L, 21.13-18.37 mmol/L and 15.39-7.56 mmol/L, respectively. The most effective GEO dose on TVFA, AA, PA and BA was determined as 1000 mg L^{-1} (P<0.05). The addition of GEO to RL affected the rumen fermentation significantly. This can be explained by the finding that GEO had an antibacterial effect on the rumen microorganisms (Tag El-Din et al. 2012; Kurniawati et al. 2018; Mekuiko Watsop et al. 2018; Mahboubi 2019). Especially, the increase in the GEO dose decreased the production of TVFA and the individual VFA. In their study, Soroor & Moeini (2015) added 30 and 60 mg of GEO to RL and reported as a result of the study that the propionic acid rate increased, but the TVFA and AA rates decreased depending on the increase in the GEO doses. Similar findings were found in the study reported by Tag El-Din et al. (2012) in relation to the TVFA, too. However, Nanon et al. (2015) reported in their study that the GEO did not affect the contents of TVFA, AA and PA.

Table 4- Effects of GEO and its different doses on the features of rumen fermentation

RL Parameters	*GEO, mg L^{-1}*							
	Control (0)	*50*	*100*	*200*	*400*	*800*	*1000*	*SEM**
pH	5.99^{f}	6.08^{e}	6.10^{e}	62.27^{d}	6.36^{c}	6.45^{b}	6.57^{a}	*0.026*
NH_3N, mg N/100 mL	31.31^{a}	31.52^{a}	29.23^{b}	28.28^{b}	24.03^{c}	23.66^{c}	21.28^{d}	*1.081*
TVFA, mmol/L	90.05^{a}	86.38^{b}	84.40^{c}	80.55^{d}	75.68^{e}	72.03^{f}	68.88^{g}	*1.068*
AA, mmol/L	47.92^{a}	46.27^{ab}	44.91^{b}	40.43^{c}	35.69^{d}	38.12^{c}	39.13^{c}	*1.348*
PA, mmol/L	20.07^{a}	19.04^{abc}	21.13^{b}	18.62^{bc}	18.37^{c}	19.65^{ab}	19.67^{b}	*0.595*
BA, mmol/L	13.55^{a}	13.29^{a}	13.39^{a}	15.39^{a}	15.15^{a}	10.68^{b}	7.56^{c}	*1.199*
OVFA, mmol/L	8.47^{a}	7.78^{ab}	7.48^{b}	6.36^{c}	6.44^{c}	3.58^{d}	2.2^{e}	*0.392*
AA/PA	2.38^{a}	2.43^{a}	2.13^{b}	2.20^{b}	1.95^{c}	1.94^{c}	1.99^{c}	*0.099*

NH_3N: Ammonia nitrogen; TVFA: Total volatile fat acids; OVFA: Other volatile fat acids; AA/PA: acetic acid/propionic acid; *: Standard error mean. Differences between the means shown with different letters on the same line are significant (P<0.05)

The rate of AA/PA determined in the study varied between 2.38 and 1.94 depending on the increase in the GEO dose and the differences between the GEO doses were found significant (P<0.05). The highest AA/PA rate was found in the control group not including GEO. The AA/PA rate determined in the study was found lower than the values of (3.33-3.34) obtained by Nanon et al. (2015) working with GEO and the ones (2.3-2.6) determined by Soroor & Moeini (2015) but higher than the values (1.8-1.7) reported by Benchaar et al. (2008) working with a different essential oil (garlic essential oil).

Depending on the GEO dose, RL pH level varied between 5.99 and 6.57 and the differences between the GEO doses were found significant (P<0.05). The highest rumen pH was found in the group including 1000 mg L^{-1}. That the pH increased depending on the increase in the GEO dose can be explained by the finding that the GEO addition decreased the VFA turning the RL into an acid character (Table 4). While the rumen pH determined in the study was found lower than the ones found by Kurniawati et al. (2018) working with GEO, it was found similar to the results reported by Busquet et al. (2006).

Moreover, ruminal NH_3N level changed between 31.31 and 21.28 mg N/100 mL depending on the increase in the GEO dose. The highest NH_3N levels were determined in the control and 50 mg L^{-1}group not including GEO with the value of 31.31 and 31.52 mg N/100 mL and the lowest NH_3N level was determined in the group including 1000 mg L^{-1} with the value of 21.28 mg N/100 mL (P<0.05). The decrease in NH_3N level resulted initially from the decrease in the activity of RL microorganisms as well as the essential oils' preventing the deamination of amino acids (Nanon et al. 2015; Soroor & Moeini 2015). It is reported that the decrease in the nitrogen loss in the rumen in the form of ammonia (NH_3) will be beneficial in terms of animal feeding and increase the benefiting from the energy and nitrogen of the feed (Nanon et al. 2015; Soroor & Moeini 2015). NH_3N level determined in the study was lower than the results reported by Nanon et al. (2015) working with GEO, but it was similar to the findings obtained by Soroor & Moeini (2015). Mekuiko Watsop et al. (2018) reported in their study that the GEO addition decreased NH_3N level. Busquet et al. (2006) reported that the GEO addition to RL did not affect NH_3N level.

3.5. Effect of GEO on CO_2 and CH_4 gas production

In the study, depending on the increase in the dose of GEO added to RL, the *in vitro* CO_2 gas production decreased (P<0.05). The highest CO_2 gas production was found in the control group with the value of 49.30 mmol/L and the lowest CO_2 gas production was determined in the 1000 mg L^{-1} group with the value of 38.11 mmol/L (P<0.05). Moreover, the *in vitro* CH_4 gas production decreased depending on the GEO dose increase, varied between 25.18 and 18.43 mmol/L and the differences between then were found significant (P<0.05).

Table 5- Effects of GEO and its different doses on CO_2 and CH_4 productions

Parameters	*GEO, mg L^{-1}*							
	Control (0)	*50*	*100*	*200*	*400*	*800*	*1000*	*SEM**
CO_2, mol/L	49.30^{a}	47.89^{a}	57.83^{a}	47.19^{ab}	45.17^{b}	39.99^{c}	35.83^{c}	1.365
CH_4, mol/L	25.18^{a}	25.01^{ab}	24.49^{b}	23.32^{c}	20.82^{d}	19.49^{e}	18.43^{f}	0.537

*: Standard error mean. Differences between the means shown with different letters on the same line are significant (P<0.05).

The CO_2 and CH_4 productions in the ruminants are made by the methanogenic bacteria existing in the rumen via the use of VFA and hydrogen ions (H^+) (Demeyer et al. 1996; Nanon et al. 2015). GEO decreases the CH_4 gas formation by showing an antimicrobial effect on methanogenic bacteria, as it has on other rumen bacteria. CH_4 is one of the most important greenhouse gases. It is reported that the greenhouse effect of CH_4 is 23 times as much as that of CO_2 (Kim et al. 2012). It is also reported that the contribution of farm animals to the emission of greenhouse gases is 18%. It was determined that about 15% of this part resulted from the fermentation occurring in the rumens and manures of ruminant animals (Takahashi et al. 2005).

It is reported that 2 - 15% of feed energy is lost in the form of CH_4 via the fermentation of feeds in rumen (Kim et al. 2012). It is stated that essential oils have a potential in decreasing energy loss and greenhouse gas emission via CH_4 gas (Chaouki Benchaar & Greathead 2011; Nanon et al. 2015; Ratika & Singh 2018). It was also revealed in many previous studies that GEO led to the decrease in CH_4 production by limiting the number of methane-producing bacteria in rumen (Tag El-Din et al. 2012; Nanon et al. 2015). In the study, the GEO addition in different doses to RL decreased the in vitro CH_4 gas production significantly (P<0.05). These results show similarity to the findings obtained by Tag El-Din et al. (2012) and Kurniawati et al. (2018) working with GEO and the ginger plant.

4. Conclusions

In conclusion, the addition of GEO in different doses to RL under *in vitro* conditions decreased the *in vitro* gas production and OMD and the ME content significantly (P<0.05). Similarly, the increase in the dose of GEO added to RL decreased the TVFA and the individual VFA, two of the rumen metabolites, and the production of NH_3N, CO_2 and CH_4 gases but increased the rumen pH. It can be stated that GEO can prevent nitrogen loss in the rumen by decreasing NH_3N level in the rumen and it can be benefited from the feed energy more effectively by decreasing the loss of CH_4 gas. However, since feeds negatively affect OMD and ME content, it is recommended to use GEO at low doses (200 mg L^{-1}) in ruminant feeding. It was concluded that more *in vitro* and *in vivo* studies with more intensive content are needed to shed light on the matter.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Hydrogeochemical Characteristics of Spring Waters for Irrigation, Gökpınar Basin Case, Denizli, Turkey

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ABSTRACT
In this study, a detailed hydrochemical evaluation has been made to determine the chemical processes of spring waters and their suitability for irrigation. The study area consists of a drainage basin of the Gökpınar dam and has fertile soils for irrigable agriculture. During the period of August 2017 and October 2018, regular samples were collected monthly from 10 spring and 140 samples in total were subjected to hydrochemical analysis. For this purpose, 11 hydrochemical parameters such as pH, EC, TDS, TH, Na%, SAR, MR, RSC, RSBC, USSL, and Wilcox were used. GIS-based spatial mapping of the hydrogeochemical parameters has been prepared using ArcGIS. The major hydrogeochemical facies of waters are Ca^{2+}, Mg^{2+}, HCO_3^- water type. Alkaline earth metals (Ca^{2+}, Mg^{2+}) and weak acid (CO_3^{2-}, HCO_3^-) dominates over the alkalies (Na^+, K^+) and strong acid (Cl^-, SO_4^{2-}) in all spring waters, respectively. Since the limit values of TDS in the samples are between 367 and 681 mgL^{-1}, the class of all samples is freshwater. The average Na% is between 1.29 and 9.28, and EC values are between 402 and 691 μScm^{-1}. For irrigation purposes, all spring waters fall within the category of "excellent to good" in the Wilcox (1955) diagram, based on the Na% and EC. Average SAR values in the range of 0.07-0.16 $meqL^{-1}$ indicate that spring water samples are excellent for irrigation purposes.

Keywords: Agricultural areas, Water quality, Catchment, GIS mapping

1. Introduction

Water is the most valuable compound on the earth, essential for humans and all other living things. The chemical composition of groundwater and their suitability for different purposes vary according to different processes in their natural environment. Continuous monitoring of the quality of natural freshwater is important for human health, agricultural production, and soil fertility. Understanding the hydrogeochemical properties and evolution of spring waters in a basin can provide important knowledge for the future management of water resources in that region (Meybeck & Helmer 1996; Kumar et al. 2009; Tiwari 2011). The dominant factors controlling the suitability of spring waters for irrigation are water chemistry, soil properties, salt sensitivity of plants, climate, and drainage (Appelo & Postma 2005). Irrigation water quality has a direct impact on the structure of the soil, and hence on crop productivity and quality (Roy et al. 2015). The prevalence and diversity of anthropogenic activities limit the water quality in nature and disrupt the ecosystem. These processes are rock-water interaction, its mix with other water sources in different facies, human factors, etc (Andrade et al. 2008). There are numerous studies on spring water characteristics' evaluation using hydrochemical techniques all around the world (Ako et al. 2012; Bhandari and Joshi 2013; Dinka et al. 2015; Nair et al. 2015; Kamtchueng et al. 2016; Batool et al. 2018).

The aim of this study is to investigate the irrigation water characteristics of spring waters, which are one of the main components of the water body feeding the Gökpınar Dam. The spring waters in the basin are used by the local people as a primary water source for drinking and domestic use as well as agriculture. Although spring waters are considered reliable since they come from groundwater, their quality is actually based on certain physicochemical parameters (Edmunds & Shand 2009). This study confirms that all spring water samples in the basin are suitable for irrigation, based on long-term physical parameters and chemical analysis results. Likewise, all springs are safe to use in terms of physical parameters, chemical composition, soil properties, salt sensitivity of plants, and drainage. During the study, the parameters controlling the chemical properties of the spring waters in the basin were analysed. The changes in the chemical properties of the spring waters in the study area are due to the rock-water interaction and oxido-reduction reactions rather than the seasons. In the past few decades, industrial development, and improved living standards have attracted many new immigrants to the study area and rapidly increased the demand for quality water in all sectors. Increasing urbanization and agricultural activities in the region have started to threaten the quality and usability of water. In this situation, using freshwater springs in the most efficient way, and finding new resources has become an urgent requirement. However, a detailed irrigation program and planning has not yet been made in the basin by

spatial planners and water managers. There is also no serious study in terms of agricultural irrigation regarding spring waters. This hydrochemical assessment is the first study on this subject in the basin and its purpose is to evaluate the suitability of these water springs for use in irrigation based on the Geographic Information System (GIS) approach.

2. Material and Methods

The methods employed for this study are field measurements, sampling, laboratory analysis, and data interpretation. The geological and hydrogeological map of the basin was prepared by GIS methods, geological, geophysical and hydrogeological evaluations and utilizing the previous researches. In order to evaluate physicochemical parameters, 140 water samples collected from 10 different sources in the period of August 2017-September 2018 were analyzed in terms of main quality parameters by following standard test procedures. Geographical positions of sampling locations were measured with a portable GPS system. In situ measurements such as temperature (T; $^{\circ}$C), electrical conductivity (EC; µS cm^{-1}), and hydrogen ion concentration (pH) of the waters were measured using a portable multi-parameter water quality monitoring and testing equipment. Total Dissolved Solids (TDS; mg L^{-1}) were calculated from the sum of the concentrations of all major ions. The analysis of the waters was carried out in accordance with the standards specified in the "Turkish Regulation on Water Intended for Human Consumption" (Official Journal Date: 17.02.2005). Cations (Sodium Na^{+}, Potassium K^{+}, Calcium Ca^{2+}, Magnesium Mg^{2+}) and anions (Chloride Cl^{-}, Sulfate SO_4^{2-}, Fluoride F^{-}, Bicarbonate HCO_3^{-}), nitrate (NO_3^{-}), nitrite (NO_2^{-}), and ammonium (NH_4^{+}) were analyzed in the Denizli Municipality Health Branch Directorate, Denizli Environmental Quality Laboratory (DENÇEV, Denizli/TURKEY, a TS EN ISO/IEC 17025:2012 accredited Laboratory). Major ion contents in spring water samples were determined using ion chromatography. The methods used for the analysis of major ions are TS EN ISO 14911 for cations and SM 4110 B-TS EN ISO 10304-1 for anions. Obtained hydrochemical results were evaluated for hydro-geochemical facies analysis by plotting on Piper (1944), Gibbs (1970), and Chadha (1999) diagram. For irrigation water samples, Electrical Conductivity (EC), Total Dissolved Solids (TDS), total hardness (TH), percent sodium (Na%), sodium adsorption ratio (SAR), magnesium ratio (MR), residual sodium carbonate (RSC), and residual sodium bicarbonate (RSBC) was assessed and compared with standard limits. Aquachem software package (version 2011.1.61) was used as a tool for hydro-geochemical calculation and evaluation for chemical data of spring waters.

Gökpınar Dam is located in Denizli City in the Aegean Region of Turkey. The catchment area of the dam is 228 km^2 between 28º 59'16" - 29º 17' 13" E longitudes and 37º 38'15" - 37º 47'14" N latitudes. It was built on the Gökpınar Creek to supply irrigation water. The dam provides irrigation services to an area of 5 824 hectares at the downstream. There are 3 100 hectares of agricultural land irrigated with spring waters in the dam basin. Western Turkey is one of the most seismically active regions in the World (Westaway 1993; Bozkurt 2003; Kocyigit 2005). Based on the hydrogeological characteristics of the lithological units in the basin, five hydrogeological units have been identified, which are Permeable rocks (Mesozoic limestone, Quaternary travertine), permeable clastic units (Quaternary alluvial fan), semipermeable rocks (Paleozoic gneiss, schist, quartzite, Eocene limestone), semipermeable clastic units (Quaternary alluvium), and impermeable units (Mesozoic clastic sediments, and Pliocene terrestrial clastic sediments) (Figure 1). The water table depth in the study area ranges between 5 and 40 meters (Taşdelen et al. 2016; Taşdelen 2018). Groundwater in the basin, as seen in the hydrogeology map, generally flows from the southeast to the northwest. Aquifers are recharged by rainfall seeping through the ground on the high elevation hills surrounding the basin. The geological units in the region have intense discontinuities due to tectonic stresses. The amount of groundwater flow in the basin is controlled by the density of the fault and fracture systems rather than the primary porosities of the units (Taşdelen et al. 2017). The highly jointed nature of the especially limestone and basement rocks makes the basin a rich hydrogeological reservoir. 10 water springs subject to this study feed the Gökpınar Creek flowing into the dam lake with a total flow of 1.5 m^3 s^{-1}. The main factor that determines groundwater hydrochemistry along the flow path from recharges to discharge areas in the aquifers of the study area is the chemical composition of the geological units. The chemical composition of the waters and the regional geological features of the basin show that groundwater chemistry in the study area is controlled by the decomposition processes of carbonates, silicates, and evaporites, which are abundant in the region. The region is very suitable for agriculture due to its mild climate, fertile soil, and the presence of water. Agriculture is the most important means of livelihood for local people in the study area. Almost all of them deal with traditional Mediterranean agriculture such as olives, grapes, figs, melons, watermelons, almonds, and pomegranates. The green vegetable and fruit agriculture of this region is also well known. The springs are also used for drinking, domestic use, and trout farming purposes in the settlements within the basin.

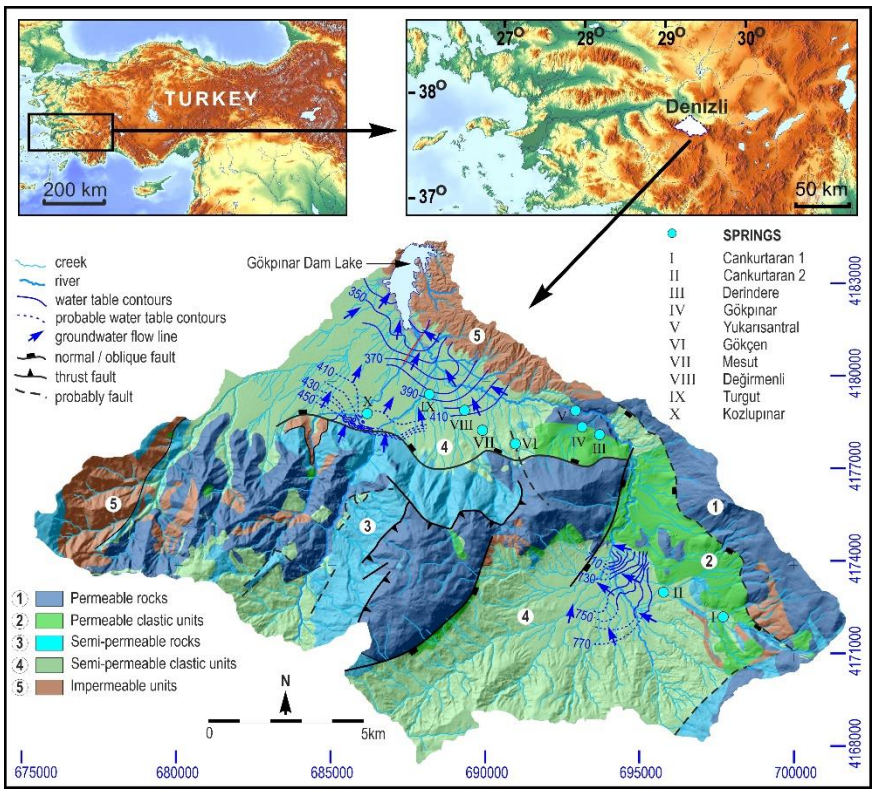

Figure 1- Hydrogeological map of the Gökpınar Dam Basin (compiled from Okay (1989), Taşdelen et al. (2017) and MTA (2018))

3. Results and Discussions

3.1. Water chemistry

The statistical summary of long-term physical parameters and chemical analyses results of each spring in the Basin (on the basis of mg L^{-1} for ions) are presented in Table 1. The long-term average major ion contents of springs based on the milliequivalent percentage are illustrated in the pH of the spring water samples in the basin ranges from 7.2 to 7.5 and with an average of 7.33 shows alkaline nature. (Table 1). The temperature of springs ranges from 14.5 to 19.7 ºC. During the monitoring period, the long-term average EC and TDS content of the spring waters in the dam basin range from 402 to 691 µS cm^{-1} and 367 to 681 mg L^{-1}, respectively. The springs having relatively high conductivity in the basin are Cankurtaran-2, Değirmenli, and Turgut. Natural waters contain some dissolved solid substances of organic or inorganic geological units with which they come into contact. The total dissolved solids (TDS) in the spring waters is a measure of organic and inorganic substances dissolved in water and in terms of groundwater depends on the solubility of geological units in water. High TDS value can restrict use for irrigation and reduce crop yield (Catroll 1962; Freeze & Cherry 1979). The alluvial aquifers are loose, unconsolidated, eroded, carried, and redeposited soil or sediment environments that made up of a variety of materials, including fine particles of clay, silt, sand, and gravel. Also, evaporation of waters from shallow alluvial aquifers, where the water table is near the land surface, increases total dissolved solids in the groundwater. Cankurtaran-2, Değirmenli, and Turgut springs are alluvial springs. Aquifers feeding the springs consist of semipermeable clastic units. The relatively high TDS values of these three sources are for the reasons mentioned above. However, this high amount is relative and there is no problem in terms of irrigation water quality. According to TDS classification (Catroll 1962; Freeze & Cherry 1979), all sources including Cankurtaran-2, Değirmenli, and Turgut are of freshwater type (TDS <1.000 mg L^{-1}) (Table 2) and suitable for irrigation purposes. Among the nutrients, the long-term average concentrations of NO_3^-, NO_2^- and NH_4^+ ions ranged from 4.2 to 16.2, 0 to 0.1 and 0 to 0.49 mg L^{-1}, respectively. Cankurtaran-2 Değirmenli and Turgut have the highest concentrations of NO_3^- in the basin (Table 1). In the alluvial environments of the basin, nitrate can be carried to surface and groundwater by direct discharge or leakage from the soil from artificial sources such as agricultural areas and settlements. These springs are vulnerable to surface contaminants, as they can recharge directly from the floor surface. Therefore, the reason for relatively high NO_3^- concentration is thought to be of an anthropogenic. Nitrate, Nitrite, and ammonium concentrations of waters were within the recommended values for drinking water (Ayers & Westcot 1985). The abundance of major ions based on the meq% in spring waters is in the following order: $HCO_3^- > Ca^{2+} > Mg^{2+} > SO_4^{2-} > Na^+ > Cl^- > NO_3^- > K^+ > F^-$. The most abundant cations (mg L^{-1}) present in waters are Ca (72.1 to 93.8), Mg (10.4 to 29.9), Na (2.6 to 6.6), and K (0.4 to 1.2); the most abundant anions (mg L^{-1}) are HCO_3^- (228.5 to 405.4), Cl^- (2.8 to 9.2), and SO_4^{2-} (4.9 to 56.7), and F (0.05 to 0.61). According to this sequence, lithological facies that are dominant in the composition of spring waters are carbonated rock minerals (Calcite, dolomite) and evaporitic sulfate minerals (gypsum, halite); secondarily, they are plagioclase minerals originating from igneous and metamorphic rocks.

Table 1- Statistical summary of the physical and chemical parameters of the spring waters (August 2017-September 2018)

	Unit		*Cankurtaran 2*	*Cankurtran 1*	*Değirmenli*	*Derindere*	*Gökçen*	*Gökpinar*	*Kozlupınar*	*Mesut*	*Turgut*	*Yukarısantral*
Parameter			15	15	16	16	14	16	9	15	16	8
Cl^-	mg L^{-1}	Avg.	4.5	3.0	8.2	2.8	3.5	4.4	5.5	7.3	8.3	4.7
	mg L^{-1}	Max.	6.4	8.9	10.3	6.7	4.4	6.4	6.1	7.9	9.2	5.6
	mg L^{-1}	Min.	3.7	2.2	7.4	2.4	3.1	3.7	3.7	6.9	8.1	4.3
		Std.	0.8	1.7	0.8	1.1	0.4	0.8	0.7	0.3	0.3	0.4
F^-	mg L^{-1}	Avg.	0.06	0.05	0.07	0.14	0.06	0.61	0.26	0.06	0.08	0.09
	mg L^{-1}	Max.	0.07	0.06	0.09	0.17	0.07	8.10	0.31	0.08	0.11	0.11
	mg L^{-1}	Min.	0.03	0.04	0.05	0.12	0.04	0.07	0.13	0.04	0.06	0.07
		Std.	0.01	0.01	0.01	0.01	0.01	2.00	0.06	0.01	0.02	0.01
HCO_3^-	mg L^{-1}	Avg.	393.4	279.1	405.4	228.5	346.5	304.0	271.5	358.8	400.0	302.3
	mg L^{-1}	Max.	464.3	293.1	439.2	239.1	361.1	319.6	315.7	372.0	418.2	312.4
	mg L^{-1}	Min.	263.0	260.6	378.4	213.1	333.6	283.5	257.6	342.5	365.1	296.8
		Std.	72.1	9.4	14.6	6.3	6.7	7.7	17.3	8.2	12.6	6.1
SO_4^{2-}	mg L^{-1}	Avg.	26.5	27.2	14.2	56.7	4.9	48.4	44.0	8.3	18.7	40.0
	mg L^{-1}	Max.	30.3	62.9	25.1	59.6	5.5	51.2	47.4	8.7	28.2	41.5
	mg L^{-1}	Min.	20.4	20.5	11.0	55.1	4.6	46.0	41.1	7.8	12.0	38.0
		Std.	3.7	10.1	3.6	1.4	0.3	1.6	1.9	0.3	4.8	1.2
Ca^{2+}	mg L^{-1}	Avg.	83.8	73.0	93.8	73.0	80.0	86.7	72.1	88.8	91.6	85.9
	mg L^{-1}	Max.	94.9	88.5	101.7	76.1	83.3	90.7	86.1	92.3	94.5	88.7
	mg L^{-1}	Min.	70.9	67.6	87.6	69.8	76.9	84.1	68.2	84.8	80.4	84.3
		Std.	8.3	5.0	3.4	1.6	1.5	1.5	5.4	2.1	3.5	1.7
Mg^{2+}	mg L^{-1}	Avg.	29.9	13.4	23.0	11.5	15.6	16.1	21.6	18.0	10.4	15.9
	mg L^{-1}	Max.	37.0	25.1	24.6	11.8	16.3	16.4	23.0	18.6	24.7	16.1
	mg L^{-1}	Min.	12.1	10.8	21.5	11.1	15.0	15.7	16.7	17.2	0.0	15.6
		Std.	6.6	3.5	0.7	0.2	0.3	0.2	1.9	0.4	8.9	0.1
K^+	mg L^{-1}	Avg.	0.9	0.9	0.8	0.4	0.5	0.5	0.6	0.6	1.2	0.4
	mg L^{-1}	Max.	1.4	1.2	1.3	0.6	1.0	0.8	0.7	0.7	2.2	0.5
	mg L^{-1}	Min.	0.7	0.7	0.5	0.3	0.4	0.2	0.5	0.4	0.7	0.4
		Std.	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.4	0.0
Na^+	mg L^{-1}	Avg.	6.6	3.4	6.3	2.6	2.8	3.6	5.9	4.6	6.3	3.5
	mg L^{-1}	Max.	12.0	6.0	6.9	2.9	3.3	4.5	6.4	4.8	6.7	3.8
	mg L^{-1}	Min.	3.4	3.1	5.7	2.5	2.6	2.9	3.3	4.2	5.9	3.3
		Std.	1,7	0.7	0.3	0.1	0.2	0.5	1.0	0.2	0.2	0.2
NO_3^-	mg L^{-1}	Avg.	13.7	4.2	16.2	9.6	4.9	7.4	4.2	10.6	15.9	8.0
	mg L^{-1}	Max.	30.7	31.2	21.4	11.2	6.1	7.9	8.7	11.4	18.0	8.7
	mg L^{-1}	Min.	1.8	0.6	14.4	9.0	0.3	7.0	2.9	9.7	12.4	7.5
		Std.	9.3	7.8	1.8	0.5	1.4	0.2	2.0	0.4	1.4	0.3
NH_4^+	mg L^{-1}	Avg.	0.23	0.19	0.17	0.49	0.22	0.46	-	0.19	-	-
	mg L^{-1}	Max.	0.25	0.23	0.17	1.12	0.29	0.46	0.00	0.19	0.00	0.0
	mg L^{-1}	Min.	0.20	0.17	0.17	0.26	0.16	0.6	0.00	0.19	0.00	0.0
		Std.	0.04	0.03	-	0.42	0.07	-	-	-	-	-
NO_2^-	mg L^{-1}	Avg.	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1
	mg L^{-1}	Max.	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.0	0.1
	mg L^{-1}	Min.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Std.	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0
TDS	mg L^{-1}	Avg.	566	406	569	387	460	475	427	499	567	462
	mg L^{-1}	Max.	681	464	622	403	478	494	482	516	590	477
	mg L^{-1}	Min.	381	372	530	367	444	461	410	476	523	454
		Std.	88	27	23	8	8	8	21	11	17	9
pH		Avg.	7.3	7.5	7.2	7.3	7.4	7.3	7.4	7.2	7.3	7.4
		Max.	7.6	8.0	7.4	7.7	7.8	7.6	7.6	7.5	7.7	7.6
		Min.	6.9	7.0	7.0	6.9	7.0	6.8	6.9	6.9	7.0	7.1
		Std.	0.2	0.3	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.2

Table 2- Irrigation water classifications of the springs (The spring numbers and names are shown in Figure 1)

Parameter	*Unit*	*Reference*	*Analytical method*	*Ranges*	*Class*	*Spring number*
TDS	mg L^{-1}	Catroll 1962; Freeze & Cherry 1979	*TDS = 0.65 x EC*	< 1000 1000–10.000 0.000–100.000 > 100.000	Fresh water Brackish water Saline water Brine water	From I to X
EC	µS cm^{-1}	Richards 1954	*pH / EC / TDS meter*	< 250 250 – 750 750 – 2000 2000 – 3000 > 3000	Excellent Good Permissible Doubtful Unsuitable	From I to X
TH	meq L^{-1}	Sawyer & Mc-Cartly 1967	*TH = (Ca^{2+}+Mg^{2+}) meq L^{-1} x 50*	< 75 75 – 150 150 - 300 > 300	Soft Moderately hard Hard Very hard	II, IV, V, VI, VII, VIII, X I, III, IX,
Na%	%	Wilcox 1955	$\%Na = \frac{rNa^+ \times 100}{rNa^+ + rCa^{2+} + rMg^{2+} + rK^+}$	0 – 20 20 – 40 40 – 60 60 – 80 > 80	Excellent Good Permissible Doubtful Unsuitable	From I to X
SAR	meq L^{-1}	Richards 1954; Todd 1960	$SAR = \frac{Na^+}{\sqrt{\frac{(Ca^{2+} + Mg^{2+})}{2}}}$	< 10 10 – 18 19 – 26 > 26	Excellent (S1) Good (S2) Doubtful (S3) Unsuited (S4-S5)	From I to X
RSC	meq L^{-1}	Eaton 1950; Richards 1954	$RSC = (CO_3^{2-} + HCO_3^-) - (Ca^{2+} + Mg^{2+})$	< 1.25 1.25 – 2.5 > 2.5	Good Doubtful Unsuitable	I, II, III, IV, V, VII, X VI, VIII, IX
RSBC	meq L^{-1}	Eaton 1950	$RSBC = HCO_3^- - Ca^{2+}$	< 5 5 – 10 > 10	Satisfactory Marginal Unsatisfactory	From I to X
MR	%	Szabolcs & Darab 1964	$MR = \frac{Mg^{2+}}{Mg^{2+} + Ca^{2+}} x100$	< 50 > 50	Suitable Unsuitable	From I to X

3.2. Water facies and hydrochemical evaluation

Piper Diagram (Piper AM 1944): A Piper diagram was plotted using the analytic data obtained from the hydrochemical analysis of the basin spring waters. Waters of similar nature will tend to be a group together on the Piper diagram. In general, the dominant water type is Ca^{2+} – Mg^{2+} – HCO_3^-. The dominant water types of springs reflect the rock interactions with limestone and dolomite dominated formations and weathering of silicate minerals in the aquifer. In addition to dolomite, the increase in Mg can result from magmatic rock minerals (olivine, biotite, hornblende, augite, etc) and minerals such as serpentine, diopside, tremolite in metamorphic rocks. All spring water samples characterized as alkaline earth metals (Ca^{2+}, Mg^{2+}) are dominant over the alkalies (Na^+, K^+). The weak acidic anions (CO_3^{2-}, HCO^{3-}) exceed the strong acidic anions (Cl^-, SO_4^{2-}) in all samples (Figure 2).

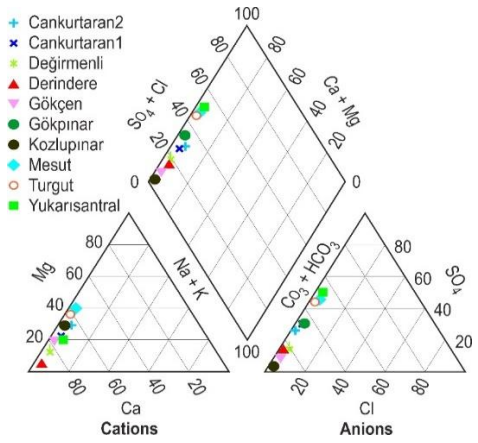

Figure 2- Piper diagram of spring waters

Chadha Diagram (Chadha DK 1999): To classify the spring waters as geochemically and to identify the hydrochemical processes based on prevailing ions, Chadha (1999) diagram is used (Figure 3a). In all the basin spring waters, alkaline earths (Ca^{2+}, Mg^{2+}) and weak acidic anions (HCO_3^-) exceed alkali metals (Na^+, K^+) and strong acidic anions (SO_4^{2-}, Cl^-). This type of water may be due to the dissolution of carbonates in the aquifer material and the decomposition of silicate minerals.

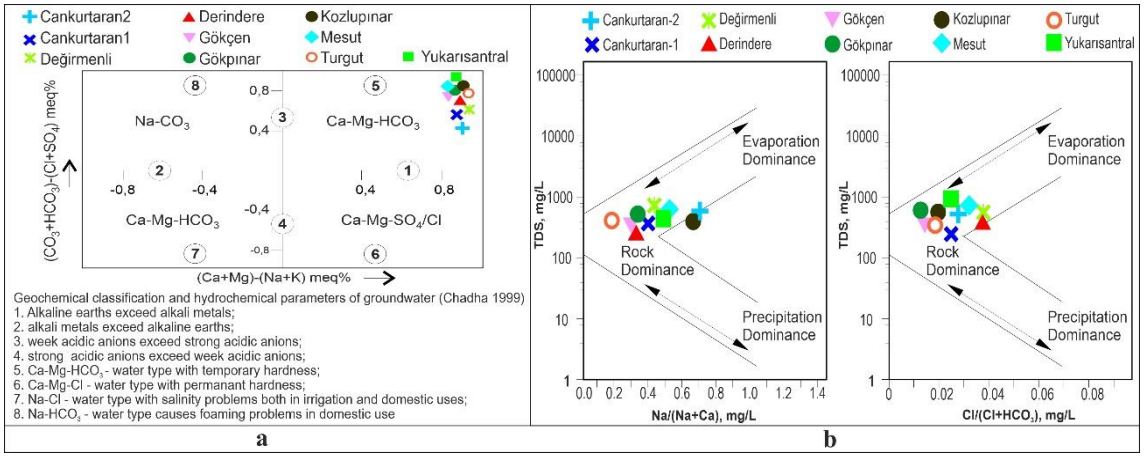

Figure 3- Chadha (1999) (a) and Gibbs (1970) (b) diagrams of spring waters

Gibbs Plot (Gibbs RJ 1970): Gibbs, proposed a diagram to identify the relationship between the water composition and properties of the aquifer units. Three distinct areas such as "precipitation dominance", "evaporation dominance" and "rock dominance" are indicated in the diagram. Gibbs plot specifies that all the basin spring water samples fall in the "rock dominance area" (Figure 3b). Falling in rock dominance area indicates that the precipitation sourced chemical weathering of rock-forming minerals are influencing the spring water geochemistry. That is, the dominant mechanism controlling groundwater major ion chemistry in the basin is rock-water interaction.

Correlation analysis: The correlation matrix has been widely used to determine the relationships between the hydrochemical components of natural waters (Tiwari 2011). If there is a good relationship between any two variables, the correlation coefficient is close to 1 or 1 (high correlation); on the contrary, if there is no relationship, the value is 0 (Table 3). The relationship between the components in the spring waters was determined using Pearson correlation analysis (Table 3). Correlation significant at the 0.01 and 0.05 levels was found between some physical parameters and major elements. There is a strong positive correlation between TDS, cations (Ca^{2+}, Na^+, Mg^{2+}), and anions (Cl^-, SO_4^{2-}, HCO^{3-}) in the spring waters. This confirms the results obtained with the Chadha and Gibbs diagrams. The dominant hydrochemical processes that determine the composition of waters are rock-water interaction and ionic changes. In particular, the strong correlation between TDS, Ca^{2+}, and HCO^{3-} may result from the presence of lithologically dominant karstic aquifers in the region. In addition, the strong correlation between TDS, NO_3^-, Na^+, and Cl^- may also be a result of human activities.

3.3. Irrigation water quality

Since only absolute ion concentration values may not be sufficient to determine the suitability of water for irrigation use, the effects of interactions between ions on the chemical properties of water should also be evaluated (Nagaraju et al. 2014). Therefore, in the next sections, the methods that include the interactions were evaluated (Table 2).

Total Dissolved Solids (TDS): The minimum and maximum values of TDS in the water samples of the springs in the basin are between 387 and 569 mg L^{-1}. According to TDS classification (Catroll 1962; Freeze & Cherry 1979), all of the springs are freshwater type (TDS < 1.000 mg L^{-1}) (Table 2) and suitable for irrigation purposes.

Electrical Conductivity (EC): According to Richards (1954), electrical conductivity classification, the lowest and highest EC values of the spring waters in the basin during the monitoring period ranged from are 402 to 691 µS cm^{-1} and "Good" class (Table 2).

Total Hardness (TH): The hardness classification of spring waters (Table 2) (Sawyer & Mc-Cartly 1967) shows that most of the samples in the region is "hard" while Cankurtaran-2 (332 mg L^{-1}), Değirmenli (329 mg L^{-1}) and Turgut Pınarı (330 mg L^{-1}) Springs are classified as "very hard". The lowest and highest values of the total hardness of the springs in the dam basin are between 213 - 389 mg L^{-1}.

Sodium Percentage (Na%): Sodium concentration is an important parameter for defining the quality of agricultural irrigation water due to specific harmful effects on soil physical properties (Eaton 1950; Doneen 1962; Raju 2007). The long-term average sodium percent (Na%) of the basin springs ranges from 1.29 to 9.28. According to the Na% classification (Wilcox 1955), all of springs classified as "excellent – S1" (Table 2).

Wilcox diagram: One of the most used methods to determine irrigation water quality is the Wilcox (1955) diagram, based on the Na% and EC graph (Figure 4a). All springs fall within the category of "excellent to good" in Wilcox (1955) Diagram according to their sodium percentage are suitable for irrigation purpose.

Sodium Absorption Ratio (SAR): The calculated long-term average SAR values as a range 0.07 - 0.16 meq L^{-1} indicate that the spring water samples are excellent for irrigation purposes (Richards, 1954). Also, all samples are "good quality - C2" due to electrical conductivity values of 438 - 617 µS/cm (Richards 1954) (Table 2). All of the water samples fall in the C2 – S1 class showing "medium salinity" hazard and "low sodium" hazard. These waters can be used to "irrigate all types of soils with little danger of exchangeable sodium" according to Richards (1954) definition (Figure 4b).

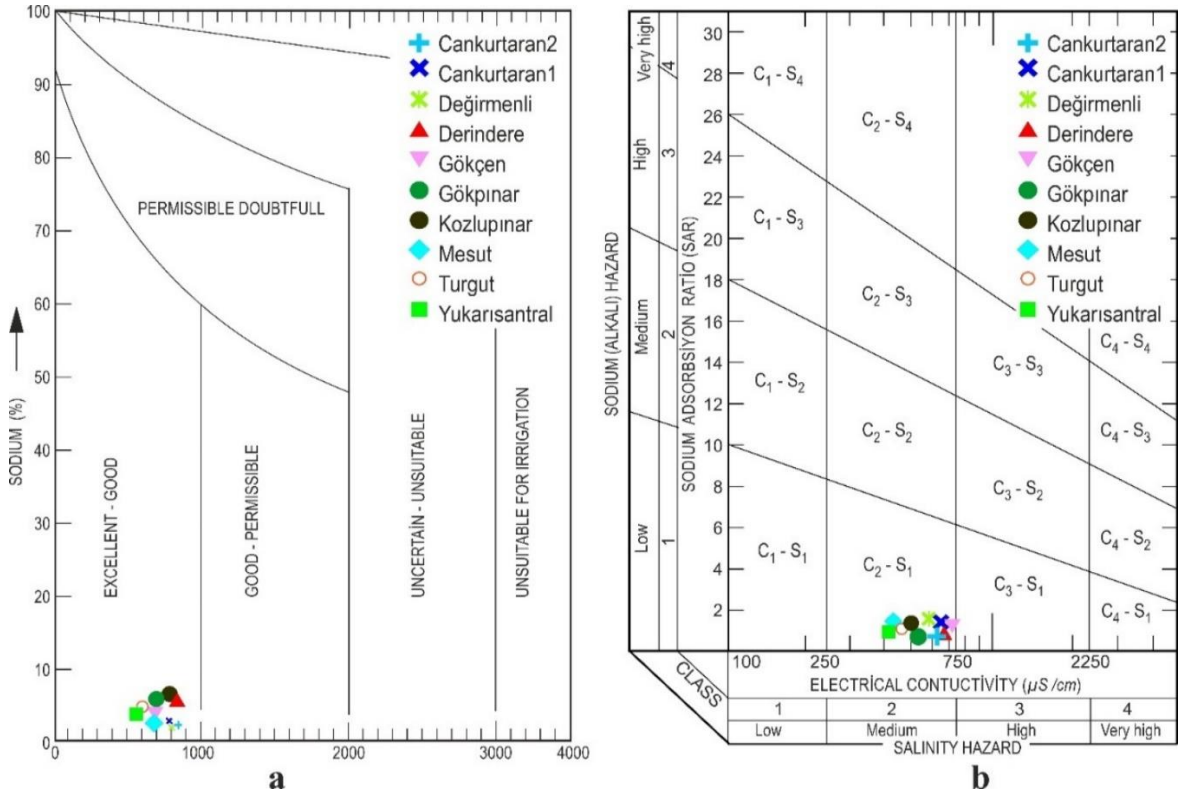

Figure 4- Wilcox (1955) (a) and USSL (Richards 1954) (b) diagrams

Residual Sodium Carbonate (RSC): The calculations showed that the long-term average RSC values of the basin spring waters range from -0.92 to 0.4 meq L^{-1} and water samples are within the safe water category "good" for irrigation (Eaton 1950, Richards 1954) (Table 2). The lowest and highest values of the RSC of the springs in the basin are between -1.3 - 0.47 meq L^{-1}. Based on the RSC, the springs in the "doubtful" category are Gökçen, Değirmenli, and Turgut Pınarı (Table 2).

Residual Sodium Bicarbonate (RSBC): RSBC values of water samples vary from 0.10 to 2.3 meq L^{-1}. Therefore, RSBC values of all spring waters are smaller than five, all of them considered "Satisfactory" for irrigation (Eaton 1950; Richards 1969) (Table 2).

Magnesium Ratio (MR): The lowest and highest values of MR of the spring samples in the basin are between 19.8% - 39.4%. The springs of Cankurtaran-2 and Kozlupınar are the waters with the highest MR. The fact that the MR value of all the spring samples is below 50 indicates that all springs in the dam basin are not harmful for irrigation in terms of magnesium content (Raghunath 1987; Szabolcs & Darab 1964) (Table 2).

Table 3- Pearson correlation coefficients of variables taken for spring waters

	Cl^-	F^-	HCO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	NO_3^-	NO_2^-	TDS	pH.
Cl^-	1											
F^-	-0.242	1										
HCO_3^-	0.847*	-0.328	1									
SO_4^{2-}	-0.440	0.574	-0.760	1								
Ca^{2+}	0.692	-0.029	0.814*	-0.484	1							
Mg^{2+}	0.592	-0.061	0.416	-0.155	0.150	1						
K^+	0.613	-0.333	0.616	-0.422	0.320	0.083	1					
Na^+	0.812*	-0.156	0.681	-0.277	0.449	0.610	0.682	1				
NO_3^-	0.807*	-0.276	0.733	-0.290	0.755	0.237	0.540	0.646	1			
NO_2^-	-0.268	-0.067	-0.059	-0.073	0.067	0.138	-0.443	-0.515	-0.067	1		
TDS	0.819*	-0.185	0.958*	-0.545	0.851*	0.466	0.610	0.773*	0.838*	-0.119	1	
pH.	-0.633	-0.043	-0.524	0.225	-0.692	-0.273	-0.063	-0.415	-0.760	-0.111	-0.612	1

*: Correlation is significant at the 0.01 level.

3.4. Spatial Distribution (GIS based spatial mapping)

GIS is frequently used in water resources management and provides fast and practical, decision making (Babiker et al. 2007; Ozelkan & Karaman 2018). The spatial distribution maps of the irrigation water quality parameters (EC, TH, SAR, RSC, RSBC, and MR) are prepared using the Spatial Analyst Extension and "spline interpolation with barriers" techniques of ArcGIS (version 10.2.2) (Figure 5). Parameters such as EC, TDS, TH, RSC, and RSBC comply with irrigation water standard values in all sources in the basin. However, it is relatively higher in urbanization and agricultural areas.

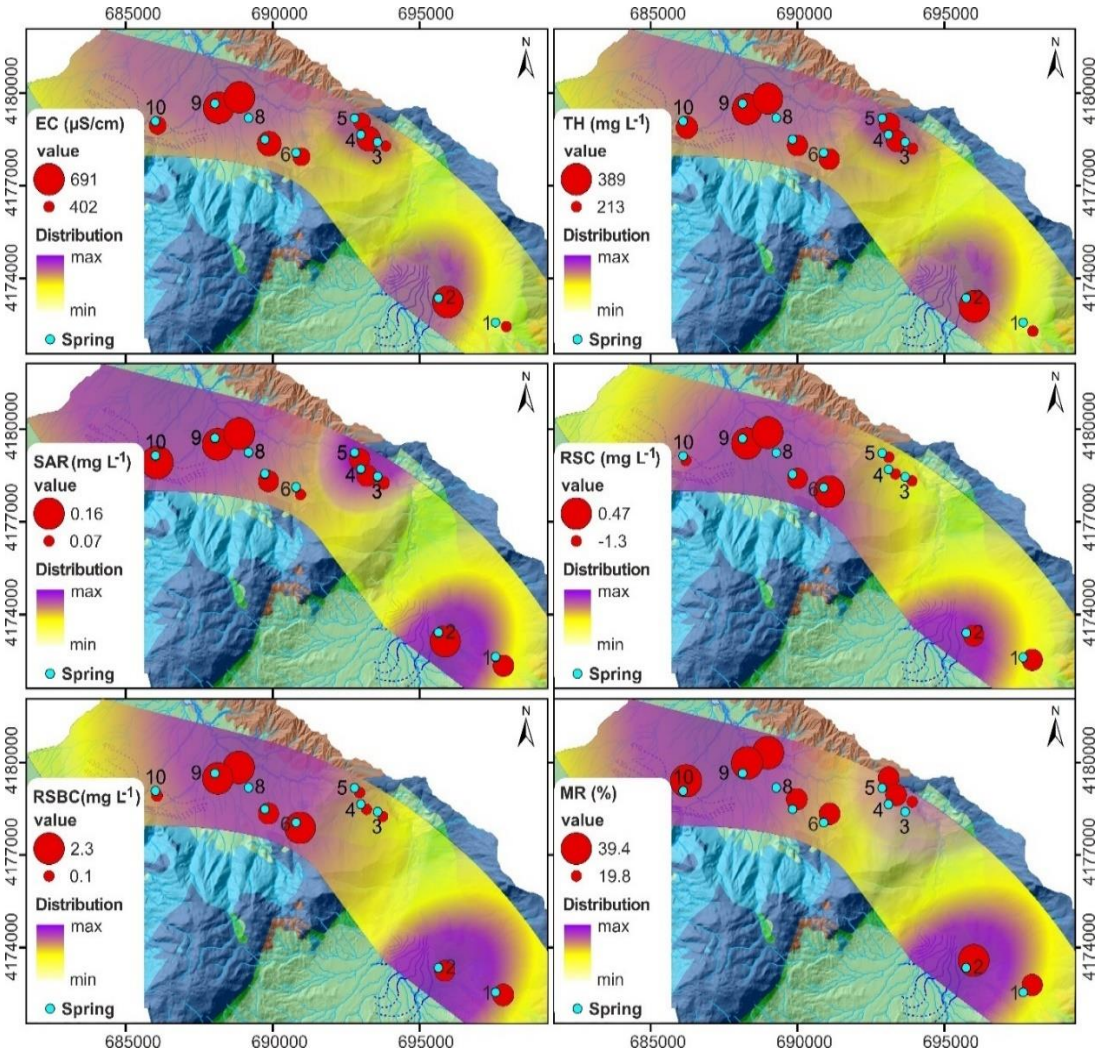

Figure 5- Spatial distribution maps of irrigation water quality of spring waters

4. Conclusions

Spring waters are characterized as fresh type, good quality, alkaline in nature, and hard-very hard based on TDS, EC, pH, and TH respectively. Generally, the domination of major ions is in the order of $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+} > F$ for cations and $HCO_3^{-} > SO_4^{2-} > Cl^{-} > NO_3^{-}$ for anions. Gibbs plot specifies that all the basin spring waters' chemical evolution is mainly controlled by the "rock dominance" process. The major hydro-geochemical facies of waters were $Ca^{2+} – Mg^{2+} – HCO_3^{-}$ water type. Alkaline earth metals (Ca^{2+}, Mg^{2+}) and weak acid (CO_3^{2-}, HCO_3^{-}) dominates over the alkalies (Na^{+}, K^{+}) and strong acid (Cl^{-}, SO_4^{2-}) in all spring waters, respectively. According to Wilcox (1955) and USSL (Richards, 1954), all of the spring waters are “excellent to good” categories for irrigation and they can be used to irrigate all types of soils with little danger of exchangeable sodium (C2-S1). Concentration levels of bicarbonate, carbonate, magnesium, and sodium in all spring waters are suitable for irrigation based on RSC, RSBC, MR, SAR, %Na classification. Consequently, the hydrogeochemical analysis of investigation in the study area displayed that concentrations of the major ions and values of important hydrochemical parameters were within the permissible limits for irrigation. The major influence on the chemistry of the basin groundwaters is naturally occurring processes carbonate, silicate, and evaporites weathering. The largest freshwater resources in the region are the aquifers in the Mesozoic marine carbonates of the pre-Neogene basement rocks. The results of this study provide valuable hydrogeochemical information on irrigation water characteristics of resources but need further data to precisely identify hydrogeological processes. Detailed hydrogeological studies including groundwater and surface water hydrogeochemical surveys will be useful for the protection and sustainable management of all water resources in the area. The results obtained in this paper can be a helpful guide to take the first step in such initiatives in the study area.

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Effects of Season and Phenology-based Changes on Soil Erodibility and Other Dynamic RUSLE Factors for Semi-arid Winter Wheat Fields

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ABSTRACT
Time-dependent and phenology-based erodibility assessments in agricultural areas are extremely important for a more accurate evaluation of erosion. This paper aims to investigate soil erodibility factor (RUSLE-K) of the "Revised Universal Soil Loss Equation (RUSLE)" model in terms of phenological and seasonal variations in the 50 different winter wheat growing parcels with the interactions other dynamic RUSLE factors (RUSLE-R, RUSLE-C). For that, parcel-based erosion assessments were performed with the help of Dynamic Erosion Model and Monitoring System, digital elevation model, and satellite images in Polatlı, Ankara. Findings showed that RUSLE-K factor varied from 0.0150 to 0.0357 t ha h ha^{-1} MJ^{-1} mm^{-1} during the period the seeding germination to the end of the tillering from autumn to spring, and the lowest RUSLE-K was obtained when the plant was in the three-leaf stage. After the frost-free period, corresponding to the flowering and fertilization stages of the wheat plant, the RUSLE-K values changed between 0.0786 and 0.0976 t ha h ha^{-1} MJ^{-1} mm^{-1}. This reveals that erodibility can vary up to nine times due to seasonality. However, the other dynamic model factors are not taken into consideration. Considering all dynamic factors on soil losses, the change coefficients from the highest to the lowest were obtained for RUSLE-R, RUSLE-K and RUSLE-C, respectively. These changes caused soil losses to change by 82% during the year. So, this study is expected to shed new light on studies of wheat or other commonly cultivated crops to accurately assess the water erosion risk as a significant land degradation problem.

Keywords: Erodibility, Modeling, Normalize different vegetation index (NDVI), Satellite images, Sustainability, Water erosion

1. Introduction

Soil erosion is known to be significant threat to sustainability in the context of ecosystem services. In particular, it is primarily responsible for land degradation in the cultivated areas located in fragile ecosystems (FAO & ITPS 2015). In Turkey, water erosion is a major problem and the predicted average soil loss rate is higher than 5 t ha^{-1} y^{-1} in the 26.4% of agricultural lands (Erpul et al. 2020). Especially in the wheat production areas, which constitute 67% of the agricultural areas in which field crops are cultivated, it leads to a significant reduction in production potentials at the national scale. However, wheat demand tends to increase due to rising population density (Anonymous 2019). Hence, accurate estimation of land productivity under the accelerated soil erosion dynamics has great importance in terms of conservation natural resources. In this context, water erosion rates have been predicted by the empirically based 'Universal Soil Loss Equation (USLE)' or its renewed version 'Revised Universal Soil Loss Equation (RUSLE)' by integrating Geographic Information Systems (GIS) as of the beginning of the 21st century (Saygin et al. 2014; Panagos et al. 2015).

But, one of the most common criticisms of the RUSLE model is the over-estimation for actual soil loss rates. Although this situation is mostly associated with the topography factor of the model due to the increase in estimated soil losses at slopes steeper than 9% (McCool et al. 1987), there are some studies that emphasize that the rate of change in erodibility is quite high (Renard et al. 1994; Panagos et al. 2014; Benavidez et al. 2018).

Soil erodibility factor (USLE-K) stands for the resistance against to erosive agents of soil, and mostly correlates with intrinsic soil properties as a significant variable of the USLE model. Thus, it can be calculated by several equations based on these examined relationships instead of through laborious field studies (Römkens et al. 1997).

On the other hand, one of the most important problems encountered in simulating the USLE-K is the lack of seasonal assessment in terms of the simulation of the antecedent water contents and soil surface variations. Originally, it was thought of as a constant parameter and dominantly controlled by some soil properties such as soil texture. But studies over the past few decades have clearly shown that soil erodibility was not a constant variable due to heterogeneity in the physicochemical structure

of soil that is influenced by many factors such as the time-dependent effects of canopy cover and climatic conditions. Indeed, seasonal variations in some soil conditions such as soil aggregation, crust formation, sealing, soil moisture contents, and freezing-thawing conditions significantly affects the shear stress of the soils (Huang & Laften 1996; Huang 1998; Auerswald et al. 2014). To increase the accuracy of the soil erodibility estimations, unlike in the USLE model, the equations were developed for calculating the semi-monthly soil erodibility factor (RUSLE-K) for a year in the RUSLE model. In this manner, the seasonal variation's effect on soil erodibility during the phenological development stages of crops can be easily simulated for agricultural areas in the RUSLE model instead of using a constant USLE-K value for a year (Renard et al. 1997).

Even though the effects of seasonal changes on soil erodibility, soil structure, crust formation and shear strength have been widely known from previous studies, RUSLE-K factor has been mostly used as a constant variable similar to USLE-K (Alewell et al. 2019). But, considering cultivated areas and phenological development stages of the different crop types, the assessment of monthly and seasonal variations in susceptibility of soils to erosive conditions is an important requirement due to strong climatic changes, especially in the fragile ecosystems characterized by irregular and intensive rainfalls, low vegetation cover, and fragile soil structure. At this point, the assessment of the time-dependent/seasonal changes' effects on the RUSLE-K values was defined as an important requirement, especially for sustainability of the cultivated areas in the arid and semi-arid climate zones (Ostovari et al. 2019). In this way, more accurate soil loss estimations can be achieved to determine the critical erosion periods if the time-dependent changes in soil erodibility are considered (Wu et al. 2018). At this point, interactions of RUSLE-K and RUSLE-C factors on erosion rates based on the rainfall erosivity changes in time periods should be taken into consideration on the axis of lumped structure of the RUSLE model. The cropping effect on soil losses is originally expressed by the RUSLE-C factor in RUSLE (Renard et al. 1997). And, it reveals the difference the soil loss rates between cropland and clean-tilled, continuous fallow field condition (Wischmeier & Smith 1978). Recently, it is determined by the help of remote sensing technology, which allows for easier and more reliable predictions of temporal and spatial differences of plant cover efficiency on soil erosion losses (Panagos et al. 2015). For example, Alexandridis et al. (2015) investigated the seasonality effect for estimating the RUSLE-C factor by using normalize different vegetation index (NDVI). They found a significant difference among their time-dependent evaluations. Similarly, Möller et al. (2017) studied on the parcel-specific NDVI profiles for phenological phases of the wheat plant, and they proposed the phenological evaluation scheme for the NDVI time series. Surely, if these and other similar studies, which are mainly related to the temporal changes in RUSLE-C and RUSLE-R factors during the year, can be developed to cover the changes in RUSLE-K factor with the time-dependent or phenology-based approaches, the accuracy rate of estimations for RUSLE model studies will be increased.

Thus, it was aimed to investigate the changes in soil erodibilities in terms of phenological development stages of winter wheat depending on the seasons in semi-arid Anatolian conditions. Regardingly, the variations among dynamic RUSLE factors (e.g. RUSLE-R, RUSLE-K and RUSLE-C factors) and their effects on soil loss rates were analysed in the semi-arid parcel-scale corresponding to the studied time periods. It was also attempted to present a more accurate and effective methodological framework to sustainably manage the soil resources by using limited databases and RUSLE methodology to evaluate water erosion risk on arid and semi-arid agricultural areas.

2. Material and Methods

2.1. Characteristics of the study area

The studied region is 78 km far from the capital of Turkey, and located in the Ilıca-Polatlı district at an average elevation of 886 m above mean sea level in the sub-agriculture basins of the Sakarya River. The geomorphological structure of the region consists of plateaus around hills and is generally comprised of conventional crop parcels under semi-arid Anatolian conditions. Winter wheat is the most common crop grown in the conventional bare fallow conditions for more than fifty years in the region. Due to climatic constraints, mostly preferred bread wheat varieties in the region were Bezostaia-1 (Spineless, white glume, hard grain, medium height), Tosunbey (Spined, white glume, white, hard grain, medium height), Esperiya (Spined, spike color white, grain color red, hard grain), Gerek-79 (Spined, glume and spike color brown, soft white grain, medium height) and Sonmez-2001 (Spineless, spike color white, grain color red, hard grain) according to the interviewed expert and farmers in the region. These types of bread are generally of very high quality and are known to be resistant to cold, drougth and bedding (Aktaş & Ünver İkincikarakaya 2010; Karabak et al. 2010; Gebremariam et al. 2020; Korkut et al. 2019; Baser 2020; Cevher et al. 2020; TGB 2020).

The climatic structure of the region was classified as cold semi-arid (type 'BSk') (Peel et al. 2007). Based on the region's 50-year average meteorological data, annual precipitation rate is 368 mm. The minimum, maximum, and average annual temperatures are -17.7 °C, 32°C, and 11.7 °C, respectively. Severe weather conditions in the region have led to freezing in the topsoil layer. The top 5 cm of soil are under frost conditions at least 41 days in a year. And, the depth of this frost layer is known to reach up to 20 cm deep from December to March (TSMS 2018). Within the scope of the study, 50 different rain-fed wheat farming parcels located around the Ilıcaozu, Hamamozu, and Korcesme streams - which pass through the region and feed the Sakarya River — were selected and sampled from 0 to 20 cm soil depth from each parcel, and, they dried and passed through a 2-mm sieve to use for chemical and physical analyses in the laboratory in order to predict the seasonal RUSLE-K values at the

parcel scale in the fields. Wheat cultivation is carried out in all of these selected parcels under fallow conditions, and the coordinates of the sampling points are provided referenced with geographical projection GCS-WGS-1984 (Table 1).

Table 1- Soil sampling points (decimal degrees)

Parcel no	*POINT_X*	*POINT_Y*	*Parcel no*	*POINT_X*	*POINT_Y*	*Parcel no*	*POINT_X*	*POINT_Y*
1	39.310	32.206	18	39.312	32.223	35	39.280	32.223
2	39.312	32.208	19	39.310	32.223	36	39.279	32.210
3	39.315	32.206	20	39.305	32.216	37	39.281	32.210
4	39.315	32.208	21	39.304	32.220	38	39.285	32.213
5	39.320	32.207	22	39.306	32.221	39	39.285	32.211
6	39.317	32.207	23	39.314	32.230	40	39.285	32.210
7	39.322	32.209	24	39.313	32.227	41	39.284	32.210
8	39.324	32.210	25	39.303	32.222	42	39.285	32.208
9	39.326	32.212	26	39.302	32.224	43	39.300	32.187
10	39.328	32.214	27	39.304	32.229	44	39.302	32.192
11	39.327	32.216	28	39.305	32.230	45	39.304	32.201
12	39.324	32.212	29	39.306	32.231	46	39.303	32.190
13	39.320	32.214	30	39.305	32.232	47	39.303	32.186
14	39.319	32.212	31	39.309	32.233	48	39.305	32.188
15	39.320	32.209	32	39.325	32.249	49	39.308	32.201
16	39.318	32.209	33	39.300	32.245	50	39.308	32.205
17	39.313	32.209	34	39.301	32.243			

2.2. RUSLE model components

The RUSLE methodology predicts soil erosion rate by evaluating rainfall erosivity, soil erodibility, topographic structure, vegetation and support practices efficiency (Renard et al. 1997; Wischmeier and Smith 1978) (Eq. (1)).

$$A = R \times K \times L \times S \times C \times P \quad (1)$$

Where, A; mean annual soil loss (t ha^{-1} y^{-1}), R; rainfall erosivity (MJ mm ha^{-1} h^{-1} y^{-1}), K; soil erodibility (t ha h ha^{-1} MJ^{-1} mm^{-1}), L; slope length, S; slope steepness, C; cover management, and P; support practice factors.

RUSLE-R (Rainfall erosivity factor) was derived from the Dynamic Erosion Model and Monitoring System in the form of annual total, monthly and semi-monthly RUSLE-R distributions (Erpul et al. 2016).

Several equations were proposed for predicting the soil's resistance in terms of RUSLE-K factor for USLE/RUSLE model. However, nomograph equation is originally proposed to estimate seasonal soil erodibility values in RUSLE methodology (Renard et al. 1997). It is clearly known that nomograph is more suitable for less aggregated and medium-textured sandy and loamy soils than the clay soils such as Turkey (Römkens et al. 1997; Baskan & Dengiz 2008; Kapur et al. 2017; Alewell et al. 2019). For these reasons, the equation (2), proposed by Torri et al. (1997, 2002), was selected to estimate annual RUSLE-K factors for the studied parcels. Besides less data requirement, this equation reveal an appropriate relationship for soils having strong aggregate formation mechanism by considering the soil's organic carbon and clay rates with other particle size classes in order to predict the RUSLE-K (Borselli et al. 2012). Also, it has been used in Turkey for generating a RUSLE-K map at the national scale (Erpul et al. 2020) and tested in different Anatolian conditions at the parcel and basin scales (Saygin et al. 2011; Yıldırım & Erkal 2013).

$$\text{RUSLE} - \text{K} = 0.0293(0.65 - \text{D}_\text{G} + 0.24\text{D}_\text{G}^2) \times \exp\left\{-0.0021\left(\frac{\text{OM}}{\text{C}}\right) - 0.00037\left(\frac{\text{OM}}{\text{C}}\right)^2 - 4.02\text{C} + 1.72\text{C}^2\right\} \quad (2)$$

Where *OM;* organic matter content, *C;* clay content; *DG;* decimal logarithm for the geometric mean of particle sizes.

The following procedures and equations were used to obtain seasonal RUSLE-K factors from the constant annual RUSLE-K value as proposed by Römkens et al. (1997).

$t_{max} < t_{min}$ If, $t_{max} < t_i < t_{min}$

$$K_i = K_{max}(K_{min}/K_{max})^{(t_i - t_{max})/\Delta t} \quad (3)$$

Where K_i = RUSLE-K factor at any time (t_i in calendar days), K_{max} and K_{min}; RUSLE-K factor at times t_{max} and t_{min}, Δt; length of frost-free period or growing period (≤ 183 days), T_{av}; average daily air temperature.

If, $t_i < t_{max}$ or $t_i > t_{min}$, then for T_{av} >-2.8 °C.

$$K_i = K_{min} exp[0.009(t_i - t_{min} + 365\delta] \quad (4)$$

With δ=1 if $(t_i - t_{min}) \leq 0$ and δ=0 if $(t_i - t_{min}) > 0$ and for $T_{av} \leq$ -2.8 °C,

$$K_i = K_{min} \quad (5)$$

If $K_i > K_{max}$ $K_i = K_{max}$ (6)

Or, If $K_i < K_{min}$ $K_i = K_{min}$ (7)

Based on the proposed relationships, the following equations were used to calculate K_{max}, K_{min} and t_{max} variables.

$$K_{max}/K_{min} = 8.6 - 0.01R \quad (8)$$

$$K_{max}/K_T = 3.0 - 0.005R \quad (9)$$

$$t_{max} = 154 - 0.44R \quad (10)$$

If, $t_{max} < 0, then$ $t_{max} = t_{max} + 365$ (11)

Where R = rainfall erosivity factor (RUSLE-R), K_T = annual RUSLE-K factor.

The time span for the phenological development stages of winter wheat was defined by taking into consideration national expert interviews and Landsat ETM + satellite images in the studied region.

The slope length (RUSLE-L) and slope steepness (RUSLE-S) factors, which are together defined as the topographic factor in the RUSLE (RUSLE-LS). This factor was obtained by an interaction between topography and flow accumulation (Eq. (12)) (Moore & Bruch 1986a, 1986b). Slope steepness was calculated by from Digital Elevation Model (DEM), and slope length for each pixel was evaluated as 15 m (Ogawa et al. 1997; Lee 2004).

$$LS = \left(\frac{\chi\eta}{22.13}\right)^{0.4} \cdot \left(\frac{\sin\theta}{0.0896}\right)^{1.3} \quad (12)$$

Where, χ; flow accumulation, η; cell size, and θ; slope steepness in degrees.

RUSLE-C factors (Crop management factor) were estimated by normalized difference vegetation index (NDVI) from Landsat ETM + satellite images. For that, 7 images which reflect to the periods of phenological development of the winter wheat were analysed for the studied fields in the 2015-2016 growing season. In there, it has been taken into consideration that conventional winter wheat cultivation in the selected parcels has been continuously carried out for at least five years. The imagery dates which includes the growing period of winter wheat plant were 10/10/2015, 26/10/2015, 20/11/2015, 03/04/2016, 28/04/2016, 30/05/2016 and 17/07/2016, respectively. In the next stage, NDVI values of the images were calculated by Eq. (13) to get RUSLE-C factors as proposed by van der Knijff et al. (2000).

$$C = \exp\left[-\alpha \times \frac{NDVI}{\beta - NDVI}\right] \quad (13)$$

Where; α and β: NDVI–C curve shape parameters (van der Knijff et al. 2000).

Since there were no agricultural and mechanical conservation practices applied for decreasing soil loss rates, the support practice factor (RUSLE-P) was assumed as 1.

Soil loss rates are estimated to correspond to satellite imaging dates and to take into account changes in other dynamic factors. In addition, these values were verified by the average area-weighted suspended sediment amounts measured from 11 different river observation stations since 1961 located in the basin. Besides that, the values were compared with the annual soil loss rate obtained from national soil erosion map statistics in Turkey (Erpul et al. 2020).

3. Results and Discussion

3.1. Soil properties

The studied soils have averagely 1.47% organic matter, 21.29% $CaCO_3$, 37.41% clay, 33.76% silt, and 28.82% sand and 0.034% salt contents with a pH of 7.79 (Table 2).

Table 2- Descriptive statistics of the primary soil properties

*Variable**	*Mean ± SE***	*StDev****	*Min*	*Max*
pH	7.79 ± 0.21	7.36	7.36	8.31
SC (%)	0.03 ± 0.003	0.02	0.01	0.09
C (%)	37.41 ± 0.93	6.54	25.35	49.93
Si (%)	33.76 ± 0.30	2.15	29.80	37.88
S (%)	28.82 ± 1.23	8.69	12.19	44.85
OM (%)	1.47 ± 0.10	0.69	0.23	3.21
$CaCO_3$ (%)	21.29 ± 1.47	10.42	4.19	58.85

*pH, Soil reaction; SC, Salt content; C, Clay; Si, Silt; S, Sand; OM, Organic matter; $CaCO_3$, Calcium carbonate; **Mean ± SE, Mean values ± standard errors; ***StDev, Standard deviation.

3.2. Time and phenology-based changes in the RUSLE model components

In these clay rich soils, the semi-monthly RUSLE-K factors demonstrated that significant changes occurred in terms of soil erodibility during the year (Table 3). The seasonal variation maps of the parcel-based RUSLE-K values are also figured out of the situation (Figure 1). Before the winter months when the soil temperature is extremely low, and wheat plant is in the three-leaf stage, RUSLE-K value was reached the lowest value (0.0108 t ha h ha^{-1} MJ^{-1} mm^{-1}) in the year. However, the highest RUSLE-K value was estimated as 0.0976 for spring season when rainfall is concentrated, corresponding to the flowering and fertilization stage of wheat.

Table 3- Seasonal and phenological RUSLE-K factors

Seasons	*Phenological periods*	*Intervals*	*Mean ± SE[a]*	*StDev[b]*	*Min*	*Max*
Winter	Vernalization & Tillering	1 - 15 Jan	0.0182 ± 0.0002	0.0011	0.0164	0.0203
		16-31 Jan	0.0210 ± 0.0002	0.0013	0.0189	0.0235
		1 - 15 Feb	0.0240 ± 0.0002	0.0015	0.0216	0.0269
		16-29 Feb	0.0270 ± 0.0002	0.0017	0.0243	0.0302
Spring	Tillering	1 -15 March	0.0309 ± 0.0003	0.0019	0.0278	0.0346
		16-31 March	0.0357 ± 0.0003	0.0022	0.0321	0.0399
	Stem elongation	1 -15 Apr	0.0408 ± 0.0004	0.0025	0.0368	0.0457
		16-31 Apr	0.0467 ± 0.0004	0.0029	0.0421	0.0523
	Booting	1-15 May	0.0535 ± 0.0005	0.0033	0.0482	0.0598
	Flowering & Fertilization	16-31 May	0.0872 ± 0.0008	0.0053	0.0786	0.0976
Summer	Milk stage	1-15 June	0.0734 ± 0.0006	0.0045	0.0661	0.0821
	Dough stage	16-31 June	0.0617 ± 0.0005	0.0038	0.0556	0.0691
	Physiological maturating	1 - 15 July	0.0520 ± 0.0005	0.0032	0.0468	0.0582
	Harvest	16-31 July	0.0432 ± 0.0004	0.0027	0.0389	0.0484
	Bare soil	1 - 15 Aug	0.0364 ± 0.0003	0.0022	0.0328	0.0407
		16-31 Aug	0.0302 ± 0.0003	0.0019	0.0273	0.0339
Autumn		1 -15 Sept	0.0254 ± 0.0002	0.0016	0.0229	0.0285
		16-31 Sept	0.0214 ± 0.0002	0.0013	0.0193	0.024
		1 -15 Oct	0.0180 ± 0.0002	0.0011	0.0162	0.0202
	Seeding & Germination	16-31 Oct	0.0150 ± 0.0001	0.0009	0.0135	0.0168
	Emergence	1-15 Nov	0.0126 ± 0.0001	0.0008	0.0114	0.0141
	Three leaf stage & Tillering	16-31 Nov	0.0120 ± 0.0001	0.0007	0.0108	0.0134
Winter	Tillering	1-15 Dec	0.0137 ± 0.0001	0.0008	0.0124	0.0154
		16-31 Dec	0.0159 ± 0.0001	0.001	0.0143	0.0178
Annual		RUSLE-K[c]	0.0528 ± 0.0005	0.0032	0.0476	0.0591

[a] Mean values ± standard errors; [b] Standard deviation; [c] Annual average RUSLE-K value.

There, the estimated average erodibility values in the autumn season from seeding to tillering stage changed from 0.0156 to 0.0194. Taking into consideration the winter season from tillering to the vernalization stage of wheat, the average RUSLE-K values varied between 0.0179 and 0.0223. However, the values in the spring season were two times higher than the winter season, ranging between 0.0442 and 0.0549 from tillering to milk stage. Similarly, in the summer season from milk stage to harvest, the RUSLE-K values in the region changed between 0.0445 and 0.0553. Annually, average RUSLE-K value of all studied parcels was found to be 0.0528 t ha h ha^{-1} MJ^{-1} mm^{-1}.

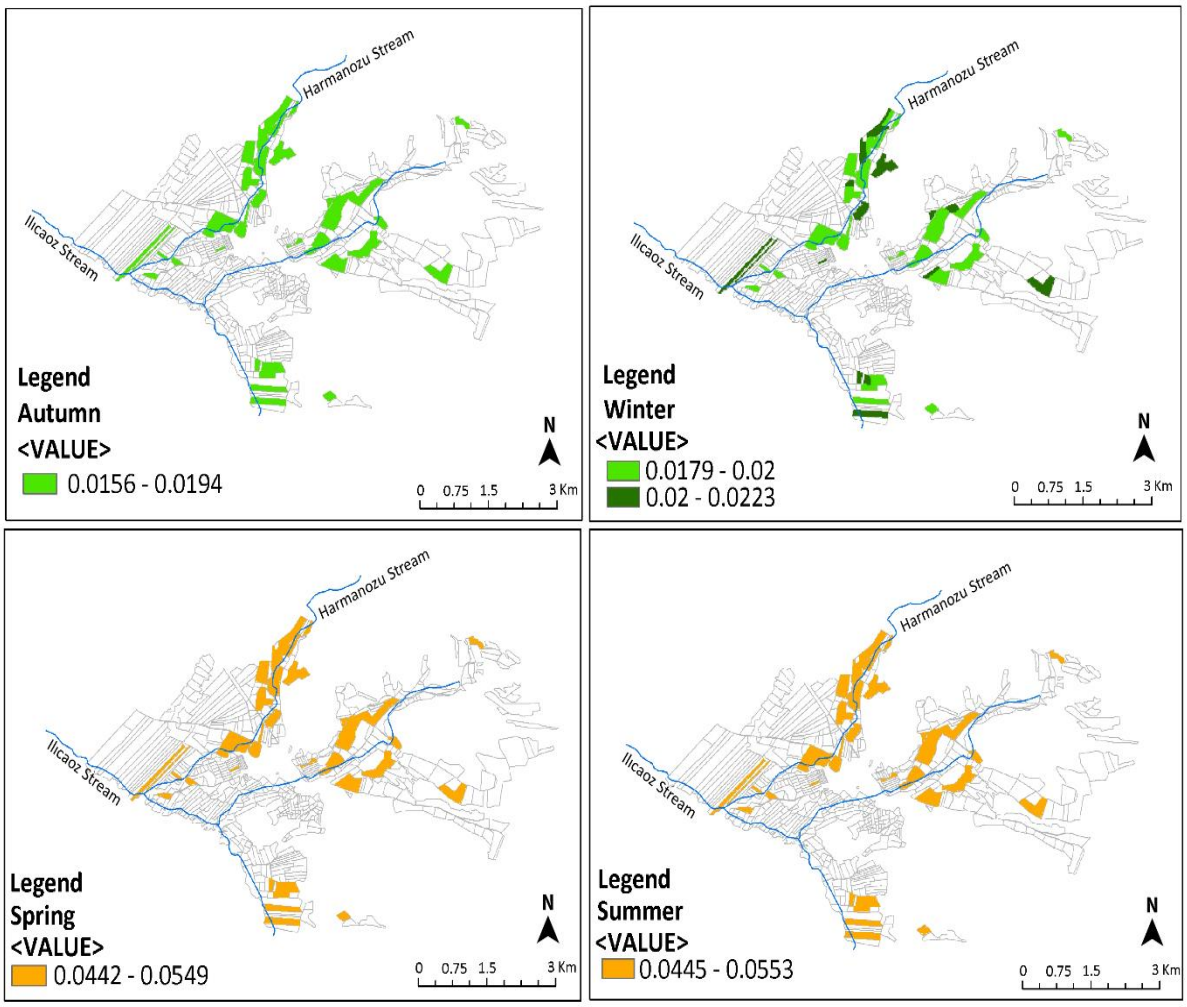

Figure 1- Prediction maps of the seasonal RUSLE-K factors

Time-dependent changes in soil erodibility are generally associated with three soil characteristics. These are freezing, soil texture and soil water (Römkens et al. 1997). In the study, it is clearly observed that freezing conditions at surface lead to lower erodibility values of the studied parcels from autumn to spring. As stated by Kværnø and Øygarden (2006), seasonal freezing lead to profound effects on soil erodibility. Phenologically, this period corresponds to a time-span covers from seeding germination to the vernalization stages in the winter wheat plant. And, RUSLE-K factors had the lowest values, ranging from 0.0108 to 0.0168. Because of decreasing soil temperatures during this period, the soils have more stable and impermeable structure (Oztas & Fayetorbay 2003).

With the end of frost conditions, higher soil moisture content increases the susceptibility of the soils against to erosive agents due to the weakening of soil strength in spring (Bajracharya & Lal 1992). This effect can be clearly seen from stem elongation to flowering and fertilization stages (1 April – 31 May). After this frost-free period, intensive rainfalls make the soils more vulnerable to detachment processes, especially in Mediterranean environments (Arnaez et al. 2007; Comino et al. 2016), and the predicted highest erodibility value in the flowering and fertilization stage (16 - 31 May) clearly reveals this situation. Thus, the soils reached the highest erodibility values, ranging between 0.0786 and 0.0976 in spring (Table 4). Certainly, the presence of canopy cover on the soil surface is an extremely important variable in this stage in which increased sensitivity has a serious effect on sediment yield (Loch, 2000). According to the typical canopy cover values of winter small grain plants (Yoder et al. 1997), 35% of the soil surface is generally covered with plant during this phenological stage. In a general mean, 65% of the field is exposed to the destructive effects of rainfall during this stage (Renard et al. 1997). Within the scope of the study, RUSLE-C values estimated from NDVI values obtained with the help of satellite images confirm the current literature (Figure 2).

Table 4- Descriptive statistics for the RUSLE variables

Variable	*Mean ± SE*[a]	*StDev*[b]	*Min*[c]	*Max*[d]	*Var*[e]	***CV***[f]
RUSLE-R	7.80 ± 2.13	5.65	0.85	14.58	31.88	**72.34**
RUSLE-K	0.0376 ± 0.0099	0.0263	0.0120	0.0872	0.00069	**69.90**
RUSLE-LS	2.95 ± 0.00	0.00	2.95	2.95	0.00	0.00
RUSLE-C	0.546 ± 0.108	0.286	0.118	0.854	0.082	**52.30**
A	0.332 ± 0.102	0.271	0.0257	0.805	0.0732	**81.49**

[a] Mean values ± standard errors; [b] Standard deviation; [c] Minimum values; [d] Maximum values; [e] Variance; [f], Coefficient of variation.

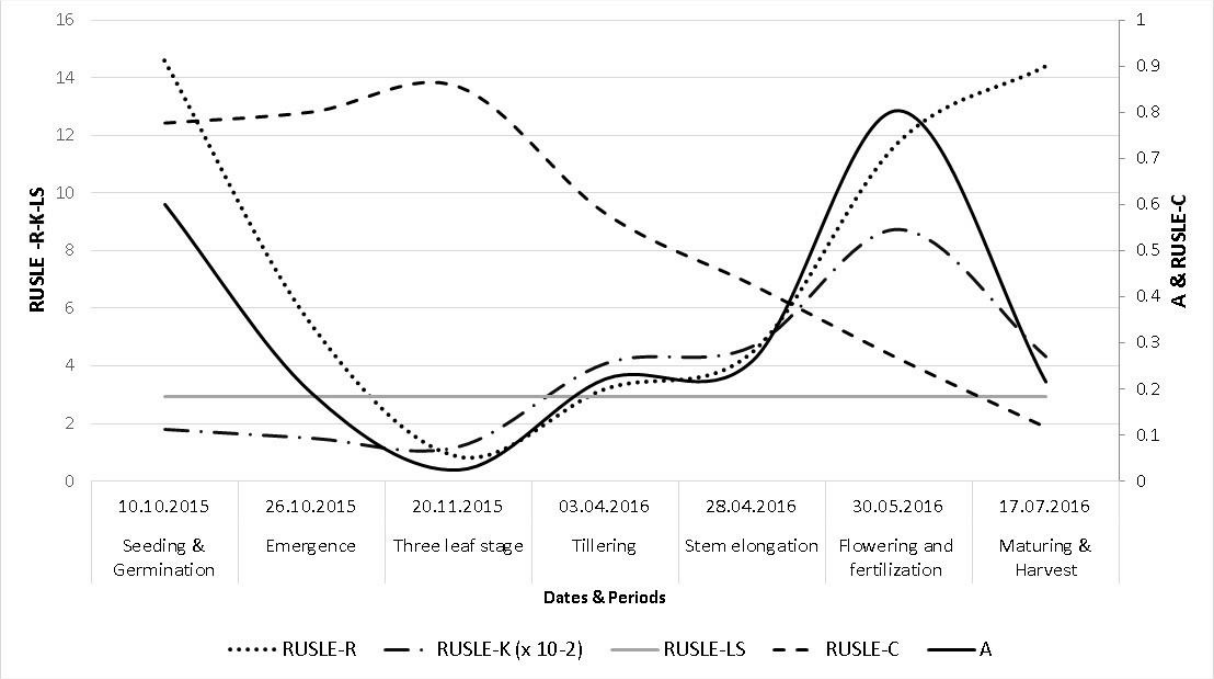

Figure 2- Predicted RUSLE sub-factors and the soil loss rates

In summer, increasing soil temperatures and decreasing soil-water contents under the fallowing typical Mediterranean semi-arid climate conditions lead to gradually decreasing erodibility potentials of the soils (López-Vicente et al. 2008). This time-span phenologically includes the period from the milk stage of the plants to the harvest. Evidently, the findings showed that the RUSLE-K values gradually decreased as a result of increasing temperatures and decreasing moisture contents of the soils. In this manner, it is obviously stated that erodibility potential of the soils changes up to nine times during a year and this situation can lead to significant changes on soil loss estimations when the other factors in the RUSLE model are not considered.

On the other hand, it is also known that changing climatic and vegetation coverage conditions lead to a temporary change in the dynamics within the RUSLE-R and the RUSLE-C factors, not only in the RUSLE-K (Ferreira & Panagopoulos 2014). For example, Baiamonte et al. (2019) investigated the RUSLE-R and RUSLE-C factor's time scale effects and their inter- and intra-annual interactions in terms of soil erosion variability. Similarly, Schmidt et al. (2018) also studied on temporal patterns of vegetation to evaluate spatial and temporal variations of RUSLE-C by measuring the temporal variation of vegetation fraction factor based on soil loss rates and RUSLE-R factor ratios. Apart from these, other model researchers have drawn attention to the same issue and pointed out that seasonal changes on soil losses are particularly closely related to the R and C factors (Panagos et al. 2015). Although the effects of climatic differentiation on model-based soil loss estimates are emphasized, it is thought that there are serious changes in soil erodibility and significant interactions with other factors (Sanchis et al. 2008). Therefore, the other RUSLE sub-factors were also estimated in the study. For this purpose, the changes in each model sub-factor were evaluated for phenological periods in which wheat plant was found in seven different dates where satellite images were taken. And, the lowest soil losses were phenologically estimated at three leaf stage of the plant, that was, at the time when rainfall erosivity and soil erodibility factors were the lowest, although RUSLE-C had the highest value in the plant growing period (Figure 2).

In a comparison to be made in terms of coefficient of variations (CV) values of the dynamic RUSLE sub-factors, the highest variance was observed in RUSLE-R and the second was RUSLE-K, and lastly RUSLE-C. The effect of these changes leads to approximately 82% change in soil losses according to the image dates and corresponding phenelogical periods (Table 4).

This situation reveals that the role of time-dependent changes in RUSLE-R and RUSLE-K factors and their interactions' effects on soil loss rates are notableand these factors should not be evaluated as a constant variable, especially in fragile

ecosystems due to the seasonal changes in soil moisture conditions (Huang 1998). In general, the direct impact of seasonal variations on soil erodibility is often overlooked in modeling studies (Sanchis et al. 2008; Alewell et al. 2019). One of the most important issues to be pointed out in the study is to reveal the effect of changes in the rainfall erosivity and erodibility on the soil loss rates by assuming no cover efficiency (representing RUSLE-C factor in RUSLE) and conservation practices (representing RUSLE-P factor in RUSLE). Certainly, the presence of canopy cover on the soil surface which means the decrease in RUSLE-C remarkably limits soil losses especially under heavy rainfall conditions where soil has higher susceptibility to erosive forces (Gallo et al. 2005). In addition, it is known that during periods when the soil surface is bare, especially in the semi-arid and arid agricultural areas of the Mediterranean climate zone, accurate prediction of the changes in RUSLE-R and RUSLE-K variables have a significant impact on combating water erosion threat (Panagos et al. 2015).

3.3. Comparing suspended sediment rates with model-based soil loss estimations

In the Sakarya basin, annual area-weighted suspended sediment rate measured regularly since 1961 from 11 observation stations is 0.79 t ha^{-1} y^{-1} and annual particle detachment rates due to water erosion processes is estimated as 4.2 t ha^{-1} y^{-1} by RUSLE model (Erpul et al. 2020). Estimated average soil loss rates from this parcel-based model study were lower than actually observed sediment yields from the region (Table 4). This is closely related to topographic conditions. Lower slope degrees in the studied parcels have caused to predict lower soil erosion rates compared to the long and steep flow paths in the regional scales or stream basins in real (Alewell et al. 2019).

Consequently, this investigation can give significant support to product-based soil erodibility assessments by evaluating the time-dependent and phenology-based variations for rain-fed, wheat-growing parcels in Anatolian conditions. In addition, seasonality in terms of the erodibility factor in the USLE/RUSLE model was not sufficiently explored for arid and semi-arid environments. In this context, findings indicate the necessity of time-dependent and phenology-based evaluations to perform more accurate soil erosion assessments for sustaining the fragile agricultural areas.

4. Conclusions

In this study, it was investigated that the changes in the RUSLE-K factor as a dynamic factor of RUSLE model depending on seasonal and product-based axis for semi-arid winter wheat parcels in the central Anatolian condition where traditional wheat production systems are widely applied. In addition, the effects of other dynamic model variables such as RUSLE-R and RUSLE-C factors on predicted soil losses were also evaluated within the RUSLE model approach. Obtained results clearly reveal that seasonal changes in the RUSLE-K factor could have quite significant effects on soil loss rates even if the changes in other dynamic factors are not considered. When all dynamic factors were considered together, the factors leading to the highest variability on soil losses were determined as RUSLE-R, RUSLE-K and RUSLE-C, respectively. Consequently, it is thought that this study can contribute to increasing the accuracy of erosion estimates even in limited soil data sets by raising awareness of similar ecosystems and regions where traditional wheat production systems are widely applied. And so, it is expected to shed new light on studies of other cultivated crop types to more accurately assess the water erosion risk as one of the most significant land degradation problems in these fragile agricultural ecosystems.

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