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Impacts of Covid-19 Pandemic on Global Agriculture, Livelihoods and Food Systems

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ABSTRACT
The Covid-19 pandemic created a massive socio-economic panic in all sectors across the world. The agricultural sector is among the most important and crucial part of developing economics in the world. Therefore, the disruption in agriculture and food systems have significant impacts on the livelihood of a large section of people in the world. With this background, this paper performs an inclusive assessment of the effect of Covid-19 on agriculture and food systems in the major part of the impacted countries. A detailed review was made on reports, scientific publications, press releases, and organizational statements etc. This review addresses and highlights the direct impacts of Covid-19 on global food systems, market access for agricultural produce, food and nutritional security, global economy, labour availability and migration, agricultural input-output connectivity, initiatives to avert the crisis and importance of information technology (IT) system in agriculture. Further, this paper suggests mitigation and coping mechanisms that could be useful to improve and sustain the livelihoods of the people.

Keywords: Food security, Food reserve, Agricultural production, Socio-economic panic, Coronavirus, Price of agri-food products

1. Introduction

The world has to encounter an outbreak of the devastating novel coronavirus (Covid-19) that has been announced by the World Health Organization (WHO) as a global public health emergency (Wang et al. 2020) which causes the huge loss of human lives. The Covid-19 pandemic has been affecting the entire economic system of the globe including trade, labour migration, employment, inflation, supply chains, market access and food security (IFPRI 2020; Galanakis 2020). This resulted in reduction of purchasing power of households and availability of foods, for instance, the postharvest losses of fruits and vegetables around 10-15% in developed countries and about 20-40% in developing countries, higher in some specific crops caused food supply shocks (Kahramanoğlu et al. 2021).

The Covid-19 is unexpected since pandemics *viz* Spanish flu, Severe Acute Respiratory Syndrome (SARS), Polio, HIV, Zika virus, Ebola and the Middle East Respiratory Syndrome (MERS) are the latest outbreak in the world (Ceylan et al. 2020a; Saqr and Wasson 2020). However, the fatality due to pandemics was far more than the major wars (Adda 2016). For instance, in the last century, Spanish flu was a serious catastrophic pandemic, which causes 50 million death worldwide. During the time, risks were minimum because urbanization, globalization and movements between the countries were not as widespread as now and therefore, the chance of disease spread could be minimized as compared to the outbreak of recent Covid-19 (Shrestha et al. 2020).

Once the first Covid-19 case was identified, many developing countries imposed a state of emergency. The precaution measures including the stay at home, country-wide lockdown, quarantine and transport termination are highly challenging for many countries (OECD 2020c). Although, the lockdown permitting movement of the essential food commodities and was supposed to allow the agri-food supply chain to operate without any obstacles. However, the agri-food supply system faces a lot of challenges, including sporadic closure of local food markets and logistics barriers between cities, states and countries (Ceylan and Ozkan 2020a; Laborde 2020; Ivanov 2020). The agri-food distribution networks of all countries across the income scale have been highly disrupted, with strong negative consequences for the economically vulnerable communities (FAO 2020b; Galanaki 2020; Hobbs 2020; Stephens et al. 2020).

In most of the countries, agri-food distribution network activities rely on migrant labours, who had left the cities during post-lockdown, which leads to operational challenges including meats, fish weekly local and farmer's markets were closed in many places (Narayanan 2020; Mahendra Dev and Sengupta 2020; Stephens et al. 2020). In an early stage, the demand and supply of agri-food systems experienced a sense of confusion due to ambiguity and anxiety. However, more groceries stores, restaurants, hotels, schools, colleges and shopping malls were shut down and the departure of migrant workers resulted in demand for many commodities including milk, meats, fresh fruits, and vegetables dropped (Narayanan and Saha 2020a). Daily wages workers loss their work which results in food insecurity among the daily wage community (Swan 2020; Seth and Vishwanathan 2020). Also, the supplies of food have been interrupted on a large scale and poor people around the world will suffer from hunger if the local bodies, NGOs, governments and other associated organizations do not take any remedial actions (Torero 2020; The Guardian 2020). Although the stages of executing Covid-19 preventing measures may differ concerning the situation and have been considerably successful in controlling the outbreak of Covid-19. However, an inclusive assessment of the global pandemic effects is absent in the developing world. Given this situation, we attempted to review the consequences of Covid-19 on global agricultural and food systems. We mainly track and focused on unexpected risks, weaknesses and systemic shifts to understanding the short-term effects as well as those that may be long-lasting or permanent.

2. Direct impacts in global food systems

At the beginning of 2020, many countries have announced the lockdown and it has extended many times, a year after so the lockdown continuing in many parts of the countries due to the second and third wave of Covid-19. The lockdown means a complete loss of income for the daily wage workers, these represented 80% of the labour force in poor countries and 76% in sub-Saharan Africa (World Bank 2020). To overcome this crisis, the governments have taken certain possible measures to protect food security but worldwide food security has been exacerbated completely due to disruption in the food supply chain. In many poor countries, people die due to a lack of awareness, hunger and poverty. The extreme poverty level is projected to increase at a rapid rate (World Bank 2020). In order to ensure the stability of food production and supply, agricultural activities have been typically exempted from lockdown. However, the harvest of fruits and vegetables that are generally perishable had declined due to labour scarcity (Stephens et al. 2020; Phillipson et al. 2020). Also, the ongoing crisis is contributing to increasing logistical problems including the distribution of food to the right place at the right time (Held 2020; Poppick 2020).

Further, interruptions to agri-food marketing systems and price volatility would be the key factors for global food insecurity. During this pandemic, food price fluctuations are an obvious consideration in the underdeveloped and developing countries for both income of farmers and purchasing power of the consumers (Barrett 2020; Bellemare 2015). The marketing cost of agricultural commodities has been increased due to difficulties in logistics linked to the pandemic (Hahn 2020) and extend the wedge between farmers and consumer prices (Reardon et al. 2020; Lusk 2020; Reardon et al. 2020; Narayan and Saha 2020a). The food price increasing trends have been noted most often for perishables such as meat, fish, and vegetables (Akter 2020; Mogues 2020; Lele et al. 2020; de Paulo Farias and de Araújo 2020; Yu et al. 2020) and declines in other cases (Harris et al. 2020; Narayan and Saha 2020b). To overcome, (I) it is important to support losses of income from workers who are unable to purchase food products, (II) minimizing the wastage of fresh vegetables, fruits, and milk by permitting food transports from point of production to local markets or supermarkets.

3. Food and nutritional security

Recent reports indicate that nearly 140 million people will extremely suffer from food access, hunger, and food insecurity due to the Covid-19 pandemic (Barrett 2020; Laborde et al. 2020). A massive of them are in the sub-Saharan Africa region, where one out of five people were in hunger in 2019, which cruxes on the ability to acquire healthy and nutritious food (FAO, IFAD, UNICEF, WFP and WHO 2019). This delicate stability between agricultural productivity, poverty and food security particularly in the economically poor sections of the community (Hajra and Ghosh 2018; Priyadarshini and Abhilash 2020; CMIE 2020). During the lockdown, food prices were declining steadily. However, supply distractions due to the lockdown have upturned the movement of food prices (FAO 2020c). The changes in food supply and price are now alarming the food insecurity of people (De Sousa 2020). These burdens on food security triggered by the pandemic may not be affected all countries at the same vigour (Evelyn 2020). The condition is even more painful in East Africa, where the coronavirus is hampering efforts to fight against locust swarms (United Nations 2020).

Further, the lockdown prompted school closures are probable to lead to nutritional insecurity for children and pregnant women since the vast majority of poor people and children depend on cooked meals provided in the school by the governments (Alvi and Gupta 2020). The closure or diminished capacity of institutions could lead to enormously increasing mortality ratios among children under five because it is precluding 300 million school children from accessing their meals from the school on which they depend (United Nations 2020). Policy measures to alleviate the impacts of the Covid-19 pandemic on food and nutritional security must be prioritized (I) the areas with lower food production differently from those with the higher production; (II) support food social safety networks including food banks and school meals programs.

4. Agri-food prices and market access

An unexpected lockdown across borders of countries and within countries has caused inputs supply, transports of agricultural products, and labour availability in the agri-food and marketing sector, which affected the food availability and prices globally (FAO 2020a). For example, in many African countries and India the agri-food commodity prices were increased by over 15% as compared with pre-Covid-19 periods (Hernandez et al. 2020). The Covid-19 has a substantial impact on agri-food systems, affecting predominately food supply and food security in the economically vulnerable region across the globe (FAO 2020c; Schmidt et al. 2020a; Alvi and Gupta 2020). The patrons are also influenced by the environments to storing food as well as sourcing the different food procurement choices due to supply chain interruptions, social distancing at the market place and reduction in grocery shops (Schmidt et al. 2020a).

Further, the retail and wholesale prices for agri-food products, including edible oils and pulses among other prices observed a sharp hike suddenly after the lockdown (Narayanan and Saha 2020a). However, there have been functional restrictions on restaurants, malls, supermarkets worldwide as a result agricultural commodities prices have been seen to be dropped by 20 percent at a later stage (Nicola et al. 2020). Supply chain interruption was a crucial factor for the short-run price instability but later the effects are less and stable (Cranfield 2020). The price stability measures including (I) increasing the communication networks and strengthening transport facilities would be the key factors in supporting food supply chain system, market access, and prices stability; (II) efforts to increase protected market yards with reduced procurement norms would enabling markets to function better during the pandemic situation. (III) It is important to have enough food reserve or storage facilities for the existing fruits and vegetables to meet the market demand. Fruits and vegetables are play a crucial role in human health during this pandemic. The postharvest handling practices must be strengthened by heat and pre-cooling treatments, curing, controlled atmosphere storage, film wrapping, edible coating materials, an optical sensor for grading, edible films etc.. (IV) It is also suggested to improve logistic and cold chain facilities during transports of the perishable commodities (Kahramanoglu et al. 2021). An increase in availability, and market access of agri-food products may stabilize the prices.

5. Global Economy

The Covid-19 also affecting the economic growth including industrial sector, agricultural production, trade and service sectors of the countries (Keogh-Brown and Smith 2008; Bloom et al. 2005; Ceylan et al. 2020a; Ceylan et al. 2020b). The global GDP contraction during 2020 was in the range of 3.0 to 7.5% and the prediction for the ensuing global GDP upturn in 2021 ranges from 2.8 to 5.8% (IMF 2020; World Bank 2020; OECD 2020a). Likewise, global trade is expected to fall over by 5.3% in 2020 but trade volume is forecasted to increase by 8.0% in 2021 (James et al. 2021). However, the world investment report (Unctad, 2020) forecasts a decline in global foreign investment by up to 40% in 2020, with a further decrease by 5-10% in 2021 (OECD 2020d). Further, the International Food Policy Research Institute (IFPRI) reported that the number of people living in extreme poverty increases by 20%, or 150 million more people fall under the category due to the global economic contraction in 2020 (Laborde et al. 2021). In many countries, the food prices are expected to be more unstable than normal (Laborde et al. 2020; Ali et al. 2020; Reardon et al. 2020) and food insecurity is anticipated to increase considerably as a result of the Covid-19 (Ceylan and Ozkan 2020b; Laborde et al. 2021; Ali et al. 2020). Hence, (I) monetary authorities and central banks and have to promote emerging market economies; (II) national governments that have to adopt fiscal policy initiatives to stimulate the economics.

6. Labour migration

The most important evolving issue is labour availability in the agri-food sector. Covid-19 preventive measures in the different countries led to a loss of productive labours. There have been extensive constraints on international labour migration and worker programs that are critical to agricultural production in some sectors or that have caused bottlenecks. In the beginning, this was may not be a primary problem in the northern hemisphere countries. Although, the harvest of fruits and vegetables that are mostly perishable had declined due to shortages of labour (Phillipson et al. 2020; Stephens et al. 2020). Farmers who are more dependent on family labour appeared to be more resilient than those relying on external labour (Seleiman et al. 2020; Cortignani et al. 2020). The lockdown limitations not only caused agricultural productivity, input supply and marketability of farm products but also led to a revenue loss. The labour shortage affected the harvest of many agricultural commodities and allied sectors in different countries across the world (Seleiman et al. 2020; Cortignani et al. 2020).

Consequently, the agricultural production system including food grains, fruits and vegetables, livestock production is relatively labour-intensive. The disrupted or delayed cultivation of agricultural produce led to a loss of agricultural products, labour income, and food insecurity (Ceylan and Ozkan 2020b; Seleiman et al. 2020). Also, international transactions of the agricultural commodity were suffered due to labour scarcity, and rising prices appeared for exporting countries. While considering the situation of farmers, this pandemic led to both failure to transfer the agri-food products and to obtain inputs like fertilizers, seeds, chemical and livestock's feeds (Seleiman et al. 2020; Abouhatab et al. 2020). However, capital-intensive techniques are usually used in the developed countries for agricultural production, whereas low-income countries are mostly labour dependent for production. Hence, the agricultural production system and supply chain should be kept running with capital-intensive technologies and sufficient labour force to meet production challenges. Also, the working conditions that are safe for workers in order to avoid terrible consequences for future food security.

7. Agricultural inputs and outputs connectivity

Farming is an input-intensive sector also each component of production can vary considerably in the agricultural production system. The inputs and connectivity including seeds, fertilizers, chemicals, irrigation equipment's and marketing of agricultural commodities were distrusted with respect to interruptions in transportation, delays in customs clearance, limitation of credit access, increased interest rates, and capital costs, which can lead to an increase the cost of the inputs. The increases in inputs cost and the perishability of agricultural products may lead to a huge loss to the farmers. The exiting situations retarded the distribution of food and agricultural inputs which created barriers in continuous food production and supply to markets (ILO 2020; FAO 2020d; FAO 2020e). Although different stages in a supply chain are strongly linked to each other in the agri-food production and processing sector, a small fault and delayed transports can prompt a "butterfly effect" and lead to a vast loss in agricultural production (FAO 2020e).

The agri-food sector is highly connected internationally, the shutdown or reduces the activity of ports and freight for agricultural goods led to supply chain disruptions (Ivanov 2020) have the potential to limit critical access to agricultural inputs and markets. Also, many countries have limited technologies and infrastructure to store food products including a lack of proper cold chain warehouses and storage godowns. This can be a major reason for food wastage and creates an impact on the resilience of the agri-foods sector (Balaji and Arshinder 2016; Raut et al. 2019). For instance, more than 18% of the horticultural product's post-harvest storage losses are incurred annually due to limited infrastructure in India (GoI MOFPI 2020; Sivaraman 2016). The situations are jeopardizing and the limitation in the agricultural production system may have negative effects on food quality including freshness, food safety, affordability and access to the markets (FAO 2020e). During the pandemic situation, it is important to make every effort to move the gears of the agricultural production system. Also, innovative measures and adaptations have to be developed by the government for effective resilience. The existing situation also leaves little scope for identifying suitable domestic substitutes in order to address emerging domestic food security concerns due to Covid-19.

8. Initiatives to avert the crisis

Social security policy measures are important for the economically vulnerable community to mitigate the impact of the Covid-19 pandemic. The government authorities in combination with the private organization must facilitate to keep the supply chain of agriculture more active and ensure to protect the agricultural and allied workers for continued food production (Hidrobo et al. 2020; Galanakis 2020). Table 1 listed Covid-19 impacts on the top five countries and their social protection measures.

Table-1: Top 5 Counties Incentives to Overcome Covid-19 Crises (Source: IMF 2021)

No	Countries	Impacts	Nature of benefits
1.	**USA**	• U.S. economy contracted around 31.4% during 2nd quarter of 2020 • The unemployment rate was 6.2% in February 2021.	• About 5731 Billion USD **(**about 27.34% of 2020 GDP) used for public health, families assistance, unemployment benefits etc.
2.	**India**	• GDP contracted sharply by 24.4% in 2nd quarter of 2020. • The advanced estimate for FY2020/21 GDP growth to be -8%.	• US$ 402.6 Billion or 15% of the country's GDP (Akanksha 2021) foregone or deferred revenues, credit provision to numerous sectors, include free food grains; foods items, cooking gas and cash transfers to lower-income households, wage support to low-wage workers, postponement of rent and utility payments etc.
3.	**Brazil**	• More than 15 million (7%) people have been infected and fatality rate is 2.8%.	• Relief package about US$ 233.8 billion (up to 12% GDP) used for health, health supplies, income supports to households, unemployment's compensation, small industries-tax reduction etc.
4.	**France**	• France's GDP contracted by 8.3% in 2020.In 2021, the economy grew by 0.4 % during the first quarter compared to the previous quarter**.**	• To about US$ 211.7 billion (8% of GDP), spent on measures include health insurance, health supplies, social security, tax payments for companies and accelerated refund of tax credits, support for wages of workers, direct financial support for microenterprises, postponement of rent and utility payments, extension of expiring unemployment benefits until the end of the lockdown etc.
5.	**Turkey**	• GDP contracted by 10% y-o-y in Q2, but with a strong rebound in Q3, growth in 2020 as a whole was +1.8%	• Amount to US$ 74 billion (12.7% of GDP) spend on measures include loan guarantees to firms and households, tax deferrals for businesses, equity injections into public banks, VAT has been reduced on food commodities, a nationwide ban on employee layoffs was in force until mid-May 2021 and short-term work allowance system for all sectors was also extended through June, as was the ban on layoffs, etc.,

The FAO has suggested ensuring staple food availability on the basis of priority and the food products to be transported to demanding regions by minimizing restrictions on trade during the pandemic (FAO 2020b; WHO/FAO 2020). Further, (i) the specific recommendations including social protection along with emergency food assistance programs need to be expanded to keep people stay at home during the lockdown period. (ii) The instant payment support with the help of e-commerce can help the farmers to continue the agricultural practices during a state of emergency.

9. Importance of IT in agriculture

The information technology platform can help to communicate and share Covid-19 medication measures including protocols, research findings, news, which was found useful in one place of the world to the other. Local, national and international organizations are adopted social media and television platforms efficiently towards curbing the impact of the pandemic. Also, social media and networks system facilitates collective action, permit knowledge diffusion and encourage social support for households and communities (Rockenbauch and Sakdapolrak 2017; Ceylan and Ozkan 2020a). Also, the IT platform has become a key actor in all the sectors including food and agricultural marketing. It has been steadily growing since the late 2000s and has now become significant market power and spread across the countries.

In recent years, the online food retailing sector has increasingly attracted scholarly attention, although a comprehensive understanding of the sector has not been achieved (De Reuver et al. 2018). For instance, the percentage of individuals purchasing food or groceries in the previous 12 months has increased from 5% to 15% in Europe (Eurostat 2019). In the European Union, overall total retail sales decreased by 17.9% however, the sales through mail orders / the Internet increased by 30% from April 2019 to 2020 (OECD 2020b). During this pandemic, in-store shopping in a grocery store would be supposed to be a risk, although consumer buying preference shifted to e-commerce / online shopping (Baker et al. 2020; Brick Meets Click 2020; Grashuis et al. 2020). The e-commerce and online delivery industry have been grown exponentially in many countries (Hobbs 2020; Information Resources Inc. 2020; Ceylan and Ozkan 2020b)). Hence, (i) to provide incentives to adopt digital technologies *viz.*, mobile applications, social media platforms, drones, robots, television, and associated technologies to overcome the pandemic impacts. (ii) Also, it is important to quickly deploy these technologies in the communication, logistics, healthcare and other related sectors.

10. Conclusions

The Covid-19 outbreak is an unexpected challenge to the global health system. It was not purely a health issue, as to the disaster of millions of people around the world; the pandemic was a shattering event for economics, food securities, government policies, world trade and financial markets. Furthermore, covid-19 complex consequences targeted economically vulnerable communities across the world in impulsive ways, which require a better understanding, mitigation and coping strategies to overcome the consequences.

The major hazards to food security because of reduced household incomes combined with higher retail prices impose the consumer to cut down on the quantity and quality of food consumption. The important actions are necessary to keep the agricultural sector and supply chains working smoothly: (I) To sustain the demand for agricultural commodities, investments in transports and logistics must be improved with a wide network in the developing countries. (II) Digital marketing and the e-commerce industry need to be exhilarated with appropriate guidelines and incentives. (III) Small and medium enterprises working with raw materials for agriculture and allied sector also need special consideration so that the rural economy doesn't collapse. (IV) To avoid the scarcity of farm labour-policies must facilitate the availability of machinery through state and block bodies including Farmer Producer Organizations (FPOs) or custom hiring centres (CHCs) with appropriate incentives. (V) Vulnerable individual and communities including landless labourers, wage earners, migrant labourers and small farmers have been supported with free food grains; foods items, cooking gas and cash incentives.

To date, in many countries, food accessibility is severely affected followed by its availability compared with other food security dimensions. In the long run or post-pandemic era, food availability could be severely impacted if necessary action has not been taken by the concerned bodies. The governments must promptly plan to enhance food production by providing access to finance for the farmers and the low-income households to ensure economic activity. Also local markets need to structure adequate conditions for storing the products during the marketing, and the consumers are also recommended to apply cold storage at favourable conditions for preventing losses.

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Effect of Different Levels of Direct-fed Microbials Plus Exogenous Fibrolytic Enzymes Additives on the Growth Traits of Dairy Calves

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ABSTRACT
The study was carried out to investigate to determine effects of the different levels of the direct-fed microbials (DFM) plus exogenous feed enzymes (EFE) on the body weights, weight gains, feed efficiency ratio, some behavioral traits as well as fecal consistency index of male Brown Swiss calves. For this purpose, 18 male Brown Swiss calves were allocated to three groups (control, 10 g and 20 g head/day of DFM plus EFE). Weights obtained at weaning time and 6 months of age of the calves in 10 g head/day of DFM plus EFE group were respectively 7.3% and 7.1% heavier than these of animals in control group. The calves in 10 g head/day of DFM plus EFE group in pre-weaning and between birth and 6 months of age periods also had respectively 16.0% and 7.3% higher total weight gains than calves in the control group. Feed efficiency ratio of the calves fed diets with 10 g DFM plus EFE had 64.2% better than that of calves in control group. Average fecal consistency score of the calves fed a diet supplemented with 10 g head/day of DFM plus EFE had the lowest score ($P<0.05$) (i.e., less scouring) compared to other treatment groups in pre-weaning period as well as between birth and 6 months of age. Furthermore, behavioral activities of the calves were not significantly influenced by DFM plus EFE additives except for the percentage of time spent for lying. The study revealed that the feeding of DFM plus EFE to male Brown Swiss calves until 6 months of age had positive but not statistically significant improvement on the growth traits and feed efficiency ratio. On the other hand, it was concluded that the level of 10 g head/day of the DFM plus EFE additives could be beneficial for reducing incidence of diarrhea in the dairy calves.

Keywords: Calves, Direct-fed microbials, Exogenous fibrolytic enzymes, Brown Swiss, Growth performance, Diarrhea

1. Introduction

In recent years, the use of antibiotics as a feed additive was banned in the European Union and some other countries including Turkey due to the determination of the adverse effects on human health. Consequently, there has been increased interest in the use of new and safe feed additives such as direct-fed microbials (DFM) and exogenous fibrolytic enzymes (EFE) in ruminant nutrition area (Ran et al. 2019).

Several studies have reported that dairy cattle diets supplemented with DFM changed positively the population of microorganisms in the small intestines, and increased resistance to diseases as well as improved animal health and yield of the ruminants (Ghorbani et al. 2002; Nocek & Kautz 2006; Weiss et al. 2008; Dutta et al. 2009; Blake & Clinon 2012; Diler et al. 2015). In addition, some benefits of the DFM can be provided through the prevention of ruminal acidosis (Seo et al. 2010), or by inhibition of food borne pathogens such as E. coli O157:H7 (Wisener et al. 2015).

On the other hand, the effects of DFM on calves have not been revealed in much clarity, and there have been reported different results on this subject (Ulger 2019). In previous studies, Adams et al. (2008), Seo et al. (2010) and Ran et al. (2019) reported that DFM reduces the incidence of diarrhea in calves along with positive effects on weight gain and feed efficiency ratio traits. However, Bakhshi et al. (2006), Frizzo et al. (2008) and Kocyiğit et al. (2015) reported no significant impact of DFM on the growth performance of the dairy calves.

Although addition of EFE into the rations of the non-ruminant farm animals have been widely practiced for a long time, their use for ruminant animals has been remaining quite limited until up to now. On the other hand, besides the increasing feed prices in many countries around the world, the EFE production costs that dropped as a result of biotechnological developments have led to the intensification of studies investigating the possibilities of using EFE in diets of the ruminant animals (Sujani & Seresinhe 2015). Especially cellulase, hemicellulase, protease and esterase among the fibrolytic enzymes are significant ones because of their potential importance in fiber digestion in ruminants (Ran et al. 2019). Jalilvand et al. (2008), Krueger et al. (2008) and Arriola et al. (2011) reported that EFE supplements added to ration for adult ruminants had positive effects on their

weight gains and feed efficiency ratio characteristics. On the contrary of the findings of these researchers, Elwakeel et al. (2007), Miller et al. (2008) and Ran et al. (2019) indicated that addition of the EFE additives to the mature bovine diets resulted in no significant difference concerning yield parameters and digestibility traits.

In literature, there is not much information about influences of the EFE on the growth parameters of the pre-ruminant calves (Ran et al. 2019). In one of few studies on the calves, Thakur et al. (2010) indicated that calf starters containing EFE at 1.5 g kg^{-1} feed dry matter level resulted in a greater weight gain of calves compared to 3.0 g kg^{-1} feed dry matter level.

DFM plus EFE combination became commercially available recently. However, reports about the effects of feeding mixtures of DFM plus EFE on the growth performance of young cattle are scarce. Therefore, effects of different doses of the DFM plus EFE combinations on weight gains, feed efficiency ratio, fecal consistency score and some behavioral traits of male Brown Swiss calves reared in Eastern Region of Turkey were investigated in this study.

2. Material and Methods

The 18 of male brown calves used in the research were obtained from the cattle breeding unit of Atatürk University Food and Animal Husbandry Research and Application Center. At the beginning of the trial, the calves were randomly allocated into three different treatment groups (control, 10 and 20 g DFM plus EFE). The calves were kept together with their mothers for the first 3 days following birth in order to receive colostrum, and then whole milk was given via calf milk bottle. Total of 4 kg whole milk was offered to calves in two meals (2 kg in the morning at 7.00 am and 2 kg in the evening at 5.00 pm), and the amount of daily milk was kept constant during the milk feeding period. They were weaned at 56 days of age. DFM plus EFE was given calves by adding to their milk during milk feeding period and then by mixing their calf starters after weaning.

During the trial, two different calf starters in ground form were used. Starter I containing 18% raw protein was offered to the calves between the seventh day and 4 months of age, while starter II having 17% raw protein was fed to the calves between 4 and 6 months of ages. Amount of the calf starters was gradually increased from the beginning of the trial, and it was restricted by 2 kg per calf as suggested by Tuzemen & Yanar (2004). Dry Hay and water were offered to the calves as *ad libitum*, and the calves were housed in individual calf pens furnished with hay and concentrate feeders, water bucket as well as calf milk bottle for the duration of the trial. Chemical compositions of the feeds used in this research are presented in Table 1. Amount of feed (whole milk, dry hay and calf starters) consumed daily by each calf was also determined throughout the trial.

Table 1- Chemical compositions of diets used in this study

Composition	*Milk*	*Starter-I*	*Starter-II*	*Dry Hay*
Dry matter (%)	12.0	88.0	88.0	87.8
Crude protein (%)	3.8	18.0	17.0	7.1
Ether extract (%)	4.1	4.8	4.5	3.8
Crude ash (%)	0.7	8.0	10.0	8.4
Crude cellulose (%)	-	12.0	12.0	28.4

The combination of DFM plus EFE utilized in this study was purchased from the market in powder form. The feed additives used in research as DFM contained microorganisms such as *Lactobacillus casei*, *Bacillus licheniformis*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Arpergillus oryzae* and *Bacillus subtilus* while EFE were composed of protease, cellulase, amylase, lipase, and pectinase.

Body weights of the calves were determined at birth, weaning, 4 and 6 months of ages. Body cleanliness scores of the calves during the trial period were evaluated by utilizing Pharmacia calf cleaning and animal health hygiene card used by Panivivat et al. (2004). Fecal consistency scores were determined using a scale whose scores ranged from 1 to 4 and developed by Larson et al. (1977). Bedding cleanliness scores of the straw bedding used on the floor of the calf pens were assessed and recorded according to a scale ranged from 1 to 4 used by Panivivat et al. (2004). In order to evaluate parameters of behaviors of the young animals, proportional calculations were made after determining the behavioral patterns (lying, standing, foraging, water drinking) that occurred at sampling time (once a week) according to the instant sampling method (Martin & Bateson 1993).

Since it was found out that all parameters investigated in this study had normal distribution, they were statistically analyzed by using the General Linear Model of SPSS statistics program (SPSS 2004). The mathematical model used for analysis of variance was as follows;

$Y_{ij} = \mu + a_i + e_{ij}$,
Where;
Y = Dependent variables
μ = Overall mean

a_j = Effect of DFM plus EFE doses [j = 1, (control); 2, (10 g/head); 3, (20 g/head)]
e_{ij} = Residual error

When F-test for main effect was statistically significant, comparison among levels of DFM plus EFE was carried out by the method of Duncan's Multiple Range Test available in SPSS program (SPSS 2004).

3. Results and Discussion

The least squares means for body weights obtained at different ages of the male Brown Swiss calves fed rations containing different levels of DFM plus EFE are presented in Table 2. The differences between DFM plus EFE doses concerning birth weight were found to be statistically insignificant. The absence of a significant difference of birth weights among DFM plus EFE groups could be due to the randomly assignment of the calves to the treatment groups. While the average birth weight of the male Brown Swiss calves was in accordance with results of Yanar et al. (1999), Guler et al. (2006), Tilki et al. (2008) and Soydan & Sahin (2016), but it was lower than finding of Kaygisiz et al. (2011).

Table 2- Least-squares means along with standard errors and results of variance analysis for weights of male Brown Swiss calves

Parameters		*Birth Weight (kg)*	*Weaning Weight (kg)*	*4 Months Weight (kg)*	*6 Months Weight (kg)*
	N	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$
Overall Mean	18	38.61±1.57	53.72±2.27	101.39±4.05	143.44±5.17
Levels of DFM plus EFE		NS	NS	NS	NS
Control	6	38.17±2.72	52.33±3.93	103.83±7.02	136.50±8.96
10 g	6	39.50±2.72	56.17±3.93	100.33±7.02	146.17±8.96
20 g	6	38.17±2.72	52.67±3.93	100.00±7.02	147.67±8.96

NS; Non-Significant

As determined in the current study, Kocyigit et al. (2015) also indicated that differences among the weaning weights of female dairy calves fed different amounts of DFM plus EFE were not statistically significant. On the other hand, Timmerman et al. (2005) indicated significantly heavier weaning weight of the calves in DFM group compared to control group. In the present study, weaning weight of the male calves in 10 g head/day of DFM plus EFE group were 7.3% heavier than these of animals in control group. Similarly, studies carried out by Jatkauskas & Vrotniakiene (2010) and Ulger (2019) reported that the weaning weights of the calves in DFM group were 9.4% and 5.2% respectively higher than these of calves in control group.

When the least squares means for 4 and 6 month weights were assessed for this study, there was no statistical differences between the control and the 10 or 20 g head/day DFM plus EFE groups (Table 2). Similarly, Isık et al. (2004) stated insignificant difference between control and DFM groups in terms of 4 months weight, while Ghorbani et al. (2007) determined that the EFE added diets did not significantly affected on the growth performance of Holstein calves at the age of 3 months.

In the current study, 6 months weight of the calves fed diets supplemented with 10 or 20 g head/day DFM plus EFE were 7.1% and 8.2% higher than calves in control group. Similar result was also reported by Kocyigit et al. (2015) who indicated that 6 months weight of crossbred calves in the DFM plus EFE group was 5.7% greater compared to that of calves in the control group. In another study that supported these results, the 6 months weight of Holstein Friesian calves in the control group was 4.25% superior to these in control group (Isık et al. 2004).

Least squares means and results of variance analysis for daily weight gains at different stages of the growth of male Brown Swiss calves are presented in Table 3. Although there were no statistically significant differences among the doses of DFM plus EFE, daily weight gains of the calves in 10 g head/day DFM plus EFE group between birth and weaning period was 16.0% higher than these in control group. Similarly, during the pre-weaning period, Gorgulu et al. (2003), Dimova et al. (2013), Kocyigit et al. (2015) and Ulger (2019) reported respectively 4.8%, 11.8%, 20.0% and 11.9% higher weight gains of the calves in DFM group compared to control group, and they also indicated that the differences in terms of weight gain at this period were not statistically significant. Furthermore, in a study comparing control group with DFM produced in laboratory conditions or sold commercially, Bayatkouhsar et al. (2013) stated that the weight gain differences among the DFM and control groups at pre-weaning period were 7.9% and 4.8% higher in favor of DFM, but the differences were not found to be statistically significant.

In the current study, male Brown Swiss calves receiving 10 g DFM plus EFE between birth and 6 months of age had superiority of 7.3% compared to the control group in terms of daily weight gains (Table 3). Similarly, between birth and 6 months of age, Bakhshi et al. (2006) and Kocyigit et al. (2015) reported respectively 4.4% and 11.7% higher weight gains of

the calves in DFM group compared to control group. They also indicated that the differences in terms of weight gain at this period were not statistically significant. However, Higginbothem & Bath (1993), Abdala et al. (2002) and Hossaini et al. (2010) stated statistically significant differences between calves in DFM and control groups concerning weight gains from birth to 6 months of age period. The differences among the results of the researches could be attributed to different type of viable cells in the DFM additives and their survivability, metabolic capacity and consistency in the host gut. Additionally, different calf rearing systems as well as Influence of feed processing (e.g., steam conditioning, pelleting) on the survivability of the DFM in the final prepared diet might also play significant roles on the inconsistent findings obtained in these studies.

Table 3- Least-squares means along with standard errors for weight gains in different parts of the growth of male Brown Swiss calves

Parameters		*Gains Between Birth and Weaning (kg)*	*Gains Between Weaning and 4 Months of Age (kg)*	*Gains Between 4 and 6 Months of Age (kg)*	*Gains Between Birth and 6 Months of Age (kg)*
	N	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$
Overall Mean	18	0.27±0.02	0.74±0.03	0.70±0.05	0.58±0.02
Levels of DFM plus EFE		NS	NS	NS	NS
Control	6	0.25±0.04	0.80±0.06	0.54±0.09	0.55±0.04
10 g	6	0.29±0.04	0.69±0.06	0.76±0.09	0.59±0.04
20 g	6	0.26±0.04	0.74±0.06	0.79±0.09	0.61±0.04

NS; Non-Significant

Least squares means and results of the variance analysis for amount of dry matter intake of milk, hay and calf starters per kg weight gain is presented in Table 4. Although the amount of dry matter of the feed per kg weight gain of the calves in the 10 or 20 g head/day DFM plus EFE groups was not significantly different from control group in the pre-weaning period, the feed efficiency ratio of the calves fed diets with 10 g DFM plus EFE had 64.2% better than that of calves in control group. Kocyigit et al. (2015) also stated that feed efficiency ratio of the female dairy calves in 10 g head/day DFM plus EFE group was 1.7 times better than that of calves in control group. Parallel findings were also reported by Jenny et al. (1991), Hamza et al. (1996), Gorgulu et al. (2003), Isık et al. (2004) and Ulger (2019), and they indicated respectively 25.1%, 25.5%, 15.2%, 11.1%, 10.5% better feed efficiency ratios of the calves in DFM group compared to control group.

Table 4- Least-squares means along with their standard errors for feed efficiency ratios of male Brown Swiss calves at different stages of the growth

Parameters		*Total Amount of Dry Matter Consumed per kg Weight Gain Between;*			
		Birth and Weaning	*Weaning and 4 Months of Age*	*4 and 6 Months of Age*	*Birth and 6 Months of Age*
	N	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$	$\overline{X} \pm S_{\bar{x}}$
Overall Mean	18	2.31±0.36	3.25±0.12	5.19±0.38	3.51±0.08
Levels of DFM plus EFE		NS	NS	NS	NS
Control	6	2.94±0.63	3.22±0.22	4.77±0.66	3.52±0.15
10 g	6	1.79±0.63	3.41±0.22	4.58±0.66	3.42±0.15
20 g	6	2.21±0.63	3.14±0.22	6.21±0.66	3.61±0.15

NS; Non-Significant

As the feed efficiency ratios of the male calves in different DFM plus EFE levels during from birth to 6 months of age were compared to each other, it was 2.9% better in favor of the calves in 10 g head/day DFM plus EFE group. Similarly, Bakhshi et al. (2006) reported statistically insignificant differences in terms of feed efficiency ratios between DFM and control groups, and they found out that calves in DFM group had 7.7% better feed efficiency ratio compared to control group. Moreover, Ran et al. (2019) stated that Charolaise x Angus calves consumed diet supplemented with DFM plus EFE had 9.6% better feed efficiency ratio than calves in control group in a period between birth and 112 days. On the other hand, Timmerman et al. (2005) and Frizzo et al. (2011) especially indicated positive improving effect of the DFM on the feed efficiency ratio of the stressful calves when they had high incidence of disease.

Least squares means and results of variance for the percentage of time spent on different activities of the male Brown Swiss calves are presented in Table 5. While the effect of the different levels of the DFM plus EFE on the percentage of time spent for lying throughout the trial (6 months) was highly significant ($P<0.01$), the rest of the behavioral traits were not significantly influenced from the treatment groups. However, the percentage of time spent for eating for calves in 10 g head/day DFM plus

EFE group had 10.3% higher compared to control group. Kocyigit et al. (2015) also reported insignificant differences in terms of percentage of time spent for standing, eating and water drinking behaviors between DFM and control groups.

Table 5- Least squares means with standard error for percentage of time spent on different activities of male Brown Swiss calves

Parameters		*Between Birth and 6 Months of Age*			
		Lying	*Standing*	*Feeding*	*Water Drinking*
	N	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$
Overall Mean	18	0.26±0.009	0.31±0.009	0.40±0.01	0.014±0.02
Levels of DFM plus EFE		**	NS	NS	NS
Control	6	0.29±0.01^{b}	0.29±0.01	0.39±0.02	0.015±0.03
10 g	6	0.22±0.01^{a}	0.33±0.01	0.43±0.02	0.013±0.02
20 g	6	0.26±0.01^{ab}	0.32±0.01	0.39±0.02	0.012±0.03

**; P<0.01, NS; Non-Significant

Least square means and results of variance analysis for fecal consistency scores, body cleanliness scores along with bedding cleanliness scores of the male Brown Swiss calves in different groups of DFM plus EFE are presented in Table 6. Statistically significant (P<0.05) differences among the levels of DFM plus EFE concerning fecal consistency scores were observed in the pre-weaning period as well as between birth and 6 months of age. During these periods, calves in 10 g head/day DFM plus EFE group had 83.4% and 38.6% lower fecal consistency scores which meant they had lower incidence of diarrhea. As a result of the decrease in the incidence of diarrhea in these calves, body cleanliness scores (4.1% and 8.9%) and bedding cleanliness scores (16.4% and 9.0%) improved compared to control group in the both milk feeding period as well as throughout the trial (Table 6). Similarly, Agarwal et al. (2002), Seo et al. (2010), Kim et al. (2011), Kocyigit et al. (2015) determined the fecal consistency scores of the dairy calves and reported a significant reduction of incidence and duration of scouring in the young animals of DFM fed groups as compared to control group. Additionally, Foster et al. (2003), Jatkauskas & Vrotniakiene (2010) have shown that supplementation of DFM into the diet of the calves caused a reduction of the incidence of diarrhea in the dairy calves.

Table 6- Least square means and standard error for fecal consistency scores of calves, body cleanliness scores, and bedding cleanliness scores

Parameters		*Fecal Consistency Scores Between Birth and Weaning*	*Fecal Consistency Scores Between Birth and 6 Months of Age*	*Body Cleanliness Score Between Birth and Weaning*	*Body Cleanliness Score Between Birth and 6 Months of Age*	*Bedding Cleanliness Score Between Birth and Weaning*	*Bedding Cleanliness Score Between Birth and 6 Months of Age*
	N	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$
Overall Mean	18	1.64±0.06	1.20±0.01	1.90±0.07	2.17±0.04	1.94±0.06	2.37±0.06
Levels of DFM plus EFE		*	*	NS	NS	NS	NS
Control	6	2.33±0.11^{b}	1.47±0.02^{b}	2.00±0.12	2.32±0.08	2.13±0.10	2.55±0.11
10 g	6	1.27±0.11^{a}	1.06±0.02^{a}	1.92±0.12	2.13±0.08	1.83±0.10	2.34±0.11
20 g	6	1.33±0.11^{a}	1.08±0.02^{a}	1.78±0.12	2.06±0.08	1.86±0.10	2.21±0.11

*; P<0.05, NS; Non-Significant

4. Conclusions

Overall results of the study revealed that even though the feeding of DFM plus EFE improved numerically weight gain as well as feed efficiency ratio of the male Brown Swiss calves, the differences were not statistically significant. However, level of 10 g head/day of the DFM plus EFE additives could be beneficial for reducing incidence of diarrhea of male Brown Swiss calves.

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Spatial Data Model for Rural Planning and Land Management in Turkey

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ABSTRACT
In Turkey, in the areas of rural planning and land management, problems regarding data retrieval, data quality, implementation scenario and legal base (law or regulation) have long been experienced. In this study, in order to contribute to resolving such problems, a conceptual/semantic data model was designed which focuses on the definition of required data, determination of their basic qualities and also their relations. As the preparation step for the model development, interviews, and discussions with authorized people were carried out. In addition, for the definitions of the data in the model, the Land Parcel Identification System and Infrastructure for Spatial Information in the European Community (INSPIRE) are considered. For the model design, an object-oriented modelling method with the Unified Modelling Language (UML) notation was used. In the model, planning activities were focused on. It is envisaged that the model will guide work for the preparation of a technical regulation which may enable a standardized implementation throughout Turkey. It has also the potential to be an example for the implementation of laws related to spatial data both in Turkey and also worldwide.

Keywords: Land use planning, Agricultural land use planning, Standardization, UML, INSPIRE

1. Introduction

The preservation, planning and sustainability of rural areas have become more important topics worldwide when compared with agricultural production (Volker 1997; Midgley et al 2005; Pasakarnis & Meliene 2010). In this context, the concept of rural development has gained importance (Banks & Marsden 2000; van der Ploeg et al 2000; Elands & Wiersum 2001; Marsden & Sonnino 2008; Naldi et al. 2015). Despite the fact that a considerable proportion (approx. %25) of the population in Turkey lives in rural areas (MARA 2006, UN 2019), except for traditional land consolidation activities, which are basically aimed at combining scattered land and improving irrigation facilities (Gun 2003; Cay & Iscan 2011; Iscan 2011), integrated and comprehensive land management activities on the preservation, planning, management and sustainability of rural texture and land have not been carried out (Inan 2010). National Rural Development Plan (MARA 2011) promotes the development of technical infrastructure and social life in rural areas rather than any improvement in rural land management tools. Regional development projects (Unver 1997) and local environmental protection applications, as part of the agricultural policy implementation (Inan 2010), make a very restricted contribution to sustainable land management. As a result, integrated rural development cannot be achieved and furthermore rural land is exposed to degradation and erosion (Sarisamur & Kilic 2011). In this context, the lack of an integrated legal base (law or regulation) was experienced until 2005. In that year, the Soil Conservation and Land Use Law (referred to hereafter in the text as the law) No 5403 (Official Gazette 2005a), which suggests integrated precautions and actions throughout the country, was enacted. This study focuses on the items (in the law) related to the inventory of rural land and soil resources, land use planning and land management. However, these items include only the main legal procedures. The definitions of required data, data quality, data processing, and data standards are not explicitly covered by the law. As for the regulation regarding the application of the law (referred to hereafter in the text as the regulation) (Official Gazette 2005b), it does not cover such definitions, either. Due to the lack of data definition, it is important, yet currently impossible, to implement the law throughout the country by complying with the same standard and quality. This study aims at providing a definition of the required data, their relations, and also the basic data processing scenario by developing a data model for rural land planning (land use plans and agricultural land use plans) and management activities in Turkey. This work was produced based on a previous study (Inan & Yomralioglu 2011) which was presented at a national meeting in Turkey.

2. Material and Methods

Data necessity analysis is the first stage of this study. In this context, the law (No 5403) and also the regulation (of the law No 5403) were analyzed; the process steps and, accordingly, the required data for these processes were determined. During this stage, processes were grouped into two classes. These were (1) compilation of available data or new data acquisition and (2) planning or land management activities.

During the necessity analysis stage, interviews, analysis, and discussions were conducted with various personnel at the Ministry of Agriculture and Forestry. In addition, common basic spatial data which are defined within the Turkish National Geographical Information System (GDLRC 2005; 2006), the Land Parcel Identification System (LPIS) as the spatial component of the Integrated Administration and Control System (Kay 2002; Goeman et al 2007; Kay & Milenov 2007; Inan et al 2010) and Infrastructure for Spatial Information in the European Community (INSPIRE) (EC 2012) were also partly considered.

In the data model design stage, considering their geometries, data were divided into datasets and each one was represented with a class. In order not to cause complexity in terms of geometry definitions, the data geometry of few classes was defined as complex (a generic geometry type specific to this study), the geometries of the majority of classes were defined by using standardized geometry types of ISO (2003; 2019). In the model design, considering their dependencies and relations, datasets were classified into nine groups (packages).

Data necessity analysis was the basic preparation step for the data model design. The required spatial or non-spatial data were determined by considering the activities of data compilation/acquisition and processing (planning and land management), and accordingly their basic qualities/characteristics were identified (see Table 1 for common data sets and data definitions below in this section for newly defined novel data sets).

For this purpose, first of all, activities (defined in the law and regulation) related to data compilation/acquisition as well as rural land planning and management were determined. These are as follows: (1) determination of soil and land resources (item no. 7 of the law and no. 8 of the regulation), (2) classification of agricultural land (item no. 8 of the law), (3) preservation of soils (item no. 9 of the law), (4) preparation of land use plans (item no. 10 of the law and no. 9 of the regulation), (5) preparation of agricultural land use plans (item no. 11 of the law and no. 10 of the regulation), (6) preparation of soil conservation projects (item no. 12 of the law and of the regulation), (7) determination of main basins which have high agricultural potential (item no. 14 of the law), (8) determination of areas with high erosion risk (item no. 15 of the law and no. 13 of the regulation), and (9) land consolidation activities (item no. 17 of the law and no. 13 of the regulation).

Land Resources data set is required for the creation of the land use plan. It was envisaged that the data set would be produced from other data sources, which are attributed in Table 1 with “Basic Functionality” column. The data set should be produced to represent different kinds of land, such as fertile land, planted land, forests, meadows, residential areas, planned urban land, nature reserves, and non-agricultural land. The INSPIRE Data Specification does not include any data theme with similar content. In the model, land resources data set is represented in the Land Resources package with the Land Resource class.

Agricultural Land Resources data set forms a specific subset of land resource data, and is required for agricultural land use planning. It specifically includes the soil properties of agricultural land. All agricultural land throughout the country was classified by the Ministry of Agriculture and Forestry within the Determination of Problematic Agricultural Land and Their Rehabilitation project (MARA 2012). In INSPIRE Data Specification, this data set is not specifically included in any data theme. However, the related data are partially included in the Land Use and Land Cover data themes (INSPIRE 2007). In the model, it is represented in the Soil Resources package with the Agricultural Land Resource class.

Land Use Plan data set is the first product of planning activities. A spatial planning unit should be chosen beforehand. This unit may be the smallest soil surveying and mapping unit or their sub-units. In INSPIRE Data Specification, the Land Use data theme may have some similar content. In the model, it is represented in the Land Use Plan package with the Land Use Plan class. With land use plans, urban and rural lands should be allocated to land use.

Physical Block data set represents the basic spatial units for the agricultural land use plan. The data set may be produced by using ortho-photos or ortho-images in a way similar to the application of LPIS (Inan & Cete 2007; Inan 2010; Inan et al 2010). The INSPIRE Data Specification does not define such geographic features. In the model, they are represented in the Agricultural Land Use Plan package with the Physical Block class.

Agricultural Land Use Plan data set is the second product of planning activities. The INSPIRE Data Specification does not define such a planning concept. In the model, it is represented in the Agricultural Land Use Plan package with the Agricultural Land Use Plan class. With agricultural land use plans, the appropriateness of physical blocks is decided for the type of agricultural products; (1) garden crops, (2) vegetable crops, (3) field crops, and (4) forage crops. Planning decisions should be taken by considering both technical and socio-economic factors. Planning maps and reports should include suggestions on appropriate agricultural activity, environmental protection precautions, and risks for each physical block.

Table 1- Basic properties of necessitated data (common data sets)

Data Type	*Basic Functionality*	*Scale Range*	*Availability & geographic coverage*	*Relation with previous studies, LPIS and INSPIRE data themes*			*Representation in the Data Model*	
				Studies	*LPIS*	*INSPIRE*	*Package*	*Class(es)*
Topographic Map	Base map for Land Resources	1/1000 1/10.000 1/25.000	-Urban Areas -Partial -Yes	Cete 2008	Yes	Partial	Topography	TopographicMap
Ortho-Photo& Image	Base map for Land Resources	1/2000 1/10.000	-No -No	Maras et al 2011; INSPIRE 2007; INSPIRE 2013c	Yes	Yes	Topography	OrthoPhoto; OrthoImage
Digital Elevation Model (DEM)	Base data for slope, aspect and basin boundary	1/25.000	-Yes	Bamber et al 2001; Fabris & Pesci 2005; Züblin et al 2008; INSPIRE 2007; INSPIRE 2013a	No	Yes	Topography	DEM
Slope	Base map for Land Resources	1/25.000	-No	INSPIRE 2013a	No	Annex	Topography	Slope
Aspect	Base map for Land Resources	1/25.000	-No	INSPIRE 2013a	No	No	Topography	Aspect
Land Parcel (Property)	Base data for Land Resources	1/1000	-Yes (with data quality problems)	INSPIRE 2007; INSPIRE 2010b	Yes	Yes	Land Resources	Cadastre & Land Registry; Forest Registry; MeadowRegistry
Nature Reserve	Base data for Land Resources	1/25.000	-Yes	INSPIRE 2007; INSPIRE 2010d	No	Yes	Land Resources	NatureReserve
Zoning Map	Base data for Land Resources	1/1000 1/10.000	-Urban Areas -Urban Areas	INSPIRE 2007; INSPIRE 2013g	No	Yes	Land Resources	DevelopmentPlan
Land Cover	Base data for Land Resources	1/100.000	-Yes	Bossard et al 2000; Büttner et al 2002; EEA 2010; INSPIRE 2007; INSPIRE 2013b	Yes (larger scales)	Yes	Land Resources	LandCover
Administrative Boundary	Subdivision of Land Resources	1/25.000	-Yes	MARA 2012; INSPIRE 2007; INSPIRE 2010a	Yes	Yes	Admin&Basin Boundary	Administrative Boundary
Basin Boundary	Subdivision of Land Resources	1/25.000	-No	INSPIRE 2007; INSPIRE 2010c	No	Conceptual	Admin&Basin Boundary	BasinBoundary
Soil Map	Soil Classification	1/10.000 1/25.000 1/100.000	-No -No -No	Dinc et al 2005; INSPIRE 2007; INSPIRE 2013d	No	Yes	Soil Resources	SoilMap
Soil Classification Map	Planning	1/10.000 1/25.000 1/100.000	-No -No -Yes	Burrough et al 1997; O'Geen et al 2008	No	Yes	Soil Resources	SoilClassification
Environ-mental Plan	anning	1/100.000	-Yes	INSPIRE 2007	No	Conceptual	Plan&Socio Economic	EnvironmentalPlan
Water Resources	Planning	1/10.000 1/100.000	-No -No	INSPIRE 2007; INSPIRE 2013e	No	Partial	WaterResources	WaterResource
Socio-Economic	Planning (Attribute Data)	Not Appl.	-Yes (by admin. unit)	INSPIRE 2007; INSPIRE 2013f	No	Partial	Plan&Socio Economic	SocioEconomic
Rural Dev. Plan	Planning (Attribute Data)	Not Appl.	-Yes (by admin. unit)	MARA 2011	No	No	Plan&Socio Economic	RuralDevelopment Plan
Climate	Planning	Not Appl. 1/25.000	-Yes (station) -No (interpol.)	Apaydin et al 2004; Jolly et al 2004	No	No	Climatic	MeteorologyStation Data; Climate

3. Results and Discussion

The data model developed in this study is presented with Unified Modelling Language (UML) class diagrams (Page-Jones 2002). In recent years, this modelling method has been used for the development of both spatial data standards (ISO TC/211 and OGC) and semantic data models for spatial data infrastructures (e.g. INSPIRE and ISO 19152 LADM).

In the data model design the classes were divided into nine groups (Figure 1). Inspired by the ISO (2003, 2019) standard, the data types of the structure attribute were defined as GM_Polygon, GM_Line, GM_Point, GM_TIN, Raster and Complex. The relations between classes were defined by appropriate relation types (see sub-sections 3.2–3.10). Dependency and use relations were used to define dynamic interactions among classes. In the cases of strong and weak interactions, use and dependency relations were used, respectively. Other relations (association, aggregation, composition, and generalization/specialization) were used to define the static (logical) interaction among classes. Six types of elements were used in the model. Of these, Package represents data groups (see Figure 1), Feature Type represents spatial data, Table represents tabular (non-spatial) data, and Enumeration and Code List represent restricted and non-restricted definition sets.

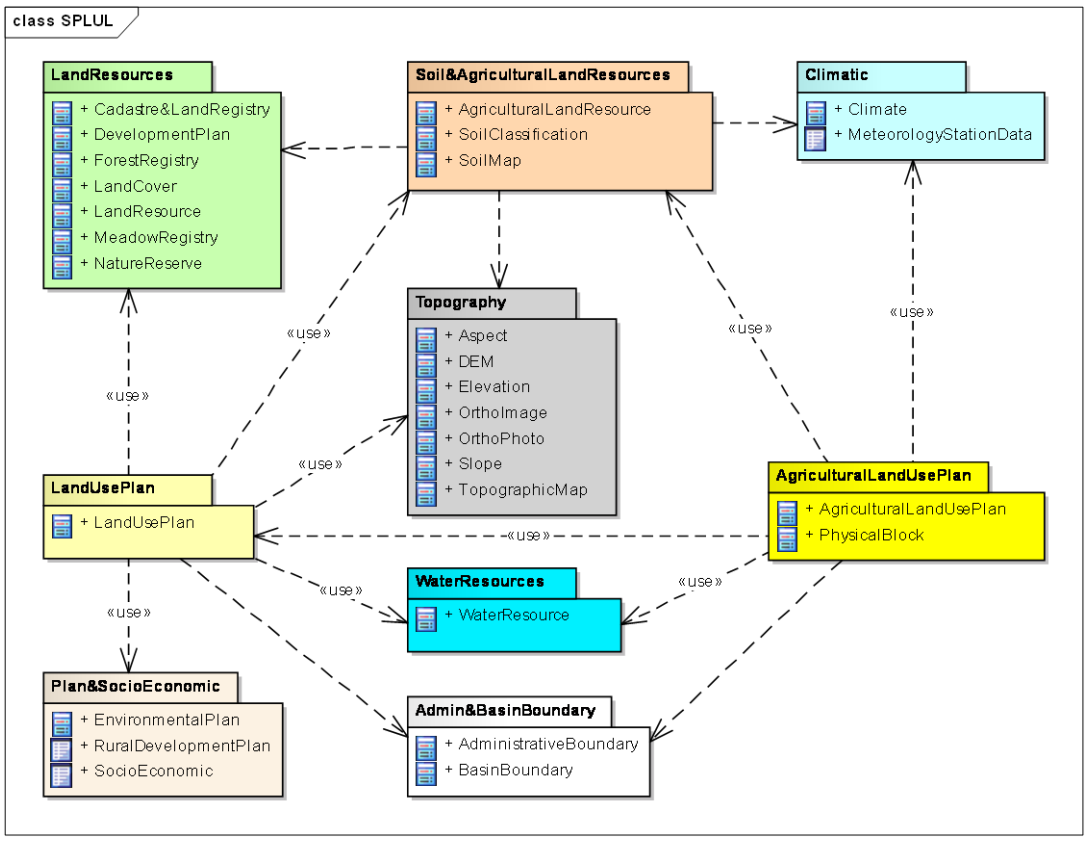

Figure 1- Data groups (packages) and their relations

3.1. General structure of the model: Packages

The model is composed of nine data packages (Figure 1). In the sub-sections, the classes in each package and their relation are presented and explained.

3.2. Land Resources package

First, the Cadastre & Land Registry, Development Plan, Forest Registry, Meadow Registry, Nature Reserve and Land Cover classes were defined to represent available data sources in the model. Afterwards, the idea of producing the land resource dataset by using available data sets was reflected in the model design through relations (Figure 2). In order to make the production of land resource data possible by administrative units or basins, the use relation among Land Resource, the Administrative Boundary and the Basin Boundary classes was defined (Figure 2). The data need in this respect was defined with dependency relations among the Land Resource class and other related classes (Figure 2).

For the classification of land resource types, the type attribute in the Land Resource class was designed. To restrict the range of data type of this attribute, the Land Resource Type definition set was defined. Similar definitions were made for other data types for different attributes of other classes (see Figure 2).

It was envisaged that the agricultural land resource dataset is produced from the land resources dataset. This was reflected in the model with the use relation defined between the Land Resource and Agricultural Land Resource classes. The idea that the agricultural land resource dataset cannot be produced without the land resource dataset was represented in the model with the composition relation (closed diamond) between the two classes (Figure 2).

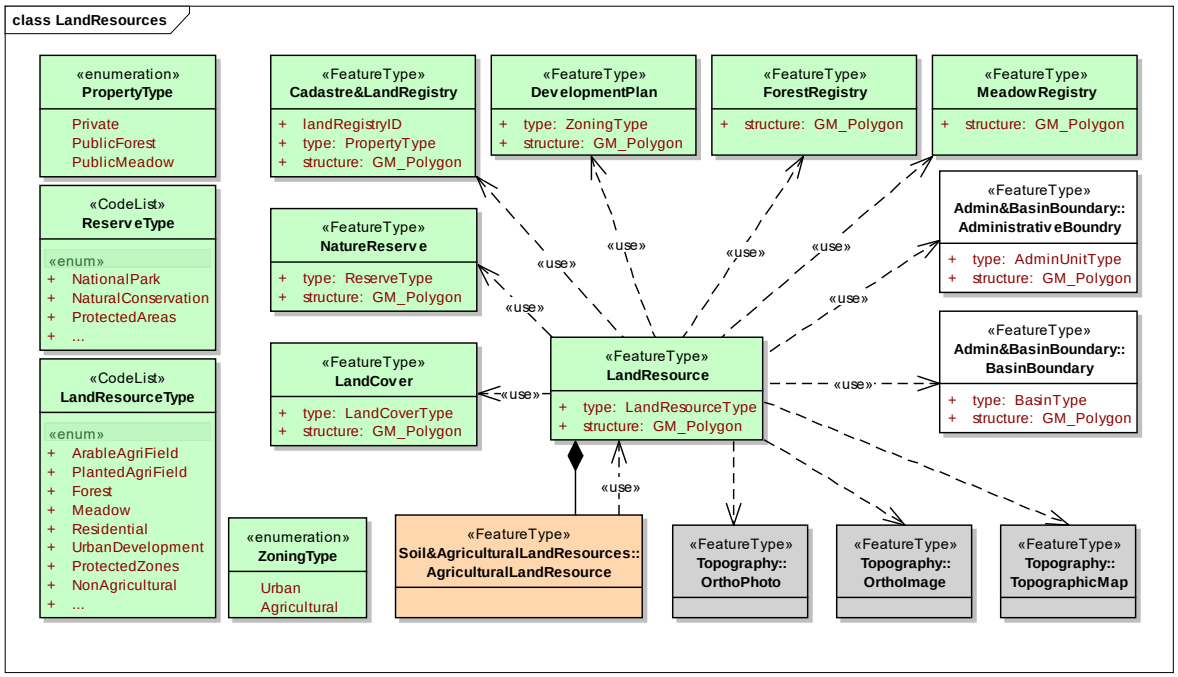

Figure 2- Land resources package

3.3. Soil & Agricultural Land Resources package

For the determination of agricultural land type (classification), soil properties are required. To represent this requirement, the use relation between Agricultural Land Resource and Soil Map classes were defined (Figure 3). The type attribute of Agricultural Land Resource class and the definition set Agricultural Land Type as its data type were defined to represent agricultural land types.

The Soil Map class represents all kinds of soil properties. It also represents all kinds of soil maps produced by using different surveying and mapping methods with its source type attribute (Figure 3). In the Soil Survey Type (Figure 3) definition set, two types of common soil surveying and mapping methods were included. The attribute soil properties (Figure 3) of this class is intended to represent all the physical, chemical and biological properties in the soil profile.

In soil surveying and mapping activities, cartographic maps are used for the establishment of mapping units. To reflect this fact in the model, the dependency relations among the Soil Map class and related classes (Topographic Map, Ortho Image and Ortho Photo) were defined (Figure 3).

In order to model the fact that a variety of different soil classification maps may be produced from the same soil map the Soil Classification class was designed. Because this class represents a kind of soil map, the generalization relation between this class and the Soil Map class was used (Figure 3).

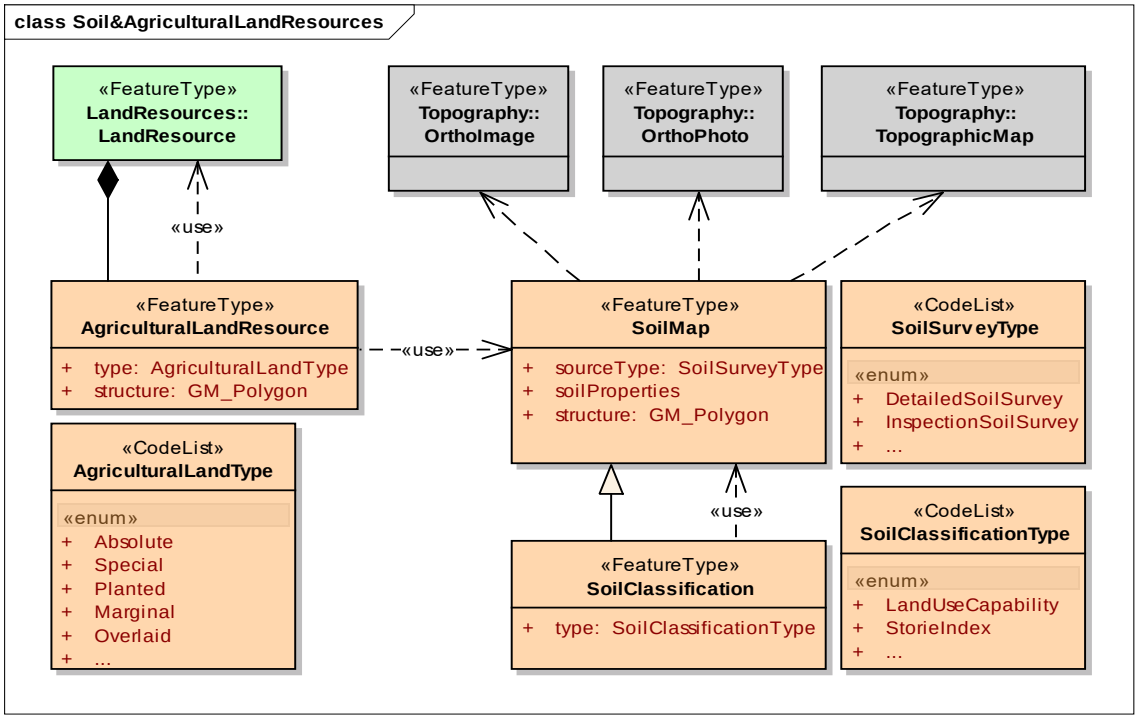

Figure 3- Soil and agricultural land package

3.4. Topography package

In this package, classes representing commonly used base maps, namely topographic map, ortho-photo and ortho-image were designed. Additionally, classes representing the Digital Elevation Model (DEM), and also slope and aspect data as the product of DEM were included (Figure 4).

Because the Topographic Map class represents data in different geometries and also in raster formats, the structure attribute of this class was defined as a complex data type (Figure 4). The attribute base scale of this class (Figure 4) represents the original production scale, which reflects data quality.

Because it was envisaged that the DEM dataset is produced from contour lines included in the topographic map, and additionally that the slope and aspect datasets would be produced from the DEM dataset, the use relations were defined between these classes (Figure 4). Because the DEM dataset was envisaged to be in TIN data structure, the data type of its structure attribute was defined as GM_TIN (Figure 4). Because the datasets represented by the Aspect, Slope, Ortho Photo and Ortho Image classes are in cell data structure, their structure attributes were defined as Raster (Figure 4). In the Aspect and Slope classes, class attributes with their Aspect Class and Slope Class data types (Figure 4) were defined to represent different slope and aspect classifications in different applications.

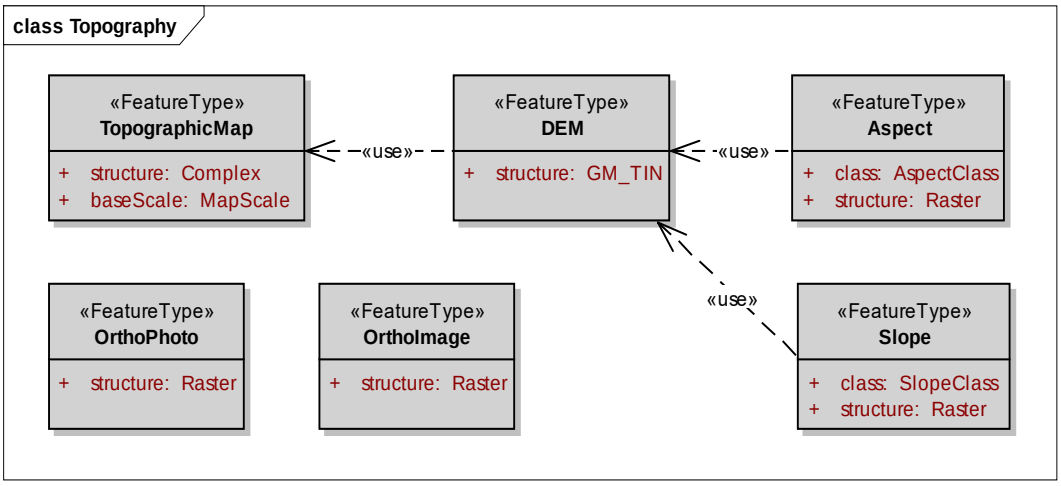

Figure 4- Topography package

3.5. Climatic package

The Meteorology Station Data class (Figure 5) was defined in order to represent raw data recorded at meteorological stations in tabular formats. The attributes of this class were designed to represent climatic data recorded systematically in different periods.

The Climate class was designed (Figure 5) in order to represent geographically continuous climatic data produced by interpolating raw and also derived (average, min and max) data. The type attribute of this class with its data type Climate Data Type (Figure 5) represents the type of data used.

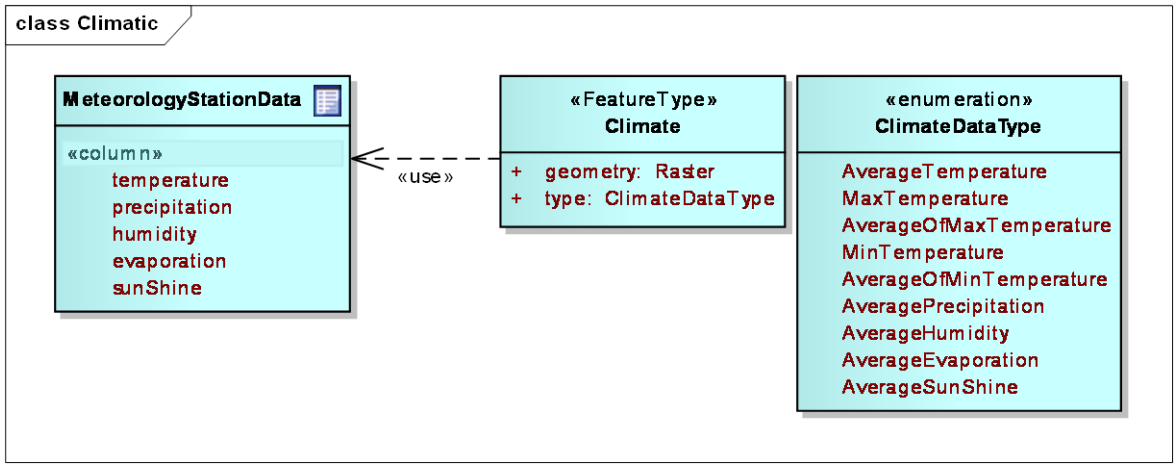

Figure 5- Climatic package

3.6. Water Resources package

The Water Resource class (Figure 6) was designed to represent all types of water resources. In order to represent water resources (WR) data in different data structures/geometries (point, line, polygon and network), the WR Structure Type definition set (Figure 6) was specially designed as the data type of structure attribute of this class. Similarly, the irrigation potential attribute was designed for the representation of information on the irrigation potential of all kinds of water resources (Figure 6).

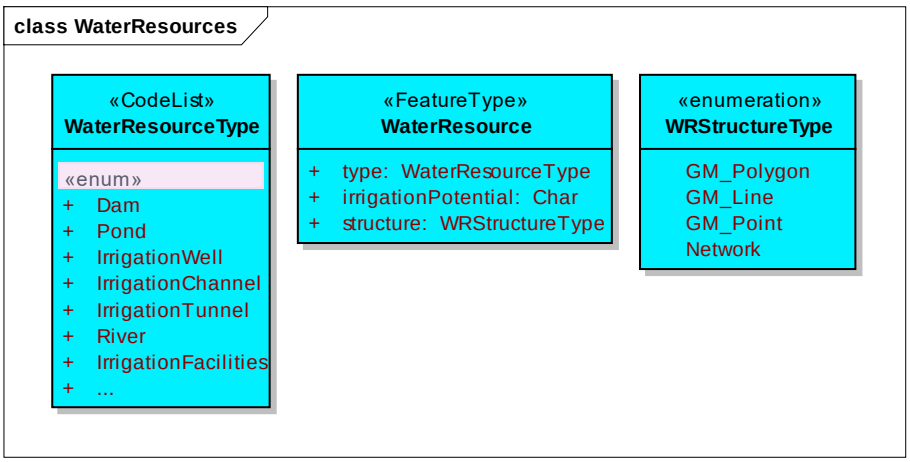

Figure 6- Water resources package

3.7. Plan & Socio Economic package

The Environmental Plan class (Figure 7) was designed to represent the basic information content of environmental plans. To represent planning decisions for basic spatial units in the map, type attribute and, accordingly, the Planned Land Use Type definition set as its data type were designed (Figure 7). Due to planning units with different geometries (polygon, line and

point), the structure attribute of this class was defined as Complex data type (Figure 7). As for the annex attribute, it represents the planning report (Figure 7).

Prepared as a report without a geographically continuous planning map, the rural development plan was represented by a class structured as an ordinary table without any geometrical structure (Figure 7). In this class, documents and maps related to planning decisions are represented by the attributes Related Documents and Related Maps (Figure 7). Because planning decisions may be linked to administrative units or basin boundaries, the association relations were defined between related classes (Figure 7).

For the representation of non-spatial socio-economic data, the Socio Economic class was designed as an ordinary table (Figure 7). To represent the link between socio-economic data and administrative units, the association relation was defined (Figure 7).

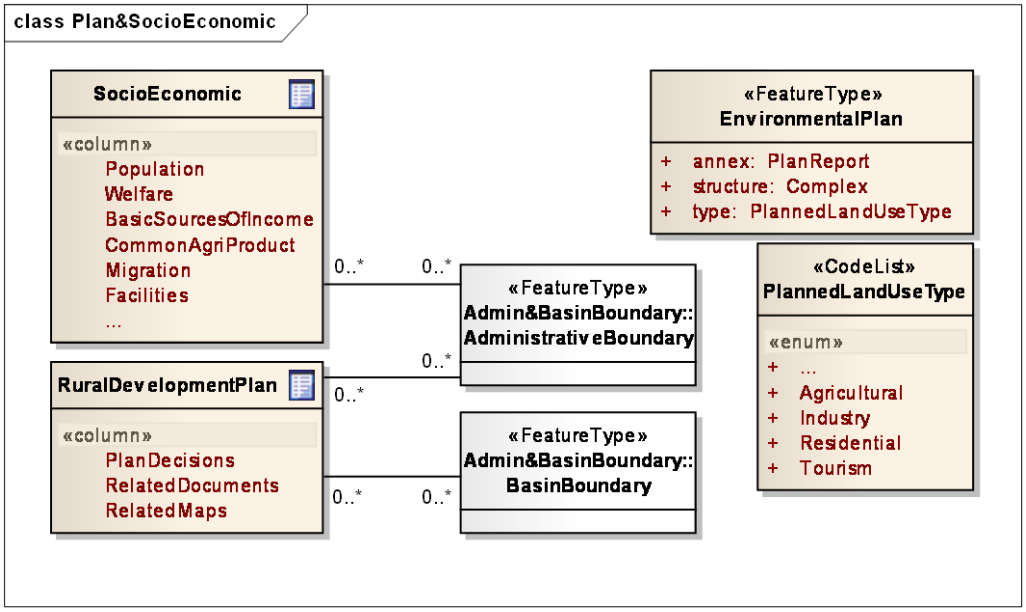

Figure 7- Plan & SocioEconomic package

3.8. Admin & Basin Boundary package

In order to represent administrative units and basins in different levels, the type attributes for the two classes were designed. For the definition of the data types of these attributes, the Admin Unit Type and Basin Type definition sets were designed (Figure 8).

Topographic data are required for the production of basin boundaries. To represent the use of DEM for this requirement, the use relation between the two classes was defined (Figure 8).

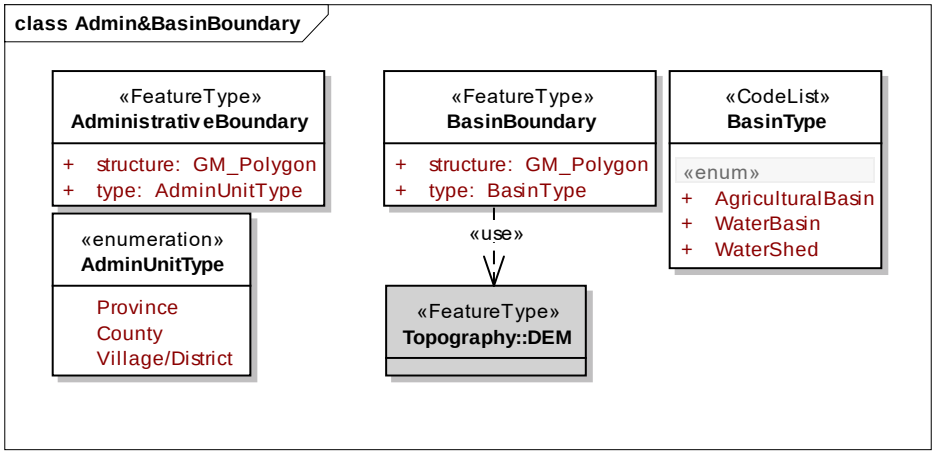

Figure 8- Admin & Basin package

3.9. Land Use Plan package

The basic dataset in this package is the land use plan, which is to be produced with the implementation of the model. The basic aim of land use planning is to determine sustainable land use decisions in conjunction with national, regional or local needs by considering current land use and all planning criteria. In this context, to represent the relation between current land use and planning decisions, the use relation was defined between the Land Use Plan and Land Resource classes (Figure 9). Indirect relations were defined among the Land Use Plan class and non-spatial data (socio-economic and rural development plan). The use relation is directly defined among the Land Use Plan class and other classes representing data on other planning criteria (soil classification, water resources, environmental plan and topography) (Figure 9).

Class attributes and the Planned Land Use Class definition set, as the data type of this attribute, were designed to represent the land use types proposed in the land use plan (Figure 9). The classes of planning decisions on this definition set were defined by inspiring earlier planning activities of the Ministry of Food, Agriculture and Livestock.

The Land Use Plan (LUP) class represents the most detailed local information. For the production of regional and national plans, the use of generalization methods was envisaged in the model. To represent this vision, the method called generalize was defined in the LUP class (Figure 9). The input data of this method are land use plans at local level, and the output data are regional or national land use plans represented by the Regional LUP or National LUP classes (Figure 9).

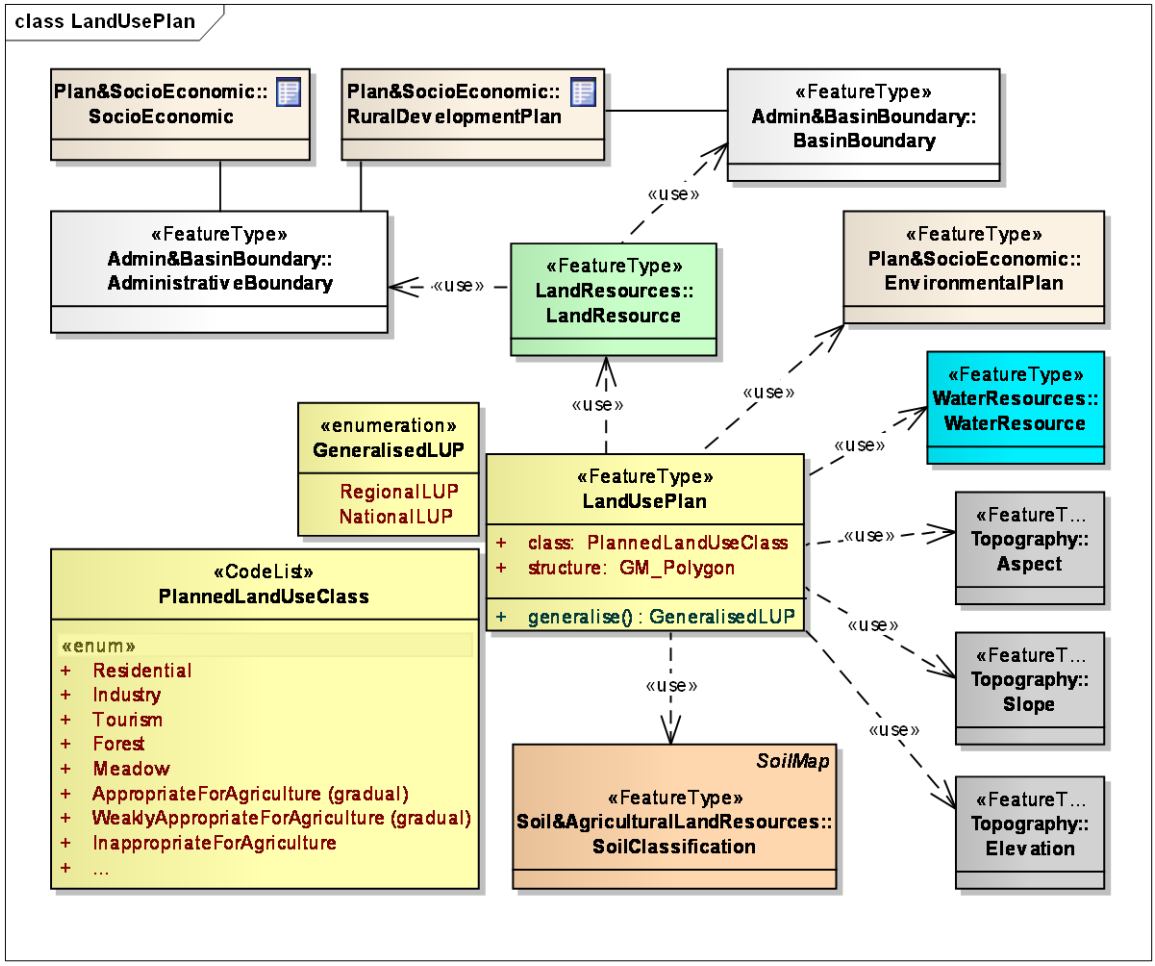

Figure 9- Land Use Plan package

3.10. Agricultural Land Use Plan package

The basic datasets in this package are the agricultural land use plan and physical block (Figure 10) which are to be produced by the implementation of the model. The production of these datasets is dependent on the use of data in other datasets. This dependency was represented by the use relation among related classes (Figure 10). The Physical Block class was designed to divide current and planned agricultural land (represented by the Agricultural Land Resource and Land Use Plan classes

respectively) into physical blocks (Figure 10). Because the spatial planning units of agricultural land use plans are physical blocks, the specialization relation was defined between related classes (Figure 10).

In the Physical Block class, the block name attribute (Figure 10) represents unique block numbers. The Agricultural Land Use Plan class inherits this attribute as well as the geometry attribute by means of the specialization relation (see Figure 10).

The attributes plan decision and suitability (Figure 10) are specific to this class, and they represent proposals or reports and crop type suitability for each planning unit (physical block). To represent the data type of the suitability attribute, the Agricultural Crop Type definition set, which is intended to include all crop types, was designed (Figure 10). When planning decisions for specific crops are needed instead of crop types, a new attribute (e.g. crop suitability) may be added to the model.

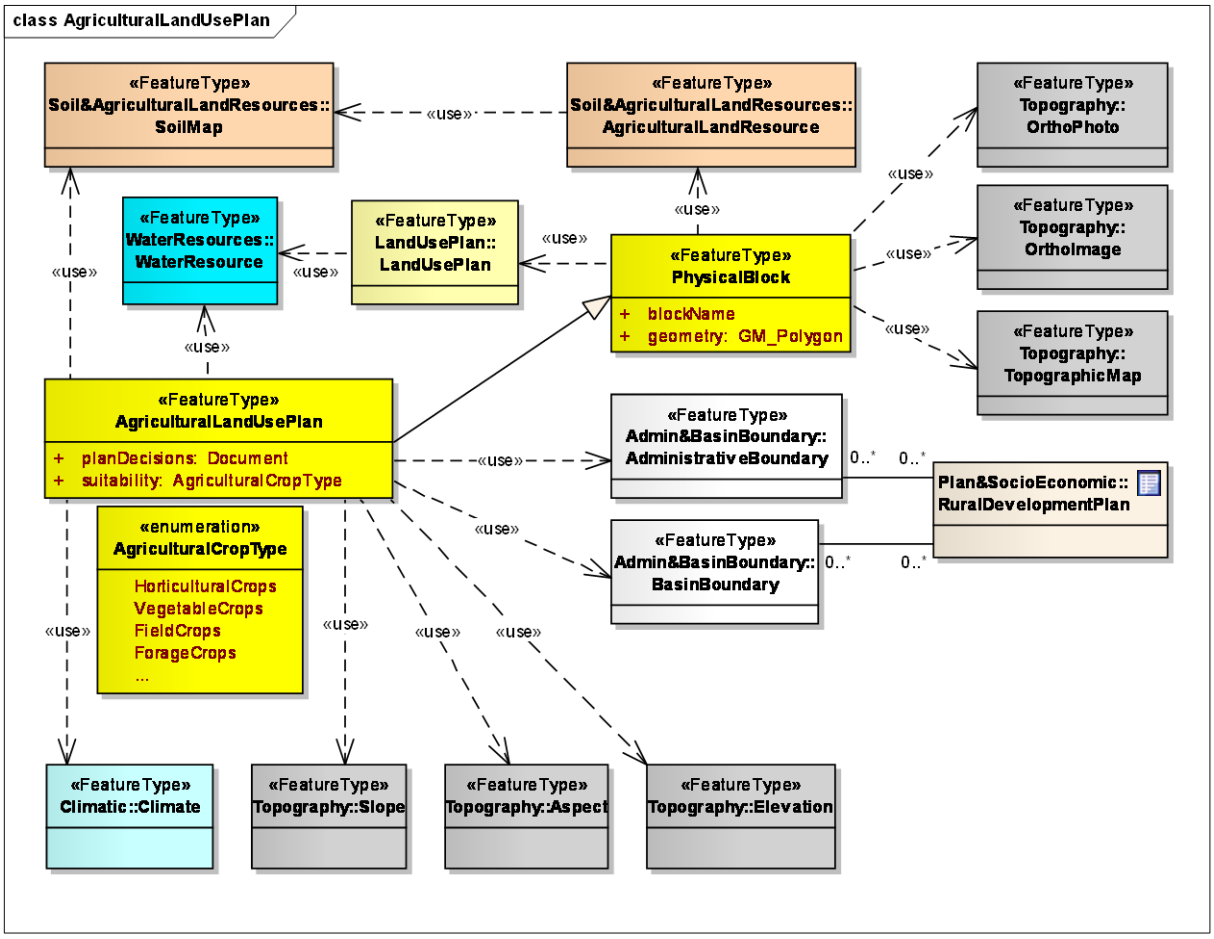

Figure 10- Agricultural Land Use Plan package

4. Conclusions

In line with the basic aim of this study, for a standardized production of Land Use Plans and Agricultural Land Use Plans, the proposed data model acts as a means to (1) understand the basic properties of the required data, (2) define the static or dynamic relations among them and also to (3) prevent different interpretations on data requirement or data processing. In the model, the complex natural relations between datasets were presented in a simple way by means of the object-oriented modelling approach using UML notation. However, it is impossible to represent all kinds of natural relations with this approach. For example, use or dependency relations are inadequate to fully model the data processing cycle. Hierarchical order in data processing should also be considered. It is possible to introduce many similar issues on data content, relations, and constraints. Yet, these issues are beyond the scope of this study.

With the model, it was aimed to contribute to the determination and development of implementation standards on rural land planning and management in Turkey. Although all the datasets required both for planning (land use plan and agricultural land use plan) and land management are included in the model, only the plan production process was focused on. The reason for this was the fact that plans are required for all kinds of land management activities. In this context, the model may be further developed for the inclusion of land management practices such as erosion prevention, soil conservation, increasing agricultural productivity and agricultural yield estimates.

To implement the model, all datasets included in the model should be produced by utilizing the data acquisition or compilation methods for new and available data, respectively. Success in this stage is dependent on many important factors related to the development of National Spatial Data Infrastructure (NSDI) such as data availability, data quality, rules for access to data and responsibilities for data production and maintenance. In this respect, the proposed model may also contribute to the development of the NSDI in Turkey. In fact, Turkish NSDI is its development phase and these kinds of related data infrastructure initiatives proposed by this study have an important role in its further development. Beyond the development of the Turkish NSDI, the proposed model is a good guide to produce a great deal of important data sets represented by classes in the model, especially the ones previously not very well known such as Land Resource, Agricultural Land Resource and Climate. After the data production stage, it is required to have a directive or regulation, which includes all the data processing stages/hierarchies in detail for the production of two basic products of the proposed model – Land Use Plans and Agricultural Land Use Plans. To meet this requirement, the proposed model is envisaged to be the basis for the preparation of such a legal document.

The modelling approach used in this study has been widely used by scientists for the development of ISO/OGC (the International Organization for Standardization / Open Geospatial Consortium) standards or similar tasks and by professionals in their commercial activities. Nevertheless, initiatives are strictly required to extend its use to the implementation of government law related to spatial data management. The proposed model is expected to contribute to such initiatives, especially in Turkey and also in other countries where such initiatives are inadequate.

To better motivate to such a modelling approach, instead of directly using class diagrams (as in this study), the use of activity and use-case diagrams prior to model development could be a convenient method, especially for those who are not adequately informed about the data management activity to be modelled.

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Role of Controlled Atmosphere, Ultra Low Oxygen or Dynamic Controlled Atmosphere Conditions on Quality Characteristics of 'Scarlet Spur' Apple Fruit

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ABSTRACT

In this study, the effects of three cold storage technologies, (i) controlled atmosphere-CA (CO_2 4%, O_2 3%), (ii) ultra low oxygen-ULO and (iii) dynamic controlled atmosphere-DCA, were investigated on fruit quality of 'Scarlet Spur' apples stored during 10 months plus 7 days of shelf life at 20 °C. After harvest, apples were stored at 0 °C and 90±5% relative humidity during 10 months in CA, ULO (CO_2 3%, O_2 1%,) and DCA (CO_2 1%, O_2 0.5%) conditions. HarvestWatch™ sensors were used for assessment of lower oxygen limit (LOL) of fruit during DCA storage. DCA was the best storage condition suppressing ethylene synthesis and respiration rate during storage. The ULO and DCA conditions showed similar results in the maintenance of firmness and TA amount. Weight loss in these conditions was also lower than CA. No significant difference was observed between storage conditions in terms of SSC. DCA technology gave better results in maintaining color of 'Scarlet Spur' than other conditions during cold storage. Result showed that; ULO and DCA conditions were more effective in maintaining quality compared to CA in terms of all quality parameters.

Keywords: Apple, Controlled atmosphere, Postharvest, Cold storage, Chlorophyll fluorescence

1. Introduction

Controlled atmosphere (CA) storage is a widely used technology for the storage of apple which is one of the most produced and consumed fruit in the world. Reducing the oxygen (O_2) concentration in the storage atmosphere and increasing the concentration of carbon dioxide (CO_2) is the most important factors for prolonging storage period in CA storage technology. Thus fruit quality is kept for longer periods, and postharvest losses during storage are reduced (Both et al. 2014). The possibility to obtain the longest storage period in CA conditions depends on (i) fruit maturity at harvest time, (ii) atmosphere composition during storage and (iii) the cultivar (Thompson 2010).

Storage under proper conditions plays an important role in maintaining product quality especially in climacteric fruit such as apples (Bertone et al. 2012). The main purpose of optimizing the CA storage conditions is to prevent ripening and aging of the fruit by decreasing O_2 level and reducing respiration rate and ethylene production (Veltman et al. 2003). Suppressing respiration rate and ethylene synthesis of fruit are the key postharvest processes throughout cold storage (Wright et al. 2015). O_2 is the most important factor to decrease metabolic activity and reduce biochemical changes after harvest. Therefore, the use of low O_2 levels in storage is an important potential (Tuna Gunes & Horzum 2017).

The equipment developed in CA storage technology has allowed to work at low O_2 or ultra low oxygen (ULO) conditions (Batu & Sen 2014). Standard CA storage involves keeping the oxygen content at 2-3% while the O_2 level in the ULO conditions can be reduced to 1%. ULO storage is more successful than standard CA technology in terms of preventing disease and physiological disorders (Balla & Holb 2007; Mattè et al. 2005). Additionally, it can protect some quality characteristics such as fruit flesh firmness and ground color better than standard CA storage procedure (Thewes et al. 2015). In developed countries, ULO storage has been extensively used in fruit industry in order to maintain fruit quality for a longer period (Watkins 2008).

Dynamic controlled atmosphere (DCA) is the new and popular technology in apple industry (Mditshwa et al. 2018). During DCA storage, O_2 level is reduced to the lowest level that the fruit can tolerate which is just above the so-called critical O_2 concentration (LOL). Quality losses related to anaerobic condition increase when fruit are stored under LOL. Ideally, fruit should be stored at levels just above the critical O_2 concentration (Gasser et al. 2008). It has been reported that fruit kept in these conditions could be stored for a long time without significant losses (Prange et al. 2007; Zanella et al. 2008; Wright et al.

2012). Researches in some apple varieties showed that DCA is more effective than CA for maintaining quality during storage (Veltman et al. 2003; Gasser et al. 2005; Delong et al. 2007; Bessemans et al. 2016; Thewes et al. 2017).

DCA technology involves monitoring of gas concentrations in the storage room via sensors. Up to now, three sensors has been developed in this technology; chlorophyll fluorescence (CF), respiration quotient (RQ) and ethanol (ET) (Thewes et al. 2018). While very little research has been done with RQ and ET sensors, CF is the most common used sensor in the pome fruit industry (Mditshwa et al. 2018).

CF technique measures the stress occurring in fruit during storage period. In this method, while the O_2 level is reduced, the CF signal on the fruit surface is measured by the sensor (Vanoli et al. 2010). Detection technology senses the response from the produce and feeds it back to an analytical software tool (HarvestWatch™) where the output is displayed in graph format (Stephens & Tanner 2005). The increase in the fluorescence signal indicates that the product enters low O_2 stress (Watkins 2008). The O_2 level is maintained over the LOL level by adapting according to fruit metabolism during storage.

In researches on effect of CA, ULO and DCA storage conditions for maintaining significant quality criteria in apples, results has changed based on cultivars (Aubert et al. 2015; Both et al. 2017; Kittemann et al. 2015; Thewes et al. 2015; Tran et al. 2015; Brizzolara et al. 2017). Therefore, in this study, the effects of CA, ULO and DCA on fruit quality of 'Scarlet Spur' apple cultivar was evaluated during a storage period of 10 months plus a shelf life period of 7 days at 20°C.

2. Material and Methods

2.1. Plant material

Experimental fruit were obtained from the commercial apple orchard located in Isparta/Eğirdir (38° 17' North, 30° 55' East), in 2012. The uniform trees were 8 years old cv. 'Scarlet Spur' apple on MM106 rootstock. Standard cultural practices were applied to the trees during fruit growth and development period.

2.2. Fruit harvest and storage conditions

Fruit were harvested at commercial harvest stage and transported to the postharvest physiology laboratory. Apples were randomly divided into three groups and stored at 0 °C and 90±5% relative humidity (RH) during 10 months in CA (CO_2 4%, O_2 3%), ULO (CO_2 3%, O_2 1%,) or DCA (0.5% O_2 and 1% CO_2) conditions, respectively. Cabinets manufactured with gas tight plastic material and each was 0.5 m^3 volume. HarvestWatch™ was used to assess lower O_2 limit (LOL) of fruit during DCA storage. LOL stress in fruit under DCA was assessed by CF sensors placed over a sample of 6 fruit each batch. LOL level in DCA was determined as 0.2%. The samples were stored at 0.5% O_2 level by adding 0.3% safety margin to the determined LOL level under DCA conditions. After cold storage, apples were kept at 20 °C and 60±5 % RH for 7 days to determine the effects of treatments on some quality parameters investigated in this research during shelf life.

2.3. Respiration rate and ethylene production

Fruit (1 kg) were kept in 5 L airtight jars at room condition (20 ºC) for determination of ethylene emission and respiration rate. After 3 h, the gas sample was taken from the closed jars by a gastight syringe and injected into loop of gas chromatography (GC) (Agilent 6840). Ethylene emission and respiration rate were measured by GC equipped with flame ionization (FID) and thermal conductivity detectors (TCD), respectively. Measurements were made in split/splitless (S/SL) of inlet in split mode with gas sampling valve with 1-mL gas sample by using fused silica capillar column (GS-GASPRO, 30 m x 0.32 mm I.D., U.S.A). Results were calculated as µL kg^{-1} h^{-1} and ml CO_2 kg^{-1} h^{-1} for ethylene production and respiration rate, respectively.

2.4. Fruit flesh firmness

Fruit flesh firmness was measured by using a texture analyzer (Güss FTA Type GS14 Fruit-Texture Analyzer Model, Strand, South Africa). The measurements were performed on both side of apple after skin removal using a stainless probe (11.1 mm). Firmness was measured over 10 fruit in each replication and results were presented in Newton (N).

2.5. Soluble solids content (SSC) and titratable acidity (TA)

The fruit juice from 10 apples in each replication was extracted with the help of a juicer for analysis. The soluble solids content (SSC) of apple juice (%) was determined with a refractometer (Digital-Atago Pocket PAL-1). The titratable acidity (TA) in apple juice was measured by titration of 10 mL of juice with NaOH solution (0.1 mol L^{-1}) to an end-point pH of 8.1 by a pH meter (Hanna pH 330 model, WTW, Germany). The results were expressed as % malic acid.

2.6. Fruit skin color

Fruit skin color of apples was measured with a colorimeter (Minolta CR 400, USA). Color measurements were made on both sides of 10 fruit in each replication along the equatorial axes. The calibration of color measurement apparatus was performed using an original calibration plate (white). The fruit colors were evaluated as CIE L*, a* and b*.

2.7. Weight loss

Weight loss of fruit was measured based on the initial weight and calculated as percent (weight loss % = [(first weight -last weight)/ first weight × 100)] during cold storage. In order to measure the weight loss during the shelf life period, weight measurements were made at the beginning and at the end of the shelf life. Weight loss of apples was measured over 10 fruit in each replicate.

2.8. Statistical analysis of results

The completely randomized design (with three replications) was chosen for this experiment. Using software package (JMP7), the general linear model was used for statistical analyses. The differences among means (at a significance level of 0.05) were analyzed using LSD test.

3. Results and Discussion

3.1. Respiration rate and ethylene production

During storage and shelf life, respiration rate increased in all storage conditions (Figure1). The differences between conditions and periods and their interactions were statistically significant in both cold storage and room conditions (P<0.001, 0.0001). The highest respiration rate during storage was determined in samples in CA (mean 9.87 mL.CO2 $kg^{-1}h^{-1}$) while the lowest respiration rate was observed in DCA (mean 7.22 $mL.CO_2\ kg^{-1}h^{-1}$). DCA was the best storage condition to suppress respiration rate. In room conditions, respiration rate values obtained from samples stored in ULO (11.86 $mL.CO_2\ kg^{-1}h^{-1}$) and DCA (11.39 $mL.CO_2\ kg^{-1}h^{-1}$) gave similar results. CA conditions were again resulted the highest (14.93 $mL.CO_2\ kg^{-1}h^{-1}$) respiration rate. It was determined that DCA storage of 'Granny Smith' apple suppressed respiratory rate better than CA storage (Eren et al. 2015). Similarly, previous studies have showed that limiting O_2 levels, significantly reduces respiration rate (Gasser et al. 2008; Wright et al. 2012; Thewes et al. 2015). Respiration is the breakdown of complex molecules (starch, sugar and organic acids) to simple molecules (CO_2 and H_2O) in the cell (Kader 2002). In the final stage of the respiratory reaction; as O_2 acts as the ultimate electron acceptor in the mitochondrial electron transport chain, the metabolism of the fruit can be slowed down by lowering the O_2 concentration in the storage (Bekele et al. 2016).

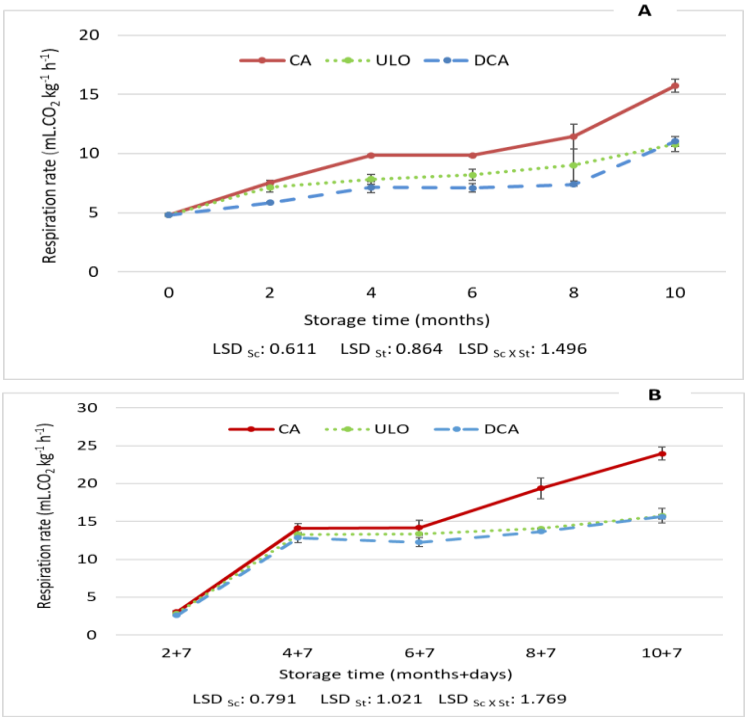

Figure 1- Respiration rate of 'Scarlet Spur' apples stored under different atmosphere conditions during 10 months (A) and plus 7 days for shelf life (B). Vertical bars represent standard error (n=3)

The effects of storage conditions and periods on ethylene production were statistically significant. The interaction between time and condition was also significant (P<0.05, 0.0001). During cold storage and shelf life, the amount of ethylene production increased in all three storage conditions (Figure 2). The highest increase was observed in CA storage. In the 8th month of storage, the ethylene production in the CA (7.46 µL.C_2H_4 kg^{-1} h^{-1}) showed a noteworthy increase compared to other conditions. This rapid increase in shelf life began to be observed since the 4th month (41.32 µL.C_2H_4 kg^{-1} h^{-1}). The highest average ethylene production during storage was obtained under CA storage. ULO and DCA caused in similar results in terms of ethylene production. For some apple cultivars, higher ethylene production was found in fruit stored in CA conditions compared to fruit stored in ULO and DCA conditions (Mattheis et al. 1998; Hennecke et al. 2008; Çalhan et al. 2012; Thewes et al. 2015). Since ethylene initiates the ripening process in fruit, its production is reduced to the lowest possible level, resulting in higher fruit quality after storage. (Watkins 2006).

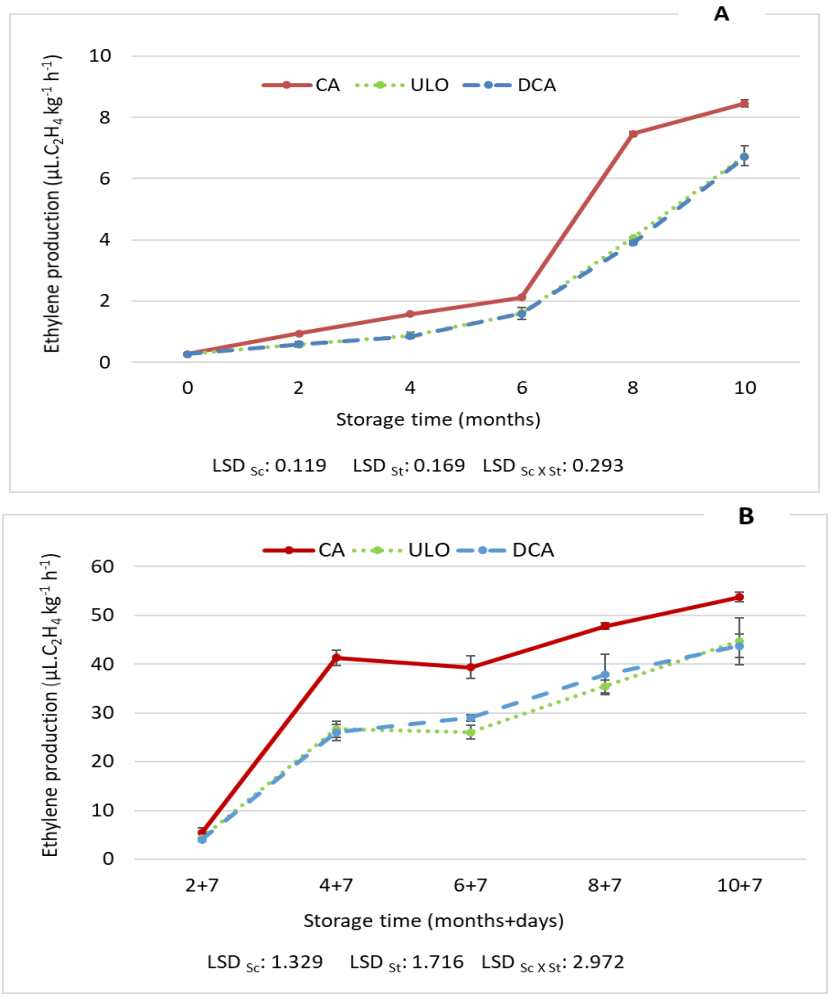

Figure 2- Ethylene production of 'Scarlet Spur' apples stored under different atmosphere conditions during 10 months (A) and plus 7 days for shelf life (B). Vertical bars represent standard error (n=3)

Reducing O_2 level in the storage atmosphere decreases the ethylene production of fruit (Gorny & Kader 1996). Storage of apples under DCA conditions significantly reduces ethylene synthesis and maintains long-term quality parameters (Watkins, 2008). DCA storage is effective in suppressing the activity of 1-Aminocyclopropane-1-carboxylate (ACC) oxidase enzyme that plays a key role in ethylene synthesis and production (Thewes et al. 2015; Weber et al. 2015; Thewes et al. 2017). ACC formed during ethylene synthesis pathway is oxidized by ACC oxidase enzyme and convert to ethylene (Nath et al. 2006). Therefore, reduction of O_2 in the storage room inhibits the activity of ACC oxidase enzyme and the conversion of ACC to ethylene.

3.2. Firmness (N), SSC (%) and TA (%)

According to the changes in fruit firmness values, the differences between the storage conditions and the storage period were statistically significant (P<0.0001). With the prolonged storage period, the firmness values of the samples decreased in all conditions. Firmness, which is one of the most important factors affecting apple quality, decreases in relation to water loss during long term cold storage (Mditshwa et al. 2017b). This decrease in the value of firmness during storage has been shown similarly in previous studies (Koyuncu & Bayındır 2013). Gwanpua et al. (2014) reported that the loss of sugar in the

'Jonagold' apples during ripening, increased pectin solubility, and the decrease in the water-soluble pectin molar mass were caused by softening. The highest average fruit flesh firmness values were obtained from the samples stored in DCA and ULO conditions during the storage (68.11 N-67.56 N) and shelf life (54.50 N -52.28 N) period. (Table 1, 2). Fruit flesh firmness was better protected in low O_2 conditions (De Castro et al. 2007). During the maturation, some enzymes cause the polymerization of pectin polymers and loosening the cohesion between the cells (Brummell & Harpster, 2001; Goulao & Oliveira 2008). This loss in cohesion of the pectin network is responsible for softening (Fischer & Bennett 1991). Many enzymes play a role in cell wall modifications during maturation of apples. The activities of these enzymes are related to ethylene production (Gwanpua et al. 2014). Ethylene signals the cell wall degrading enzymes and triggers their activity (Payasi et al. 2009). The lower firmness loss in DCA is related to the low amount of ethylene produced in this condition. It is reported that DCA storage suppresses the enzymes responsible for softening (Mditshwa et al. 2018). Studies on apples have shown that the firmness of fruit flesh in DCA conditions is better protected than CA conditions (Mattheis et al. 1998; Zanella et al. 2005; DeLong et al. 2007; Zanella et al. 2008; Tran et al. 2015; Thewes et al. 2015 Bessemans et al. 2016; Mditshwa et al. 2017a). The ULO conditions also yielded better results than CA storage in maintaining firmness. Similar findings were obtained from previous studies on 'Royal Gala' apple variety (Thewes et al. 2015; Weber et al. 2015; Both et al. 2017).

Table 1- Firmness (N), SSC (%) and TA (% malic acid) of 'Scarlet Spur' apples during cold storage

Storage conditions (Sc)		*Storage time (St)* 0	2	4	6	8	10	*Mean*
	CA	76.60	64.84	62.81	60.21	55.25	47.76	61.25B[1]
Firmness	**ULO**	76.60	72.47	68.12	66.35	62.54	59.30	67.56A
(N)	**DCA**	76.60	71.46	70.18	66.16	65.18	59.08	68.11A
	Mean	76.60a	69.59b	67.03c	64.24d	60.99e	55.38f	
P values		Sc ***	St ***	Sc X St *				
	CA	12.57	14.70	15.60	15.80	15.27	15.93	14.98[NS]
	ULO	12.57	15.30	15.33	15.70	14.97	15.70	14.93
SSC (%)	**DCA**	12.57	15.00	15.23	15.63	15.73	15.57	14.96
	Mean	12.57 [NS]	15.00	15.39	15.71	15.32	15.73	
P values		Sc NS	St NS	Sc X St NS				
	CA	0.36	0.33	0.30	0.29	0.27	0.23	0.30B
TA (% malic acid)	**ULO**	0.36	0.33	0.31	0.31	0.30	0.26	0.31A
	DCA	0.36	0.33	0.31	0.30	0.29	0.27	0.31A
	Mean	0.36a	0.33b	0.31c	0.30c	0.29cd	0.25e	
P values		Sc ***	St ***	Sc X St NS				

*, P<0.05-0.01; **, P<0.01-0.001; ***, P<0.0001; NS, nonsignificant. [1]Means followed by different letters with in the same row and column are significantly different.

Table 2- Firmness (N), SSC (%) and TA (%) of 'Scarlet Spur' apples during shelf life after cold storage

Storage conditions (Sc)		*Storage time (St)* 2+7	4+7	6+7	8+7	10+7	*Mean*
	CA	45.03	52.65	50.13	37.26	24.92	42.00B[1]
Firmness	**ULO**	55.58	63.40	56.30	44.84	41.27	52.28A
(N)	**DCA**	62.38	56.43	62.41	52.03	39.26	54.50A
	Mean	54.33cd	57.49a	56.28b	44.71d	35.15e	
P values	Sc ***	St ***	Sc X St *				
	CA	16.03	15.47	15.40	16.33	16.73	15.99 [NS]
	ULO	15.83	15.67	16.20	15.30	15.80	15.76
SSC (%)	**DCA**	15.03	15.63	15.93	15.13	15.67	15.48
	Mean	15.63 [NS]	15.59	15.84	15.59	16.07	
P values	Sc NS	St NS	Sc X St NS				
	CA	0.28	0.24	0.25	0.21	0.17	0.23B
TA (%)	**ULO**	0.32	0.28	0.27	0.25	0.23	0.27A
	DCA	0.31	0.31	0.30	0.25	0.22	0.28A
	Mean	0.30a	0.28b	0.27b	0.24c	0.20d	
P values	Sc ***	St ***	Sc X St NS				

*, P<0.05-0.01; **, P<0.01-0.001; ***, P<0.0001; NS, nonsignificant. [1]Means followed by different letters with in the same row and column are significantly different.

In comparison with the DCA and ULO conditions in terms of fruit flesh firmness, it is better protected in the apple cultivars of 'Gloster' (Köpcke 2015), 'Granny Smith' and 'Red Delicious' (Mditshwa et al. 2017a, 2017b; Brizzolara et al. 2017) in the DCA conditions while similar results obtained from the studies regarding apple cultivars 'Golden Delicious' and 'Pinova' (Kittemann et al. 2015), 'Fuji' and 'Gala' (Zanella & Rossi 2015). This is mainly because of different metabolic reactions of apple genotypes (Brizzolara et al. 2017).

The SSC of fruit increased at the end of the storage period compared to initial values with fluctuation during cold storage. The amount of SSC was 12.57% at harvest. At the end of the cold storage period, SSC amount was determined as 15.93% in CA, 15.70% in ULO and 15.57% in DCA condition. No significant difference was observed between technologies in terms of SSC (Table 3, 4). TA values decreased significantly during cold storage and shelf life (Table 3, 4). The decrease of TA during storage is due to the consumption of malic acid as a metabolite substrate in fruit respiration (Ackerman et al. 1992). TA was significantly lower in samples stored in CA compared to other conditions (P<0.0001). The ULO and DCA conditions showed similar results in the maintenance of TA amount. Similarly, 'Granny Smith' (Eren et al. 2015), 'Cortland' (DeLong et al. 2007), 'Royal Gala' (Weber et al. 2015), and 'Red Delicious' (Brizzolara et al. 2017) apple cultivars have been reported to maintain better TA levels under the DCA and ULO than CA conditions. The CA conditions with low O_2 are advantageous in maintaining TA values (Özer 2002). Generally, a decrease in the concentration of O_2 in atmosphere causes a decrease in consumption rates of citrate and malate in the formation of organic acids in the tricarboxylic acid cycle (Mir & Beaudry 2002).

Table 3- Fruit skin color changes of 'Scarlet Spur' apples during storage and shelf life

Storage conditions (Sc)		*Storage time (St)(months)*						
		0	***2***	***4***	***6***	***8***	***10***	***Mean***
L*	**CA**	30.53	30.66	29.64	29.59	27.02	27.21	29.11A[1]
	ULO	29.71	29.74	27.89	28.60	25.07	26.50	27.92B
	DCA	29.93	30.43	28.88	28.56	25.49	25.38	28.11B
	Mean	30.06a	30.28a	28.80b	28.92b	25.86c	26.36c	
P values	Sc***	St***	Sc X St	NS				
a*	**CA**	19.14	20.80	23.31	23.21	24.66	23.52	22.44A
	ULO	19.10	20.12	22.81	22.53	24.81	24.68	22.34A
	DCA	18.44	19.63	21.72	21.68	23.53	23.40	21.40B
	Mean	18.89e	20.18d	22.62bc	22.47c	24.33a	23.87ab	
P values	Sc*	St***	Sc X St	NS				
b*	**CA**	8.98	9.90	11.06	10.94	11.54	11.63	10.68A
	ULO	8.44	9.13	10.22	10.20	11.21	11.69	10.15AB
	DCA	8.33	9.12	9.99	9.80	10.51	10.65	9.73B
	Mean	8.58c	9.38c	10.42ab	10.32b	11.08ab	11.32a	
P values	Sc*	St***	Sc X St	NS				
Storage conditions (Sc)		*Storage time (St)(months+days)*						
			2+7	***4+7***	***6+7***	***8+7***	***10+7***	***Mean***
L*	**CA**		33.97	30.72	33.27	34.78	33.02	33.15[NS]
	ULO		32.37	30.63	31.70	33.07	32.70	32.10
	DCA		32.66	29.15	32.39	34.41	31.03	31.93
	Mean		33.00b	30.17d	32.46bc	34.08a	32.25c	
P values	Sc NS	St**	Sc X St	NS				
a*	**CA**		25.01	23.65	23.10	25.33	22.52	23.92 [NS]
	ULO		24.35	23.26	24.01	21.99	22.05	23.13
	DCA		24.22	23.86	23.09	24.33	22.16	23.53
	Mean		24.53 [NS]	23.59	23.40	23.88	22.24	
P values	Sc NS	St NS	Sc X St	NS				
b*	**CA**		13.61	12.33	11.56	12.46	10.81	12.16 [NS]
	ULO		12.23	11.83	11.78	11.23	10.79	11.57
	DCA		11.51	11.26	11.41	10.77	10.57	11.10
	Mean		12.45 [NS]	11.81	11.58	11.49	10.72	
P values	Sc NS	St NS	Sc X St	NS				

*, P<0.05-0.01; **, P<0.01-0.001; ***, P<0.0001; NS, nonsignificant. [1]Means followed by different letters with in the same row and column are significantly different.

Table 4- Weight loss (%) of 'Scarlet Spur' apples stored in different conditions

Storage conditions (Sc)		*Storage time (St)*					*Mean*
		2	***4***	***6***	***8***	***10***	
Cold Storage (0 ºC)	**CA**	0.44	0.85	1.21	1.51	2.08	1.22A[1]
	ULO	0.36	0.61	0.87	1.08	1.57	0.91B
	DCA	0.36	0.63	0.88	1.07	1.61	0.90B
	Mean	0.38e	0.70d	0.99c	1.22b	1.75a	
Shelf Life (+7 days at 20 ºC)	**CA**	1.71	2.17	2.06	2.76	3.76	2.49A
	ULO	1.57	1.83	1.69	2.16	2.82	2.02B
	DCA	1.58	1.95	1.57	2.18	2.91	2.03B
	Mean	1.62e	1.98c	1.77d	2.36b	3.16a	
P values	**Cold Storage**	Sc St Sc X St	*** *** ***	Shelf Life	Sc St Sc X St	* *** NS	

*, P<0.05-0.01; ***, P<0.0001; NS, nonsignificant. [1]Means followed by different letters with in the same row and column are significantly different.

3.3. Fruit skin color

L* value, which expresses brightness during storage and shelf life, generally decreased according to initial value. Whereas red color (a*) and yellow ground color (b*) increased due to maturation (Table 3). This increase is caused by the decomposition of chlorophyll forming the green color in the fruit during the storage and turning the color of the green in the fruit to yellow (Çalhan et al. 2012). The effect of different storage conditions on color values during storage was statistically significant ($P<0.001$). The interaction between the storage conditions and storage time was insignificant in both cold storage and shelf-life conditions. The lowest average a* and b* values (21.40-9.73) were obtained from the samples stored in DCA conditions during the storage period. DCA preserves the quality of fruit better, by contributing to the preservation of the fruit color during storage and shelf life (Zanella et al. 2008; Veltman et al. 2003). Previous studies showed that DCA technology gave better results in maintaining color of 'Granny Smith' than CA (Bessemans et al. 2016) and ULO in 'Elstar' (Veltman et al. 2003). Additionally, it was reported that DCA delays chlorophyll degradation (Tran et al. 2015).

3.4. Weight Loss

The weight loss during storage and shelf life of 'Scarlet Spur' apple samples kept under different atmospheric composition was increased continuously as shown in Table 4. This change was statistically significant ($P<0.0001$). The maximum weight loss occurred in CA for both storage (1.22%) and shelf life (2.49%) period while the weight loss observed in ULO (0.91%-2.02%) and DCA (0.90%-2.03%) conditions were similar. Weight loss is associated with the respiratory rate of the product. Increases in weight loss are due to the removal of water from the tissues along with the CO_2 released as a result of the respiration of the product during storage (Erbaş et al. 2014). The gas composition in the ULO and DCA storage conditions suppressed the respiration rate better compared to the CA storage and thus the weight loss in these conditions was also lower. The interaction between storage time and storage conditions was statistically significant ($P<0.0001$) during storage and insignificant during shelf life.

4. Conclusions

Result of this study conducted with 'Scarlet Spur' apple showed that ULO and DCA conditions were more effective in maintaining quality compared to CA in terms of all quality parameters. DCA was the best storage condition suppressing respiration rate and ethylene production that expressed maturation during storage. Additionally, DCA was found to be more effective than other conditions to preserve important quality parameters in apple fruit such as color and firmness. As a result, the storage of 'Scarlet Spur' apples under DCA was found to be more successful than ULO and CA conditions in terms of preservation of quality criteria in long-term cold storage and during shelf life.

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Effects of Different Irrigation Levels on Fruit Yield and Quality of Valencia Late Orange Under Northern Cyprus Conditions

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ABSTRACT

This study was carried out to define different drip irrigation approaches on fruit yield and quality parameters of orange (*Citrus sinensis* cv. Valencia Late) trees during 2014-2016 at the private farm in Güzelyurt, Northern Cyprus. The amount of irrigation water was applied based on the total evaporation amount obtained from the Class A pan (K_{cp1}:1.25, K_{cp2}:1.00, K_{cp3}:0.75 and K_{cp4}:0.50) and experimental plots were irrigated when the total evaporation of Class A pan was about 35 ± 5 mm. It was determined that irrigation treatments affect yield, weight, length, width and juice of fruit, total soluble sugar, total acidity, pH and vitamin C content except for peel thickness. The average evapotranspiration values were 1343.5 mm for K_{cp1}, 1135.0 mm for K_{cp2}, 956.0 mm for K_{cp3}, and 787.3 mm for K_{cp4} irrigation treatments. According to the average data of 2 years, yearly yield for K_{cp1}, K_{cp2}, K_{cp3}, and K_{cp4} irrigation levels were 45.0, 47.1, 38.7, and 19.2 t ha^{-1}, respectively. It is determined that Valencia Late can be irrigated by means of the volume of irrigation equivalent to 75% of Class A Pan in Güzelyurt region in Turkish Republic of Northern Cyprus.

Keywords: Citrus, Drip irrigation, Water use, Yield, Total soluble sugar

1. Introduction

Nowadays, water shortage represents a real environmental concern. According to the climate change simulation forecasts, the problem of water shortage will be greater than before in the future in Mediterranean countries. After Sicily and Sardinia, Cyprus is the third largest island, with an area of 9,251 km^2, in the Mediterranean Sea. Cyprus, possesses a coastline of 1,364 km. It is located in the Eastern Mediterranean Basin at the crossroads of Asia, Africa, and Europe continent. Turkish Republic of Northern Cyprus (TRNC) is the northern part of the island with 3,355 km^2, approximately one third of the whole island (Gozen & Turkman 2008). Its economy is dominated by sectors like industry, tourism, public, education, and agriculture sectors. The backbone of the economy of TRNC is agricultural sector that plays a decisive role for the trade and industry development, and provides employment area and income for a significant part of labour force.

Turkish Republic of Northern Cyprus faces a severe water scarcity problem because of the climate conditions. Drought has affected negatively the agricultural production and the decreasing drop in precipitation during winter and increasing warm temperature in summer have caused deficiency in water resources. Especially, Morphou (Güzelyurt) area was famous with orange production but the increasing water scarcity problem and the increasing salt level in surface and underground water have dried trees over time. Citrus is the main crop that could be exported to other countries in TRNC. Citrus yields are affected by soil conditions, weather conditions, growing season, and water availability. Citrus are very sensitive to irrigation water deficit and deficit irrigation effects are several, which include leave loss, reduction in CO_2 assimilation, stomatal conductance, water potential and transpiration. Consequently, plant cell development decreases leading to plant growth inhibition and reproductive failure (Arbona et al. 2005).

Especially in arid and semi-arid regions, the amount of irrigation water plays numerous roles in the productive yield of oranges by affecting the yield, morphological and physiological chemical processes (Gozen & Ergil 2006). Treeby et al. (2007) reported that low irrigation water in the soil profile may denote a main restrictive reason for orange yield and quality in these regions and irrigation water deficiency losses crop loads of oranges. Perez-Perez et al. (2009) stated that if water stress is occurred

at late growing periods, water deficiency in orange tree is related positively to titratable acids and total soluble solids and negatively to juice percentage, with no overall effect on maturation index in oranges.

Some researchers determined that the effects of irrigation water deficiency on orange yield and quality in the literature. Toledo et al. (1982) reported that the amount of irrigation water applied at 85% field capacity was the best results whereas the amount of irrigation water applied at 65% field capacity. Low field capacity lead to less water consumption, drought injury symptoms and excessive defoliation. Ghadekar et al. (1989) found that the irrigation water amounts were 651.9, 849.0 and 997.8 mm for young, middle age and mature trees, respectively. Eliadses (1998) stated that 780 mm of irrigation water was adequate for best yield and growth of 6-years-old Washington Navel orange and reducing irrigation water by 37% decreased yield by 10.7%, while further reduction to 26% reduced the yield by 5.8%. Garcia-Tejero et al. (2010), studied 4 approaches of different deficit irrigation levels based on a different water deficiency ratio in Spain. They reported that different deficit irrigation water amounts affect orange yield and quality. Shahabian et al. (2012), determined that the effects of three irrigation levels (75% and 50% of the full irrigation) on yield and quality orange and stated that water deficiency reduced yield by around 30% compared with full irrigation treatments, but deficit irrigation treatments caused no negative impact on quality. Consoli et al. (2014) concluded that the application of irrigation water to 75% of the plant needs did not cause significant harmful effects on orange tree and allowed water savings. Stagno et al. (2015) determined that the orange became low sensitive to moderate water stress (70% ETc) allowing nearly 80 mm of irrigation water saving in Sicily (Italy).

The irrigation and public water shortage problem on TRNC cannot be ignored, especially when future predictions of irrigation water needs show a gap of 32 million cubic meters (MCM) by 2035. To solve the problem of water scarcity in the TRNC, The Government of Republic of Turkey has experienced an under-sea Water Supply Project with a length of 80 km for the first time in the world. The project targets to transfer about 75 MCM of water annually (37.24 MCM for irrigation purpose and 37.76 MCM for domestic purposes) from Turkey to TRNC (Gozen & Turkman 2008; Gungor 2016). In order to deal with irrigation water shortage and to use water properly devoid of reducing agricultural productivity, it has become necessary to use water resources used in agriculture in the most efficient way and to increase water use and irrigation water productivity.

As seen from the researches, though there are some researches relating to irrigation of young and old orange trees, there is no research carried out the assessment of different irrigation levels on orange in TRNC. For that reason, this research was conducted to define the influence of deficit and excessive irrigation amounts on water use efficiency, yield and quality parameters of Valencia Late orange trees and to define the lowest irrigation threshold for orange trees and to predict the amount of irrigation water saving for the Mediterranean area and climate conditions.

2. Material and Methods

2.1. Research area and climatic parameters

The study was carried out on orange trees (*Citrus sinensis* cv. Valencia Late, twenty years old) located at the private farm (35°12'N, 33°0'E, 54 m above sea level) during the growing seasons between 2014-2016 years in Güzelyurt, TRNC. The climatic properties of the region are extreme Mediterranean type with very hot and dry summers and mild winters. Most of the rainfall is concentrated between December and February. The long term monthly mean temperature, relative humidity, rainfall, and wind speed averagely ranged from 15.2 to 32.4°C, from 62.2% to 72.6%, from 10.0 to 129.0 mm, and from 2.5 to 3.7 m s^{-1}, respectively. The annual average evaporation according to Class A Pan readings reaches up to 1807.0 mm $year^{-1}$. During the experimental years, climatic data obtained from Güzelyurt weather location were similar values. The climatic variables for long-term (1976-2013) means and experimental years in 2014-2015 and 2015-2016 are given in Table 1.

Table 1- Long-term monthly and growing season climatic data of the experimental area

Years	*Months*	*Temperature (°C)*	*Rainfall (mm)*	*Evaporation (mm)*	*Wind ($m\ s^{-1}$)*	*Relative humidity (%)*
1976-2013	Jan.	15.2	126.0	42.0	3.5	68.4
	Feb.	16.1	105.0	52.0	3.5	67.2
	Mar.	18.4	78.0	86.0	3.4	67.5
	Apr.	22.5	54.0	143.0	3.6	68.1
	May	25.6	37.0	215.0	3.5	68.3
	June	28.8	17.0	268.0	3.6	69.1
	July	31.2	12.0	301.0	3.7	71.7
	Aug.	32.4	10.0	266.0	3.6	72.6
	Sep.	30.8	24.0	197.0	3.2	65.4
	Oct.	27.1	54.0	128.0	2.5	62.2
	Nov.	22.4	88.0	67.0	2.9	63.1
	Dec.	17.3	129.0	42.0	3.1	69.2
2014-2015	Apr.	17.4	6.8	167.5	2.7	68.4
	May	14.1	38.2	225.6	2.8	71.9
	June	16.8	11.3	298.6	3.2	61.7
	July	26.9	0.0	308.9	3.1	68.3
	Aug.	21.9	0.0	291.0	2.7	68.4
	Sep.	19.0	0.0	264.9	3.1	62.0
	Oct.	20.7	28.8	118.4	2.1	67.7
	Nov.	16.0	45.6	97.4	2.1	65.4
	Dec.	14.2	41.3	30.0	2.2	77.5
	Jan.	10.3	71.6	36.9	2.5	74.5
	Feb.	10.9	67.8	35.6	2.6	75.8
	Mar.	13.9	49.0	122.2	2.8	70.6
2015-2016	Apr.	15.5	23.3	137.8	2.7	65.0
	May	20.9	82.1	212.2	2.7	66.9
	June	23.5	0.0	292.7	3.1	67.8
	July	26.8	0.1	298.5	2.6	63.7
	Aug.	28.4	0.0	313.5	2.7	61.8
	Sep.	26.1	14.4	203.4	2.5	66.4
	Oct.	22.4	19.8	126.2	2.1	69.4
	Nov.	17.7	28.6	72.6	2.1	60.9
	Dec.	12.0	3.9	73.3	2.0	68.9
	Jan.	12.8	52.5	32.0	2.4	72.4
	Feb.	12.1	14.1	60.3	2.3	70.8
	Mar.	14.2	24.8	129.8	3.4	63.4

2.2. Properties of soil and irrigation water

The soil properties of the study area is clay (C) in texture, non-saline (0.42 dS m^{-1}), and rich in alkaline and calcium carbonate. The water content in 0-30, 30-60, 60-90 and 90-120 cm soil profile (cm cm^{-3}) at field capacity (FC, 1/3 atm. pressure) was 36.8, 36.1, 37.4, and 39.6 and at permanent wilting point of soil (PWP, 15 atm. pressure) was 19.1 19.4, 20.1, and 20.4, respectively. The bulk density, pH and $CaCO_3$ of experimental soil were 1.29, 1.25, 1.27 and 1.30 g cm^{-3}, 8.0, 8.3, 8.7, and 8.6 and 8.7%, 11.3%, 10.9%, and 13.0% in 0-30, 30-60, 60-90 and 90-120 cm soil profile, respectively. The electrical conductivity (EC) of irrigation water used in study was 1.66 dS m^{-1}. The electrical conductivity value does not threat for oranges and sodium absorption rate (SAR) was 5.2 (Ayers & Westcot 1985).

2.3. Experimental design

Randomized block (RB) with three replications was applied as an experimental design and each treatment has eighteen trees (Gomez & Gomez 1984). The plant material (*Citrus sinensis* cv. Valencia Late, grafted on Sour orange rootstock, 20 years old) are implanted at 6 m × 6 m in row spacing and in-row spacing. There were 12 plots in the study and each experimental plot consisted of 18 orange trees. Four orange trees in the middle of the each experimental plot were used for obtaining yield and quality data.

2.4. Irrigation system and treatments

Surface drip irrigation system was used and each orange tree row contained two drip irrigation laterals. The drip laterals were placed 0.50 m from afar the orange tree. The in-line drippers having discharge rate of 2 L h^{-1} on laterals were located 0.50 m apart. The irrigation water amount applied to each plot was controlled using a water counter. Each experimental plot has valves located on the main pipeline.

Soil water content in each experimental plots was observed gravimetrically before each irrigation treatments throughout the growing season. Irrigation applications were applied according to the data (E_{pan}, mm) achieved from a Class A Pan located in study area (Doorenbos & Pruitt 1977). When the cumulative amount of Class A pan evaporation was about 35 ± 5 mm, the experimental plots were irrigated. Irrigation levels were implemented, i.e. K_{cp1} = 1.25 E_{pan}, K_{cp2} = 1.00 E_{pan}, K_{cp3}=0.75 E_{pan}, and K_{cp4} = 0.50 E_{pan}. The amount of applied irrigation water (L) was determined according to the Kanber et al. (1996):

$$I = A \times E_{pan} \times K_{cp} \times P \quad (1)$$

Where I = total irrigation water amount (L); A = experimental plots (m^2); E_{pan}= the amount of Class A pan evaporation (mm); K_{cp} = crop-pan coefficient value (0.50, 0.75, 1.00 and 1.25); and P = wetted area (%, According to the Keller & Bliesner (1990) P was taken as 40%). The initial irrigation application was applied 1 April 2014 and 2015 when the orange trees were at the harvesting stage and study plots were irrigated when total Class A pan evaporation reached 35 ± 5 mm throughout the year.

2.5. Water use parameters

Evapotranspiration (ET) was determined using the soil-water balance method (Doorenbos & Pruitt 1977) for each growing period.

$$ET = I + P - D \pm \Delta W \quad (2)$$

Where ET = evapotranspiration (mm); I = total irrigation water applied in growing period (mm); P = total precipitation (mm); D = total deep percolation (mm); and ΔW = change in soil moisture. Total irrigation water (I) was measured using water counters, and P was measured at the weather station in the study area. Soil water measurement taken before some irrigation applications were compared to the amount of evaporation occurred. It was accepted that there was no deep percolation, since no soil moisture increase was observed in the lower soil profile during moisture control prior to irrigation.

Irrigation water use efficiency (IWUE, kg da^{-1} mm^{-1}) and water use efficiency (WUE, kg da^{-1} mm^{-1}) were explained as yield (Y, kg da^{-1}) divided by the evapotranspiration (ET, mm) and total irrigation water (I, mm) applied in the plots (Howell 2001).

$$WUE = (Y / ET) \times 100 \quad (3)$$

$$IWUE = (Y / I) \times 100 \quad (4)$$

2.6. Yield and fruit quality parameters

The Valencia Late fruits were harvested according to fruit maturity on March (March 14, 2015 and 2016) in experimental years. During the harvest, 4 trees at the edges of the experimental plots were left as a side effect and total yield (t ha^{-1}) was determined by harvesting all the fruits. Fruit weight (g), fruit length (mm), fruit width (mm), peel thickness (mm), total soluble solids (TSS, %), total acidity (TA, %), pH, vitamin C values were determined in the end of the study for each growing season. Variance analysis (ANOVA) was used to calculate the assessment of irrigation levels on parameters of Valencia Late. Duncan's multiple range tests was used to compare the averages (Gomez & Gomez 1984).

3. Results and Discussion

3.1. Water use parameters and yield

The values related to I, P, D, ΔW, ET, IWUE, and WUE were given in Table 2. The experimental plots were irrigated 58 and 56 times in 2014-2015 and 2015-2016, respectively. The total irrigation water applied to K_{cp1}, K_{cp2}, K_{cp3}, and K_{cp4} treatments were 1103.5, 903.8, 704.1, and 504.4 mm in 2014-2015, 976.2, 780.9, 585.7, and 390.5 mm in 2015-2016, respectively. Evapotranspiration ranged from 794.9 to 1347.8 mm in 2014-2015, and from 779.7 to 1299.1 mm in 2015-2016 growing season and ET rates were decreased with decreasing applied irrigation water and the peak ET rates in both of the years were obtained from K_{cp1} treatment.

Table 2- The parameters of water balance and water use efficiency in the experiment

Parameters	*2014-2015*				*2015-2016*			
	K_{cp1}	K_{cp2}	K_{cp3}	K_{cp4}	K_{cp1}	K_{cp2}	K_{cp3}	K_{cp4}
Irrigation events	58.0	58.0	58.0	58.0	56.0	56.0	56.0	56.0
Irrigation water (I, mm) [1]	1103.5	903.8	704.1	504.4	976.2	780.9	585.7	390.5
Rainfall (P, mm) [2]	360.4	360.4	360.4	360.4	263.6	263.6	263.6	263.6
Soil water depletion (ΔS, mm) [3]	-116.1	-111.6	-89.4	-69.9	59.3	72.9	87.6	125.6
ET (mm) [4]	1347.8	1152.6	975.1	794.9	1299.1	1117.4	936.9	779.7
Yield (t ha^{-1})	42.1a^{x}	45.5a	37.7a	18.2b	47.9a	48.6a	39.6a	20.1b
Relative yield decrease	7.5	-	17.2	60.0	1.5	-	18.6	58.7
WUE (kg da^{-1} mm^{-1})	3.12	3.95	3.86	2.29	3.69	4.35	4.23	2.58
IWUE (kg da^{-1} mm^{-1})	3.82	5.04	5.35	3.61	4.91	6.22	6.77	5.15

x; The different letters indicate the important differences according to the Duncan test,[1] Irrigation periods are from 01 April 2014 to 31 March 2015 (first year) and 01 April 2015 to 25 March 2016 (second year), [2] Total rainfall received from 01 April 2014 to 31 March 2015 (first year) and 01 April 2015 to 25 March 2016 (second year) all the rainfall has been accepted to be effective, periodically, [3] Soil water depletion values are from 01 April 2014 to 31 March 2015 (first year) and 01 April 2015 to 25 March 2016 (second year), [4] Evapotranspiration values are from 01 April 2014 to 31 March 2015 (first year) and 01 April 2015 to 25 March 2016 (second year).

Water is important for orange trees (or for any citrus) because it is the transporter that transfers plant nutrients and other materials throughout the tree, it is an integral part of the biochemical reactions that take place within the plant, and it helps maintain plant temperature through transpiration. Ghadekar et al. (1989) were found irrigation water requirements as 651.9, 849.0 and 997.8 mm for young, middle and mature orange trees, respectively. Kanber et al. (1996) reported that irrigation water amounts were 1290 and 921 mm for sprinkler and drip irrigated orange trees, respectively. Treeby et al. (2007) determined that irrigation water amount was 1000 mm of Bellamy Navel orange in Australia. Eliadses (1998) reported that 780 mm of irrigation water was adequate for best yield and plant growth in the coastal region of Cyprus for Washington Navel orange trees and while Hussien et al. (2013) determined that 1024 mm irrigation water was adequate for Washington Navel orange. Additionally, Nizinski et al. (2017) reported that irrigation and evapotranspiration values of Valencia Late were 994.3 and 1271.5 mm under Egypt conditions.

The results obtained from the study indicated that different irrigation water amounts statistically ($P < 0.01$) effected orange yield in the study. Orange yields ranged from 18.2 t ha^{-1} to 45.5 t ha^{-1} and 20.1 t ha^{-1} to 48.6 t ha^{-1} for 2014-2015 and 2015-2016 growing season, respectively. In the study, maximum and minimum yield was obtained from K_{cp2} and K_{cp4} treatment. Orange yields were testified to ranged from 7.7 t ha^{-1} to 13.4 t ha^{-1} for drip and sprinkler irrigation system by Kanber et al. (1996), from 17.5 t ha^{-1} to 37.0 t ha^{-1} for deficit and full irrigation application conditions by Petillo & Castel (2004), from 29.1 t ha^{-1} to 35.2 t ha^{-1} for deficit and full irrigation conditions by Al-Rousan et al. (2012).

Data obtained study for both years on IWUE and WUE are shown in Table 2. In the study, IWUE and WUE were determined statistically not significant. The WUE values ranged from 2.29 to 3.95 kg da^{-1} mm^{-1} in 2014-2015 and from 2.58 to 4.35 kg da^{-1} mm^{-1} in 2015-2016. In both years, WUE values were lower than IWUE values. The IWUE values were between 3.61 and 5.35 kg da^{-1}mm^{-1} in 2014-2015 and from 4.91 to 6.77 kg da^{-1} mm^{-1} in 2015-2016. Goodwin & Boland (2000) informed that excessive or deficit irrigation water level causes stomatal closure, stomatal closure leads to water stress in plants and thereby improving the WUE. Meshram et al. (2010) determined that the water shortage causes lower WUE values and therefore lower yields are obtained from water stressed plants. The results obtained this study are in consistent with literature studied by Kanber et al. (1996), Perez-Perez et al. (2009), Hussien et al. (2013), Zapata-Sierra & Manzano-Agugliaro (2017), and Silveira et al. (2018). Some differences can be attributed to the cultivar of orange used in the study and climatic conditions of the experimental area.

3.2. Irrigation water (I)-yield (Y) and evapotranspiration (ET)-yield relationship

The relationship between orange yield and water use was assessed for each growing season (Figure 1). It was found second degree-polynominal relationship between amount of I and Y and between ET and Y were established, as shown in Figure 1. Yield in Kcp_2 treatment is higher than excessive and deficit irrigation treatments. Water requirement varies considerably with climate, soil type and orange variety. When a tree suffers from lack of water, its yield decreases even it may recover after irrigation. On the other hand, increasing the number of irrigations (or water quantity) may result in injuring the crop and the soil besides being a waste of water and labour (Hussien et al. 2013). Compared to K_{cp2} strategy, decreases in the orange yield were determined as 7.5%, 17.2%, and 60.0% for K_{cp1}, K_{cp3}, and K_{cp4} treatments for 2014-2015 growing season, and 1.5%, 18.6%, and 58.7% for K_{cp1}, K_{cp3}, and K_{cp4} treatments for 2015-2016 growing season, respectively. Zekri (2011) reported that a good irrigation management is required for maximum and quality yield in citrus. Citrus trees that are irrigated and nourished adequately grow stronger, tolerate biotic and abiotic stress parameters, and produce consistent yield and fruit quality. Conversely, excessive irrigation water or water shortage will leads to low yield and poor fruit quality. Similar relationships between yield and irrigation

water for orange was recognized in other researches having a report a rather strong linear connection between the irrigation water applied and yield (Shalhevet & Levy 1990; Ali & Lovatt 1996; Goldhamer & Salinas 2000).

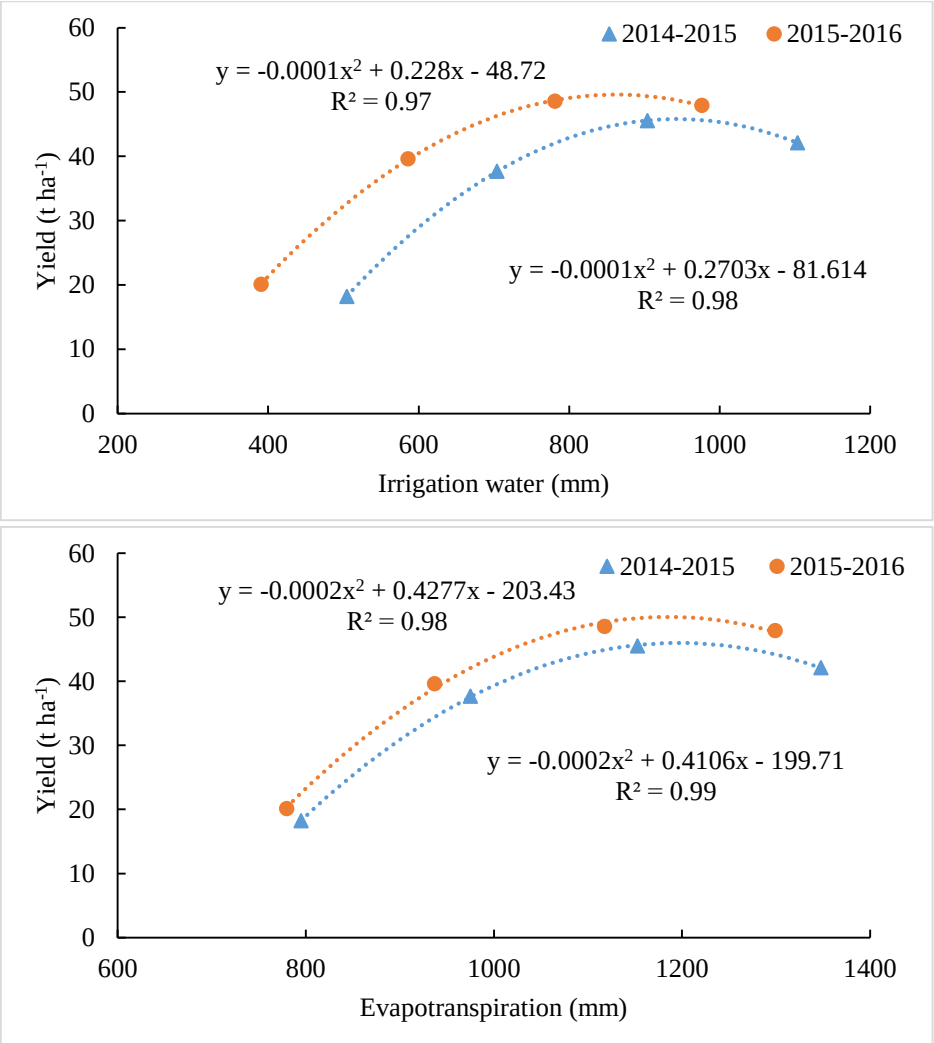

Figure 1- The relationship between orange yield and amount of irrigation water and evapotranspiration.

3.2. Fruit quality parameters

The effects of treatments on fruit weight, fruit length, fruit width, juice, TSS, pH, TA, vitamin C, except for peel thickness were statistically significant (Table 3). Except vitamin C, other parameters were higher in Kcp_1 treatments as compared to the other treatments in the study.

In both experimental years, it was found significant differences between water treatments in fruit weight, length, and width. These parameters significantly decreased with the decrease in water levels (Table 3). The fruit obtained from the K_{cp4} irrigation application were lesser than fruit obtained from the K_{cp3}, K_{cp2}, and K_{cp1} irrigation treatments, respectively. The highest fruit weight (173.4 and 173.9 g), fruit length (70.4 and 73.6 mm) and fruit width (70.2 and 69.8 mm) were obtained from K_{cp1} irrigation treatments in both years. The lowest fruit weight (89.2 and 111.3 g), fruit length (58.6 and 63.6 mm) and fruit width (56.7 and 59.7 mm) were obtained from K_{cp4} irrigation treatments in both years. Shalhevet & Levy (1990) and Castel & Buj (1990) concluded that fruit size is the main quality characteristic in citrus and this characteristic is greatly affected by water management. Treeby et al. (2007) stated that water stress reduced fruit size values in Navel orange. Nagaz et al. (2015) argued that irrigation water deficiency leads to a significant decrease in the fruit weight. Esmail et al. (2016) determined that the highest fruit weight and fruit length were obtained 240 g and 80.0 mm in full irrigation, respectively, while the lowest fruit weight and fruit length were obtained 120 g and 73.0 mm in deficit irrigation conditions for Valencia orange variety.

The irrigation levels (deficit or excessive) did not have a significant influence on peel thickness in two growing seasons (Table 3). The highest peel thickness (6.0 and 6.2 mm) was determined the least irrigation treatments (Kcp_4) in where the differences between irrigation treatments were insignificant in both seasons, whereas the lowest peel thickness (5.0 and 5.8 mm) was obtained from the highest irrigation treatment for both years. Hilgeman & Sharp (1970) El-Gazzar et al. (1986) and

Chartzoulakis et al. (1999) stated that low supply water increased peel thickness on Valencia, Washington and Bonanza orange trees. Similar effects were determined in this study for Valencia Late orange trees.

Table 3- The average values of some fruit quality components and Duncan test groups in different irrigation treatments

*Fruit weight (g)***	*Irrigation treatments*			
	K_{cp1}	K_{cp2}	K_{cp3}	K_{cp4}
2014-2015	173.4 a[x]	159.3 ab	137.0 b	89.2 c
2015-2016	173.9 a	152.7 ab	144.0 b	111.3 c
Fruit length (mm)**				
2014-2015	70.4 a	70.0 a	66.5 a	58.6 b
2015-2016	73.6 a	70.9 a	68.9 ab	63.6 b
Fruit width (mm)**				
2014-2015	70.2 a	68.0 a	65.1 a	56.7 b
2015-2016	69.8 a	65.6 b	65.5 b	59.7 c
Peel thickness (mm)[ns]				
2014-2015	5.0	5.3	5.4	6.0
2015-2016	5.8	6.0	6.1	6.2
Juice (%)**				
2014-2015	56.2 a	45.4 b	44.8 b	36.1 c
2015-2016	50.2 a	48.0 a	48.5 a	39.9 b
TSS (%)**				
2014-2015	10.2 c	12.0 b	12.3 b	13.2 a
2015-2016	10.3 c	13.2 b	13.8 b	15.5 a
pH**				
2014-2015	3.3 a	3.2 b	3.1 bc	3.0 c
2015-2016	3.4 a	3.3 b	3.2 bc	3.1 c
TA (%)**				
2014-2015	1.2 b	2.0 a	2.1 a	2.3 a
2015-2016	1.6 a	2.1 a	2.4 ab	2.6 b
Vitamin C (mg 100 mL^{-1})**				
2014-2015	37.2 b	50.8 ab	56.8 a	63.0 a
2015-2016	39.8 b	51.2 ab	57.4 a	64.2 a

ns and **; not significant and significant %1 level, respectively. [x]; Means different according to Duncan test at 5% confidence level are shown using different letters

The fruit juice, TSS, pH, TA and vitamin C were influenced by irrigation treatments (Table 3). The fruit juice was affected by different irrigation levels in experimental years, statistically. The fruit juice values decreased with decreasing level of irrigation in 2014-2015 and 2015-2016 study years. The average fruit juice of K_{cp1} (50.2%), K_{cp2} (48.0%), and K_{cp3} (48.5%) irrigation applications were in the same statistical group in 2015-2016 except for K_{cp4} treatments while K_{cp2} (45.4%), and K_{cp3} (44.8%) irrigation applications were in the similar statistical sets in 2014-2015. These results were similar Perez-Perez et al. (2009) and Gasque et al. (2016).

The influence of treatments on the TSS, TA, and pH were statistically significant in the study. Total soluble sugar values increased with decreasing level of irrigation in 2014-2015 and 2015-2016 study years. The average TSS values were changed between 10.2 and 13.2% in 2014-2015, and 10.3 and 15.5% in 2015-2016. The highest pH was determined 3.3 in 2014-2015 growing season and 3.4 in 2015-2016 growing season for K_{cp1}. Titratable acidity values increased with decreasing amount of water applied in both experimental years. Titratable acidity values varied from 1.2 to 2.3% and 1.6 to 2.6 for first and second year of experiment, respectively. K_{cp4} irrigation treatment gave the highest TA value while K_{cp1} treatment gave the lowest TA value in both experimental years. There is a conflict concerning water stress and TSS and TA level in the literature. Mohsen et al. (1989), El-Hanawy (2006) and Ghosh & Pal (2010) reported that TSS increased with increasing soil moisture level, while Ginestar & Castel (1996), Yakushiji et al. (1998), Gonzalez-Altozano & Castel (1999), Hockema & Etxeberria (2001); Ali & Gobran (2002), Romero et al. (2006), Perez-Perez et al. (2009), Garcia-Tejero et al. (2010), Ballester et al. (2011); Esmail et al. (2016) and Yang et al. (2018) reported that irrigation water deficiency causes an increase in TSS and TA level. The majority of published study results conform with those of the present research in that the basic influence of decreasing the amount of irrigation water is a enhance of the TSS and TA values.

Other significant results obtained from this study relate to the vitamin C content. Vitamin C values increased significantly decreasing irrigation water amounts. The vitamin C values for K_{cp1}, K_{cp2}, K_{cp3}, and K_{cp4} were 37.2, 50.8, 56.8, and 63.0 mg 100 mL^{-1} in 2014-2015 and 39.8, 51.2, 57.4, and 64.2 mg 100 mL^{-1} in 2015-2016, respectively. Seung and Adel

concluded that high vitamin C content may function as a protective strategy against water stress and drought injury. Whereas, El-Zawily (2004), El-Hanawy (2006) reported that increasing irrigation water applied increased vitamin C of orange fruit juice. On the other hand, Perez-Perez et al. (2009), Yang et al. (2018) determined that the irrigation water deficiency enhanced vitamin C content in citrus fruit. In our study of Valencia Late orange fruit, reduced water amount was associated with higher vitamin C levels in fruit.

4. Conclusions

The influence of excessive and deficit irrigation treatments in Valencia Late orange grown in Güzelyurt region in TRNC on fruit yield and quality characteristics were studied in the research. The influence of irrigation levels on yield, fruit weight, fruit length, fruit width, juice, total soluble sugar, total acidity, pH and vitamin C content were statistically significant, nevertheless peel thickness was statistically insignificant under the experimental conditions. The maximum fruit yield was achieved from the K_{cp2} irrigation level, followed by K_{cp1} level. Fruit weight, fruit length, fruit width, and juice values increased as the amount of water deficit decreased. Conversely, TSS, TA, and vitamin C values decreased as the amount of water deficit decreased. This study showed that the yields obtained from K_{cp1} and K_{cp3} treatments did not decrease significantly compared to the K_{cp2} treatment with the highest yield. Regarding the combined effect of water use efficiency and yield reduction, the K_{cp3} treatments could be recommended in semiarid regions where the irrigation water resources are inadequate, as K_{cp3} treatments could protect about average 18% of water with only a 17.9% relative yield reduction in two experimental years. According to the all results obtained from the study, it can be stated that Valencia Late trees can be irrigated as much as the 75% of evaporation measured in Class A pan in Güzelyurt region of Turkish Republic of Northern Cyprus.

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Rainwater Harvesting with Polyethylene Film Covered Ridges for Pumpkin (*Cucurbita pepo* L.) Seed Production Under Semiarid Conditions

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ABSTRACT

The aim of the study is to evaluate effectiveness of a rainwater harvesting technique with polyethylene film covered ridges (RHCR) on pumpkin seed production under rain-fed conditions in Kayseri/Turkey. For this purpose, a two-year experiment, of which were consisted three covered ridge widths (R_1= 0.5, R_2= 0.7, and R_3= 0.9 m) and a control treatment, was conducted. The experimental design was completely randomized plots in blocks with three replications. Significantly higher seed yields were obtained from R_2 and R_3 (202 and 208 kg ha^{-1}) in first year and from R_2 (660 kg ha^{-1}) in second year. Although excessive drought conditions were experienced during pumpkin growing period in those years, especially R_2 treatment resulted significantly higher yield. Higher plant density in R_1 and lower density in R_3 negatively affected seed yield especially in water scarce second year. In second year, leaf area, mean fruit weight, fruit yield, seed yield and 1000-seed weight were found higher than ones in first year because of application of nitrogen a whole at sowing. We concluded that RHCR with optimum plant density and proper covered ridge wide, and application whole nitrogen at sowing under rain-fed conditions are effective ways to obtain higher pumpkin production in semiarid regions.

Keywords: Water harvesting, Micro catchment, Rainfed agriculture

1. Introduction

Generally, pumpkin seed consumed as snack in Turkey but has been also used as medicinal purposes in other countries (Yavuz et al. 2015; Babayee et al. 2012). Pumpkin seed production is one of the important livelihood ways of semiarid Middle Anatolian Regions of Turkey. Turkey pumpkin seed production was 41,326 tons from 64,964 ha in 2014 and great amounts of that production (71.8%) were supplied in semiarid Middle Anatolian Region of Turkey, especially in Kayseri and Nevşehir provinces (TSI 2017). Since pumpkin seed can be stored for long durations such as 1 or 2 years, the farmers in the region can sell their production without experiencing considerable marketing problems (Yanmaz 2014).

Better rainwater management foresaw considerable scope for increased food production and poverty alleviation while minimizing additional water use and can help to upgrading rainfed agriculture (De Fraiture et al. 2010). The net return for a unit water used was described briefly as water productivity. Producing more food, income, better livelihoods and ecosystem services by using less water could be succeeded by improvement of water productivity. Practices that contribute the improvement in water productivity include water harvesting, supplemental irrigation, deficit irrigation, precision irrigation techniques and soil-water conservation practices (Molden et al. 2010).

Capturing precipitation from one part of the land and transfer it to another part is the basic principle of agricultural water harvesting that differs from traditional soil-water conservation practices, and water availability is increased, thereby (Oweis et al. 2012). A lot of techniques were experienced to harvest rainwater (Boers & Ben-Asher 1982; Boers et al. 1986; Abu-Awwad 1999). Rainwater harvesting can be classified as flood-water, macro-catchment, micro-catchment, rooftop and courtyard water harvesting (Studer & Liniger 2013). The main advantages of micro-catchment systems are simple, cheap, replicable, efficient and adaptable (Reij et al. 1988). Polyethylene (PE) covered ridge and furrow rainwater harvesting (RHCR) systems that were called as "ridge-furrow rain water harvesting" were classified as micro-catchment water harvesting techniques and considered as one of the most efficient harvesting methods. Ridges and furrows are built parallelly to counters on field surface for this technique and harvested rain waters from the covered ridge were concentrated to the furrows on where plants were grown.

Different ridge and furrow ratios were used according to rain amount, rain intensity, crops and soil characteristics (Li et al. 2000; Tian et al. 2003; Wang et al. 2009).

The purpose of this study was to increase pumpkin seed yield and quality for rain-fed farming under semiarid conditions by harvesting rain water with polyethylene covered ridges and find out proper ridge width for Central Anatolian Region of Turkey.

2. Material and Methods

This research conducted two years by a collaboration of Erciyes University Agricultural Faculty and Ankara Soil, Fertilizer and Water Resources Central Research Institute to improve pumpkin seed yield and quality under semiarid rain-fed conditions in Develi/Kayseri/Turkey. Develi Research Station of the Agricultural Faculty is at 38°23′ N latitude and 35°27′ E longitude 1190 m above sea level. Long-term annual temperature of Kayseri province is 10.7 °C and monthly mean temperatures varied from -1.7 °C for January and to 22.6 °C for July. Total rainfall is 384.9 mm of which 28% fall in pumpkin growth period from 1 May until 31 August (TSMS 2017). Reference evapotranspiration values (ETo), that represent atmospheric evaporative demand and estimated according to Allen et al. (1998), and some meteorological parameters for trial years were shown in Table 1. Total atmospheric evaporative demands (ETo) were 587 and 557 mm in 2013 and 2015, respectively. Therefore, ETo in 2013 growing season was 30 mm higher than one in 2015.

Table 1- Reference evapotranspiration and some meteorological parameters for the trial years

	2013				*2015*			
	May	*June*	*July*	*August*	*May*	*June*	*July*	*August*
T_{min} (°C)	11.1	13.1	14.7	14.7	9.6	12.3	14.5	16.7
T_{max} (°C)	24.2	27.8	29.7	30	22.2	25.1	30.3	31.5
RH_{min} (%)	26.2	19.4	18.7	17	29.2	32.5	20.7	21.9
RH_{max} (%)	70.4	65.9	60.1	58.1	76.8	85.7	66.8	70.4
n (hour)	287.6	363.2	395.9	383.3	285.6	276.9	391.8	370.9
U_2 (m/s)	1.4	1.3	1.3	1.2	1.4	1.3	1.3	1.2
ETo (mm)	123	153	171	140	122	127	168	140

T_{min} and T_{max}; Monthly mean minimum and maximum temperature, RH_{min} and RH_{max}; Monthly mean minimum and maximum relative humidity, n; Monthly total sunshine duration, U_2; Monthly mean wind speed at 2 m height, ETo; Reference evapotranspiration or atmospheric evaporative demand

Soil samples from the soil surface to 0.9 m deep for each 0.3 m soil layer were taken and analyzed in the collaborated Institute laboratories according to Tüzüner (1990). Soil texture was loamy sand and sandy loam with 175 mm m^{-1} available water holding capacity. Soil salinity was low (ECe< 0.7 dS m^{-1}) and soil reaction was about 7.5 (Table 2).

Table 2- Some soil characteristics of Seyrani Agricultural Faculty Develi Research Station Area in Kayseri/Turkey

Soil Layers (cm)	*Field capacity (%)*	*Wilting point (%)*	*Soil density (g cm^{-3})*	*pH*	*EC_e (dS m^{-1})*	*Sand (%)*	*Silt (%)*	*Clay (%)*	*Soil tex.*
0-30	24.5	8.9	1.15	7.50	0.628	79.6	13.5	6.9	LS
30-60	24.1	8.4	1.13	7.45	0.453	72.0	18.5	9.5	SL
60-90	24.6	9.4	1.12	7.58	0.462	70.3	19.5	10.2	SL

ECe; Electrical conductivity of soil saturation paste extract, LS; Loamy sand, SL; Silt loam

There were three rainwater harvesting treatments consisted of three different PE film covered ridge widths and a control treatment. PE film covered ridge widths were 0.5 m, 0.7 m and 0.9 m for R_1, R_2 and R_3 treatments, respectively. Pumpkin plants were grown on 0.3 m-width furrow areas also used for infiltration of harvested rainwater. Therefore, plant row spaces were 0.8 m, 1.0 m and 1.2 m for R_1, R_2 and R_3 treatments, respectively (Figure 1). Rainwater harvesting with PE covered ridges treatments were compared to control treatment which represents conventional pumpkin cultivation in the region. Plant row space in control was 1.0 m and pumpkin plant spaces at rows for all treatments were 1.2 m in 2013 and 1.0 m in 2015. Narrower plant distance in the second year was used to increase plant density and to obtain higher seed yield. The experimental design was completely randomized plots in blocks installed parallelly to counters. Three times replicated each treatment' plot had three rows and each row had 15 pumpkin plants. To avoid the side effect, the middle row of each replications was considered at harvest. Widely used Develi pumpkin population (*Cucurbita pepo* L.) called "pumpkin seed with frame" was utilized in the experiment. Polyethylene cover (PE+UV) was 0.1 mm thickness and resistant to ultraviolet radiation of sun (Figure 1).

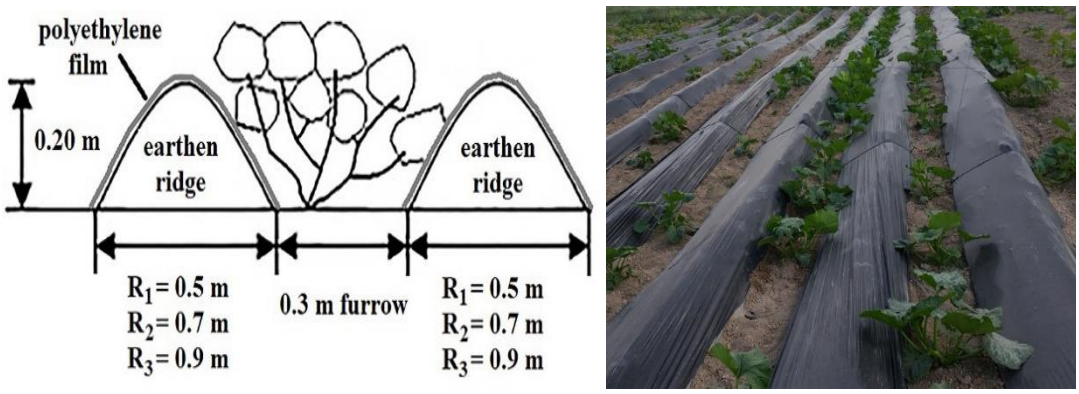

Figure 1- Rainwater harvesting with polyethylene film covered ridge (RHCR) techniques and experimental treatments

After soil tillage and preparation of ridge furrow system, 100 kg ha^{-1} phosphorus (P_2O_5), 100 kg ha^{-1} potassium (K_2O) and 50% of 100 kg ha^{-1} nitrogen (NH_3-NH_4) applied at sowing (Vural et al. 2000) according to soil analysis and mixed in to the soil by hoeing manually in 2013. The rest of 50 kg nitrogen was applied at 3-4 true leaf stage. Because of light rains or possible non-rainy periods during the vegetative growth stage of pumpkin, all of the fertilizers applied at sowing time below seed bed in 2015. Sowing and harvesting were performed on 26 April and 28 August in 2013 and on 1 May and 26 August in 2015, respectively. Pumpkin seeds were sowed about 3 cm below soil surface manually and 3 seeds were used for each point. After germination, one seedling at 1 true leaf stage was left by thinning.

Soil moisture of the treatments was monitored by 503 DR Hydroprobe neutron moisture meters. Soil moisture measurements were taken at 0.2, 0.4, 0.6, and 0.8 m soil deeps in a 50 mm-diameter aluminum access tubes by neutron probe. Soil moisture measurements were performed 4 times in 2013 and 9 times in 2015 (Figure 2). Neutron meter calibration and measurements were carried out according to the method offered by Evett (2007). Rain amounts were measured by pluviometers next to the experimental area. Plant water consumptions were determined by following Equation 1 based on soil water budget (James 1988; Wang et al. 2009):

$$ET = I + P \pm \Delta W - d_p \quad (1)$$

In where, ET pumpkin evapotranspiration (mm), I irrigation water amount (mm), P is rainfall amount (mm), ΔW is stored soil water difference between the sowing and the harvest (mm) and d_p is deep percolation below root zone (mm). Irrigation amount (I) was considered zero because of rainfed conditions. Deep percolation was neglected due to light rains.

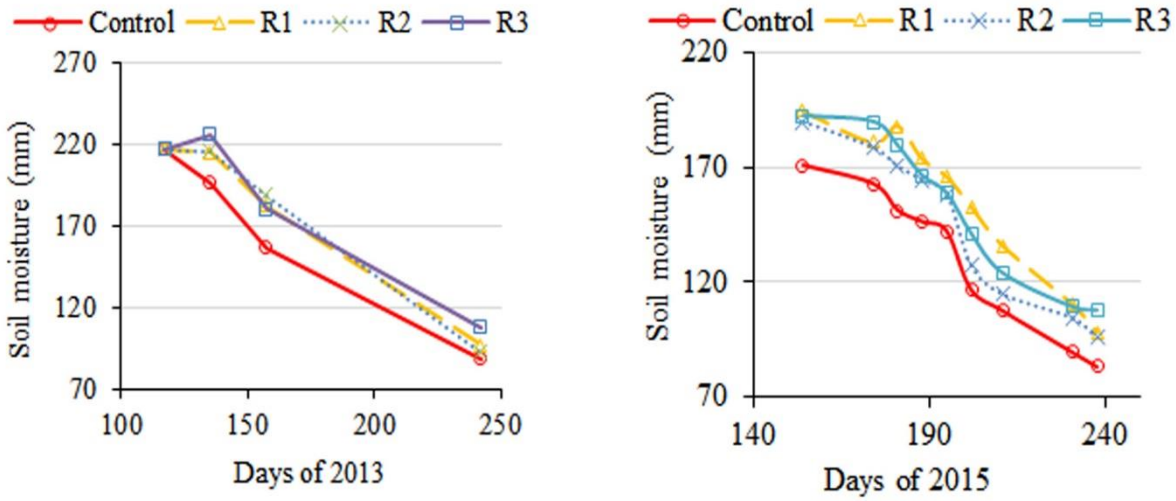

Figure 2- Soil moisture changes along growing season of pumpkin in 2013 and 2015

Mid-season leaf area of pumpkin was determined as a plant growth parameter by non-destructive method (Cemek et al. 2011). Relationships between leaf area and leaf dimensions (leaf width and length) were found out by regression and correlation analysis using 100 pumpkin leaves. Whole leaf dimensions (length and width) of five pumpkin plants for each treatment were measured at mid-season and then leaf areas were calculated based on these relationships.

Fruit numbers and fruit weight of the middle rows were recorded at harvest. Pumpkin seeds were extracted from fruits manually and dried under sun light in plastic pans. Empty or partly empty pumpkin seeds were removed and then seed weight was determined. 1000 seeds were weighted as a seed quality parameter.

All data was subjected to variance analysis and means were compared at 0.05 probability level by Duncan Multiple Separation Test by SPSS 11.5 statistical software. Microsoft Excel 2016 software was used for regression and correlation analysis.

3. Results

3.1. Rainfall and plant water consumption

Precipitation date and rain amounts during pumpkin growth season were presented in Table 3. Although the average rainfall of 55 years was 117 mm for the growing period (between 5th and 8th months), the recorded rainfalls for the growing period were only 24.2 and 13.8 mm, in 2013 and 2015, respectively. Consequently, lower rainfall than regular precipitation during pumpkin growth season was occurred.

Table 3- Pumpkin evapotranspiration, soil moisture differences at the sowing and the harvest dates, rainy days and rain amount during pumpkin growth season in 2013 and 2015

	Rain dates	*1 May*	*2 May*	*3 May*	*12 May*	*14 May*	*18 May*	*4 June*	*10 June*	*Total*
	Rain amounts	*0.1*	*3.3*	*7.7*	*6.8*	*1.6*	*1.0*	*2.7*	*1.0*	*24.2*
	Treatments	*P (mm)*		*ΔW (mm)*						*ET (mm)*
2013	Control	24.2		127.7						151.9
	R_1	24.2		119.1						143.3
	R_2	24.2		123.7						147.8
	R_3	24.2		108.3						132.4
	Rain dates	*24 May*	*31 May*	*1 June*	*3 June*	*12 June*	*16 June*		*Total*	
	Rain amounts	*2.8*	*2.2*	*0.7*	*5.3*	*2.3*	*0.5*		*13.8*	
	Treatments	*P (mm)*		*ΔW (mm)*						*ET (mm)*
2015	Control	13.8		87.2						101
	R_1	13.8		96.2						110
	R_2	13.8		93.8						107
	R_3	13.8		84.2						98

P; Rainfall (mm), ΔW; Soil water difference between the sowing and the harvest (mm), ET; Pumpkin evapotranspiration (mm), R_1, R_2 and R_3; Rainwater harvesting treatments with 0.5, 0.7 and 0.9 m polyethylene covered ridge widths, respectively

Water consumption of control, R_1, R_2 and R_3 RHCR treatments were determined as 152, 143, 148, and 132 mm in 2013 and 101, 110, 107, and 98 mm in 2015, respectively. Not significant differences in plant water consumption were occurred among the treatments in both years. Although lower rainfall amounts were recorded during pumpkin growth season, pumpkin consumed higher than 98 mm water because of storing pre-season precipitations in the soil profile of its rooting depth. Averaged initial soil moistures for 0.9 m soil depth were 216 mm in 2013 and 196 mm in 2015, and averaged soil moisture at the harvest were around 97 mm in 2013 and 95 mm in 2015 (Figure 2). Therefore, great amounts of water for plant consumption were supplied from stored soil water in the rooting depth of pumpkin in the both of years (Table 3). Soil moistures along growing season in control treatment were lower than ones in RHCR treatments in 2013 and also lower in 2015 until 190th day of that year (Figure 2).

3.2. Leaf area

Leaf area, some yield components and 1000-seed weight presented in Table 4 and variance analyze results were shown in Table 5. Leaf area was considered as a growth parameter and determined at the full growth stage of pumpkin in a non-destructive manner. Stronger relationships (R^2= 0.97) between leaf width-leaf area (LA= 0.6397 W^2-1.6551 W+19.467) and leaf length-leaf area (LA= 0.3709 L^2+10.859 L-54.463) were determined. Leaf area changed significantly for both years (Table 4). Leaf area varied from 0.151 to 0.256 m^2 in 2013 and from 0.333 to 0.464 m^2 in 2015. Maximum leaf area (0.256 m^2) was observed for R_2 treatment and minimum leaf area (0.151 m^2) for the control in 2013. R_3 produced maximum leaf area (0.464 m^2) and other treatments produced minimum leaf area (0.333 to 0.355 m^2) in 2015.

Table 4- Some growth and yield parameters of pumpkin plants grown under rainwater harvesting with polyethylene film covered ridge treatment and conventional cultivation in 2013 and 2015

Year		*Leaf area (m^2)*	*Mean fruit wgt. (kg)*	*Fruit yield (tha^{-1})*	*Fruit seed yield (g)*	*Seed yield ($kg\ ha^{-1}$)*	*1000-seed wgt. (g)*
2013	Control	0.151 c	0.67 b	3.85 b	9.7 c	55 b	156 b
	R_1	0.189 bc	0.73 b	4.20 b	17.0 bc	112 ab	179 ab
	R_2	0.256 a	1.31 a	9.85 a	27.4 ab	202 a	199 ab
	R_3	0.235 ab	1.14 ab	7.89 ab	30.0 a	208 a	210 a
2015	Control	0.333 b	1.87 c	20.50 b	46.2 b	512 ab	204 bc
	R_1	0.355 b	1.57 c	20.43 b	30.3 c	397 b	190 c
	R_2	0.338 b	2.60 b	29.28 a	60.7 a	660 a	234 ab
	R_3	0.464 a	3.33 a	28.73 a	54.9 ab	478 b	264 a
2013		0.208 b	0.96 b	6.45 b	21.0 b	144 b	186 b
2015		0.372 a	2.34 a	24.48 a	48.0 a	512 a	223 a

Table 5- Variance analyze results

	2013			*2015*		
	Std. error	*F*	*Sig.*	*Std. error*	*F*	*Sig.*
Leaf area	0.0098	5.754	0.021	0.0148	4.306	0.044
Mean fruit wgt.	0.0726	4.567	0.038	0.108	13.403	0.002
Fruit yield	0.616	5.585	0.023	1.186	3.818	0.05
Fruit seed yield	1.894	6.184	0.018	1.978	11.232	0.003
Seed yield	21.284	3.038	0.05	20.869	6.924	0.013
1000-seed wgt.	5.205	5.302	0.026	5.297	9.601	0.005

3.3. Pumpkin fruit yield

Maximum fruit yield obtained from R_2 (9.85 t ha^{-1}) and R_3 (7.89 t ha^{-1}) while minimum ones from the control (3.85 t ha^{-1}) and R_1 (4.20 t ha^{-1}) in 2013 (Table 4). Minimum fruit yields in the second year were harvested again from the control as 20.5 t ha^{-1} and R_1 as 20.43 t ha^{-1}. R_2 and R_3 treatments produced maximum fruit yields as 29.28 and 28.73 t/ha, respectively (Table 4). Similar results were obtained for mean fruit weights (Table 4). Mean fruit weight was changed significantly. In the first year, the control and R_1 treatments produced the lower fruit weights as 0.67 and 0.73 kg $fruit^{-1}$, respectively while the heavier ones were produced by R_2 and R_3 as 1.31 and 1.14 kg $fruit^{-1}$, respectively. In second year, mean fruit weights were obtained as 2.34 kg $fruit^{-1}$ and the heaviest fruits were harvested from R_3 (3.33 kg). R_1 and the control treatments produced the smallest fruits (Table 4).

3.4. Seed yield

Significant differences in seed yield among treatments both in 2013 and 2015 were found. Maximum seed yields as 202 and 208 kg ha^{-1} were obtained from R_2 and R_3, respectively, while minimum seed yield was 55 kg ha^{-1} in the control treatment in 2013 (Table 4). In 2015, maximum seed yield as 660 kg ha^{-1} was harvested from R_2 and minimum seed yield as 397 kg ha^{-1} was harvested from R_1. Higher seed yields were obtained from R_2 treatment for both years.

3.5. 1000 seed weight

In both years, 1000-seed weight was affected significantly by the treatments. In 2013, the highest 1000-seed weights as 210 g was recorded in R_3 water harvesting treatment and the least one as 156 g was recorded in the control (Table 4). In 2015, the highest 1000-seed weight 264 g from R_3 and the least 1000-seed weight 190 g from R_1 were obtained.

4. Discussion

Pumpkin water consumption in this region was estimated 430 mm under full irrigation conditions according to FAO-56 Penman-Monteith method (Ünlükara 2014). Pumpkin mean water consumption under full irrigation and rainfed conditions were determined as 474 and 293 mm in Kayseri (Kirnak et al. 2019), as 645 and 201 mm in Konya (Yavuz et al. 2015) and as 539 and 336 mm for squash in Van (Ertek et al. 2004). Water stress free squash (*C. pepo L.*) consumed 304 mm and 344 mm water under trickle and furrow irrigation for spring period in Egypt (Amer 2011). Pumpkin water consumptions in the current experiment were found less than the ones above mentioned because lower rainfalls were recorded in pumpkin growing season for both experimental years. However, Zotarelli et al. (2008) reported 108 to 171 mm water consumption for zucchini cultivated with a plastic mulch bed system in Florida for two years under full irrigation. Plastic mulching considerably reduced water consumption of pumpkin in that experiment.

In 2013, leaf area, mean fruit weight, fruit yield, seed yield per fruit, seed yield and 1000-seed yield were higher in RHCR treatments than ones in the control treatment. In 2015, maximum leaf area, fruit weight and 1000-seed weight were found in R_3, maximum fruit yield in both R_2 and R_3, maximum seed yield per fruit and seed yield in R_2. The purpose of pumpkin farming in semiarid middle Anatolian region of Turkey is to produce pumpkin seed for confectionary consumption. Especially, RHCR treatment with 0.70 m covered ridge (R_2) improved pumpkin seed yield and nearly all growth and yield parameters for both experimental years. In severely drought 2015, higher plant density in R_1 reduced both seed yield and quality because of competition for water. R_3 treatment improved 1000-seed weight but reduced seed yield because of its lower plant density in unit area.

Fruit yields in R_1, R_2 and R_3 were found 9%, 156% and 105% higher than the control in 2013 and 0%, 43% and 40% higher in 2015. RHCR treatments improved fruit yield considerably. Mean fruit yields of RHCR treatment for two years were 6.45 and 29.28 t ha^{-1}. In researches in semiarid conditions in Turkey, 14.8 and 7.3 t ha^{-1} pumpkin fruit yields for two years were obtained (Yavuz et al. 2015). Comparing to results reported by Yavuz et al. (2015), we obtained higher fruit yields in second year. Tian et al. (2003) also reported that larger potato tubers obtained from RHCR and smaller tubers from control treatment.

R_1, R_2 and R_3 treatments improved seed yield 104%, 267%, and 278% compared with the control in 2013. Mean seed yield increase for R_2 was 29% according the control in 2015. Similar to our findings, Tian et al. (2003) found 219% yield increase in potato grown in RHCR system. Wang et al. (2009) also determined sweet sorghum biomass yield increases from the RHCR technique with covered both ridge and furrow and the RHCR technique with covered ridge compared with conventional cultivation and non-covered ridge and furrow technique.

Kirnak et al. (2019) obtained 470 and 427 kg ha^{-1} pumpkin seed yield against 256 mm and 227 mm water consumption and, Yavuz et al. (2015) reported 545 and 247 kg ha^{-1} seed yield against 194.2 mm and 208.2 mm water consumption under rainfed conditions. We obtained 606 kg ha^{-1} seed yield against 110 mm water consumption for R_2 in 2015. Covered ridges concentrate harvested rainwater to furrow areas and allow harvested water to percolate and to store in the soil profile deeply. Undesirable evaporation losses from relatively narrow furrow surface beneath crop canopy decreased greatly in RHCR. Furthermore, covered ridges keep soil moisture before and during the growing season and promote plant transpiration. The main reason for yield increase of RHCR system resulted from preventing evaporation and improving available water for plant transpiration.

All parameters considered in the current experiment were found higher in second year. The main reason was changing nitrogen application. In 2013, nitrogen divided into two parts and the first part was applied at sowing and the second part was applied at 3 or 4 leaf stage. But in 2015, whole fertilizers were applied during the sowing. We observed that nitrogen application by dividing several parts in rain-fed conditions was not efficient due to low rainfall characteristic of the region. Drier soil surface during the application of second nitrogen part resulted inefficient use of nitrogen. Similar result also reported by Dumanlar (2018) for pumpkin even in full irrigation. Maximum pumpkin growth and yield characteristics were obtained by application whole nitrogen need at sowing. Although plant densities for control, R_1, R_2 and R_3 treatments increased 20% in the second year, seed yield increased 831%, 254%, 227% and 130%, respectively. We concluded that great amount of seed yield increase resulted from application whole fertilizers at sowing.

5. Conclusions

Effects of rainwater harvesting technique with PE film covered ridges were investigated for pumpkin growth and yield under semiarid rainfed conditions of Develi/Kayseri in Turkey for two years. RHCR treatments had plastic covered ridges with 0.5, 0.7 and 0.9 m widths and all treatments had constant 0.3 m furrow width between these covered ridges. RHCR treatments and conventional pumpkin farming treatment (the control) in the region were compared. We concluded according to our findings that:

- RHCR technique improved pumpkin growth, fruit yield, seed yield and 1000-seed weight considerably by relatively preventing evaporation from soil surface and by increasing transpiration. Because of these positive effects of RHCR, we concluded that RHCR is one of ways to increase pumpkin production and quality in rainfed farming under semi-arid regions of Turkey.

- Considering seed yield of two trial years, polyethylene film covered ridges with 0.7 m width came in prominence with higher pumpkin yield and quality despite scarce rainfall in pumpkin growing season. It is suggested to use 0.7 m covered ridge and 0.3 m furrow widths to decrease plant competition for soil water and prevent plant failure and to obtain higher seed yield under RHCR technique.

- Fertilizer need of pumpkin should be applied at sowing stage as a whole to improve efficient utilization under semi-arid rainfed conditions. Under semi-arid rainfed conditions, applying nitrogen requirement of pumpkin by dividing in to several parts caused poor growth and yield because of possibly insufficient and lower rainfalls during growth stage of pumpkin.

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Effects of Honey Bee Race and Season on Propolis Composition

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ABSTRACT
Honey bees collect the main material of propolis from the buds, leaves, branches, and barks or other botanical sources and mixed that resinous material with beeswax produced from abdominal exocrine glands, mandibular gland secretions, and pollen to produce propolis. The composition of propolis changes depends on multiple factors such as honey bee races, geographical locations, phytogeography, harvesting seasons, extraction methods and solvents. In this study, two different studies were conducted in two different locations in order to reveal the effect of race and season variables on the composition and antioxidant value of propolis. The effect of race factor was studied on three different honey bee races (*Apis mellifera caucasica*, *Apis mellifera syriaca* and *Apis mellifera carnica*) and two different ecotypes (Muğla ecotype and Yığılca ecotype) to investigate the effects of race factor on propolis composition in Central Anatolia by under the controlled conditions. The effect of seasonal change was determined by Yığılca ecotype of *A. melifera anatoliaca* in the apiary located in Yığılca, Düzce location. Studied samples were harvested by propolis trap between May and October. Total phenolic content, total antioxidant capacity and chemical profiles of propolis samples were determined using HPLC-UV. The obtained results showed that honey bee race and season have an effect on the antioxidant capacity and chemical composition of propolis.

Keywords: Antioxidant capacity, Honey bee races, Phenolic compounds

1. Introduction

Propolis is a resinous, waxy substance collected by honey bees from the trees, and other plant sources. It is used as a sealant and antimicrobial agent inside the hive. Moreover, it has gained popularity as a natural health product, used extensively in the food industry as supplementary food to improve human health and prevent disease (Eroglu et al. 2008; Ozkul et al. 2005; Uzel et al. 2005). Propolis contains biological active compounds like phenolics. Unfortunately, the chemical composition of propolis varies depending on the plant material, geographical origin, honey bee subspecies and harvest season (Bankova et al. 2006; Miguel et al. 2011).

There are 27 honey bee races that have been identified all over the world. Five honey bee races (*Apis melifera caucasica*, *A. m. syriaca*, *A. m. anatoliaca*, *A. m. meda*, *A. m. carnica*) distributes in Turkey due to its special geographical location on the migration routes and climatic range (Ruttner 1988). *A. mellifera* races adapted to the climatic conditions and floral structure of the regions where they spread on. It has been known that these adaptations have an effect on morphological and physiological structure of the honey bees and it may result as different races and ecotypes. These adaptations of different races can also affect the glands sizes and secretions, also it connectedly affects the composition and activity of the honey bee products such as propolis and royal jelly, which produces by or mixing with honey bees secretions (Silici & Kutluca 2005, Bankova et al. 2006; Miguel et al. 2011). Al-Ghamdi et al. (2011) were compared the development of hypopharyngeal glands (HPG) of *Apis mellifera jemenitica* and Carniolan hybrid bees. They detected the staining of cell cytoplasm by hematoxylin and eosin as similar but, secretory cell numbers were found more in Carniolan hybrid than the *A. m. jemenitica*. Even though all these findings, there is limited study found for honey bee race effect on propolis compound. Brazilian green and red propolis originating from Africanised *A. mellifera* is rich in prenylated phenylpropanoids and isoflavonoids, respectively (Teixeira et al. 2005; Daugsch et al. 2008). C-methylated flavanones, terpenic acids and phenolic acids such as gallic acid and diterpenic acids, the p-coumaric and abietic types are the predominant chemicals in the cerumen propolis from stingless bees, but it lacks the characteristic flavonoids and prenylated phenolics found in propolis from other honey bee species in Australia (Massaro et al. 2011; Massaro et al. 2014).

In present study, two different research were conducted in two different regions to reveal the effect of season and race factors on the propolis content. (1) Race factor: We investigated the effect of honey bee races and ecotypes which distribute in Turkey,

A. m. caucasica, A. m. syriaca, and A. m. carnica, Yığılca ecotype and Muğla ecotype on propolis composition under the controlled conditions in central Anatolia, (2) Season factor: The effect of season on the composition of propolis collected by Yığılca ecotype were investigated in the Düzce University Beekeeping Research Development and Aplication Centre (DAGEM) apiary in Düzce, Turkey.

2. Material and Methods

2.1. Propolis collection

To determine the effect of the honey bee race and ecotypes of *Apis mellifera* L. on collected propolis composition we maintained three indigenous races (*A. m. caucasica*, *A. m. syriaca*, and *A. m. carnica*) and two ecotypes (Yığılca and Muğla) in the same apiary, under the controlled conditions in central Anatolia. 12 and 15 colonies were represented three honey bee races and two ecotypes in Central Anatolia (Yığılca ecotype (N=15) *A.m. caucasica* (N=15), *A. m. anatolica* (N=13) *A. m. syriaca* (N=14) *A. m. carnica* (N=12)). Propolis samples were collected three times from each colony.

In addition, the seasonal effect on the propolis phenolic composition was also investigated. Therefore, samples harvested in different seasons from May to October were examined to detect any variations in the chemical composition of the propolis collected from the same area and by the same honey bee ecotype habited in the DAGEM apiary in Düzce, Turkey.

Propolis samples were collected with propolis traps in the early spring season and samples were collected regularly every month from May to October 2016. The samples were stored in a deep freezer at -20 ºC until further processing.

2.2. Propolis extraction

Methanolic propolis extracts were used for analyses. Approximately 3.0 g propolis was extracted with 99% methanol. The maceration technique was used for extraction. After 24h, the mixture was filtered and the final volume of the solution was adjusted with methanol.

2.3. Determination of total phenolic content (TPC) and total flavonoids

The TPCs of the methanol extracts were determined following the Folin–Ciocalteu method (Singleton et al., 1999). Gallic acid was used as a standard and the phenolic content was measured at 760 nm. The amount of total flavonoid content was measured by the $AlCl_3$ spectrophotometric method at 415 nm, as reported previously (Fukumoto et al. 2000), using quercetin as the standard.

2.4. Determination of total antioxidant capacity

The total antioxidant capacity was determined by using ferric-tripyridyltriazine (Fe-III-TPTZ) complex (Benzie & Strain 1996). Trolox was used as a positive control in order to construct a reference curve (62.5–1000 M). 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging method (Molyneux 2004). The equal milliliter of propolis extracts and fresh DPPH solution was mixed and measured at 517 nm after 50 min using a spectrophotometer. The DPPH scavenging activity was calculated as SC_{50} (mg of sample per mL), too.

2.5. Determination of phenolic compounds by RP-HPLC-UV

Fourteen standards of phenolic compounds were analysed using HPLC (Elite LaChromHitachi, Japan) with a UV–Vis detector. The phenolic profile was determined according to Can et al. (2015) using a Fortis C18 column (150 mm × 4.6 mm, 5µm). The mobile phase consisted of (A) 2% acetic acid in water and (B) 70% acetonitrile in water. The injection volume of the samples was 20 µL with the flow rate at 0.75 mL/min. The eluent was monitored at 280 nm at 30 °C. For quantitative determination, the calibration curves of each phenolic component were between 0.998 and 0.999 (Table 1).

Table 1- RP-HPLC-UV validation parameters of the phenolic compounds

No	*Compounds*	R^2	*%RSD (Retention Time)*	*%RSD (Area)*	*LOD* (mg L^{-1})	*LOQ* (mg L^{-1})
1	Gallic Acid	0.999	0.210	1.941	0.022	0.067
2	Protocatequic Acid	0.999	0.871	1.920	0.042	0.128
3	*p*-OH Benzoic Acid	0.998	0.351	3.055	0.036	0.109
4	Catechin	0.998	0.492	4.279	0.040	0.121
5	Vanillic Acid	0.999	0.828	2.066	0.025	0.075
6	Caffeic Acid	0.998	0.179	4.039	0.062	0.187
7	Syringic Acid	0.998	0.550	0.848	0.009	0.027
8	Epicatechin	0.999	0.429	3.819	0.030	0.090
9	*p*-Coumaric Acid	0.999	0.204	1.562	0.010	0.030
10	Ferulic Acid	0.999	0.222	1.301	0.011	0.033
11	Rutin	0.999	0.234	3.139	0.041	0.123
12	Daidzein	0.998	0.174	1.545	0.018	0.054
13	*t*-Cinnamic Acid	0.998	0.262	1.071	0.014	0.042
14	Luteolin	0.994	0.229	5.833	0.043	0.130
15	CAPE	0.998	0.228	2.129	0.023	0.068

The analyses of the caffeic acid phenethyl ester (CAPE) compound was carried out using HPLC with UV-Vis detection (Elite LaChromHitachi, Japan) and a Fortis C18 column (150 mm × 4.6 mm, 5µm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The sample injection volume was 25µL at a flow rate of 1.0 mL/ min, and the eluent was monitored at 270 nm at 30 °C. The programmed solvent used began with a linear gradient held at 90% A for 3 min, decreasing to 70% A at 10 min, 50% A at 20 min, 10% A at 40 min, 40% A at 45 min, and finally, 75% A at 50 min.

2.6. Statistical analyses

The statistical analyses were performed using SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SE. One-way variance analysis (ANOVA) and the Tukey test were used for differences regarding the honey bee race and season with $P<0.05$ considered as statistically significant.

3. Results and Discussion

The aim of the present study was to detect the effect of season and race factors on propolis phenolic content, chemical compositions and antioxidant value. To identify the race effect, propolis samples were gathered in the central Anatolia region of Turkey and variants such as plant material and harvest time were kept constant. Total phenolic and total flavonoid contents were found to vary according to the honey bee races. The propolis samples of the Yığılca honey bee colonies contained the highest phenolic content (18.24±0.56 mg GAE.g^{-1}), followed by *Apis mellifera syriaca* propolis (16.92±0.40 mg GAE.g^{-1}). Total flavonoid content was found to be the highest in the *A. m. syriaca* propolis samples (8.60±3.80 mgQE.g^{-1}), whereas propolis of Muğla honey bee ecotype contained the lowest phenolic content (4.25±0.55 mg GAE.g^{-1}). The highest antioxidant capacity (FRAP) was detected in the *A. m. syriaca* propolis samples (2125±86.20 $FeSO_4.7H_2O$µl moL.g^{-1}) and lowest value detected in the Muğla ecotype (1442±20.50 $FeSO_4.7H_2O$µl moL.g^{-1}). The radical scavenging activity showed different ranking, while the highest score detected in Muğla ecotype (0.40±0.18(µg.mL^{-1}), the lowest score found in Yığılca (0.05±0.09 µg.mL^{-1}) and *A. m. syriaca* (0.06±0.02 µg.mL^{-1}) respectively (Table 2).

Table 2- The effect of honey bee races on total phenolics and antioxidant values

Parameters	***Muğla ecotype***	***A. m. caucasica***	***A. m. carnica***	***Yığılca ecotype***	***A. m. syriaca***
Total phenol contents (mg GAE.g^{-1})	12.50±0.33^a	13.25 ±1.54^a	12.31±0.14^a	18.24±0.56^b	16.92±0.40^b
Total flavonoids (mg QE.g^{-1})	4.25±0.55^a	5.41±0.65^b	4.85±2.44^c	5.30±1.56^d	8.60±3.80^e
FRAP ($FeSO_4.7H_2O$µl mol.g^{-1})	1442±20.50^a	1688±38.40^b	1960±120.30^c	1850±130.60^c	2125±86.20^d
DPPH (µg.mL^{-1})	0.40±0.18^c	0.14±0.15^b	0.08±0.03^a	0.05±0.09^a	0.06±0.02^a

FRAP: ferric reducing antioxidant power, **DPPH**: 2-diphenyl-1-picrylhydrazyl radical scavenging activity. The same letter is not significantly different ($P<0.05$)

Phenolic composition of propolis samples collected by different honey bee races were compared. Gallic acid, proto-catechuic acid, p-OH benzoic acid weren't determined in propolis samples. Vanillic acid and catechin were determined only in propolis

samples of Muğla ecotype. (Table 3). In the previous study, Vanillin was detected in *A. m. anatoliaca* and *A. m. carnica* propolis samples from the same apiary in Erzurum, though it was not found in *Apis mellifera caucasica* propolis samples (Silici & Kutluca, 2005). Greenaway et al., (1987) determined vanillin and vanillic acid in England propolis but they did not report catechin in propolis samples. Similar with our findings, Eroğlu et. al. (2021) determined that honey bee races used neither the same nor the single propolis source. The plant choice differences of honey bee races affect the antioxidant value of the propolis samples due to the different ingredients of plants.

Table 3- Phenolic composition of propolis samples depend on honey bee races and ecotypes (µg phenolic compound.g propolis sample^{-1})

Races µg.g^{-1}	*Ga*	*Proto Cat*	*p-OH BA*	*Cat*	*Va*	*Caff*	*Syr*	*EpCa*	*p-Cou*	*Fer*	*Ru*	*Dai*	*t-Cinn*	*Lut*	*Cape*
A.m.caucasia	-	-	-	-	-	390.20 ±22.30^{c}	-	190.30 ±24.30^{a}	137.60 ±33.60^{c}	77.50 ±3.36^{b}	-	-	7.08 ±1.20^{b}	2541 ±15.46^{b}	390.05 ±32.30^{ab}
A.m.syriaca	-	-	-	-	-	85.60 ±12.40^{a}	-	-	24.25 ±5.77^{a}	381.82 ±26.50^{c}	-	17.2 ±2.30^{b}	38.24 ±5.06^{c}	11453 ±23.88^{d}	818.52 ±28.99^{c}
A.m.carnica	-	-	-	-	-	1013 ±15.50^{d}	-	324.33 ±16.05^{c}	291.30 ±22.05^{d}	10.23 ±1.30^{a}	713.80 ±56.30^{b}	5.66 ±2.41^{a}	41.78 ±5.33^{c}	4304 ±28.05^{c}	291.60 ±5.60^{a}
Muğla ecotype	-	-	-	222.30 ±13.40	9.40 ±0.20	214.80 ±30.06^{b}	-	253.62 ±17.50^{b}	90.70 ±20.30^{b}	17.03 ±2.34^{a}	10.60 ±1.40^{a}	-	2.45 ±0.66^{a}	1497 ±16.30^{a}	410.10 ±20.02^{b}
Yıgılca ecotype	-	-	-	-	-	2086 ±12.30^{e}	-	-	704.30 ±23.33^{e}	18.60 ±4.40^{a}	-	32.6 ±4.60^{c}	57.40 ±6.71^{d}	4683 ±38.60^{c}	211.23 ±17.56^{a}

The same letter is not significantly different (P<0.05). Gallic acid (Ga), proto-catechuic acid (ProtoCat), p-OH benzoic acid (p-OH BA), catechin (Cat), vanillic acid (Va), caffeic acid (Caff), syringic acid (Syr), Epicatechin (EpCa), p-coumaric acid (*p-Cou*), ferulic acid (Fer), rutin (Ru), daidzein (Dai)t-cinnamic acid (*t*-Cinn), luteolin (Lut) and caffeic acid phenethyl ester (Cape).

Honey bee race is one of the important variable for the propolis composition, due to the substances secreted (wax and saliva) and plant choice differences of honey bees (Gomez-Caravaca et al. 2006; Da Cunha et al. 2013; Wilson et al. 2013; Dutra et al. 2014; Silici & Kutluca 2005). Race differences also have an effect on average propolis yield as stated in previous literature; highest to lowest respectively as; *A. m. anatoliaca*, *A. m. ligustica*, *A. m. carnica*, *A. m. caucasica* in literature (Kutluca 2003; Şahinler & Gül 2005). Şahinler & Gül (2005) compared bee races (*Apis mellifera lingustica*, *Apis mellifera anatoliaca*, *Apis mellifera caucasica*, *Apis mellifera carnica)* for propolis collection amounts and, they did not determine significant differences between the races, though highest propolis collection amount determined in *Apis mellifera anatoliaca*. In the later study, it was determined that *A. m. carnica*, Yığılca ecotype, *A. m. caucasica* which distributes on Northern Turkey have better propolis collecting ability than Muğla ecotype and *A. m. syriaca* which distribute on Southern of Turkey (Kekecoglu et al. 2020). Despite all this information, there is still limited study for honey bee race effect on propolis composition.

In addition to honey bee race, the effect of season on propolis composition was investigated. The total phenolic and flavonoid contents were significantly reduced between May+June and September+October (P<0.05). The highest phenolic and flavonoid values of the samples were found in the spring season as 27.29 mg GAE.g^{-1} and 9.25 mgQE.g^{-1} respectively. The higher antioxidant capacities for the propolis samples observed in the spring season were reduced in summer and then increased again in autumn. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities showed parallel fluctuation with the flavonoid amounts in all season (Table 4).

Table 4- Seasonal Impact on total polyphenols content and antioxidant activity of propolis samples by the month

Parameters	***May+June***	***July+August***	***Sep+october***	***Mean values***
Total Phenolic contents (mg GAE.g^{-1})	34.85±4.32^{c}	25.82±3.08^{b}	21.26±2.4^{a}	27.29±1.2
Total flavonoids (mgQE.g^{-1})	9.25±1.02^{b}	4.91±0.60^{a}	5.37±0.7^{a}	6.51±0.52
FRAP ($FeSO_4.7H_2O$µl moL.g^{-1})	2861±12,56^{c}	2117.55±55.5^{a}	2668.66±20,40^{b}	2548.88±11
DPPH (µg.mL^{-1})	0.02±0.0^{a}	0.03±0.0^{a}	0.03±0.0^{a}	0.04±0.0

FRAP: ferric reducing antioxidant power, **DPPH:** 2-diphenyl-1-picrylhydrazyl radical scavenging activity, n=45 for each season. The same letter is not significantly different (P<0.05)

The composition of propolis from sources within short distances of each other in the same region can dramatically differ due to plant diversity and the limited travel distance of the bees from the propolis collection field to the place of deposition (Marcucci 1995). The results of the present study showed that propolis samples collected from May to October had unstable compositions due to the changing seasonal flora. In the Düzce region, the richest phenolic compounds were found in the early spring season (May+June). The antioxidant capacity of the propolis samples was also found to be higher in May+June than in the other seasons. The high phenolic content of the propolis samples may be explained by the plants accessible in the region during the spring period. As with the phenolic content, total antioxidant capacity and DPPH radical scavenging activity were found to be higher

in the May+June season than at other harvest times. In most cases, the higher phenolic content cause higher antioxidant activity, as well as an antimicrobial and anti-inflammatory activities (Can et al. 2015; Baltas et al. 2016).

When the propolis phenolic profiles were examined according to the collection time, some differences were observed among the months. Except for the proto-catechuic acid, and p-OH benzoic acid, all the phenolic compounds were detected in propolis samples harvested in the spring and summer seasons. Surprisingly, CAPE was detected in all propolis samples harvested in all season. Although some phenolic components were at high levels in May+June, some were found to be lower. Gallic acid and catechin were detected in May+June, but not in September+October, while daidzein was not detected in May+June (Table 5).

Table 5- Effect of seasonal changes of phenolic profile collected from Düzce by Yıgılca honey bee ecotype (µg phenolic compound.g propolis sample^{-1})

Seasons µg.g^{-1}	*Ga*	*Proto Cat*	*p-OH BA*	*Cat*	*Va*	*Caff*	*Syr*	*EpCa*	*p-Cou*	*Fer*	*Ru*	*Dai*	*t-Cinn*	*Lut*	*Cape*
May+June	13.30 ±2.35^{a}	-	-	1.23 ±0.52	18.10 ±3.2 ^{a}	950.64 ±26.30^{a}	96.65 ±13.0^{b}	837.40 ±88.60^{a}	398.03 ±22.30^{a}	109.70 ±13.30^{a}	364.70 ±34.56^{a}	-	43.47 ±5.66^{b}	3223.02 ±460.30^{c}	120.5 ±18.30^{a}
July+Aug	7.70 ±1.5^{b}	-	-	-	98.20 ±12.4^{c}	5122.0 ±48.50^{c}	14.05 ±0.56^{a}	3943.20 ±506.60^{c}	1553.60 ±460.30^{b}	158.02 ±22.30^{b}	733.33 ±68.55^{b}	23.50 ±3.40^{a}	59.08 ±8.50^{b}	2178.10 ±544.05^{b}	250.2 ±16.05^{b}
Sep+Oct	-	-	-	-	31.04 ±3.2^{b}	2300.20 ±387.44^{b}	11.03 ±0.5^{a}	1980.06 ±136.30^{b}	1230.06 ±408.80^{b}	746.04 ±38.60^{c}	342.00 ±18.80^{a}	683.60 ±22.60^{b}	27.74 ±5.30^{a}	642.56 ±209.30^{a}	820.2 ±39.60^{c}

N=45 for each season. The same letter is not significantly different (P<0.05), Gallic acid (Ga), proto-catechuic acid (ProtoCat), p-OH benzoic acid (p-OH BA), catechin (Cat), vanillic acid(Va), caffeic acid(Caff), syringic acid (Syr), Epicatechin(EpCa), p-coumaric acid (*p-Cou*), ferulic acid (Fer), rutin (Ru), daidzein (Dai)t-cinnamic acid (*t*-Cinn), luteolin (Lut) and caffeic acid phenethyl ester (Cape).

A lot of research has been done in different countries about the change of propolis composition according to the season; in a study where Brazilian propolis was investigated monthly for a year; as a result of HPLC analysis, significant differences were detected in aromadendrin-4-methyl ether, baccharin andartepillin C values of propolis samples harvested at different times of the year. It was also determined that extracts with the highest antioxidant activity were detected on May, June and August productions (Simoes-Ambrosio et al. 2010). Salas et al. (2016) compared propolis samples which produced in Argentina in March and December, found no difference in antifungal and antibacterial performance of samples. In addition, it was found that Mexican propolis harvested over a four-month period did not show significant differences in phenolic and flavonoid values (Valencia et al. 2012). In a study which conducted in Poland; spring, summer and autumn propolis samples were compared in many respects. Although there was not determined significant difference on the chemical profile and antioxidant potential of propolis extracts obtained from these samples, but within the slight differences the highest values were determined in spring and the lowest values in autumn (Wozniak et al. 2019). The Season is an important factor determining propolis composition, since phenological factors influence biosynthesis of plant secondary metabolites. In addition to the seasonal changes and harvesting methods there is also honeybee race factor on propolis composition.

4. Conclusions

The results of the present study clearly revealed that honey bee races and different seasons have an effect on compound and connectedly on antioxidant property of the propolis. As we declared in our results; propolis samples which collected by different races and different seasons has also different antioxidant and radical scavenging activity due to their different compositions which shows us the medicinal value differences of the propolis. It was not surprised to see the effect of season on propolis composition but the race effect in the same apiary was gave us new information on propolis composition variables since there was no many studies on this area. Hence, propolis composition of each honey bee races and seasonal effect on propolis medicinal value should be studied by complex researches to clarify all the unanswered questions.

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Increasing of Phenolic Compounds by Brassinosteroid Applications in Immobilized Cell Suspension Cultures of *Vitis vinifera* L. cv. Cinsault

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ABSTRACT
In this paper, the effects on secondary metabolite accumulation of brassinosteroid (BR) (24-epibrassinolide (24-eBL) on immobilized cells that were obtained from *Vitis vinifera* cv. Cinsault was investigated. 24-eBL was treated to immobilized cells covered calcium alginate beads at concentrations of 0, 0.25, 0.50, 0.75 and 1.0 mg L^{-1} for one month. As a result of this study, it was found that 24-eBL applications modified secondary metabolite accumulation and had positive effects on secondary metabolite production when the suitable concentration was used. While the highest total phenolic, catechin, *p*-coumaric acid and chlorogenic acid contents were found in immobilized cells treated 0.75 mg L^{-1} 24-eBL, the highest epicatechin, quercetin, *trans*-resveratrol contents were obtained in immobilized cells treated 0.50 mg L^{-1} 24-eBL and the highest gallic acid content was determined in immobilized cells treated 0.25 mg L^{-1} 24-eBL. Moreover, the highest 24-eBL concentration (1 mg L^{-1}) decreased the content of secondary metabolite compared to the control (0 mg L^{-1} 24-eBL) except total phenolic and catechin content. To conclude, 0.50 and 0.75 mg L^{-1} 24-eBL concentrations were the most suitable concentrations for immobilized cell culture to provide the highest secondary metabolite accumulation.

Keywords: 24-eBL, Immobilization, Grapevine, Phenolic compounds

1. Introduction

Phenolic compounds ranging from simple phenolic molecules to highly polymerized compounds, constitute major class of plant secondary metabolites (Dai & Mumper 2010). Secondary metabolites are not correlated with photosynthesis, reproduction, respiration or other primary functions required for growth and development of the plants. Therefore, they have been considered waste products without significant use for plants for many years (Verpoorte 2013). However, nowadays, much attention has been devoted to secondary metabolites, due to they are valuable and important raw materials for many areas such as cosmetics, pharmaceuticals, antioxidants, insecticides and organoleptic properties of food industries (Keskin & Kunter 2010; Pehlivan et al. 2016). Plants are grown at certain development stages under natural conditions in which they occupy small quantities and agricultural areas and the material required for obtaining secondary products is mostly achieved from plants collected from natural cultivation areas (Keskin & Kunter 2008). The constant collection of some plant species from nature may come to the threat of extinction, it is difficult and expensive to collect some of them (Gonçalves & Romano 2018; Karaboyacı & Kılıç 2018). Furthermore, the amount and quality of secondary metabolites are influenced by climate conditions and the amount of the pure matters varies according to the quantity and quality of the plant material (Keskin & Kunter 2010).

In recent years, several strategies have been developed to increase secondary metabolite production such as use of cell culture, selection of high productivity cell lines, media modification, permeabilization, nutrient and precursor feeding, plant cell immobilization, elicitation and biotransformation methods (Zhang et al. 2002; Murthy et al. 2014). By immobilization technique, plant cells are fixed to a suitable matrix such as agar, agarose, algae, calcium alginate, gelatine, and polyacrylamide in cell cultures (Pras & Woenderbag 1999). However, alginate gels have received much attention due to their simplicity and relative lack of toxicity (Smetanska 2008; Nielsen et al. 2019). Since plant cells are very sensitive to chemical and physical stress, some biological and technological factors must be considered (Guardiola et al. 1996). In order to improve such processes and overcome the limitations of plant cell culture, immobilization has been considered as a tool for protecting cells against stress factors and enhancing the production of secondary metabolites (Choi et al. 1995). Elicitor applications are another effective strategy for secondary metabolite production in plant cell cultures (Shumakova et al. 2011). Elicitors affect the production of most commonly used secondary metabolites for stress tolerance and plant defense (Zhao et al. 2005). For this purpose Brassinosteroids (BRs), the sixth class of phytohormones could be used. BRs play diverse roles for physiological and developmental processes in plant growth and also respond to various biotic and abiotic stresses (Bajguz & Hayat 2009, Ahammed et al. 2012). 24-eBL is the most

effective and stable BR analog and has been found to high stimulatory effect in enzymatic activity and antioxidant systems in the majority of studies (Hayat et al. 2010). Moreover, 24-eBL also promote the production of secondary metabolites in several plants (Çoban & Baydar 2017; Asci et al. 2019) and the exogenous application of BRs has been determined to increase antioxidant capacity and phenolic content in grapes (Luan et al. 2013; Xi et al. 2013; Ghorbani et al. 2017; Wang et al. 2019; Babalık et al. 2020). Although there are few studies conducted the impact of immobilization on the increased accumulation of secondary metabolites in several plants (Choi et al. 1995; Gillet et al. 2000; Dornenburg 2004) and grapevine (Iborra et al. 1994; Guardiola et al. 1996), there is no study to determine on the effect of brassinosteroid applications on phenolic contents of grapes in immobilized cultures. For this reason, more detailed studies are needed to investigate. This study was performed to provide a better understanding the effects of 24-eBL and immobilized cells on the accumulation of phenolic compounds. This is also the first report to our knowledge of the use of 24-eBL in immobilized cell culture in grapes.

2. Material and Methods

In the research, petioles belonging to *Vitis vinifera* cv. Cinsault preferred in red wine making due to its low tannin and rich aromatic components, were used as plant material. Cuttings were provided from Tekirdağ Viticultural Research Institute. Single node cuttings were planted in pots containing sand, perlite and torf (1:1:1) and then incubated to a controlled environment chamber at 25 °C with cool fluorescent daylight (16 h photoperiod). When 8-10 leaves were formed on the shoots, petioles were taken. Petioles were washed with tap water 3-5 times and then petioles were treated with 70% ethanol for 70 s under laminar airflow cabinet. After pretreatment with ethanol, petioles were rinsed with sterile distilled water for three times and surface-sterilized with commercial bleach (22.5%) for 18 min and last rinsed with sterile distilled water again. Then, petioles were cut into 1 cm pieces and inoculated on MS medium (Murashige & Skoog 1962) containing 1 mg L^{-1} benzylaminopurine (BAP) and 0.1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 30 g L^{-1} sucrose and 7 g L^{-1} agar. The pH of the medium was adjusted to 5.85 prior to autoclaving. Cultures were incubated at 25±1 °C under dark conditions. Calli were transferred to the same fresh media every 40-45 days in order to maintain adequate stock cultures.

2.1. Immobilization of cells

Approximately 1 g of cells from 25 days of callus cultures were mixed with 50 mL of 2% sodium alginate and 25 mL of liquid MS media containing 1 mg L^{-1} BAP, 0.1 mg L^{-1} 2,4-D and 30 g L^{-1} sucrose. The cell/alginate suspension was then added drop by drop through a 5 mL pipette tip into 0.2 M $CaC1_2$ solution, and almost homogeneous size beads (~4 mm in diameter) were formed. The beads were left to harden in $CaCl_2$ solution for 30 min (Sajc et al. 1995), rinsed with sterile water and transferred into MS medium containing different concentrations of 24-eBL.

2.2. Application of 24-eBL to immobilized cells

Immobilized cells were added to 50 mL liquid MS nutrient media containing 1 mg L^{-1} BAP, 0.1 mg L^{-1} 2,4-D and 30 g L^{-1} sucrose with different concentrations of 24-eBL (0, 0.25, 0.50, 0.75 and 1 mg L^{-1}). While choosing these concentrations, the previous study we made on vineyards (Babalık et al. 2020) is taken as reference. The 24-eBL stock solution was prepared by dissolving in dimethyl sulfoxide (DMSO). The immobilized cell cultures were incubated under dark conditions at 25±1 °C on the rotary shaker at 100 rpm for one month. There were 3 replicates per treatment and 5 culture flasks (eg. erlenmayer) per replicates. At the end of the incubation, 1% sodium citric acid solution was added to the medium to dissolving calcium-alginate and they were incubated at 25 °C for 30 min. Then, free cells were washed with sterile water and dried in a 45 °C oven until the constant mass is obtained.

2.3. Extraction of phenolics compounds

For extraction of phenolic compounds 0.1 g of powdered callus was extracted with 10 ml 0.1% HCl in 70% methanol. Extraction was ensured in the ultrasonic bath for 30 min and then centrifuged at 4000 rpm for 15 min. The residue left over from the first extract was washed with HCl:methanol solution once again and the supernatant was collected (it was done for three times total) and evaporated in a rotary evaporator at 45 °C until dry.

2.4. Determination of phenolic compounds

Folin-Ciocalteu reagent assay was used to determine phenolic compounds (Singleton & Rossi 1965). The absorbance was determined by spectrophotometer at 765 nm. Results were expressed as mg gallic acid 100 g^{-1} dry weight (DW).

High-performance liquid chromatography (HPLC) analyses were performed according to the modified procedure of Caponio et al. (1999). Phenolic compounds were determined using the HPLC system including a pump (LC-10 ADvp), auto-sampler (SIL-10 ADvp), column oven (CTO 10Avp) and diode-array UV/VIS detector (DAD λ_{max}:278). The separation was performed on an Agilent Eclipse XDB-C18 (5 µm, 250 x 4.60 mm). Mobile phase A contained 3% acetic acid in water; solvent B contained methanol. The gradient was: 93% A and 7% B for 0.01-0.10 min, 72% A and 28% B for 0.10-20 min, 75% A and 25% B for 20-28 min, 70% A and 30% B for 28-35 min, 70% A and 30% B for 35-50 min, 67% A and 33% B for 50-60 min, 58% A and 42%

B for 60-62 min, 50% A and 50% B for 70-73 min, 30% A and 70% B for 73-75 min, 20% A and 80% B for 75-80 min, 0% A and 100% B for 80-81 min, 93% A and 7% B for 81-90 min. The flow rate was 0.8 mL min^{-1} and the injection volume was 20 µL. Standard solutions, mobile phases, and samples were filtered through a 0.45 µm pore size membrane filter. The detection UV wavelength was 278 nm. The temperature of the column oven was 30 °C. Catechin, chlorogenic acid, epicatechin, gallic acid, *p*-coumaric acid, quercetin, and *trans*-resveratrol contents were expressed as µg g^{-1} DW.

2.5. Statistical analysis

Descriptive statistics were presented as mean and standard deviation. Treatment effects were determined using one-way ANOVA. Duncan's multiple range test (significance level $p<0.05$) was used to compare mean. All statistical analyses were conducted using the software package SPSS (ver:18).

3. Results and Discussion

Phenolic compounds changed significantly according to different concentrations of 24-eBL applications ($p\leq0.05$). The results pointed out that the 24-eBL application caused an increase in phenolic compounds compared to control (Figure 1). As shown in Figure 1A, the highest total phenolic content was observed from the immobilized cells treated 0.75 mg L^{-1} 24-eBL, while the lowest values were recorded from the immobilized cells treated 1 mg L^{-1} 24-eBL. The amount of total phenolic compounds increased 1.9-fold compared to the control. Ahammed et al. (2013) stated that BR applications regulate secondary metabolism. 24-eBL has been reported to increase the activity of secondary metabolism-related enzymes such as phenylalanine ammonialyasethe first enzyme involved in flavonoid biosynthesis) and flavonoid 3-O-glucosyltransferase, which modulate the phenylpropanoid metabolism. Thus, it promotes the synthesis of phenolic compounds (Xi et al. 2013; Li et al. 2016). Asci et al. (2019) reported that exogenous applied BR acts as a signalling molecule to increase phenolic biosynthesis and enhances metabolite accumulation by affecting enzymes and genes involved in biosynthesis. In previous studies, it was found that BR applications increased total phenol and antioxidant capacity compared to control treatment (Xi et al. 2013; Ghorbani et al. 2017).

In our study, chlorogenic acid contents significantly changed depending on the applications. Based on the results, the highest chlorogenic acid was obtained the immobilized cells treated 0.5 mg L^{-1} 24-eBL, while the immobilized cells that treated 1 mg L^{-1} 24-eBL application was the lowest (Figure 1C). Compared to the control, chlorogenic acid contents of immobilized cells treated 0.5 mg L^{-1} 24-eBL application increased 1.45-fold. 24-eBL applications had significant effects on the amount of catechin compared to control cells. The highest catechin accumulation was found in immobilized cells that treated 0.75 mg L^{-1} 24-eBL. However, control cells had the lowest values. Catechin content increased 4.76-fold compared to the control group (Figure 1B). Epicatechin content was ranged from 36.33 to 369.52 µg g^{-1}. The concentration of 0.5 mg L^{-1} of 24-eBL was given the highest epicatechin value. However, 24-eBL at higher concentration (1 mg L^{-1}) caused a significant decline in epicatechin content. Epicatechin contents of immobilized cells treated with 0.5 mg L^{-1} 24-eBL application increased 6.24-fold compared to the non treated 24-eBL cells (Figure 1D). The changes of gallic acid content in *V. vinifera* cv. Cinsault immobilized cell culture subjected 24-eBL application were also shown in Figure 1E. Gallic acid content was low in immobilized cells treated with 1 mg L^{-1} 24-eBL. The highest gallic acid content was found in the immobilized cells treated with 0.25 mg L^{-1} 24-eBL. In this medium, the amount of gallic acid was 2.85-fold higher than the control. The content of *p*-coumaric acid ranged from 2.23 to 15.30 µg g^{-1}. According to data in Figure 1F, the highest amount of *p*-coumaric acid was obtained from the application of 0.75 mg L^{-1} 24-eBL. However, the lowest values were detected in 1 mg L^{-1} 24-eBL application. The amount of *p*-coumaric acid increased 3.57-fold over control. Quercetin contents significantly changed depending on the 24-eBL concentrations. The highest quercetin accumulation (68.38 µg g^{-1}) was observed from 0.5 mg L^{-1} 24-eBL application (Figure 1G). There is currently no literature on the effects of BRs on catechin, chlorogenic acid, epicatechin, gallic acid and *p*-coumaric acid metabolism in grapevines, and therefore it is important to learn more to investigate the mechanism of action of BRs on grapes. This study was performed to eliminate the deficiency in the literature and to understand better the effects of BRs. It was also the first report determining the effects of BRs on catechin, chlorogenic acid, epicatechin, gallic acid and *p*-coumaric acid contents of grapes.

Immobilization is an effective method for enhancing secondary metabolite production in plant cell cultures. In this method agar, agarose, calcium alginate, gelatine, carrageenan or polyacrylamide are used as a matrix to fix plant cells (Pras & Woenderbag 1999; Smetanska 2008). Gillet et al. (2000) stated that immobilized cells have created the formation of aggregate during the growth of cells, moreover, the presence of aggregates can modify cell to cell and cell to matrix interactions. Researchers also reported that synthesis of secondary metabolite could be stimulated as a result of increased gene expression due to cell-cell interaction in cells maintained in the culture medium.

Resveratrol is a phytoalexin that a stilbene derivative and has a potential protective role against cardiovascular disease. It is produced by plants and notably present in grapes (Bonnefont-Rousselot 2016). In the current research, the accumulation of the *trans*-resveratrol in immobilized cell cultures was changed according to the 24-eBL concentrations. The highest *trans*-resveratrol content (16.37 µg g^{-1}) was recorded from the immobilized cells treated with 0.5 µg g^{-1} 24-eBL. In this medium, the amount of *trans*-resveratrol was 1.1-fold higher than the control (Figure 1H). There is only one study conducted on the effects of exogenous brassinosteroid applications on *trans*-resveratrol in grapevine (Babalık et al. 2020). Researchers indicated that *trans*-resveratrol content increased 2.67-fold compared with control vines. The increase in *trans*-resveratrol accumulation through BR applications

was thought to be result from the stimulating effects of BRs on the expression of genes encoding enzymes such as stilbene synthase that function in *trans*-resveratrol synthesis (Babalık et al. 2020). Keskin & Kunter (2009) stated that *in vitro* techniques in grapevine, especially callus and cell suspension cultures, have several advantages to increase productivity in the production of *trans*-resveratrol. In this study, it was also deduced that callus cultures can be used as model systems for the stimulation and determination of trans-resveratrol production in grapevines.

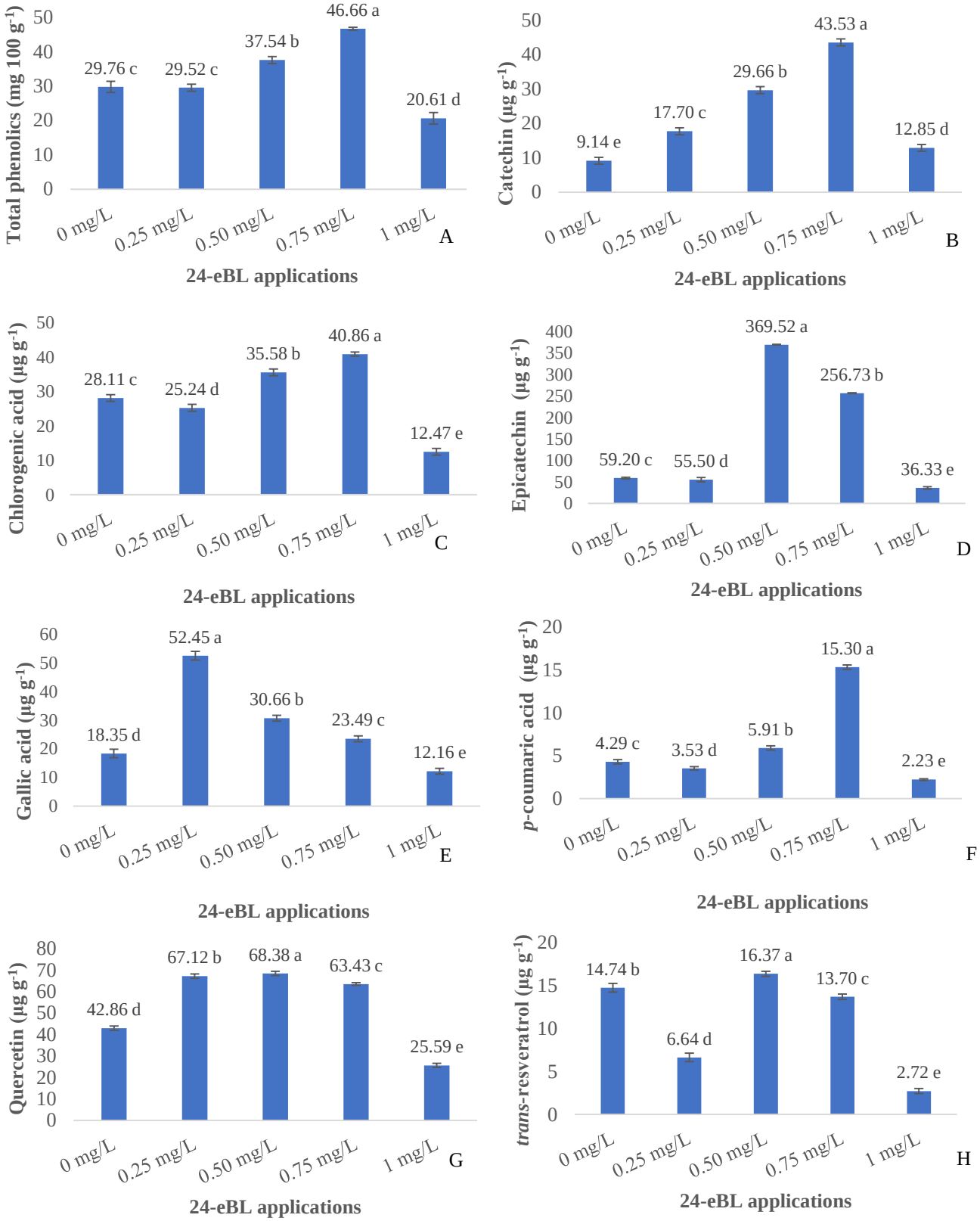

Figure-1. Effects of 24-eBL applications on the total phenolic content (A), catechin (B), chlorogenic acid (C), epicatechin (D), gallic acid (E), *p*-coumaric acid (F), quercetin (G) and *trans*-resveratrol (H) in Cinsault immobilized cells. Different letters indicate statistically significant differences among the applications (p≤0.05)

4. Conclusions

The present study demonstrated that 24-eBL application to immobilized cells has an advantageous effect on secondary metabolite production of cv. Cinsault, effectively. The best results were obtained from 24-eBL applications performed at concentrations of 0.5 or 0.75 mg L^{-1}. However, it has been determined that the application of high concentrations of 24-eBL (1 mg L^{-1}) reduced the secondary metabolite production even compared to control, because of this reason; higher doses of BR have been evaluated not effective.

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Impact of Hot Water and Modified Atmosphere Packaging Treatments on the Postharvest Quality of Pomegranate Fruit (*Punica granatum* cv. 'Hicaznar')

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ABSTRACT
Hot water (HW) and modified atmosphere packaging (MAP) treatments were evaluated to maintain postharvest quality of pomegranate fruit (*Punica granatum* cv. 'Hicaznar'). Pomegranates were subjected to hot water (HW) treatment (at 50 °C for 3 min) and packaged with or without MAP bags. Fruit was then kept at 6 °C for 6 months and at 20 °C for 7 days after cold storage period. The untreated and unpackaged fruit was served as a control treatment (C). MAP and HW+MAP treatments was more effective in reducing weight loss, fungal decay and husk scald, compared to HW and C treatments. The lightness and red color intensity of husk and aril (higher values of L* and C* and lower values of h°) were maintained better in the packaged fruit with MAP (MAP + HW+MAP treatments). The unpackaged fruit from HW and C treatments became unmarketable while those from MAP and HW+MAP treatments were still marketable after 6 months of cold storage and shelf life period. Although fungal decay incidence was low in HW-treated fruit, relatively high scald incidence and weight loss had adverse effect on overall visual acceptability of HW-treated fruit. Hot water dipped pomegranate fruit cv. 'Hicaznar' (50 °C for 3 min) could be kept in MAP bags for 6 months at 6 °C and for 7 days 20 °C without adverse effect on quality.

Keywords: MAP, Shelf life, Cold storage, Chilling injury, Husk scald

1. Introduction

Pomegranate (*Punica granatum* L.) production and export of Turkey has been reached over 500 thousand tons and 200 thousand tons, respectively (TSI 2018). 'Hicaznar' is the dominated cultivar and produced in mostly the Mediterranean and Aegean regions. Major destination markets of Turkish pomegranate export are the European Union countries beside to Russian Federation and other Middle East countries. Commercial storage of pomegranates is advised to prolong until late March (Selçuk & Erkan 2015) when price of pomegranate fruit in European markets reaches the highest level (Rymon 2012). Postharvest quality of pomegranate fruit is often impaired by the visible shriveling symptoms, chilling injury, husk scald, fungal decay beside to deterioration in aril color and taste of pomegranate fruit during long-term cold storage (D'Aquino et al. 2010; Selçuk & Erkan 2015; Porat et al. 2016; Candir et al. 2018; 2019).

Postharvest heat treatments such as curing, intermittent warming and hot water (HW) dips (45 °C to 55 °C for 1 to 5 min) have been studied to reduce chilling injury and fungal decay and improve nutritive and functional properties of pomegranates (Artés et al. 2000a; Mirdehghan & Rahemi 2005; Mirdehghan et al. 2006; 2007; Ramezanian & Rahemi 2010; Moradinezhad & Khayyat 2014). Modified atmosphere packaging (MAP) has been reported to be effective in maintaining the external and internal quality of pomegranate fruit by controlling weight loss, fungal decay and husk scald, and during cold storage period (Artés et al. 2000b; Nanda et al. 2001; D'Aquino et al. 2010; Selçuk & Erkan 2014; 2015; Porat et al. 2016). Combination of hot water, salicylic acid and MAP were reported to be more effective in reducing decay and chilling injury of pomegranate fruit cv. 'Sheshi-kab' in compared to individual application of each treatment (Moradinezhad et al. 2013). In this study, we investigated the combined effects of HW and MAP treatments on postharvest quality characteristics of pomegranate fruit cv. 'Hicaznar' during cold storage and shelf life period.

2. Material and Methods

Pomegranates (cv. 'Hicaznar') were taken from the local commercial orchard where the trees were planted at 5 m × 5 m spacing and were 9-year-old. Fertilizers (160 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 140 kg K_2O ha^{-1}) were applied under drip irrigation system. The orchard with loamy-clayey and slightly alkaline of soil was located in Antakya-Hatay in the Eastern Mediterranean region of Turkey (36°12′59′′ N, 36°25′43′′E, at altitude of 88 m). The typical Mediterranean climate prevails

in this region with annual 1.126 mm precipitation, 69% average annual relative air humidity and annual average temperatures ranged from 8.2 °C to 27.7 °C.

Fruit was hand-harvested when titratable acidity (TA) and soluble solids content (SSC) were <1.85% and >17%, respectively during the 2015-2016 season and were then immediately transported to the storage and laboratory facilities of the Horticultural Department at Hatay Mustafa Kemal University. Pomegranate fruit in uniform size and maturity without defects and blemishes was subjected to the following treatments: (1) Fruit was dipped in hot water (at 50 °C for 3 min) and stored in 52 × 36 × 30 cm plastic boxes (HW); (2) fruit dipped in hot water was packaged with MAP (HW+MAP); (3) fruit without hot water dip was packaged in modified atmosphere packages (MAP); (4) fruit was dipped in water at 24 °C for 3 min and stored in plastic boxes (W) and (5) Control fruit without HW and MAP treatments was stored in plastic boxes (C). Hot water dip temperature was chosen according to the findings of previous studies conducted on 'Hicaznar' and 'Sheshi-kab' pomegranate cultivars (Kipri & Dündar 2011; Moradinezhad & Khayyat 2014). In MAP and HW+MAP treatments, the 5 kg Life Pack® (Patent No.: 2007 45625, Aypek Ambalaj Co., Bursa) bags were used as MAP. The HW and W treated fruits were allowed to dry on a paper towel at room temperature for 1 hour before packaging and storage. Packaged fruits were cooled to 6 °C for 24 hours before sealing the MAP bags and then stored together with the fruits from other treatments at 6±0.5 °C and 90±5% RH for 6 months. Fruit was also kept at 20 ± 1 °C and 70 ± 5% relative humidity for 7 days after 2, 4 and 6 months of cold storage period.

Postharvest quality was evaluated by two months intervals. Weight loss was determined as percentage by weighting of each fruit at harvest and after every 2 months. Check Point model O_2/CO_2 analyzer (PBI-Dansensor America Inc., NJ) was used to monitor headspace O_2 and CO_2 concentration of the bags. Husk color was measured at three points on the equatorial region of each individual fruit using the CIE L*a*b* color space with a CR-300 Minolta Chroma Meter (Osaka, Japan). Arils color was determined according to Artés et al. (1998). Chroma (C*) values were calculated as $C^*= (a^{*2}+b^{*2})^{1/2}$ and hue angle values as (h°) $h°=\tan^{-1}$ (b*/a*). The juice was obtained by squeezing of arils of five fruit per replicate through cheesecloth with hand press and used to determined total soluble solids (TSS) content and titratable acidity (TA). The TSS content was measured using a Atago Model ATC-1E refractometer. The five mL of juice was titrated with 0.1 N NaOH to a pH of 8.1 to determine TA (citric acid equivalents). The panelists evaluated overall visual quality using a 5 point scale, where: 1=very poor; 2=poor (limit of marketability); 3= good; 4= very good; 5=excellent (Selçuk & Erkan 2015), and taste using a hedonic scale , where 1= disliked to 9= liked. The fruit was examined visually for fungal decay and chilling injury and husk scald symptoms according to Defilippi et al. (2006). Fungal decay and scald incidence was calculated as a percentage of the fruit affected by decay or and scald. Severity of scald was assessed using a 6 point scale, where 1=no scald, 2=<10%, 3=11 − 250%, 4=25 − 50%, 5=50 − 75 and 6=75 − 100% of the surface affected.

The data were analyzed using SAS software (SAS 2019) according to a completely randomized design with five treatments, and three replications for each treatment. Each replication contained the 5 kg of fruit. Fisher's least significant difference (LSD) test was performed at a $P<0.05$ level for mean separation using the SAS Proc GLM procedure.

3. Results and Discussion

3.1. Headspace O_2 and CO_2 concentration

Figure 1a and 1b presents changes in O_2 and CO_2 concentrations, respectively, inside the MAP bags. Effects of treatments × storage period interaction on the changes in headspace O_2 and CO_2 concentration were significant ($P<0.05$). In both treatments, except for a slight increase in O_2 concentration and a slight decrease in CO_2 concentration after 4 months, O_2 concentrations inside MAP decreased while CO_2 concentration increased during cold storage period. After 6 months of cold storage, final headspace O_2 and CO_2 levels were 15.30% and 7.77% in MAP treatment and 16.73% and 6.57% in HW+MAP treatment, respectively. MAP treatment resulted in a lower O_2 and a higher CO_2 levels than HW+MAP treatment during cold storage. The MAP bag tested in this study ensured a proper modified atmosphere for pomegranate fruit cv. 'Hicaznar' since previous studies suggested 13.50-17.60% of O_2 and 4.40-8.10% of CO_2 (Selçuk & Erkan 2014; 2015), 13.63% of O_2 and 7.85% of CO_2 (Candir et al. 2018) and 15.30% of O_2 and 7.45% of CO_2 (Candir et al. 2019) for pomegranate fruit for long term storage at 6 °C. HW treatment did not have any improving effects on the headspace gas concentration inside MAP.

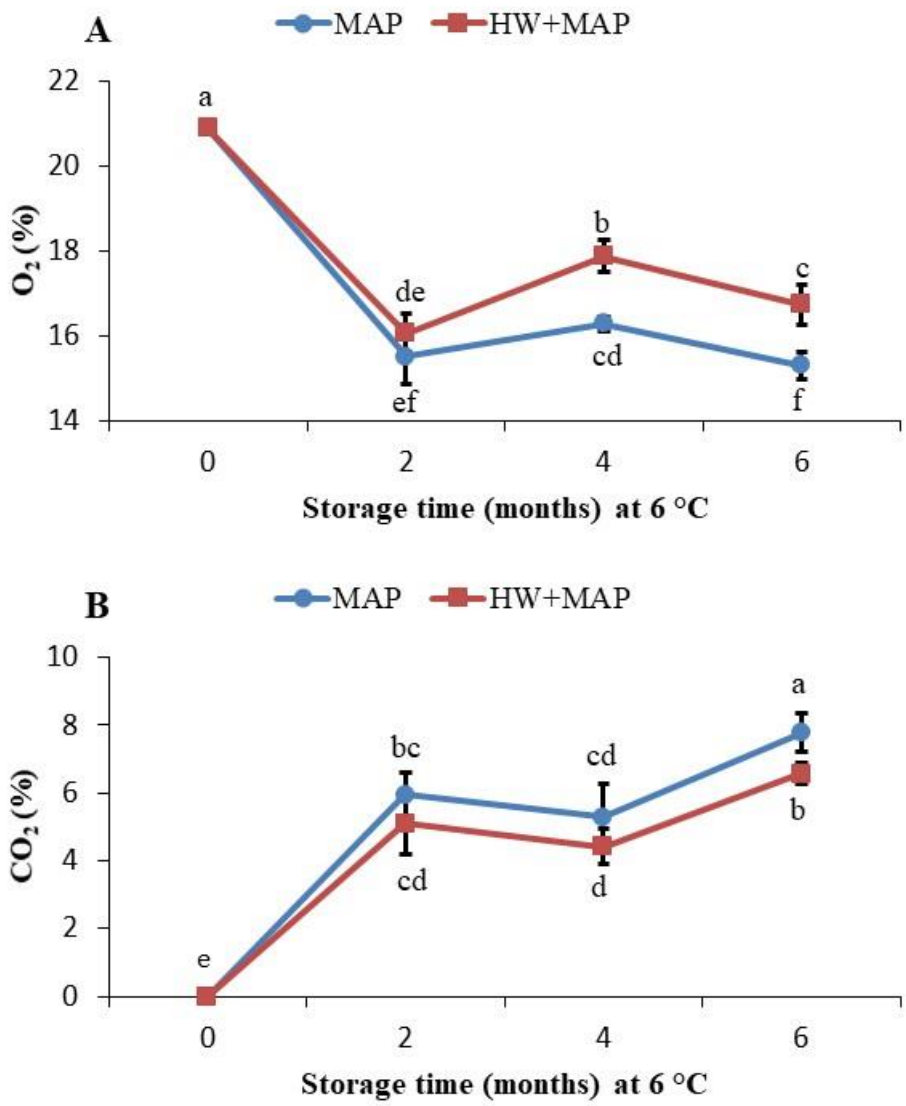

Figure 1- Changes in headspace O_2 (A) and CO_2 (B) concentration (%) inside the MAP containing hot water treated- or not treated pomegranate fruit cv. Hicaznar during storage at 6 °C. HW: Hot water; MAP: Modified atmosphere packaging

3.2. Weight loss

Effects of treatments × storage period and treatments × shelf life period interactions on the changes in weight loss were significant ($P<0.05$). MAP and HW+MAP treatments resulted in a significant reduction in weight loss during cold storage and shelf life period, compared to HW, C and W and treatments (Figure 2). Percent weight loss in MAP and HW+MAP treatments ranged from 9.09% to 9.64% after cold storage and from 11.91% to 12.52% after shelf life period. HW treatments also lead to reduction in weight loss, but it was not as much as MAP and HW+MAP treatments. The 18.51 and 20.27% of weight loss occurred in HW treated-fruit after cold storage and shelf life period, respectively. HW dip contributed a slight effect in controlling weight loss in HW+MAP treatment. Percent weight loss were highest in C and W treatments and reached to 20.36% and 22.11% respectively, after 6 months of storage and exceeded 20% occurred during shelf life period following cold storage. No shriveling was observed in MAP packaged fruit with or without HW dip. In case of the unpackaged fruit of C, W and HW treatments, the husk became hard and darkened, indicating severe shriveling at the end of cold storage and shelf life period. MAP have been suggested to minimize weight loss of pomegranate fruit during cold storage (Artés et al. 2000b; Nanda et al. 2001; D'Aquino et al. 2010; Selçuk & Erkan 2014, 2015, 2016; Porat et al. 2016). In agreement with our results, Mirdehghan & Rahemi (2005) found a lower weight loss occurred in pomegranate fruit from HW treatment at 50 °C than control fruit during 3 to 4 months of cold storage. In contrast to our findings, Moradinezhad & Khayyat (2014) reported that HW treatment alone had no significant effect on weight loss in cold stored pomegranate fruit, compared to control.

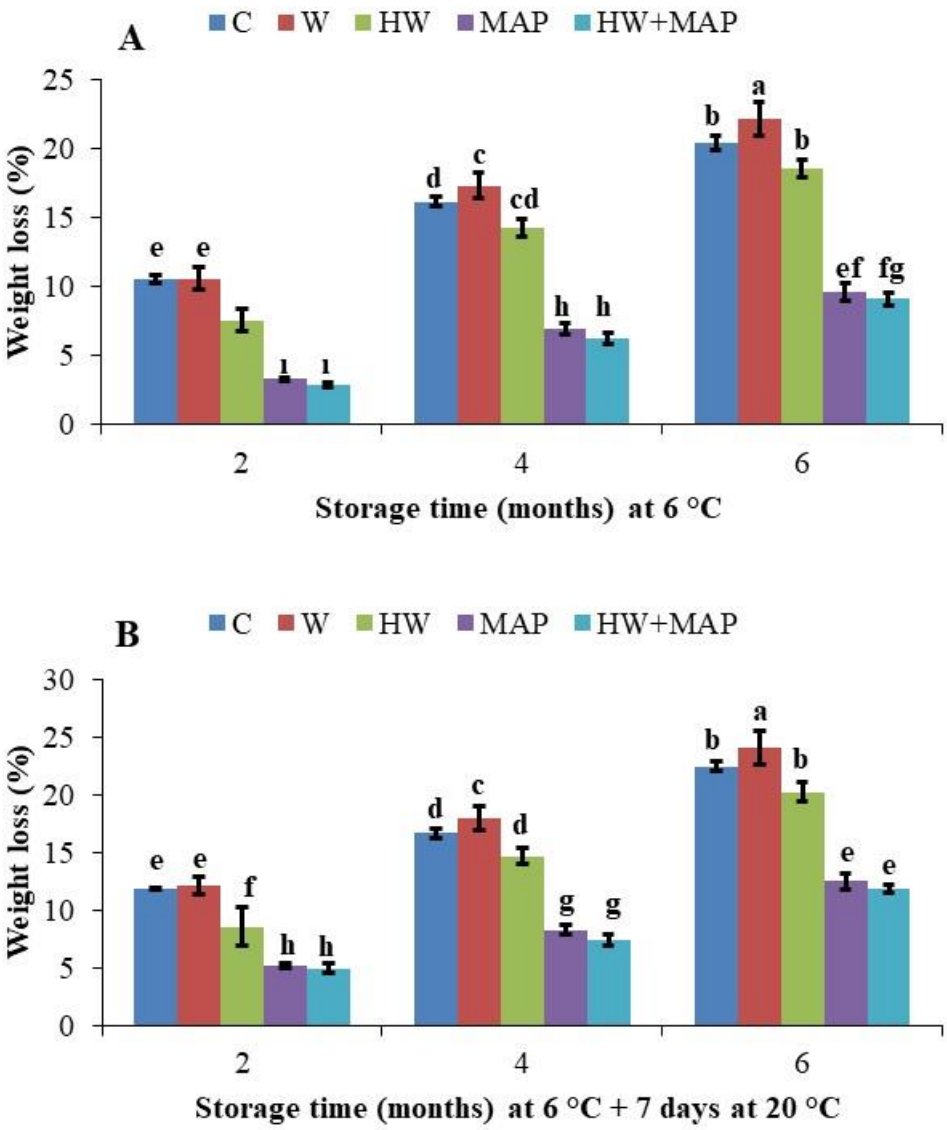

Figure 2- Effects of how water and MAP treatments on the changes in weight loss of Hicaznar pomegranate fruit during storage at 6 °C (A) and subsequent shelf life period for 7 days at 20 °C (B). C: Untreated; W: Water; HW: Hot water; MAP: Modified atmosphere packaging

3.3. SSC and TA

Treatments × storage period and treatments × shelf life period interactions significantly affected on changes in SSC and TA. ($P<0.05$). A decrease in SSC and TA occurred in all treatments after cold storage and shelf life period, compared to the values at harvest (Table 1). Pomegranates are non-climacteric fruit and the consumption of acids and sugars by pomegranate fruit via respiration process lead to decrease in SSC and TA during postharvest period (Kader et al. 1984; D'Aquino et al. 2010; Selçuk & Erkan, 2015, 2016). SSC was similar among the treatments during cold storage period and shelf life period following 2 and 4 months of storage (Table 2). However, control and water treated fruit had higher SSC than the fruit of HW, MAP and HW+MAP treatments after shelf life period following 6 months of storage due to a concentration effect of water loss on sugars (Selçuk & Erkan 2015). In compared to C treatment, TA was maintained better in HW, MAP and HW+MAP treatments throughout cold storage period, but there is no significant difference in TA among treatments after shelf life period. In pomegranate fruit, TA and SSC were reported not to be affected significantly by HW (Mirdehghan & Rahemi 2005; Ben Abda et al. 2010; Kipri & Dündar, 2011; Ramezanian & Rahemi 2010; Sepahvand et al. 2013; Moradinezhad & Khayyat 2014), MAP (Artés et al. 2000b; D'Aquino et al. 2010; Selçuk & Erkan 2015; 2016) or HW+MAP (Moradinezhad et al. 2013) treatments according to the results some studies. In contrast, other studies showed that TA was maintained better in pomegranate fruit dipped in hot water (Mirdehghan et al. 2006) or packaged MAP (Nanda et al. 2001; Selçuk & Erkan 2014; Candir et al. 2018; 2019) in comparison to control during cold storage and shelf life period. In our study, HW+MAP treatment did not have additive beneficial effect in maintaining SSC and TA, compared to individual application of each treatment.

Table 1- Effects of how water and MAP treatments on some quality parameters of Hicaznar pomegranate fruit during 6 months of storage at 6 °C

Treatments[1]	*SSC (%)*	*TA (%)*	*Husk color*			*Aril color*		
			*L**	*C**	*h°*	*L**	*C**	*h°*
At harvest	*17.62a*[2]	*1.38a*	*43.90a*	*49.68a*	*25.11g*	*32.70a*	*24.51e*	*29.73a*
2 months of cold storage at 6 °C								
C	17.30abc	1.20cd	42.32 bc	44.92cde	27.99bcd	31.39a	27.84cd	27.04b
W	16.87cde	1.12ef	41.46 cd	44.29def	27.90bcd	27.76a	26.99d	25.66bcd
HW	17.40ab	1.23bc	41.64 cd	44.97cd	25.60 fg	31.60a	27.89cd	27.02b
MAP	17.33ab	1.24bc	43.79 a	49.38a	25.65 fg	32.62a	26.70d	26.13bc
HW+MAP	17.23abc	1.25b	43.79 a	49.71a	25.82 fg	31.53a	26.62d	26.23bc
4 months of cold storage at 6 °C								
C	17.23abc	1.06g	40.61 d	43.03fgh	28.87 b	28.28cd	30.35a	25.22cde
W	16.70def	0.98h	40.57 d	43.17fg	28.77 b	24.81e	27.78cd	26.08bcd
HW	17.23abc	1.16de	41.19 cd	43.0efg	27.35cde	29.05cd	28.62bc	26.34bc
MAP	17.07bcd	1.13e	43.07 ab	46.74b	26.22efg	32.75a	28.34bc	26.27bc
HW+MAP	17.07bcd	1.16de	43.22 ab	46.14bc	27.22 de	31.15ab	28.73bc	25.72bcd
6 months of cold storage at 6 °C								
C	16.57ef	1.01h	40.80 d	42.22ghı	31.20 a	25.57c	30.69a	24.55de
W	16.73def	1.00h	40.78 d	41.16ı	30.93 a	20.33de	29.41ab	24.12e
HW	16.50ef	1.07g	41.31 cd	41.48hı	30.84 a	25.10c	27.56cd	24.87cde
MAP	16.33f	1.08fg	43.74 a	47.40b	26.49 ef	29.71b	26.84d	25.08cde
HW+MAP	16.67def	1.06g	42.99 ab	45.05cd	28.59 bc	28.00b	27.40cd	25.43cde

[1] C: Untreated; W: Water; HW: Hot water; MAP: Modified atmosphere packaging,[2] Means (n=3) followed by different letters within a column are significantly different according to Fisher's LSD test at P<0.05

Table 2-Effects of how water and MAP treatments on some quality parameters of Hicaznar pomegranate fruit after shelf life period for 7 days at 20 °C following 2, 4 and 6 months of storage at 6 °C

Treatments[1]	*SSC (%)*	*TA (%)*	*Husk color*			*Aril color*		
			*L**	*C**	*h°*	*L**	*C**	*h°*
At harvest	*17.62a*[2]	*1.38a*	*43.90ab*	*49.68a*	*25.11c*	*32.70a*	*24.51d*	*29.73d*
Shelf life for 7 days at 20 °C after 2 months of cold storage								
C	17.27abc	1.20cd	43.17bcd	44.83cd	25.69c	25.58ef	27.05bc	30.83c
W	17.10c	1.23cd	43.13bcd	44.98cd	25.64c	21.27g	27.17bc	31.05bc
HW	17.20bc	1.24c	43.75ab	45.44bc	25.59c	26.65d	26.20c	29.47de
MAP	17.17bc	1.31b	44.11a	46.87b	23.82c	30.51b	27.68bc	29.46de
HW+MAP	17.17bc	1.25bc	43.83ab	45.85bc	24.55c	28.81c	26.98bc	29.06def
Shelf life for 7 days at 20 °C after 4 months of cold storage								
C	17.35abc	1.01f	42.58de	41.52fg	29.06b	25.96de	31.24a	31.27bc
W	17.10c	1.06f	42.73cde	41.44fg	29.02b	21.78g	30.84a	31.93ab
HW	17.23bc	1.13e	43.43abc	42.46ef	24.26c	26.43de	27.13bc	28.70efg
MAP	17.20bc	1.25bc	43.70ab	45.14cd	25.41c	30.24b	26.59bc	28.09g
HW+MAP	17.13c	1.17de	43.88ab	45.42bc	25.44c	28.33c	27.75bc	29.07def
Shelf life for 7 days at 20 °C after 6 months of cold storage								
C	17.00c	1.02f	38.89f	39.74h	32.03a	22.14g	30.24a	32.47a
W	17.53ab	1.02f	38.97f	39.87h	32.41a	20.08h	30.48a	31.91ab
HW	15.53d	1.03f	39.66f	40.53gh	31.38a	24.90f	28.01b	29.79d
MAP	14.98e	1.03f	42.67cde	43.81de	27.78b	28.67c	26.67bc	28.15fg
HW+MAP	15.27de	1.01f	41.96e	43.84de	29.33b	28.82c	27.62bc	29.29de

[1] C: Untreated; W: Water; HW: Hot water; MAP: Modified atmosphere packaging, [2] Means (n=3) followed by different letters within a column are significantly different according to Fisher's LSD test at P<0.05

3.4. Husk and aril color

Husk and aril color were significantly affected by treatments × storage period and treatments × shelf life period interactions (P<0.05). Husk color L* (lightness) and C* (intensity) values were lower while h° values were higher after 6 months of cold storage and shelf life period, compared to the initial values (Table 1 and 2). Similar changes in husk color of cold stored pomegranate fruit have been previously reported (Fawole & Opara 2013; Selçuk & Erkan 2013; 2014; 2015; 2016; Candir et al. 2018; 2019). A significantly higher water loss resulted in loss of husk color lightness (lower L* values) in the unpackaged fruit from C, W and HW treatments. In comparison to the unpackaged fruit (C, W and HW treatments), the lightness and red color intensity of husk (higher values of L* and C* and lower values of h°) were maintained better in the packaged fruit with MAP (MAP + HW+MAP treatments) as reported in previous studies (Artés et al. 2000b; D'Aquino et al. 2010; Selçuk & Erkan 2013; 2014; 2015; 2016; Candir et al. 2018; 2019). Stand-alone MAP treatment was found to be effective in maintaining

husk color. HW treatment was not successful in preventing husk color loss as C and W treatments. Therefore, we concluded that there is no improvement in husk color due to HW or HW+MAP treatments. Consistent with our findings, Kipri & Dündar (2011) reported no significant effect of HW treatments at 50 °C to 55 °C for 1-2 min on husk color. Sepahvand et al. (2013) found no significant differences in husk color a* and b* values between HW-treated and control pomegranate fruit cv. 'Malas Saveh', but HW treatments resulted in lower husk color L* values than control treatment after storage and shelf life period.

Aril color L* and h° values decreased and aril color intensity (C*) increased after 6 months of storage, compared to values at harvest (Table 1), indicating resulting in more intense aril color. Arendse et al. (2014) found similar changes in aril color L*, C* and h° values of pomegranate fruit cv. 'Wonderful' kept at 5 °C for 5 months. They reported that anthocyanin synthesis and accumulation is continued in the cold stored pomegranate fruit. Fruit from C and W treatments had darker (lower L*) and more intense (higher C*) aril color than those from HW, MAP and HW+MAP treatments after cold storage and shelf life period. Aril color h° values were similar among the treatments after 6 months of cold storage. However, C and W treatments resulted in higher h° values than HW, MAP and HW+MAP treatments during shelf life period following 6 months of cold storage (Table 2). This indicates discoloration of aril color in C and W treatments. Previous studies reported that aril color was not affected by HW treatment (Ben Abda 2010; Kipri & Dündar 2011). However, we found that stand-alone HW treatment and combination with MAP treatment was successful in maintaining aril color as MAP treatment did. Candir et al. (2019) reported a delay of discoloration of aril color in pomegranate fruit packaged with MAP compared to unpackaged control fruit during prolonged cold storage.

3.5. Husk scald, fungal decay, visual quality and taste

Husk scald, fungal decay visual quality and taste were significantly affected by treatments × storage period and treatments × shelf life period interactions ($P<0.05$). Husk scald symptoms and fungal decay were not observed until 6 months of storage and subsequent shelf life period, except for control treatment (Table 3 and 4). After 4 months of storage plus 7 days at 20 °C, scald incidence of 17.78% was observed only on control fruits. Incidence of husk scald was lower in MAP and HW+MAP treatments than HW, C and W after 6 months of cold storage and shelf life period. Severity of scald was low since only <10% of the skin's surface area covered with and scald symptoms. Fungal decay incidence was significantly reduced by HW, MAP and HW+MAP treatments compared to C and Win both cold storage and shelf life period. How water dip at 50°C for 3 min was found previously effective in reducing chilling injury of pomegranate fruit packaged with low-density polyethylene bags during 10 weeks of cold storage (Moradinezhad & Khayyat 2014). In our study, chilling injury symptoms were not observed in any of treatments. We found that stand-alone HW treatment was not effective as MAP and HW+MAP treatments in controlling husk scald. There is no improvement in preventing husk scald when combined HW treatment with MAP treatment. Ben-Arie & Or (1986) suggested that oxidation of phenolic compounds on the husk of pomegranates may resulted in husk scald when stored at >5 °C. Lower scald incidence was reported in 'Wonderful', 'Primosole' and 'Hicaznar' pomegranates stored in MAP bags for 12 to 16 weeks in comparison to unpackaged control fruit (D'Aquino et al. 2010; Porat et al. 2016; Candir et al. 2019). According to D'Aquino et al. (2010), lower O2 levels in MAP bags may reduce or delay oxidation of phenolic compounds on the husk and consequently could control scald incidence in pomegranate fruits packaged with MAP bags.

Table 3- Effects of how water and MAP treatments on the incidence of husk scald and fungal decay and visual quality and taste of Hicaznar pomegranate fruit after 6 months of storage at 6 °C

Treatments[1]	*Husk scald(%)*	*Severity of scald* [3]	*Fungal decay (%)*	*Visual quality* [4]	*Taste* [5]
At harvest	*0.00d*[2]	*1.00b*	*0.00c*	*5.00a*	*9.00a*
2 months of cold storage at 6 °C					
C	0.00d	1.00b	0.00c	4.87a	7.00cde
W	0.00d	1.00b	0.00c	5.00a	7.17cd
HW	0.00d	1.00b	0.00c	4.93a	7.67bc
MAP	0.00d	1.00b	0.00c	5.00a	8.50ab
HW+MAP	0.00d	1.00b	0.00c	5.00a	7.08cde
4 months of cold storage at 6 °C					
C	0.00d	1.00b	0.00c	2.20e	6.17e
W	0.00d	1.00b	0.00c	2.73d	6.17e
HW	0.00d	1.00b	0.00c	1.53f	6.50de
MAP	0.00d	1.00b	0.00c	4.77a	7.00cde
HW+MAP	0.00d	1.00b	0.00c	4.73a	7.00cde
6 months of cold storage at 6 °C					
C	41.48a	2.12a	37.78a	1.00g	6.42de
W	37.78a	2.11a	41.48a	1.00g	6.44de
HW	23.71b	2.12a	0.00c	1.00g	6.39de
MAP	13.34c	2.14a	0.00c	3.59b	6.89cde
HW+MAP	12.2c	2.15a	7.78b	3.15c	6.72de

[1] C: Untreated; W: Water; HW: Hot water; MAP: Modified atmosphere packaging, [2] Means (n=3) followed by different letters within a column are significantly different according to Fisher's LSD test at $P<0.05$, [3] Assessed based on a 1–6 scale, (1=no scald; 2=<10%; 3=11-25%; 4=25-50%; 5=50-75%; 6=%75-100 of the fruit surface affected), [4]Evaluated based on a 5 point scale, where: 1=very poor; 2=poor (limit of marketability); 3=good; 4=very good; 5=excellent. [5]Evaluated based on a hedonic scale of 1=disliked extremely to 9=liked extremely

Table 4- Effects of how water and MAP treatments on the incidence of husk scald and fungal decay and visual quality and taste of Hicaznar pomegranate fruit after shelf life period for 7 days at 20 °C following 2, 4 and 6 months of storage at 6 °C

Treatments[1]	*Husk scald(%)*	*Severity of scald* [3]	*Fungal decay (%)*	*Visual quality* [4]	*Taste* [5]
AT	*0.00d*	*1.00b*	*0.00b*	*5.00a*	*9.00a*
Shelf life for 7 days at 20 °C after 2 months of cold storage					
C	0.00d	1.00b	0.00b	4.87ab	8.17b
W	0.00d	1.00b	0.00b	4.73ab	6.20de
HW	0.00d	1.00b	0.00b	4.87ab	7.50c
MAP	0.00d	1.00b	0.00b	5.00a	6.00ef
HW+MAP	0.00d	1.00b	0.00b	4.93ab	6.33de
Shelf life for 7 days at 20 °C after 4 months of cold storage					
C	17.78c	2.27a	0.00b	4.27c	6.38de
W	0.00d	1.00b	0.00b	2.48f	6.61d
HW	0.00d	1.00b	0.00b	2.85ef	5.89ef
MAP	0.00d	1.00b	0.00b	4.57bc	7.38c
HW+MAP	0.00d	1.00b	0.00b	3.53d	7.22c
Shelf life for 7 days at 20 °C after 6 months of cold storage					
C	40.00a [2]	2.43a	36.67a	1.00g	5.28g
W	37.50a	2.38a	40.00a	1.00g	4.45hı
HW	36.67a	2.37a	0.00b	1.00g	4.06ı
MAP	27.41b	2.23bc	2.22b	3.20de	5.44fg
HW+MAP	28.34b	2.33ab	0.00b	2.93e	4.97gh

[1] C: Untreated; W: Water; HW: Hot water; MAP: Modified atmosphere packaging, [2] Means (n=3) followed by different letters within a column are significantly different according to Fisher's LSD test at P<0.05, [3] Assessed based on a 1–6 scale, (1=no scald; 2=<10%; 3=11-25%; 4=25-50%; 5=50-75%; 6=%75-100 of the fruit surface affected), [4]Evaluated based on a 5 point scale, where: 1=very poor; 2=poor (limit of marketability); 3=good; 4=very good; 5=excellent, [5]Evaluated based on a hedonic scale of 1=disliked extremely to 9=liked extremely

HW and HW+MAP treatment was more effective in reducing decay, compared to MAP treatment. Effectiveness of HW treatment and its combination with MAP treatment in controlling fungal decay of pomegranate fruit was previously reported (Ben Abda et al. 2010; Kipri & Dündar 2011; Moradinezhad et al. 2013; Moradinezhad & Khayyat 2014). MAP treatment did not affect decay incidence in pomegranate fruits cv. Mollar de Elche (Artés et al. 2000b) and Wonderful (Porat et al. 2016) during cold storage and shelf life period. In contrast, Candir et al. (2019) observed lower decay percentage in 'Hicaznar' pomegranates packaged with MAP bags than control.

The unpackaged fruit from HW, C and W treatments became unmarketable while those from MAP and HW+MAP treatments were still marketable after 6 months of cold storage and 7 days at 20 °C. Higher incidence of fungal decay, weight loss and husk scald impaired visual quality in the fruit of C and W treatments. Although fungal decay incidence was low in HW-treated fruit, relatively high scald incidence and weight loss had adverse effect on overall visual acceptability of HW-treated fruit. Taste of fruits received lower scores as storage time extended in all treatments. The taste of fruits was rated as acceptable (>5) after 6 months cold storage plus shelf life period except for W and HW treatments. MAP, HW+MAP and control treatments received higher taste score than W and HW treatments after shelf life period following 6 months of cold storage.

4. Conclusions

MAP and HW+MAP treatments was effective in reducing weight loss, husk scald and fungal decay, and maintaining husk and aril color and overall visual quality at 6 ± 0.5 °C and 90 ± 5% relative humidity for 6 months of cold storage and the subsequent shelf life period at 20 ± 1 °C and 70 ± 5% relative humidity for 7 days. Although HW treatment reduced weight loss and fungal decay, was not effective as MAP and HW+MAP treatments to maintain quality parameters.

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GE Interaction and Stability Analysis in Some Basma Type Oriental Tobacco (*Nicotiana tabacum* L.) Lines

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ABSTRACT

Turkey has long been the leader in oriental tobacco (*Nicotiana tabacum* L.) production in the world. Standard cultivars are needed to increase the yield and quality of tobacco production. This study aimed to determine the most stable cultivar candidates by evaluating the performances of tobacco genotypes grown in different environmental conditions. Field trials were carried out in Bafra district of Samsun Province, the district with most tobacco production in Mid-Black Sea Region, and Evciler, Karayaka and Gümüşhacıköy where the Basma type oriental tobaccos are produced. The experimental design was randomized complete blocks with three replications in 2017. The study material consisted of 21 lines selected by morphological characteristics and identified by DNA fingerprinting analysis and four standard cultivars/lines. Chemical analyses were carried out using the HPLC method. The stability of genotypes was determined by regression coefficient (b_i), regression constant (a), determination coefficient (r^2), coefficient of variation (CV) and deviation from regression (S^2d) parameters using the leaf yield, quality grade index, nicotine and sugar content values. The ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 lines were considered the prominent candidates based on the stability parameters and other traits investigated. Therefore, future studies should be continued using the aforementioned lines. In conclusion, much more detailed studies are needed on hopeful cultivar candidates determined as stable for production areas of the Basma type oriental tobacco.

Keywords: Adaptability, HPLC, Nicotine, Quality, Sugars, Yield

1. Introduction

Turkey is the largest (30%) known producer of high-quality aromatic oriental tobacco (*Nicotiana tabacum* L.). It produces 93.665 tons of leaf tobacco in 99.528 ha areas with 64.541 farmers in 25 provinces, 98 districts and 1.942 villages in Turkey, and its production that a total value of approximately 250 million dollars is made 66% in the Aegean, 15% in Southeast Anatolia and 10% in Black Sea region, the remaining 9% are made in Marmara, Eastern Anatolia and Mediterranean regions according to Republic of Turkey Ministry of Agriculture and Forestry Statistics (Yilmaz et al. 2020). The oriental tobacco has sufficient sugar content with less carcinogen and nicotine, as well as a more favorable aroma than the other tobacco types. Therefore, oriental tobacco is often blended with Virginia and Burley tobaccos, which have a stronger effect, and used in American blend type cigarettes (Darvishzadeh et al. 2013). In this respect, tobacco must be regularly analyzed and screened for proper use. Alkaloids are important components of tobacco and have a marked influence on tobacco quality and consumption (Andersen et al. 1991). nicotine is the most abundant alkaloid among more than the (20) alkaloids in tobacco and causes extensive consumption of tobacco products worldwide (Xia et al. 2014). Sugars are the primary metabolites that contribute to the growth of tobacco plants (Cai et al. 2015). Sugar composition is directly related to the taste and aroma of the tobacco (Nagai et al. 2012). The amount of sugar in tobacco types is quite variable and primarily depends on the curing process. Glucose and fructose, called reducing sugars, are the most important of soluble sugars (Leffingwell 2001; Roemer et al. 2012). Therefore, the chemical structure should be reliably determined in defining leaf tobacco for blenders and determining potential toxicity in terms of health (Cai et al. 2015).

Adaptation strength and stability of the varieties at different districts are very important for breeders to ensure optimum yield. The stability, as well as agronomic, morphological, pathological and technological traits (Zencirci et al. 1990), and the initiative of the breeder (Keser et al. 1999) should be considered in line selection. The characteristics of tobacco genotypes as in all plants are the consequence of the common effect of the genotype (G), environment (E) and GE interaction (Ekren & Sekin 2008).

Genotypes tested in different environments show variations in yield and other characteristics due to environmental factors such as soil, climate and presence of disease pathogens. These fluctuations are often attributed to the effect of GE interactions, which is widely used in many plants. Many researchers use the terms of "stability" and "compatibility" to consistently address the high genotype efficiency in different environments (Romagosa & Fox 1993). Lin & Binns (1994) have identified two types of stable genotypes, with stable performance in all environments and high performance in specific environments (with specific adaptation).

The GE interactions of genotypes can be determined by classical statistical analyzes, which do not provide information about the stability of genotypes (Kilic et al. 2003). Therefore, various statistical methods have been proposed to assess the stability of genotypes in changing environments. Dehghani et al. (2006) emphasized that a single method cannot adequately explain the efficiency of genotypes in different environments. The most commonly used approach is based on a linear regression of genotype yield on an environmental index derived from the average performance of all genotypes in each environment (Finlay & Wilkinson 1963). This model provides two stability parameters. The first one is the mean linear regression coefficient (b_i) of the genotypes in the environmental index and the second one is a deviation from regression (S^2d) for each genotype. The richness of origin in the region is controlled by the tobacco producers due to the exchange of seeds and seedlings. Mixed cultivation of the different tobacco types restrains obtaining the product with the desired characteristics. Breeding studies are needed to develop high-yield and high-quality varieties to eliminate the problems arising from especially in terms of yield and quality. In this study, field experiments have been carried out using (21) the Basma type tobacco lines, which have some prominent characteristics, in four districts where the most common tobacco production takes place to determine the performance and the stabilities.

2. Material and Methods

2.1. Material

Cultivation areas of the Basma tobacco (*Nicotiana tabacum* L.) types in Turkey (especially in Tokat province) have been screened in 2015, morphologically different plants were identified and seeds were collected. The material of the study composed of (25) tobacco genotypes including (21) Basma type tobacco lines identified as the Basma type in Turkey by Kurt (2019) and (4) standard tobacco varieties/lines.

2.2. Field Experiments

Seedlings of the 25 genotypes were grown in a float system with peat medium within foam viols. Composite fertilizer containing 20.10.20 (N, P, K) + micronutrients (Iron 0.4%, Manganese 0.4% and Zinc 0.4%) was mixed with 500 g t^{-1} water in float pond water to supply nutrients for the seedlings. The experimental fields were chosen from different altitudes where the study material could be cultivated. Field studies were carried out in the Evciler (40°36'43.48" N, 36°36'5.25" E, 581 m) and Karayaka (40°44'16.45" N, 36°33'58.31" E, 302 m) villages of Tokat-Erbaa, in Samsun-Bafra (41°33'45.29" N, 35°52'18.35" E, 26 m) and Amasya-Gümüşhacıköy (40°53'1.03" N, 35°12'47.98" E, 848 m) districts in 2017. Before the planting of seedlings, 60 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O were applied to the experimental fields (Yilmaz & Kinay 2011). The field experiments were carried out in a randomized block design with three replications. The seedlings were planted with 45 cm inter-row and 12 cm intra-row spacings on 5 m long plots. The seedling planting was performed on May 21, 2017, in Evciler, May 19, 2017, in Karayaka, July 4, 2017, in Bafra and June 29, 2017, in Gümüşhacıköy.

Table 1- Soil analysis results

Properties	*Districts*			
	Evciler	*Karayaka*	*Gümüşhacıköy*	*Bafra*
P_2O_5 (kg da^{-1})	5.13 Low	6.18 Modarate	4.85 Low	3.45 Low
K_2O (kg da^{-1})	169.70 High	175.30 High	156.80 High	137.17 High
Lime (%)	10.2 Modarate calca.	2.39 Calcareous	5.17 Modarate calca.	12.73 Modarate calca.
Org. Mat. (%)	0.95 Very low	1.43 Low	2.36 Modarate	1.76 Low
pH	7.99 Slightly alkaline	7.81 Slightly alkaline	7.98 Slightly alkaline	7.61 Slightly alkaline
EC (dS m^{-1})	0.25 Very low	0.13 Very low	1.12 Very low	0.72 Very low
Texture	Clay loam	Sandy loam	Sandy loam	Sandy loam

The soil in Evciler experimental field had clay loam texture and the other three districts had sandy loam texture. Electrical conductivity indicated a very low salinity level in all experimental fields. The soils were slightly alkaline and the highest organic matter content was recorded in the Gümüşhacıköy. The experimental fields in Bafra and Karayaka fields had low and Evciler had very low organic matter content. The soils in Evciler, Gümüşhacıköy and Bafra districts were moderately calcareous while in the Karayaka was calcareous. The potassium contents of all experimental fields were high. Plant available phosphorus content of an experimental field in Karakaya was moderate while the other three fields had low in phosphorus content (Table 1). The temperature values during the seven months covering the seedling, field and curing periods of tobacco were similar to the long-

term averages, while the relative humidity values were higher than the long-term averages. The average relative humidity during this period was 69.86% in Erbaa, 64.43% in Gümüşhacıköy and 82.24% in Bafra. The total precipitation during the vegetation period was 222.2 mm in Erbaa, 256.5 mm in Gümüşhacıköy and 222.1 mm in Bafra. The precipitation was 36.1 mm lower in Erbaa, 47.1 mm lower in Gümüşhacıköy and 152.2 mm lower in Bafra compared to the long-term averages.

2.3. Investigated Parameters

A genotype is defined as stable when an S^2d value is close to zero, a b_i value is close to 1.0 and mean value (X_{mean}) is higher than the overall mean value (Eberhart & Russell 1966). A b_i value higher than 1.0 indicates the adaptation to a good environment, and a b_i value lower than 1.0 indicates the adaptation to a poor environment. The low S^2d values indicate that genotypes are not affected by the changing environmental conditions (Albayrak et al. 2005). The coefficient of variation (CV) for genotypes in different environments can be used as a stability parameter (Francis & Kannenberg 1978). Positive and high-value of regression constant (a), which represents the first point in the regression line (Finlay & Wilkinson 1963) indicates the high efficiency of a genotype in poor environmental conditions. Therefore, a stable genotype is expected to have a positive high constant value and a high coefficient of determination (r^2) (Eberhart & Russell 1966). The adaptation of genotypes was separated into nine regions using the general experimental mean, regression coefficient and confidence limits (Confidence interval = $X_{mean} \pm t\alpha.Sx$) in terms of the parameter considered (Uzun et al. 2012) (Figure 1). The significance of genotype x environment interactions and stability of genotypes in yield, quality grade index, nicotine and reducing sugar characteristics were evaluated by analysis of variance (ANOVA) using SAS 9.0 software.

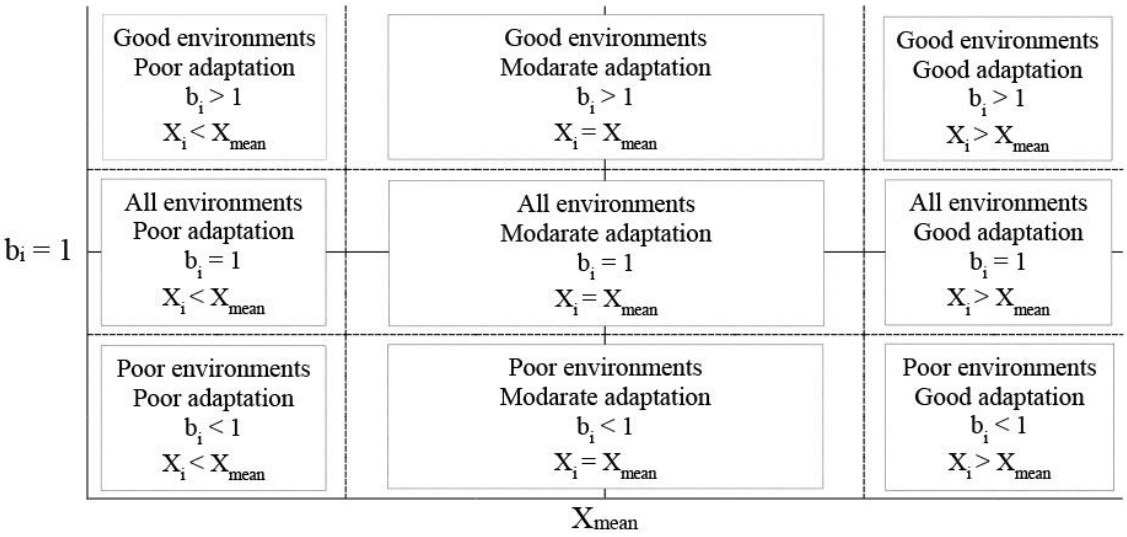

Figure 1- Mathematical and verbal expression of genotypic adaptation regions

Yield and quality grade index: All leaves harvested from the experimental plots in 3 different periods (Table 2) were dried by the sun-cured method, the moisture content was fixed to 17% and calculated as kg da^{-1}. The yield values were determined according to the American Grading method in dried leaf samples of each plot.

Table 2- Priming dates

Priming	***Districts***			
	Evciler	*Karayaka*	*Gümüşhacıköy*	*Bafra*
1. priming	03.07.2017	27.06.2017	03.08.2017	15.08.2017
2. priming	18.07.2017	11.07.2017	23.08.2017	04.09.2017
3. priming	12.08.2017	08.08.2017	23.09.2017	26.09.2017

Chemical analysis: Nicotine, glucose and fructose contents were determined by using HPLC and the sum of (glucose + fructose) was evaluated as reducing sugar. In the nicotine analysis (%), moisture-free tobacco samples were ground, 200 mg of powdered samples were put into 50 mL falcon tubes, 1% Acetic acid and Acetonitrile were added and left in the ultrasonic water bath for 30 minutes (Kinay 2018; Kurt 2019). Samples removed from the water bath were centrifuged at 4000 rpm for 10 minutes. The supernatant remaining above the precipitated sample was removed by an injection. The solution was passed through the filter (Nylon 0.45 µm) and placed into the vial with the sample code written. The extracts were analyzed by HPLC with a diode array detector at a flow rate of 1 mL min^{-1} and a column temperature of 35 °C with a C18 column (Moghbel et al. 2015). For glucose and fructose analysis (percentage), one gram of powdered sample was weighed into the falcon tubes and (1%) acetic acid and methanol were added. After the addition of solvents, they were mixed and placed in an ultrasonic water bath. The ultrasonic water bath was allowed to stand for 30 minutes and the samples removed from the water bath were centrifuged at 4000 rpm for

eight minutes. The supernatant above the precipitated sample was removed by an injection. The removed solution was passed through the filter (Nylon 0.45 µm) and placed into the vial with the sample code written. The solution was analyzed using an HPLC with a refractive index detector in the carbohydrate column with 1.5 mL flow and column temperature at 40 °C (Nagai et al. 2012). The peaks obtained from the sample chromatograms were identified by comparing the peaks obtained from the standards and peak areas were calculated according to their standard calibrations (r^2; 0.999 and 1.0). Extraction recovery ratios indicating the reliability of the analyzes were 101% in nicotine, 106% in glucose and 102% in fructose (Kinay 2018; Kurt 2019).

3. Results and Discussion

3.1. Yield

The results of variance analysis for the yield were given in Table 3, and the results of the stability parameters related to yield were presented in Table 4. The graph for adaptation classes determined using the regression coefficients and the mean yield values were shown in Figure 2. The mean yield of the experiment was 178.72 kg da^{-1}. High yield is a desired characteristic; thus, genotypes with higher yield values than average yield were considered to meet the first condition of the stability. The confidence interval for the regression coefficients of the genotypes was ±0.11, and the confidence interval for the mean yield was calculated as ±5.41, and the lower and upper limits were given in Figure 2. The genotypes with b_i values between 0.89 and 1.11 indicated a stable performance in all environments and those with b_i values lower or higher than these values pointed out the stable performance in certain environments. The ERB-16, ERB-18 and ERB-30 lines, which had a yield above the average and a b_i value equal or close to 1.0, were classified as stable in all environments. The ERB-16 line was stable with a positive regression constant (21.67) and a low coefficient of variation (9.17) value. However, the r^2 value of this line is expected to be higher than 0.88, and the S^2d value is expected to be lower than 309.12 to meet all the conditions of the stability. Similarly, the ERB-18 line had a regression constant (-4.04) closest to the positive, despite the high r^2 (0.98), low CV (4.06) and low S^2d (57.87) values. In this group, the ERB-30 line was a stable genotype with positive regression constant (36.74), high r^2 (0.97), low CV (3.83) and low S^2d (56.08) values in addition to a bi value in high yield and confidence interval (Table 4; Figure 2).

Table 3- The results of the analysis of variance

Variation sources	*D.F.*	*Yield*	*Quality Grade Index*	*Nicotine*	*Reducing sugar*
Environment (E)	3	133967.78**	17598.68**	34.37**	25.17**
Genotype (G)	24	2063.72**	939.18**	0.32**	12.30**
G x E	72	908.22**	177.89**	0.30**	9.55**
Error	198	409.34	53.43	0.05	0.43
C.V. (%)		11.32	10.46	21.53	7.70

D.F.; The degree of freedom, C.V.; Coefficient of variation, **; Indicates the significance level at P<0.01

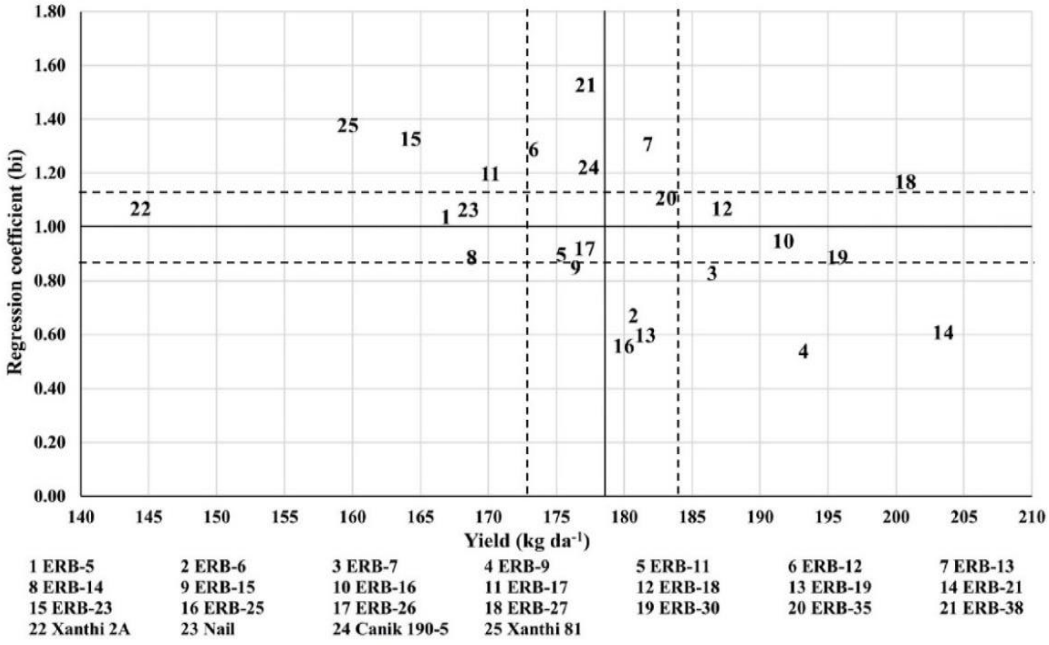

Figure 2- Stability conditions of different tobacco genotypes according to yield and the regression coefficient

Table 4- Values of the stability parameters for yield and quality grade index

No	*Genotypes*	*Yield (kg da^{-1})*						*Quality grade index (%)*					
		X_{mean}	b_i	*a*	r^2	*CV*	S^2d	X_{mean}	b_i	*a*	r^2	*CV*	S^2d
1	**ERB-5**	167.95	1.02	-13.84	0.96	6.36	114.02	76.34	0.73	24.64	0.67	12.58	92.17
2	**ERB-6**	180.82	0.66	63.24	0.73	11.42	426.61	72.34	1.05	-1.13	0.94	6.37	21.21
3	**ERB-7**	186.50	0.83	38.00	0.98	2.50	21.73	78.03	0.91	14.07	0.94	5.40	17.78
4	**ERB-9**	193.20	0.54	97.35	0.96	2.92	31.90	56.92	1.07	-18.12	0.75	20.34	134.07
5	**ERB-11**	175.80	0.90	14.63	0.99	1.35	5.65	57.58	0.92	-6.49	0.75	17.21	98.23
6	**ERB-12**	173.35	1.29	-58.12	0.99	3.76	42.60	71.55	1.11	-6.12	0.93	8.04	33.09
7	**ERB-13**	181.74	1.31	-53.28	0.92	11.03	401.99	78.65	0.88	16.77	0.88	7.78	37.48
8	**ERB-14**	168.79	0.89	8.54	0.95	5.75	94.13	67.44	0.95	1.29	0.79	13.60	84.18
9	**ERB-15**	176.48	0.87	20.93	0.70	16.46	843.52	77.76	1.16	-3.91	0.82	13.40	108.50
10	**ERB-16**	191.74	0.95	21.67	0.88	9.17	309.12	67.01	1.29	-23.68	0.90	12.39	68.94
11	**ERB-17**	170.19	1.20	-44.56	0.94	8.67	218.08	60.20	1.05	-13.24	0.80	16.58	99.62
12	**ERB-18**	187.24	1.07	-4.04	0.98	4.06	57.87	55.90	1.14	-23.84	0.93	10.64	35.36
13	**ERB-19**	181.33	0.60	73.50	0.78	8.94	263.07	79.70	0.85	20.49	0.74	11.96	90.88
14	**ERB-21**	203.52	0.61	94.23	0.99	0.86	3.08	72.62	0.57	32.51	0.51	14.68	113.68
15	**ERB-23**	164.31	1.33	-73.81	0.93	10.79	314.50	73.34	0.78	18.81	0.67	13.90	103.97
16	**ERB-25**	179.99	0.56	79.89	0.73	9.64	301.44	59.78	0.86	-0.48	0.99	1.75	1.09
17	**ERB-26**	177.30	0.91	15.25	0.94	6.68	140.25	69.97	1.19	-13.29	0.79	16.32	130.36
18	**ERB-27**	200.72	1.17	-7.86	0.85	12.48	627.60	46.73	1.40	-51.47	0.98	7.03	10.79
19	**ERB-30**	195.72	0.89	36.74	0.97	3.83	56.08	75.56	1.17	-6.54	0.77	15.98	145.85
20	**ERB-35**	183.11	1.11	-15.68	0.87	12.13	492.99	75.39	0.65	30.20	0.55	14.47	118.92
21	**ERB-38**	177.96	1.53	-94.80	0.95	9.98	315.60	78.09	1.13	-0.80	0.92	8.07	39.67
22	**Xanthi 2A**	144.45	1.07	-47.61	0.99	2.37	11.73	77.07	1.20	-7.12	0.91	9.31	51.45
23	**Nail**	168.25	1.06	-20.38	0.92	9.17	237.94	71.58	0.92	7.54	0.80	11.89	72.51
24	**Canik 190-5**	177.87	1.23	-42.62	0.93	9.18	266.79	70.45	0.99	1.34	0.81	12.66	79.59
25	**Xanthi 81**	159.69	1.38	-87.46	0.91	13.96	497.15	77.67	0.98	8.84	0.97	4.15	10.39
	Mean	178.72						69.91					
	Confidence Interval	±5.41	±0.11					±3.65	±0.08				

The genotypes moderately adaptable to all environments were ERB-11, ERB-26 and ERB-35 lines; thus, these lines were considered as stable genotypes. The yields of ERB-11 and ERB-26 lines were lower than the average yield but remained within the confidence limits (175.80 and 177.30 kg da^{-1}). The ERB-11 and ERB-26 lines had positive regression constants (14.63 and 15.25), high coefficient of determination (0.99 and 0.94) and low coefficient of variation (1.35 and 6.68) values. The deviation from near-zero regression was recorded for the ERB-11 line (5.65). The ERB-35 line had high yield (183.11 kg da^{-1}) and a low coefficient of variation (12.13) value, while the regression constant was negative (-15.68), the coefficient of determination (0.87) was low and the deviation from the regression was high (492.99). The variability in yield can be mostly attributed to the differences in the environmental conditions of the experimental fields (Sadeghi et al. 2011). The ERB-11 and ERB-30 lines met all the conditions of the stability parameters used in the study in terms of yield values. The ERB-7, ERB-9, ERB-14, ERB-18 and ERB-21 lines also did not meet the requirements in terms of a single parameter (Table 4; Figure 2).

3.2. Quality Grade Index

The results for the variance analysis of the quality grade index were given in Table 3 and the results of the stability parameters related to yield were presented in Table 4. The graph of the adaptation classes determined using the regression coefficients and the mean yield values were shown in Figure 3. The mean quality grade index of the experiment was 69.91%. Since high-quality grade index is a desirable property, genotypes with performance higher than the overall mean value have been considered to provide the first condition of the stability. The Xanthi 81 genotype, which had a quality grade index value (77.67%) higher than the mean quality grade index value and a b_i value of 0.98, was the only genotype placed in a class suitable to all environments. The Xanthi 81 line had a positive regression constant (8.84), high r^2 value (0.97), low CV (4.15) and low S^2d (10.39) value; therefore, considered as a stable genotype. The ERB-6, ERB-14, Nail and Canik 190-5 genotypes were defined as stable genotypes since they are moderately suitable to all environments. Regression coefficients and mean yield values of these genotypes were within the confidence intervals. The ERB-6 line is remarkable with a close to zero but negative regression constant (-1.13) as well as a high coefficient of determination (0.94) and low CV (6.37) and S^2d (21.21) values. The ERB-14

line in this group stands out with a positive regression constant (1.29) and a low CV (13.60) despite the highest S^2d (84.18) and the lowest r^2 (0.79) values (Table 4; Figure 3).

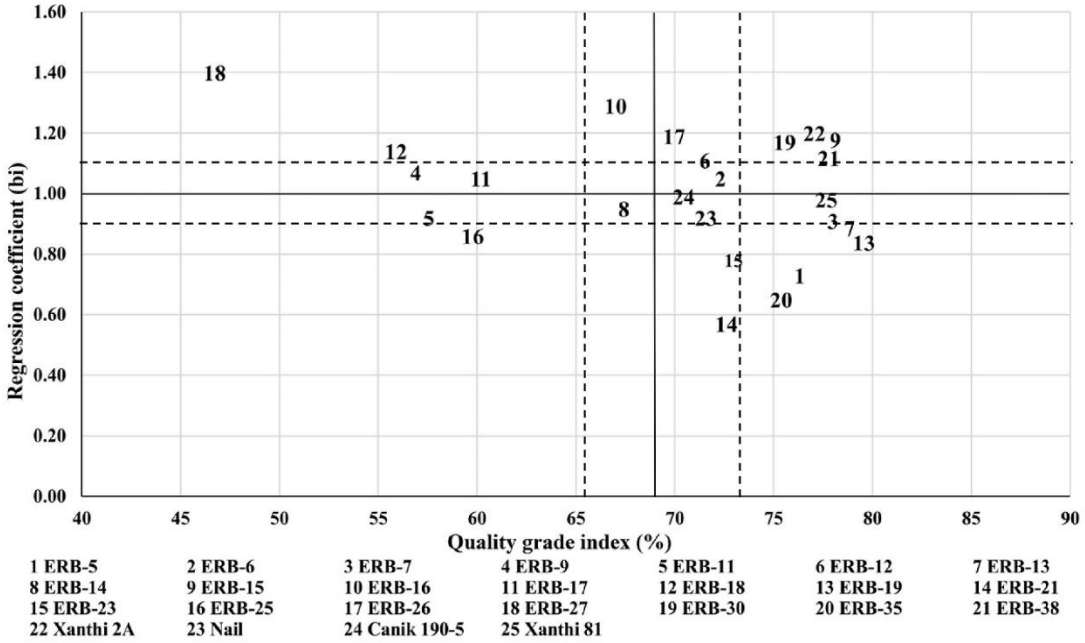

Figure 3- Stability conditions of different tobacco genotypes according to quality grade index and the regression coefficient

The quality grade index corresponds to the quantitative equivalent of quality; therefore, the value of a crop is estimated accordingly. The decline in quality is sometimes neglected under reduced production conditions; however, the quality will always be important in oriental tobacco production. Therefore, the adaptability and stability of the genotypes to different districts in terms of quality grade index are very important for tobacco breeders. In this study, Xanthi 81 was the only genotype that corresponded to all the conditions of the stability in terms of quality grade index values; thus, it was placed in the "good adaptation to all environments" class. The ERB-6 and ERB-7 lines did not meet the conditions in terms of a single parameter but attracted the attention (Table 4).

3.3. Nicotine

The results of the variance analysis for nicotine content were given in Table 3 and the related stability parameters were presented in Table 5. Adaptation classes determined using the regression coefficients and mean yield were shown in Figure 4. The low nicotine content is considered as a quality indicator for oriental tobacco. The nicotine content of Basma type tobacco demanded by the tobacco industry is between 2.00 and 2.75% (Yilmaz & Kinay 2011). The nicotine content of tobacco can be increased by cultural measures such as nitrogenous fertilization (Kinay 2010), wide planting distances (Bilalis et al. 2015) and topping (Camas et al. 2009) to meet the market demand. Therefore, the genotypes having nicotine performance over the mean value (>1.09%) have been accepted to meet the first condition of the stability (Table 5). The ERB-15 line with a nicotine content (1.19%) over the mean value and a b_i value of 1.12 was placed in the "good adaptation for all environments" class. The ERB-15 line can be considered as a stable genotype with a high r^2 (0.99), low CV (7.31) and low S^2d (0.007) values when negative regression constant (-0.037) is ignored.

The ERB-7, ERB-18 and ERB-19 lines were moderately adaptable to all environments, therefore, accepted as stable genotypes. Regression coefficients and mean nicotine contents of these genotypes were within the confidence intervals. The ERB-19 genotype in this class had the highest coefficient of determination, and the lowest CV (7.81%) and deviation from regression (S^2d; 0.007) values in this class, despite its negative regression constant (a; -0.049). The ERB-18 line was the only genotype that met all the conditions of stability parameters calculated with the nicotine values of 25 genotypes investigated. The ERB-15 and ERB-19 lines met all conditions except one parameter (Table 5; Figure 4).

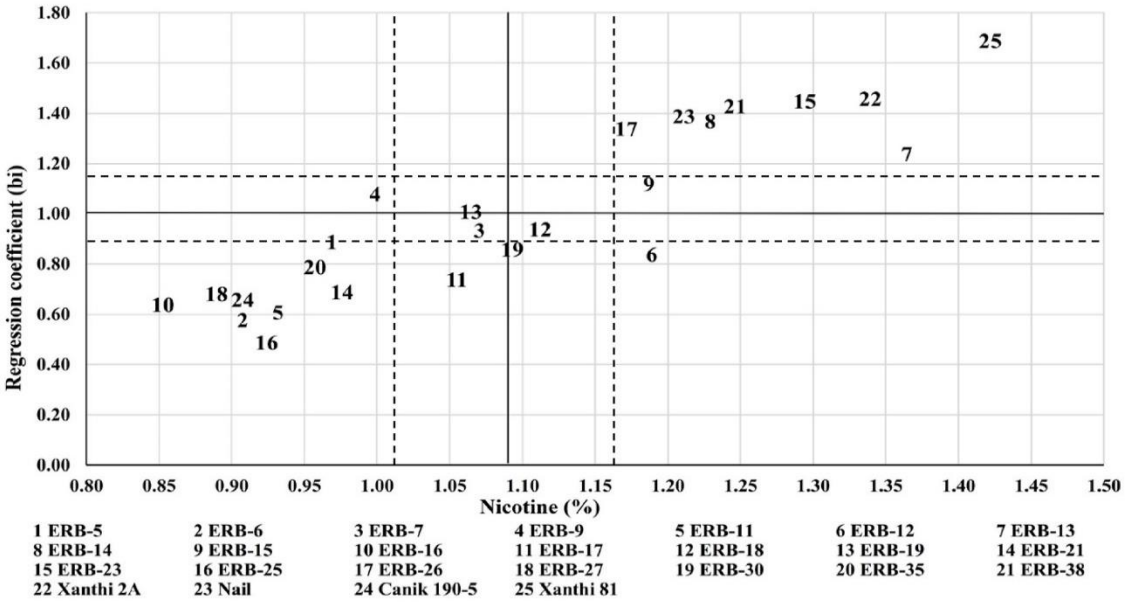

Figure 4- Stability conditions of different tobacco genotypes according to nicotine and the regression coefficient

Table 5- Values of the stability parameters for Nicotine and reducing sugar

No	*Genotypes*	*Nicotine (%)*						*Reducing sugar (%)*					
		X_{mean}	b_i	*a*	r^2	*CV*	S^2d	X_{mean}	b_i	*a*	r^2	*CV*	S^2d
1	**ERB-5**	0.97	0.89	-0.004	0.93	20.54	0.040	7.15	1.11	-2.37	0.81	13.78	0.97
2	**ERB-6**	0.91	0.60	0.256	0.84	23.91	0.047	9.27	0.65	3.67	0.75	7.45	0.48
3	**ERB-7**	1.07	0.97	0.005	0.91	23.87	0.065	9.16	0.85	1.87	0.96	2.65	0.06
4	**ERB-9**	1.00	1.08	-0.186	0.99	4.47	0.002	8.36	0.42	4.80	0.95	2.05	0.03
5	**ERB-11**	0.93	0.61	0.265	0.96	10.87	0.010	7.93	0.76	0.41	0.98	0.82	0.05
6	**ERB-12**	1.19	0.84	0.270	0.90	19.73	0.055	7.85	1.44	-4.49	0.87	12.81	1.01
7	**ERB-13**	1.36	1.24	-0.001	0.96	15.28	0.043	7.99	1.11	-1.51	0.94	6.35	0.26
8	**ERB-14**	1.23	1.37	-0.271	0.96	18.22	0.050	8.26	1.23	-2.32	0.96	5.64	0.21
9	**ERB-15**	1.19	1.12	-0.037	0.99	7.31	0.007	8.78	0.65	3.18	0.84	5.90	0.27
10	**ERB-16**	0.85	0.64	0.158	0.94	15.70	0.018	6.60	1.43	-5.70	0.93	11.00	0.53
11	**ERB-17**	1.06	0.74	0.246	0.90	19.51	0.042	8.99	0.81	2.05	0.99	2.02	0.03
12	**ERB-18**	1.11	0.94	0.078	0.95	17.03	0.036	7.94	0.93	-0.04	0.93	5.87	0.22
13	**ERB-19**	1.06	1.01	-0.049	0.99	7.81	0.007	8.92	0.57	4.01	0.97	2.05	0.03
14	**ERB-21**	0.98	0.69	0.219	0.90	19.98	0.038	10.12	1.85	-5.79	0.79	17.28	3.06
15	**ERB-23**	1.29	1.45	-0.297	0.99	3.92	0.002	8.16	0.75	1.69	0.87	6.56	0.29
16	**ERB-25**	0.92	0.49	0.391	0.85	18.11	0.028	8.15	0.69	2.18	0.88	5.75	0.22
17	**ERB-26**	1.17	1.34	-0.302	0.98	13.21	0.024	9.35	0.99	0.84	0.99	2.82	0.07
18	**ERB-27**	0.89	0.67	0.160	0.99	4.48	0.002	8.03	0.22	6.13	0.84	2.19	0.03
19	**ERB-30**	1.09	0.86	0.152	0.97	11.03	0.014	10.61	1.93	-5.96	0.95	7.24	0.59
20	**ERB-35**	0.96	0.79	0.087	0.90	7.03	0.004	7.39	0.72	1.17	0.68	12.15	0.81
21	**ERB-38**	1.25	1.43	-0.318	0.99	7.61	0.009	8.08	0.65	2.47	0.78	2.99	0.06
22	**Xanthi 2A**	1.34	1.46	-0.259	0.98	11.79	0.025	7.96	0.57	3.09	0.91	4.12	0.19
23	**Nail**	1.21	1.39	-0.322	0.99	9.87	0.014	10.57	2.35	-9.56	0.90	13.72	2.10
24	**Canik 190-5**	0.90	0.66	0.176	0.94	14.35	0.017	9.92	2.22	-9.13	0.88	14.68	2.12
25	**Xanthi 81**	1.42	1.69	-0.435	0.95	23.08	0.108	8.84	1.43	-3.39	0.83	13.06	1.33
	Mean	1.09						8.57					
	Confidence Interval	±0.07	±0.13					±0.42	±0.23				

3.4. Reducing Sugars

The results of the variance analysis for the reducing sugars were given in Table 3, and the stability parameters related to reducing sugars were presented in Table 5. The adaptation classes determined using regression coefficients and the mean reducing sugars values were shown in Figure 5. The reducing sugar ratio required by the tobacco industry for Basma type tobacco ranges between 8.00 and 13.00% (Yilmaz & Kinay 2011). Therefore, the genotypes which had reducing sugar performance higher than the mean value (>8.57%) considered meeting the first condition for stability. The ERB-7 and ERB-26 lines which had a reducing sugar value (8.99%) higher than the mean reducing sugar content and a b_i value above 1.23 were included in a "good adaptation for all environments" class. Although the regression coefficient of ERB-7 and ERB-26 lines was above the confidence intervals, they can be considered as stable genotypes due to the positive regression constants, high r^2 (0.96-0.99), low CV (2.65-2.82) and S^2d (0.06-0.07) values. The ERB-14 and ERB-17 lines, which were moderately adaptable to all environments, were stable genotypes only when evaluated in this aspect. The regression coefficients and mean reducing sugar values of these two lines were within the confidence intervals. The ERB-17 line in this group was the most stable genotype due to the positive regression constant, high coefficient of determination (r^2; 0.99), low CV (2.02%) and deviation from regression (S^2d; 0.03) values. The ERB-14 line is another genotype within the "moderately adaptable to all environments" class. Despite the negative regression constant (a; -2.32), the ERB-14 which had a high coefficient of determination (r^2; 0.96), low coefficient of variation (CV; 5.64) and low deviation from the regression (S^2d; 0.21) values, met all the conditions of the stability. The ERB-7, ERB-17 and ERB-26 genotypes met all conditions of stability parameters calculated by reducing sugar ratios of 25 genotypes. The ERB-14 line also met the conditions for all parameters except the negative regression constant (Table 5; Figure 5).

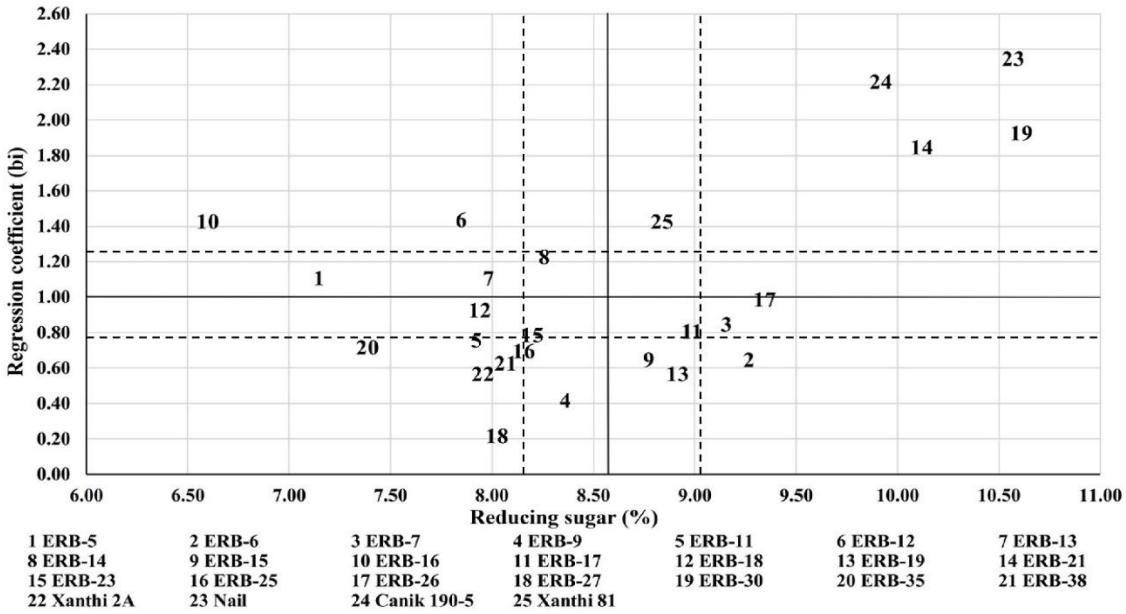

Figure 5- Stability conditions of different tobacco genotypes according to reducing sugar and the regression coefficient

4. Conclusions

The ERB-11 and ERB-30 lines met all the conditions of the stability parameters used in the study in terms of yield values. In addition, the ERB-7, ERB-9, ERB-14, ERB-16, ERB-18 and ERB-21 lines did not meet the requirements in terms of a single parameter. The ERB-6 and ERB-7 lines did not meet the stability conditions in terms of a single parameter but attracted the attention along with ERB-12, ERB-13 and ERB-38 lines in terms of quality grade index. The ERB-18 line was the only genotype that met all the conditions of stability parameters calculated by the nicotine values. The ERB-15 and ERB-19 lines met all conditions except one parameter. The ERB-7, ERB-17 and ERB-26 genotypes met all conditions of stability parameters calculated by reducing sugar. The stability results indicated that ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 lines are slightly affected by the environmental conditions. The results revealed that the development of (hopeful) tobacco candidates to meet the needs of producers and the sector could be continued with the aforementioned eight lines.

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Determination of the Seedling Reactions of Some Turkish Bread and Durum Wheat Cultivars to Stem Rust Races TTTTF, RTTTC and RTTTF

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ABSTRACT
Seedling resistance of 46 bread wheat and 14 durum wheat cultivars grown commonly in Turkey to stem rust races TTTTF, RTTTC, and RTTTF, the most common races in the Kastamonu region of Turkey, was determined under greenhouse conditions. Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 were found to be resistant to three stem rust races. Durum wheat cultivars Sarı Çanak 98 and Fırat 93 were resistant to stem rust race TTTTF. Durum wheat cultivars Eminbey, Altıntaş 95, Zühre and Sarı Çanak 98 were resistant to stem rust race RTTTC whereas durum wheat cultivars Eminbey, Altıntaş 95, İmren, Yelken 2000 and Zühre exhibited resistant reaction to stem rust race RTTTF. The majority of the wheat cultivars tested showed susceptible reactions to these stem rust races.

Keywords: Bread wheat, Durum wheat, *Triticum* spp., Stem rust, *Puccinia graminis* f. sp. *tritici*, Kastamonu, Turkey

1. Introduction

Wheat (*Triticum* spp.) is one of the most strategic crops in the world. It is the staple food in many countries because of its good food value, easy storage, handling, and processing (Geçit 2016). Wheat contributes greatly to the economy. It provides raw material to the agricultural industry and it is the main source of income for many rural areas. Wheat is commonly planted in Turkey (Taşçı et al. 2017). Rust diseases are among the most important biotic factors affecting the yield and quality of wheat plants. Stem rust disease caused by *Puccinia graminis* Pers. f. sp. *tritici* Erick & Henn. is one of the oldest plant diseases known and causes significant losses in many areas of the world (Agrios 2005; Bockus et al. 2010; Singh et al. 2011). Although the disease is more common in stems of wheat, other above-ground portions of the plant could also be affected. The pathogen can form races and new races may render wheat cultivars susceptible to the disease. The Ug99 race emerged in Africa caused big losses in wheat production (Singh et al. 2011). Identification of the races of the pathogen and determination of the response of wheat cultivars to these races will have a great value in wheat production. The use of resistant cultivars is one of the most important control methods of stem rust disease (Knott 1989, Roelfs et al. 1992).

In a study conducted in Turkey, 21 different stem rust races of wheat were determined. Race TKTTC was the most common stem rust race (Mert et al. 2012a). Using the races TKTTC, RTKTF, and RTTTC, the seedling response of 97 bread wheat and 41 durum wheat cultivars were determined (Mert 2010). Also, seedling reactions of 28 bread wheat genotypes and 10 durum wheat genotypes were determined using these 3 races (Mert et al. 2012b).

In a study conducted in Kastamonu, Turkey, stem rust races TTTTF, RTTTC and RTTTF were found as the most common races (Akci and Karakaya 2021a). Stem rust races RTTTC and RTTTF have been reported from Kastamonu, Turkey (Mert et al. 2012a), previously and stem rust races TTTTF and RTTTF have been reported from Sinop, Turkey (Akci & Karakaya 2019, 2021b). In this current study, under greenhouse conditions, the seedling response of 60 Turkish bread and durum wheat cultivars to these races was determined. The reactions of Turkish bread and durum wheat cultivars to stem rust races TTTTF and RTTTF were not determined previously.

2. Material and Methods

Under greenhouse conditions, seedling reactions of 46 bread wheat cultivars and 14 durum wheat cultivars grown in Turkey were determined against wheat stem rust races TTTTF, RTTTC and RTTTF. Bread and durum wheat seeds were planted into

150 mL pots containing soil: sand: animal manure (1:1:1). Each pot for inoculum enumeration and cultivar resistance studies included 8 plants. There were 6 plants in each pot for obtaining single pustule isolates. Plants were maintained in a controlled room at 15-20 ºC with 16 hours light/8 hours dark conditions. For single pustule isolation and inoculum enumeration susceptible cv Demir 2000 was used. Plants were treated at the emergence stage with maleic hydrazide (0,001 g maleic hydrazide dissolved in 30 ml water) for growth retardation and spore production was stimulated (Knott 1989). For inoculation, uredospores obtained from single pustules of the stem races TTTTF, RTTTF and RTTTC suspended in Soltrol 170® mineral oil were used (Akci and Karakaya 2021a). Inoculated plants were maintained in a humidity chamber with 95% relative humidity (RH) for 16 hours and then transferred to a greenhouse with a temperature regime of 20-25 ºC and 60-80% RH. Inoculation was accomplished when plants were at the Zadoks growth stage 11 (Zadoks et al. 1974).

First disease evaluations were carried away 10 -12 days after inoculation. Two to three days later, a second disease assessment was accomplished. For disease assessment, a 0-4 scale was used (Roelfs & Martens 1988). Infection types 0, ;, 1, and 2 were considered low infection responses, and infection types 3 and 4 were considered high infection responses.

3. Results and Discussion

Seedling reactions of 46 bread wheat and 14 durum wheat cultivars to stem rust races TTTTF, RTTTC, RTTTF were determined under greenhouse conditions. Both resistant and susceptible cultivars and cultivars showing different reactions to these races were present (Table 1, Figures 1 and 2).

Table 1- Assessment of the seedling reactions of bread wheat/durum wheat cultivars to stem rust races TTTTF, RTTTC, RTTTF. For disease assessment, a 0-4 scale was used (Roelfs & Martens 1988). Infection types 0, ;, 1, and 2 were considered low infection responses, and infection types 3 and 4 were considered high infection responses

No	*Bread wheat/Durum wheat*	*Cultivars*	*Developer*	*Stem rust race TTTTF*	*Stem rust race RTTTC*	*Stem rust race RTTTF*
1	Bread wheat	İKİZCE 96	TARM- ANK*	3+	3	3+
2	Bread wheat	AKSEL 2000	TARM- ANK	4	3+	3
3	Bread wheat	BAYRAKTAR 2000	TARM- ANK	4	3+	3+
4	Bread wheat	DEMİR 2000	TARM- ANK	3	3	3
5	Bread wheat	ATLI 2002	TARM- ANK	4	3	3+
6	Bread wheat	ZENCİRCİ 2002	TARM- ANK	3	3+	3+
7	Bread wheat	ESER	TARM- ANK	3+	3+	3+
8	Bread wheat	SEVAL	TARM- ANK	3	3	3
9	Bread wheat	TOSUNBEY	TARM- ANK	3	3+	3
10	Bread wheat	KENANBEY	TARM- ANK	3+	3+	3+
11	Bread wheat	LÜTFİBEY	TARM- ANK	3	3	3+
12	Bread wheat	ALTAY 2000	GKTAEM-ESK	4	3+	3+
13	Bread wheat	ÇETİNEL 2000	GKTAEM-ESK	3+	3	3
14	Bread wheat	ALPU 2001	GKTAEM-ESK	2+	1+	2
15	Bread wheat	İZGİ 2001	GKTAEM-ESK	3+	3+	3+
16	Bread wheat	SÖNMEZ 2001	GKTAEM-ESK	3+	3+	3+
17	Bread wheat	SOYER02	GKTAEM-ESK	3	3	3
18	Bread wheat	MÜFİTBEY	GKTAEM-ESK	4	3+	3
19	Bread wheat	NACİBEY	GKTAEM-ESK	3	3	3
20	Bread wheat	ES 26	GKTAEM-ESK	3	3+	3+
21	Bread wheat	YUNUS	GKTAEM-ESK	3	3+	3+
22	Bread wheat	MESUT	GKTAEM-ESK	4	3	3+
23	Bread wheat	BAĞCI 2002	BDUTAEM-KNYA	4	3	3
24	Bread wheat	KONYA 2002	BDUTAEM-KNYA	3	3	3+
25	Bread wheat	AHMETAĞA	BDUTAEM-KNYA	3	3+	3
26	Bread wheat	EKİZ	BDUTAEM-KNYA	3+	3+	3
27	Bread wheat	ERAYBEY	BDUTAEM-KNYA	3	3	3

Table 1 (Continued) - Assessment of the seedling reactions of bread wheat/durum wheat cultivars to stem rust races TTTTF, RTTTC, RTTTF

No	*Bread wheat/Durum wheat*	*Cultivars*	*Developer*	*Stem rust race TTTTF*	*Stem rust race RTTTC*	*Stem rust race RTTTF*
28	Bread wheat	KATE A-1	TTAEM-EDRN	4	3+	3+
29	Bread wheat	PEHLİVAN	TTAEM-EDRN	3	3+	3+
30	Bread wheat	SELİMİYE	TTAEM-EDRN	3+	3	3+
31	Bread wheat	BEREKET	TTAEM-EDRN	4	3+	3+
32	Bread wheat	CEMRE	GAPUTAEM-DYBKR	3+	3	3+
33	Bread wheat	BEZOSTAJA-1	MAEM-SKRY	4	3+	3+
34	Bread wheat	TAHİROVA 2000	MAEM-SKRY	2+	2	2
35	Bread wheat	BEŞKÖPRÜ	MAEM-SKRY	4	3	3
36	Bread wheat	HANLI	MAEM-SKRY	3+	3	3
37	Durum wheat	KIZILTAN 91	TARM- ANK	3+	3	3
38	Durum wheat	ÇEŞİT 1252	TARM- ANK	4	3+	3+
39	Durum wheat	MİRZABEY 2000	TARM- ANK	4	3	3+
40	Durum wheat	EMİNBEY	TARM- ANK	3+	2	2+
41	Durum wheat	İMREN	TARM- ANK	3+	3	2
42	Durum wheat	ALTINTAŞ 95	GKTAEM-ESK	3-	2	;
43	Durum wheat	KÜMBET 2000	GKTAEM-ESK	3+	3+	3+
44	Durum wheat	YELKEN 2000	GKTAEM-ESK	3+	No plants	2++
45	Durum wheat	DUMLUPINAR	GKTAEM-ESK	3+	3+	3+
46	Durum wheat	MERAM 2002	BDUTAEM-KNYA	3	3	3+
47	Durum wheat	SARI ÇANAK 98	GAPUTAEM-DYBKR	2	2	3-
48	Durum wheat	FIRAT 93	GAPUTAEM-DYBKR	2+	3-	3-
49	Durum wheat	ZÜHRE	GAPUTAEM-DYBKR	4	2+	2++
50	Durum wheat	GÖKGÖL 79	TTAEM-EDRN	4	3	3+
51	Bread wheat	DAPHAN	DATAE-ERZRM	4	3+	3+
52	Bread wheat	YILDIRIM	DATAE-ERZRM	1	2+	2
53	Bread wheat	AYYILDIZ	DATAE-ERZRM	3+	3	3+
54	Bread wheat	CANİK 2003	KTAEM-SMN	2++	2	1+
55	Bread wheat	ALTINDANE	KTAEM-SMN	3	3+	3+
56	Bread wheat	CUMHURİYET 75	ETAEM-İZM	3+	3+	3+
57	Bread wheat	BASRİ BEY 95	ETAEM-İZM	1+	1+	2+
58	Bread wheat	KAŞİF BEY 95	ETAEM-İZM	3	3	3-
59	Bread wheat	CEYHAN 99	DATAEM-ADN	3+	3+	3+
60	Bread wheat	PANDAS (PANDA)	DATAEM-ADN	3+	3+	3+

BDUTAEM/KNYA; Bahri Dağdaş International Agricultural Research Institute/Konya, DATAEM/ADN; Eastern Mediterranean Agricultural Research Institute./Adana, DATAE/ERZRM; Eastern Anatolia Agricultural Research Institute/Erzurum, ETAEM/İZM; Aegean Agricultural Research Institute/İzmir, GAPUTAEM/DYBKR; Southeast Anatolia Agricultural Research Institute/Diyarbakır, GKTAEM/ESK; Transitional Zone Agricultural Research Institute/Eskişehir, KTAEM/SMN; Black Sea Agricultural Research Institute /Samsun, MAEM/SKRY; Maize Research Institute/Sakarya
*TARM/ANK; Central Research Institute for Field Crops/Ankara, TTAEM/EDRN; Trakya Agricultural Research Institute/Edirne

Five bread wheat cultivars (Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95) and 2 durum wheat cultivars (Sarı Çanak 98 and Fırat 93) were found to be resistant to stem rust race TTTTF. Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 and durum wheat cultivars Eminbey, Altıntaş 95, Zühre and Sarı Çanak 98 showed resistant reactions to the stem rust race RTTTC. Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 and durum wheat cultivars Eminbey, Altıntaş 95, İmren, Yelken 2000 and Zühre exhibited resistant reactions to stem rust race RTTTF. Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri

Bey 95 were found to be resistant to the 3 stem rust races used in our study. However, majority of the cultivars were susceptible to these stem races. Durum wheat cultivars Altıntaş 95, Eminbey and Zühre showed resistant reactions to stem rust races RTTTC and RTTTF. Durum wheat cultivar Sarı Çanak 98 showed resistant reaction to stem rust races TTTTF and RTTTC.

Figure 1- Reactions of some wheat cultivars to stem rust race TTTTF

Figure 2- Reactions of some wheat cultivars to stem rust race RTTTF

Mert (2010), under greenhouse conditions, assessed the seedling stage reactions of 138 registered wheat cultivars in Turkey against wheat stem rust races TKTTC, RTKTF and RTTTC. Twelve bread wheat cultivars (Alpu 2001, Ahmetağa, Yıldırım, Karacabey 97, Tahirova 2000, Canik 2003, Köksal 2000, İzmir 85, Basri Bey 95, Menemen, Seri 82 and Carisma) and 9 durum wheat cultivars (Diyarbakır 81, Fırat 93, Artuklu, Eyyubi, Turabi, Amanos 97, Balcalı 2000, Zenit and Svevo) were found resistant to these 3 races. In our study, bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 were found resistant to stem rust races TTTTF, RTTTC and RTTTF. In Mert (2010) study, bread wheat cultivars Ahmetağa, Alibey, Alpu 2001, Altay 2000, Ata 81, Atay 85, Aydın 98, Bağcı 2002, Basribey 95, Canik 2003, Carisma, Cemre, Çukurova 86, Dariel, Eser, Genç 99, Göksu 99, Gönen 98, Gün 91, Hanlı, İzmir 85, Karacabey 97, Karacadağ 98, Kaşifbey 95, Kınacı 97, Kırkpınar 79, Köksal 2000, Menemen, Nacibey, Nurkent, Pamukova 97, Saroz 95, Seri 82, Seyhan 95, Sultan 95, Süzen 97, Tahirova 2000, Tosun 144, Tosunbey, Uzunyayla, Yakar 99, Yıldırım and Yıldız, durum wheat cultivars Amanos 97, Artuklu, Balcalı 2000, Ceylan 95, Diyarbakır 81, Eyyubi, Fırat 93, GAP, Gediz 75, İmren, Özberk, Pınar 2001, Sarı Çanak 98, Svevo, Turabi, Yelken 2000, Yılmaz 98 and Zenit exhibited resistant reactions to stem rust race TKTTC. In our current research stem rust race TKTTC was not used. In our current study, bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 were resistant to the 3 stem rust races. These cultivars also exhibited resistance to race TKTTC

(Mert 2010). In Mert (2010) study, bread wheat cultivars Ahmetağa, Alibey, Alpu 2001, Ankara 093/44, Ata 81, Aytın 98, Basri Bey 95, Bayraktar 2000, Beşköprü, Canik 2003, Carisma, Çukurova 86, Dariel, Genç 99, İkizce 96, İzmir 85, Karacabey 97, Kenanbey, Kırgız 95, Kırkpınar 79, Köksal 2000, Menemen, Nurkent, Özcan, Seri 82, Tahirova 2000, Uzunyayla, Yıldırım and Ziyabey 98, durum wheat cultivars Amanos 97, Ankara 98, Artuklu, Balcalı 2000, Ceyhan 95, Diyarbakır 81, Eyyubi, Fırat 93, Özberk, Pınar 2001, Salihli 92, Sham 1, Svevo, Şahinbey, Turabi, Tüten 2002 and Zenit showed resistant reactions to stem rust race RTKTF. In our current research stem rust race RTKTF was not used. In our current study, bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003, and Basri Bey 95 showed resistant reactions to the 3 stem rust races. These cultivars also exhibited resistance to race RTKTF (Mert 2010). In Mert (2010) study, bread wheat cultivars Ahmetağa, Alpu 2001, Ankara 095/44, Basribey 95, Canik 2003, Carisma, Esperia, İzmir 85, Karacabey 97, Karacadağ 98, Köksal 2000, Menemen, Seri 82, Tahirova 2000 and Yıldırım, durum wheat cultivars Amanos 97, Ankara 98, Artuklu, Balcalı 2000, Diyarbakır 81, Eyyubi, Fırat 93, GAP, İmren, Kümbet 2000, Pınar 2001, Salihli 92, Svevo, Şahinbey, Şölen 2002, Turabi, Tüten 2002, Yelken 2000, Yılmaz 98 and Zenit showed resistant reactions to race RTTTC. In this study, relatively low numbers of resistant bread wheat cultivars to race RTTTC were observed as compared to bread wheat cultivars resistant to races TKTTC and RTKTF. In our current study we found the bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 and durum wheat cultivars Eminbey, Altıntaş 95, Zühre and Sarı Çanak 98 resistant to the stem rust race RTTTC. All bread wheat cultivars identified as resistant in our current study were also resistant in Mert (2010) study. Durum wheat cultivars Eminbey, Altıntaş 95 and Zühre were not used in Mert (2010) study. These cultivars were identified as resistant to race RTTTC with our current study for the first time. Mert (2010) study listed cv. Sarı Çanak 98 as susceptible (scale value 3). This cultivar showed a resistant reaction in our study (scale value 2). On the other hand, cv Fırat 93 was reported as resistant (scale value 2+) in Mert (2010) study. This cultivar showed a susceptible reaction (scale value 3-) in our study. These cultivars showed reactions at the borderline between resistance/susceptibility. Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 were resistant stem races TKTTC, RTKTF, RTTTC, TTTTF and RTTTF ((Mert (2010) and this study)), These bread cultivars can be planted in areas where these races are common. Also durum wheat cultivar Sarı Çanak 98 was identified as resistant to races TKTTC and RTTTC ((Mert (2010) and this study)). This cultivar can be planted in the areas where these stem rust races occur. Mert et al. (2012b) assessed the reactions of 28 bread wheat genotypes and 10 durum wheat genotypes to stem rust races TKTTC, RTKTF, and RTTTC. One bread wheat genotype and 2 durum wheat genotypes were resistant to three stem rust races. Twenty-three genotypes exhibited susceptible reactions to three stem rust races. The other twelve genotypes exhibited resistant and/or susceptible reactions to these stem rust races.

Stem rust race TTTTF was common in Kastamonu and Sinop provinces of Turkey (Akci & Karakaya 2021a, 2021b). In 2008, stem rust races RTTTC and RTTTF were reported from Kastamonu-Ağlı and Kastamonu-Seydiler regions, respectively (Mert et al. 2012a). However, race TTTTF was not reported in their study. Recently, in addition to Turkey, this race has been reported from Italy (Patpour et al. 2018), Kenya (Wanyera et al. 2018) and Ethiopia (Abera et al. 2018). The reasons for the widespread appearance of this race in the Kastamonu and Sinop provinces of Turkey should be investigated.

4. Conclusions

Bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003, and Basri Bey 95 were resistant to stem rust races TTTTF, RTTTC and RTTTF. While durum wheat cultivars Sarı Çanak 98 and Fırat 93 were resistant to stem rust race TTTTF, durum wheat cultivars Eminbey, Altıntaş 95, Zühre and Sarı Çanak 98 were resistant to stem rust race RTTTC and durum wheat cultivars Eminbey, Altıntaş 95, İmren, Yelken 2000 and Zühre exhibited resistant reaction to stem rust race RTTTF. Most of the cultivars exhibited susceptible reactions to these 3 stem rust races, however, resistant bread and durum wheat cultivars were also found. We identified resistant bread and durum wheat cultivars to stem rust races TTTTF and RTTTF for the first time in Turkey. Also, new cultivars resistant to race RTTTC were found. Resistant cultivars can be planted in the areas where these stem rust races occur.

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Evaluation of Some Operational Parameters of a Vacuum Single-Seed Planter in Maize Sowing

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ABSTRACT
The objective of the study was to evaluate the performance of a vacuum single-seed planter in field conditions to optimize some operating parameters in maize production. Three forward speed of the tractor (4.0, 5.4 and 7.9 km h^{-1}) and five target seed spacing (102, 147, 195, 247 and 309 mm) were evaluated by examining the mean seed spacing, coefficient of precision in spacing, miss index, multiple index, quality of feed index, sowing depth, deviation from the row (inter-row spacing), mean emergence time, emergence rate index and percentage emergence. The point dropped in a furrow of seed, depth of seed placement, emergence rate and three indices of uniformity in seed spacing and precision coefficients of sowing quality were determined. The planter performed the best performance at the lowest forward speed and the highest target seed spacing. However, the deviation of seeds from the intended point in intra-row spacing was significantly affected only by the forward speed ($P<0.001$). Improvement in the larger seed spacing was due to the lower variation. Increasing the forward speed resulted in a shallower sowing depth. The desired planting depth was also obtained at 4.0 km h^{-1} in all plots. Increasing target seed spacing increased the emergence percent by about 35% while increasing forward speed decreased the emergence percent by 10%. Sowing at 4.0 km h^{-1} resulted in the lowest miss, multiple and precision indices (5.1% 2.9%, and 15.3%, respectively), and a quality of feed index as high as 92% was obtained in similar conditions. The results indicated that, with single-seed planters, success may be achieved in a conventional tillage maize production system at a target seed spacing of more than 102 mm and tractor forward speeds of less than 7.9 km h^{-1}, and thus satisfying farmers who carry out maize sowing by conventional tillage.

Keywords: Plant spacing, Tractor forward speed, Sowing depth, Deviation from a row, Sowing uniformity

1. Introduction

The aim of sowing is to dropped seeds at an asked seed spacing (intra-row spacing), inter-row spacing and target seed depth. This process is one of the most major missions that growers take upon oneself. Therefore, a planter should place seeds in an environment for reliable germination and emergence.

Intra-row spacing (plant spacing), inter-row spacing (deviation from row), sowing depth and percentage emergence are the most known features used by growers to measure planter performance. One of the reasons for reduced yield is irregular seed germination (Nafziger 1996). Therefore, uniform seed spacing, inter-row spacing, and sowing depth result in better sprouting and emergence and increase output by decreasing competition among plants for existing moisture, light and nutrients (Griepentrog 1998; Karayel & Özmerzi 2002).

Various factors such as seed metering unit, furrow opener, tractor forward speed, seed quality, and soil conditions affect seed distribution in soil. However, producers have a trend towards both higher plant distribution uniformity and faster planter forward speeds.

In the present, the yield has been increased by reducing the variation of plant spacing within rows. Kachman & Smith (1995) reported that the mean plant spacing and the standard deviation of plant spacing are beneficial for precision planters. However, they reported that these parameters do not entirely describe the distribution of plant spacing. In addition to the standard deviation of the row spacing and the mean, the uniformity indicators (The multiple index, the miss index, the quality of feed index and coefficient of precision) of the plant spacing should also be considered. This is because the space between seeds within a furrow is affected by several parameters including multiple seeds drop or single-seed drop failure, drop failure of a seed, failure in germination and displacement of seeds in the furrow. Nafziger (1996) reported that multiple seeds drop (Multiple index) may increase yields by 6%, however, losses in seed drop failure (miss index) may decrease yields by about 7%.

Nielsen (1995) reported that the loss of yield is at least 78 kg per hectare due to the increase in speed in the range of 1.8 to 3.1 m s^{-1} forward speed. Nielsen (1995) also determined that forward speed significantly affects the variation in plant spacing. Panning et al. (2000) evaluated the performance of a planter designed for row crops and two sugar beet planters in laboratory and field conditions. They reported that with increasing forward speed, the precision coefficient did not change for a planter and decreased for another. However, in field tests, they found the most uniform seed distribution for each planter at the lowest forward speed (3.2 km h^{-1}). As the forward speed increased from 3.2 to 8.0 km h^{-1}, plant distribution uniformity decreased for all planters. They reported that the field and laboratory test results were not alike and laboratory test results could not be used in practice.

It is a concern for maize growers that the plant emergence time is nonuniform. Nafziger (1996) reported that nonuniform plant emergence time reduces yield in maize. However, Nafziger (1996) suggested that the yield loss would not exceed 3% if plant emergences were completed in shorter than two weeks. Erbach (1982) developed the Emergence Rate Index (ERI) to evaluate maize and soybean plant stands. The ERI is an indication of how rapid and uniform germination of seeds from the soil. He reported that the ERI ranged from 4.9% to 12.7% for soybean and 4.9% to 11.0% for maize.

Singh et al. (2005) reported that seed distribution uniformity is affected when the seed metering unit fails to drop a seed or drops multiple seeds. Therefore, to achieve accurate seed spacing, on needs to optimize different parameters that affect seed placement such as tractor forward speed for appropriate planter performance and target seed spacing to regulate seed spacing. Problems due to seed plate operational speed, furrow openers and planter speed range from insufficient seed placement in the target seed spacing to the incorrect seed placement depth, which are problems that may contribute to reducing the emergence rate of maize, and finally, its yield. Additionally, some physical properties such as shape, size, and weight of the seeds, operation parameters such as the forward speed of the tractor and tillage system are among the important factors that affect planter performance and sowing quality (Ivancan et al. 2004; Staggenborg et al. 2004). The specific objective of this study was to evaluate the influence of different tractor forward speeds and target seed spacings on maize plant spacing variation, sowing depth, deviation from the row of plants, mean emergence time (MET), ERI, and percentage emergence (PE).

2. Materials and Methods

The field experiment was conducted on a research area in Erzurum province in Turkey in the growing season of 2014. The soil properties of the research area are given in Table 1. In the experiments used maize (Zea mays) seeds, with 326 g of thousand-grain weight, 74% of sphericity, 0.9 g cm^{-3} of bulk density and 7.5 mm of geometric mean diameter. A vacuum single-seed planter was used in the sowing operation, with air suction, four-rows and 70 cm each of row spacing. The planter was composed of shoe furrow openers and 30-holes (each of hole diameter 5.0 mm) metering plates (Figure 1). The seeds were sowed at 60 mm of sowing depth determined as the most suitable value for maize by Özmerzi et al. (2002). The ability to hold the vacuum plate of the seeds was driven by tractor PTO. The negative air pressure generated by the fan was used 8.8 kPa suggested by Önal (2011) for maize. The hole diameters of the seed metering plate were determined depending on the geometric mean diameter of maize seeds. The geometric mean diameter of the seeds was calculated by Equation (1). The geometric mean diameter was measured from 100 samples randomly selected from each kernel (Mohsenin 1986).

$$d = (lwt)^{1/3} \quad (1)$$

In this Equation, d is the geometric mean diameter, and l, w, and t are the length, width, and thickness, respectively.

Table 1- Physical soil properties for the 0 to 0.15 m depth range in the experiment area

Physical property	***Value***
Moisture content (% d.b.)	23.86
Bulk density (g cm^{-3})	0.93
Porosity (%)	64.90
Penetration resistance (MPa)	0.64
MWD* (%)	15.67
Roughness (%)	3.16
Sand (%)	38.7
Clay (%)	37.8
Silt (%)	23.5
Texture class	Clay loam

*MWD; Mean weight diameter of soil aggregates

The experimental setup was a complete factorial design (3x5) with three repetitions. For optimization of the operational factors affecting the performance of the vacuum planter, the experiment field was divided into 45 plots, including five target seed spacings (102, 147, 195, 247 and 309 mm), three tractor forward speeds (4.0, 5.4 and 7.9 km h^{-1}), and three replicates. The plots were 40 m in length and 3 m in width. All plots in the field were uniform in terms of physical soil properties. A space between the plots was allowed for turning the tractor into and out of the plot. The plots were treated by conventional tillage. The

soil was tilled by a moldboard plow and a disc harrow combined by a float, consecutively. The tillage depth was set at 250 mm considering previous studies carried out by Peterson et al. (1983), Raoufat & Mahmoodieh (2005), Stipesevic et al. (2009) and Topakci et al. (2011). The experiment area had been also processed in the previous year. The initial moisture content of the soil was measured at a soil depth of 20 cm by a TDR 300 device (Time Domain Reflectometry). The moisture content was about 28%.

Figure 1- Single-seed planter (a), Sowing (b) and plant emergences (c)

The practical spacing between plants within a row ranges between 100 and 300 mm for maize as a fraction of inter-row spacing (Heege & Billot 1999). Target seed spacings were determined based on different chain drive ratios of a planter, following the values used in practice. The forward speeds were determined using different gear stages of the tractor. The determination of the forward speed was carried out by measuring time at a distance of 30 meters in the experiment area.

To carry out measures of intra-row spacing (plant spacing), inter-row spacing (deviation from rows) and sowing depth, the sub−plots with lengths of 15 m were established in the four rows at the center of each treatment (Staggenborg et al. 2004). The sub-plots were determined considering the center of the 45 main plots. The measurements of intra-row spacing, deviation from rows and sowing depth were performed on three rows randomly selected from each plot. As a result of these measurements, sowing quality values were determined considering the spacing between plants, deviation from rows of plants, sowing depth, and the variation coefficient values of plant spacing and sowing depth. The seed spacings were analyzed using the performance indices of multiple index, miss index, quality of feed index, and precision which were described by Kachman & Smith (1995).

Intra-row seed distribution was characterized using the mean plant spacing and the coefficient of variation in plant spacing. The intra-row spacing was determined by measurement of 100 consecutive plant spacing in each of the sub-plots, previously also used by Staggenborg et al. (2004) (Figure 2a). The distances between consecutive plants were measured 16 days after sowing for each plot. Using these values of measurement, the mean ($\bar{x}$) spacing between plants, the standard deviation (s) of intra-row spacing of plants and the variation coefficient of intra-row spacing (CV) were computed by Equations 2, 3 and 4, respectively (Kachman & Smith, 1995). In computing the coefficient of variation, those which were double or more than the theoretical spacing between plants from the measured values were not taken into consideration (ISO 1984).

$$\bar{x} = \sum_{i=1}^{N} \frac{x_i}{N} \quad (2)$$

$$s = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}} \quad (3)$$

$$CV = \frac{s}{\bar{x}} \quad (4)$$

Where; x_i is the spacing between two consecutive plants, and N is the total plant spacings measured.

After sowing, the amounts of deviation from the row axis of plants were measured to determine the inter-row distribution uniformity of the plants. A rope was tied up between two iron bars on each row of the sub-plots to measure the amounts of deviation from the row of the plants. The mean deviation amount was determined by measuring the distance of the plants from the right and left to the rope (Figure 2c). The measurements were carried out for 75 plants randomly selected from the rows for each repetition. However, the plant depth below the soil surface was measured to determine the depth at which the seed dropped. After the plant's germination was completed, it was dug out from the soil. Sowing depth was carried out by measuring the mesocotyl length of the plant reported by Özmerzi & Keskin (1983). The mesocotyl lengths were measured for 75 maize plants in each repetition (Figure 2b). Depending on these measurements, the mean ($\bar{x}$) sowing depths of the plants were calculated. After then, the coefficient of variation of sowing depth was computed by the Equation (4).

Figure 2- Measurements of intra-row spacing (a), sowing depth (b) and deviation from the row (c)

The measurement methods described by Kachman & Smith (1995) were used to evaluate the performance of the single-seed unit. These were multiple index, miss index, quality of feed index and coefficient of precision (MULT, MISS, QFI and PREC, respectively). The multiple index is the spacing percent between consecutive plants in a row, equal to or fewer than half of the target seed spacing (Z) (*MULT ≤ 0.5Z*). The miss index is the spacing percent greater than 1.5 times the target seed spacing (*MISS > 1.5Z*). The quality of feed index is the plant spacing percent that is greater than 0.5 times but not greater than 1.5 times the target seed spacing *(0.5Z<QFI≤1.5Z*). In the sowing process, the lower the miss and multiple indices are, the better is the sowing quality. The precision coefficient index is the variation coefficient of seed or plant spacing in a row (Kachman & Smith 1995; Singh et al. 2005). The limit values of these indices are shown in Table 2 (Önal 2011; Yazgı & Degirmencioglu, 2014).

Table 2- Performance criteria to evaluate sowing quality in single-seed sowing

*QFI (%)	MISS (%)	MULT (%)	Performance of planter
>98.6	<0.7	<0.7	Very good
>90.4 – ≤98.6	≥0.7 – <4.8	≥0.7 – <4.8	Good
≥82.3 – ≤90.4	≥4.8 – ≤10.0	≥4.8 – ≤7.7	Moderate
<82.3	>10	>7.7	Insufficient

*QFI; quality of feed index, MISS; miss index, MULT; multiple index

The SPSS package program was used for the statistical analysis of the data. The data were evaluated by analysis of variance (ANOVA) to determine the effects of target seed spacings and tractor forward speeds on the sowing quality and performance of the single-seed planter. Additionally, Duncan multiple comparison (Post-Hoc) tests were used to determine the significant differences and similarities, according to the significance levels of 0.01 and 0.05, between the groups in the experiments.

3. Results and Discussion

From the data analyzed, it was found that the effect of seed spacing on MISS, MULT, QFI, and MSS / TSS ratio was statistically significant and the effect on DTP was also insignificant (Table 3). Each mean seed spacing (MSS) was higher than the target seed spacing (TSS) to which it belonged, whereas, MSS/TSS ratio decreased with increasing target seed spacing. Although the drift and rolling of the seeds in the furrow are generally close to each other in all TSSs, this result can be explained by the decrease of variation in intra-row seed spacing due to the increase of TSS. The MSS closest to the TSS was obtained at the 247 and 309-mm intra-row target seed spacings. However, as TSS decreased, the seed distribution deteriorated. This was because the MSS / TSS ratios became distant from 1.0. There was little MISS (≤8%) and MULT (≤5%) in any seed spacing except the 102 mm target intra-row seed spacing. The sowing performance at the 247 and 309-mm target seed spacings of the vacuum single-seed planter was better than other seed spacings. When the performance of the planter was compared with the limit values of the performance criteria given in Table 2; sowing quality was considered "insufficient" at 102 mm TSS, "moderate" at 147 and 195 mm and "good" at 247 and 309 mm.

PREC decreased as the intra-row plant spacing uniformity increased, with increased target seed spacing. The best PREC was obtained at the 247 and 309-mm spacings. The PREC values at all spacings were <29% (the highest acceptable limit for field trials reported by Kachman & Smith (1995) and considered acceptable precision for the vacuum single-seed planter. Bracy et al. (1999) reported that MISS, MULT, and QFI were affected by variation in seed spacing of a vacuum planter. Seeds at all target seed spacings except 147 mm fell away by about 42-45 mm from the intended point. However, there was no also statistical

difference between the deviation from the intended point (34 mm) at 147 mm TSS and other DTP values. Wanjura & Hudspeth (1969) reported that trajectories of falling seed varied and caused the targeted fall point to vary by 25 mm. Bracy et al. (1999) reported that, if the loss in uniformity is at the seed release point or is an effect of seed bounce, the absolute variability would be relatively constant over seed spacing, and precision would be greater at wider spacings.

Table 3- Sowing uniformity in different target seed spacings

TSS (mm)	*Measures*[+]						
	MSS (mm)	*DTP (mm)*	*MSS /TSS*	*MISS (%)*	*MULT (%)*	*QFI (%)*	*PREC (%)*
102	122	45	1.20 a	18.0 a[x]	6.8 a	75.3 c	23.1
147	154	34	1.05 b	7.6 b	4.1 b	88.3 b	19.0
195	200	42	1.03 b	7.0 b	3.5 b	89.5 b	16.4
247	252	42	1.02 b	4.9 b	2.2 c	93.0 ab	13.2
309	315	45	1.02 b	4.4 b	1.3 c	94.3 a	12.5
Significance		NS	0.000	0.000**	0.000	0.000	
Significance$_{(FS \times TSS)}$ [z]		NS	NS	0.043*	0.023	0.015	

[+] TSS; target seed spacing, MSS; mean seed spacing, MSS/TSS; the ratio of mean seed spacing to target spacing, DTP; mean deviation from the intended point at intra-row seed spacing, MISS; miss index, MULT; multiple index, QFI; quality of feed index, PREC; precision (variation of the spacings within target range), [x] ; In each group, the differences between the means followed by the same letter are insignificant at the 95% probability level, NS ,* ,**; Nonsignificant at P≤0.05, significant at P≤0.05 and 0.001, respectively, [z] FS x TSS; interaction of tractor forward speed and target seed spacing

All measures of sowing uniformity and precision of the vacuum single-seed planter were affected by the tractor forward speed (Table 4). The DTP increased as the tractor forward speed increased. MISS ranged from 11.5% for the highest forward speed to 5.1% for the smallest forward speed. MULT seed drops were significantly lower at the 4.0 and 5.4 km h^{-1} forward speeds than at the 7.9 km h^{-1} forward speed. QFI was the greatest (>90%) at the 4.0 km h^{-1} forward speed. However, compared to the limit values in Table 2, the performance of the single-seed planter was sufficient for all forward speeds. PREC increased as the tractor forward speed increased. However, PREC decreased with increasing seed spacing at each forward speed (Figure 3). The loss of sowing uniformity of the single-seed planter was probably caused by a combination of target seed spacing and forward speed factors. The forward speed and TSS interaction found statistically significant and supported this result (Table 3 and 4). And also, since the transmission of motion was achieved with a sprocket, narrower seed spacing required a higher plate operational speed. Therefore, the high plate speed required to achieve the narrower seed spacings resulted in higher MISS and MULT. Wanjura & Hudspeth (1969) in determining the seed distribution efficiency of the vacuum wheels, the most different results found at lower vacuum pressures and higher wheel speeds.

Table 4- Sowing uniformity at different tractor forward speeds

Tractor forward speed (km h^{-1})	*Measures*[+]					
	DTP (mm)	*MSS /TSS*	*MISS (%)*	*MULT (%)*	*QFI (%)*	*PREC (%)*
4.0	33 c	1.01 b	5.1 c[x]	2.9 b	92 a	15.3
5.4	42 b	1.07 ab	8.5 b	3.3 b	88 b	16.7
7.9	50 a	1.10 a	11.5 a	4.5 a	84 c	18.5
Significance	0.000	0.019*	0.000***	0.002**	0.000	
Significance$_{(FS \times TSS)}$[z]	NS	NS	0.043	0.023	0.015	

[+] DTP; mean deviation from the intended point at intra-row seed spacing, MISS; miss index, MULT; multiple index, QFI; quality of feed index, PREC; precision (variation of the spacings within target range), [x] ; In each group, the differences between the means followed by the same letter are insignificant at the 95% probability level, NS ,* ,**, ***; Nonsignificant at P≤0.05, significant at P≤0.05, 0.01 and 0.001, respectively, [z] FS x TSS; interaction of tractor forward speed and target seed spacing

The effect of target seed spacing on MET, ERI, and PE was statistically significant (Table 5). However, the MET value obtained from only the largest TSS was significantly different from the others, while it was significant the difference between any two values of ERI depending on the TSS. As an expected result, the ERI decreased as the target seed spacing increased. There were no significant differences between 147 to 195 mm, between 147 to 247 mm and between 247 to 309 mm, while the values of PE obtained from the 102 mm seed spacing were significantly different from the others. In general, PE increased as TSS increased. The increase rate was about 35%. The highest emergence percentage (94.5%) depending on target intra-row seed spacing in the study was obtained from the 309 mm seed spacing. PE values can be influenced by the variation of sowing depth and miss index. Kuş (2014) reported that with the increase of deviation from the row, the seeds remained at the furrow edge

without dropped to the targeted depth and disrupted the sowing depth. In this case, the seed may not be in contact with moisture and the germination rate may decrease. However, sowing depth values in the current study were not statistically different from each other. Therefore, the increase of PE values depending on the increase of TSS values was related to the decrease of the misses in these spacings. This means that the lower plate peripheral speeds required to achieve the greater seed spacings could have resulted in fewer misses in the rate of holding of seeds to the holes of the single-seed metering plate. However, the results obtained from this study on the misses were similar to those of the study conducted by Barut & Özmerzi (2004).

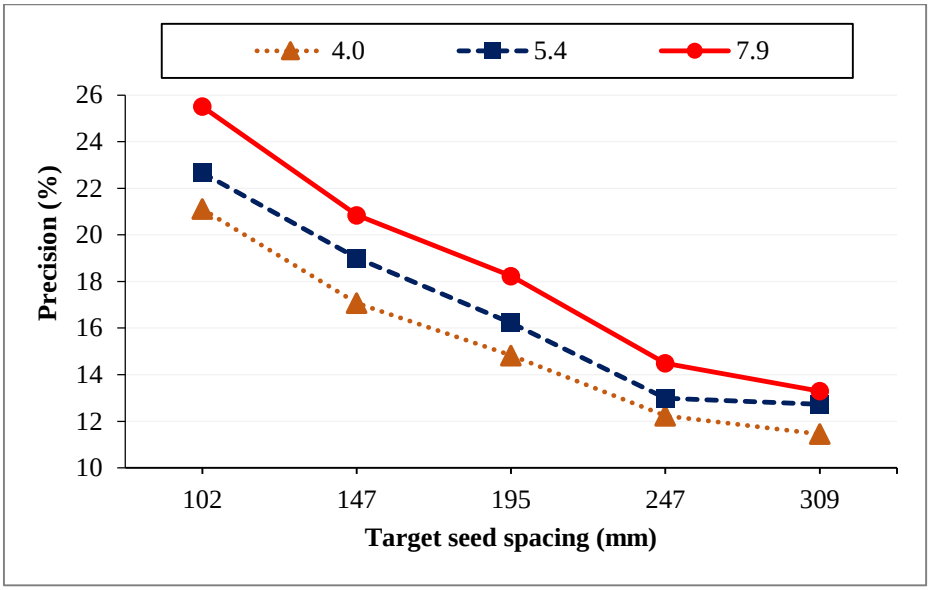

Figure 3- The precision of intra-row plant spacing depending on forward speed

Table 5- Results related to plant emergence depending on target seed spacings

TSS (mm)	*Measures*[+]					
	CV (%)	*Mean Sowing Depth (mm)*	*Mean deviation from row (mm)*	*MET (%)*	*ERI (%)*	*PE (%)*
102	12.0	52.1	9.1 a[x]	13.7 a	0.62 a	70.2 d
147	12.2	53.2	8.5 a	13.6 a	0.48 b	87.6 bc
195	12.2	52.3	7.6 ab	13.7 a	0.36 c	84.8 c
247	12.1	54.5	6.8 b	13.7 a	0.29 d	92.3 ab
309	12.6	53.2	6.7 b	13.4 b	0.23 e	94.5 a
Significance		NS	0.011*	0.000**	0.000	0.000
Significance(FS x TSS)[z]		NS	NS	0.000	NS	0.033

[+] TSS; target seed spacing, CV; coefficient of variation of sowing depth, MET; mean emergence time, ERI; emergence rate index, PE; percent emergence, [x]; In each group, the differences between the means followed by the same letter are insignificant at the 95% probability level, NS, *,**,***; Nonsignificant at P≤0.05, significant at P≤0.05 and 0.001, respectively, [z] FS x TSS; interaction of tractor forward speed and target seed spacing

The sowing depth, deviation from the row (inter-row spacing), MET, and PE were affected by increasing the tractor forward speed, while ERI was not affected (Table 6). With the increase in the forward speed, the mean sowing depth decreased and the variation coefficient of the sowing depth increased. However, mean sowing depth values obtained only at 4.0 and 7.9 km h^{-1} forward speeds were statistically different from each other. The deviation from the row of the plants also increased significantly as increased the forward speed. The deviation values were statistically different from each other. The mean deviation from the row of the plants at the smallest forward speed was 4.4 mm, while this value increased by 240% by doubling the forward speed. According to these results, the increase in the forward speed increased the displacement of seeds in the furrow. The displacement occurred as bouncing or dragging. This situation caused the seed to remain on the edge of furrow without falling to the target depth. It was concluded that the seed, which did not fall to the target depth, both increased the amount of deviation in plant germination and decreased the sowing depth.

The effect of the 7.9 km h^{-1} forward speed on MET and PE was significant in comparison to the 4.0 and 5.4 km h^{-1} forward speeds. However, there was no significant difference between 4.0 and 5.4 km h^{-1}. Increasing forward speed decreased the MET about 2.2% and PE values 10.0% also. It is thought that the decrease in PE with the increase in the forward speed was due to the increase of MISS and the decrease of ERI. It was assumed that the decrease in the MET with increasing of the forward speed was also caused by decreasing the mean sowing depth. Additionally, the effect of the forward speed on ERI was insignificant.

Table 6- Results related to plant emergence depending on tractor forward speeds

		Measures[+]				
Tractor forward speed (km h^{-1})	*CV (%)*	*Mean Sowing Depth (mm)*	*Mean deviation from the row (mm)*	*MET (%)*	*ERI (%)*	*PE (%)*
4.0	11.5	55.0 b	4.4 c[x]	3.7 a	0.40	90.0 a
5.4	12.3	53.0 ab	8.2 b	3.6 a	0.39	86.8 a
7.9	12.8	51.2 a	10.6 a	3.4 b	0.38	81.0 b
Significance		0.003**	0.000***	0.000	NS	0.001
Significance$_{(FS \times TSS)}$ [z]		NS	NS	0.000	NS	0.033*

[+]CV; coefficient of variation of sowing depth, MET; mean emergence time, ERI; emergence rate index, PE; percent emergence, [x] In each group, the differences between the means followed by the same letter are insignificant at the 95% probability level, NS ,*,**,***; Nonsignificant at P≤0.05, significant at P≤0.05, 0.01 and 0.001, respectively, [z] FS x TSS: interaction of tractor forward speed and target seed spacing.

4. Conclusions

The seed distribution uniformity of the vacuum single-seed planter was affected by both the seed spacing and the forward speed of the tractor. The uniformity (expressed as a percentage of target seed spacing) increased by increasing the seed spacing. Nonuniformity probably occurred due to the higher plate speeds and decreased seed spacing. However, it was assumed to occur as a result of the combination of tractor forward speed and seed spacing. Because the effect of forward speed and seed spacing interaction on MISS, MULT, and QFI was found significant.

Improvement of seed distribution, in addition to dropping the seed to the intended point in the intra-row spacing, it is possible by moving the seed down in the furrow and dropping to the target point. Bouncing and rolling in the furrow of the seed disrupts the seed spacing uniformity, and failure to fall to the bottom of the furrow also disrupts the sowing depth uniformity. The performance of the planter was sufficient for all of the others except 102 mm TSS. However, although increasing the forward speed of the tractor reduces planter performance, the primary detriment of higher tractor forward speeds is the risk of increasing the amount of deviation from the rows of plants and decreasing the seed depth uniformity. These results were supported in the study by the variation coefficient and the data of deviation from rows. This shows that the target seed spacing and tractor forward speed must be correctly selected to achieve desired planter performance.

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Investigation of Tomato Ringspot Virus (ToRSV) by Real-Time TaqMan RT-PCR in Hakkari Province, Turkey

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ABSTRACT
Tomato ringspot virus (ToRSV) belongs to the Nepovirus genus in the family *Secoviridae*. It has a wide host range and is listed as a quarantine virus in Turkey. In this study, 80 leaf samples were collected from tomato, pepper, cucumber and grapevine cultivation sites located in three different parts of Hakkari province: Şemdinli, Çukurca and Center districts. Real-time TaqMan reverse transcription-polymerase chain reaction (RT-PCR) method was used for the detection of the virus. Amplification was carried out in reaction mix including QuantiNova Probe RT-PCR kit (Qiagen, Germany) using primers and TaqMan probe based on 3'-UTR (untranslated region) of virus, which amplified a 182 bp product of the genome. ToRSV was detected in 13 of the 80 samples and threshold cycle (CT) values ranged from 23.9 to 37.4. It was found that 16.25% of the samples collected from the districts of Hakkari province were found to be infected with ToRSV whereas no ToRSV was detected in the samples collected from the center of the city. The virus was detected on pepper and cucumber samples in Çukurca district, and it was also detected in tomato, pepper, cucumber and grapevine samples in Şemdinli district. To our knowledge, this study is the first report of molecular detection of ToRSV by real-time TaqMan RT-PCR in Turkey.

Keywords: ToRSV, Molecular detection, Tomato, Pepper, Cucumber, Grapevine, Turkey

1. Introduction

ToRSV (tomato ringspot virus, genus Nepovirus, subgroup C, family *Secoviridae*) is a bipartite single-stranded, positive sense RNA virus (Sanfaçon et al. 2006; 2009). ToRSV primarily infects perennial plants such as tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum*), grapevine (*Vitis vinifera*), blueberry (*Vaccinium corymbosum*), strawberry (*Fragaria vesca*), geranium (*Pelargonium domesticum*), raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus, Rubus sp*), walnut (*Juglans regia*) and ornamental plants and causing diseases that results in great economic losses. Experimental host diversity of ToRSV is also very high and about 35 families are susceptible to this virus (Samuitiene et al. 2003; OEPP/EPPO 2005; Fuchs et al. 2010; Sneideris et al. 2012; Tzanetakis & Martin 2013; Zindovic et al. 2014). The most typical symptom of ToRSV infection in plants is the presence of annular spots on the leaves. It has also other conspicuous symptoms in fruit trees and grapevines. In the grapevines, the virus manifests itself especially with necrotic pitting, spongy phloem tissue, fall of fruit, the rosette formation of leaves, ring spots on the leaves and general decrease in yield (OEPP/EPPO 2013). In infected plants, the effect of the virus can be seen as, pale yellow and pale green spots on the leaves that develop along the major side veins or the main vein of the leaves and causing systemic chlorotic or necrotic ring stains and deformation as well as inhibition of the fruit growth. In certain cases the virus does not show any visible symptoms, being usually characterized by a decrease in the yield. ToRSV is transmitted by natural ways, such as seeds, transplantation, pollen, vegetative organs and different species of *Xiphinema* (Bitterlin et al. 1987; Pinkerton et al. 2008).

The objective of this research is to determine the presence of ToRSV in tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*) and grapevine (*Vitis vinifera*) samples,collected from three different districts of Hakkari province, by real-time TaqMan RT-PCR method.

2. Material and Methods

2.1. Field surveys and sample collection

In early autumn of 2014 and summer of 2015, 80 leaf samples of tomato, pepper, cucumber and grapevine plants were collected from Çukurca, Şemdinli and Center districts of Hakkari province (Durankaya, Kırıkdağ, Üzümcü, Çimenli, Geçitli

villages in the Center; Narlı, Geçimli, Kayalı villages in Çukurca; Bağlar, Şapatan, Güzelkonak, Yukarıyokuş, Balova villages in Şemdinli district). The samples were collected from various plant species based on the presence of suspicious viral symptoms at the time of sampling, such as necrosis, chlorosis, mosaic, and ring stains and transported to the laboratory in cool conditions and stored at 4 °C until tested.

2.2. Preparation of primers and total nucleic acid extraction

A pair of primers and probe were synthesized to amplify the 182-bp region in 3'-UTR of ToRSV RNA1 genome and used at the real-time TaqMan RT-PCR method (Table 1). RNA extraction from leaf samples of tomato, pepper, cucumber and grapevine were conducted by using the RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) as specified in the manufacturer's protocol. Total nucleic acids were extracted from 80 samples and tested for the presence of ToRSV along with positive and negative controls.

Table 1- List of primers and probe used for detecting ToRSV

Primer/Probe		*Sequence*	*Target gene and position*	*Reference*
ToRSV-UTR (Forward primer)	F	5'-GAATGGTTCCCAGCCACTT-3'	3' –UTR 7686-7704 bp of RNA1	Tang et al. 2014
ToRSV-UTR (Reverse primer)	R	5'-AGTCTCAACTTAACATACCAC-3'	3' –UTR 7847-7867 bp of RNA1	
ToRSV-UTR (Probe)	P	FAM-5'-AGGATCGC-TACTCCTCCGTCAAC-3'-BHQ-1	prob –7746–7768 bp	

2.3. The real-time TaqMan RT-PCR

The 3'-UTR sequence of RNA1 genome of ToRSV was amplified by real-time TaqMan RT-PCR method. Positive control was used to obtain high accuracy and optimization in real-time TaqMan RT-PCR. The plant sample (the original host: *Pelargonium* sp) obtained from Leibniz-Institut DSMZ German Collection of Microorganisms and Cell Cultures (Germany) was used as positive control. Nuclease-free water was used as negative control. The total RT-PCR reaction mix was prepared by using QuantiFast Probe PCR (Qiagen, Germany) and it consisted of 1 µl forward primer (0.3 µM), 1 µl reverse primer (0.3 µM), 1 µl prob (0.5 µM), 10 µl 2xProbe RT-PCR Mix, 0.2 µl QuantiFast RT Mix, 4.4 µl $MgCI_2$ (5.5 µM) and 1.4 µl RNase-free water. 5 µl of RNA isolated from leaf samples was added to the mix, amounting a total of 20 µl. For amplification, complementary DNA (cDNA) was synthesized at 50 °C for 10 min, initial denaturation was conducted at 95 °C for 5 min and amplification step were performed in a total of 40 cycles at 95 °C for 15 min, and at 60 °C for 45 sec. Real time RT-PCR analyses were performed using Rotor-Gene Q (Qiagen, Germany) and Rotor Gene Q Series Software (version 2.3.1).

3. Results and Discussion

3.1. Field observation

Field surveys were conducted in Çukurca, Şemdinli and Center districts of Hakkari province during the 2014-2015 growing season. It was observed during the surveys that vegetable farming is generally done without the use of pesticide in these areas and so that plants are susceptible to viral and other infectious agents such as bacteria, fungi etc. The samples were collected in accordance with the common symptoms that are known to be caused by ToRSV on tomato, pepper, cucumber and grapevine (Figure 1). The plants showing no apparent known symptoms were also sampled for control. Eighty samples were collected from 13 villages in study area (Table 2).

Figure 1- Symptomatic plants collected in the field survey from Hakkari province: a) Tomato b) Grapevine c) Pepper d) Cucumber

Table 2- List of plant samples collected from Hakkari province for real-time TaqMan RT-PCR analyses

No	*Host*	*Location*	*No*	*Host*	*Location*
1	Tomato	Çukurca-Narlı	41	Cucumber	Şemdinli-Balova
2	Tomato	Çukurca-Narlı	42	Grapevine	Şemdinli-Bağlar
3	Tomato	Çukurca- Narlı	43	Grapevine	Şemdinli-Bağlar
4	Tomato	Çukurca-Narlı	44	Grapevine	Şemdinli-Bağlar
5	Tomato	Çukurca-Geçimli	45	Grapevine	Şemdinli-Şapatan
6	Tomato	Çukurca-Geçimli	46	Grapevine	Şemdinli-Şapatan
7	Tomato	Çukurca-Kayalı	47	Grapevine	Şemdinli-Şapatan
8	Tomato	Çukurca-Kayalı	48	Grapevine	Şemdinli-Yukarıyokuş
9	Pepper	Çukurca-Narlı	49	Grapevine	Şemdinli-Yukarıyokuş
10	Pepper	Çukurca-Narlı	50	Grapevine	Şemdinli-Yukarıyokuş
11	Pepper	Çukurca-Geçimli	51	Grapevine	Şemdinli-Yukarıyokuş
12	Pepper	Çukurca- Geçimli	52	Grapevine	Şemdinli-Yukarıyokuş
13	Pepper	Çukurca-Narlı	53	Grapevine	Şemdinli-Yukarıyokuş
14	Pepper	Çukurca-Narlı	54	Grapevine	Şemdinli-Şapatan
15	Pepper	Çukurca-Narlı	55	Grapevine	Şemdinli-Şapatan
16	Pepper	Çukurca-Narlı	56	Grapevine	Şemdinli-Şapatan
17	Cucumber	Çukurca-Narlı	57	Cucumber	Şemdinli-Güzelkonak
18	Cucumber	Çukurca-Narlı	58	Cucumber	Şemdinli-Güzelkonak
19	Cucumber	Çukurca-Kayalı	59	Cucumber	Şemdinli-Güzelkonak
20	Cucumber	Çukurca-Kayalı	60	Tomato	Center-Durankaya
21	Tomato	Şemdinli-Balova	61	Tomato	Center-Durankaya
22	Tomato	Şemdinli-Balova	62	Tomato	Center-Durankaya
23	Tomato	Şemdinli-Güzelkonak	63	Tomato	Center-Durankaya
24	Tomato	Şemdinli-Güzelkonak	64	Tomato	Center-Durankaya
25	Tomato	Şemdinli-Güzelkonak	65	Tomato	Center-Durankaya
26	Tomato	Şemdinli-Yukarıyokuş	66	Tomato	Center-Durankaya
27	Tomato	Şemdinli-Yukarıyokuş	67	Pepper	Center-Durankaya
28	Tomato	Şemdinli-Yukarıyokuş	68	Pepper	Center-Durankaya
29	Pepper	Şemdinli-Güzelkonak	69	Pepper	Center-Durankaya
30	Pepper	Şemdinli-Güzelkonak	70	Pepper	Center-Durankaya
31	Pepper	Şemdinli-Güzelkonak	71	Tomato	Center-Üzümcü
32	Pepper	Şemdinli-Balova	72	Tomato	Center-Üzümcü
33	Pepper	Şemdinli-Balova	73	Grapevine	Çukurca-Narlı
34	Pepper	Şemdinli-Balova	74	Cucumber	Center-Kırıkdağ
35	Pepper	Şemdinli-Balova	75	Cucumber	Center-Kırıkdağ
36	Pepper	Şemdinli-Balova	76	Pepper	Center-Kırıkdağ
37	Cucumber	Şemdinli-Balova	77	Pepper	Center-Kırıkdağ
38	Cucumber	Şemdinli-Balova	78	Cucumber	Center-Çimenli
39	Cucumber	Şemdinli-Balova	79	Grapevine	Center-Çimenli
40	Cucumber	Şemdinli-Balova	80	Cucumber	Center-Geçitli

3.2. Molecular detection

The CT value of the positive control was 15.6. After determining the appropriate program for real-time TaqMan RT-PCR with the positive control, the procedure was applied to the other samples. After the tests for optimization, a total of 80 samples were evaluated by real-time TaqMan RT-PCR. The real-time TaqMan RT-PCR tests conclusively proved the presence of ToRSV in the province. Real-time TaqMan RT-PCR analysis of 80 samples collected in the field surveys revealed that 13 (16.25%) samples were infected with ToRSV. According to the real-time TaqMan RT-PCR results, CT value of ToRSV infected samples ranged from 23.88 to 37.41 (Table 3). Samples with CT value greater than 38 were ignored.

Table 3- CT (cycle threshold) values obtained from real-time TaqMan RT-PCR analyses of different plant samples collected from Hakkari province

The collected field	*Host*	*No of infected ToRSV the sample*	*CT value*
Çukurca-Geçimli	Pepper	12	33.49
Çukurca-Kayalı	Cucumber	20	35.99
Şemdinli-Balova	Tomato	21	32.66
Şemdinli-Balova	Tomato	22	34.09
Şemdinli-Güzelkonak	Tomato	24	30.9
Şemdinli-Güzelkonak	Tomato	25	37.29
Şemdinli-Yukarıyokuş	Tomato	26	33.49
Şemdinli-Yukarıyokuş	Tomato	27	37.41
Şemdinli-Yukarıyokuş	Tomato	28	32.48
Şemdinli-Balova	Pepper	34	34.96
Şemdinli-Balova	Pepper	35	37.04
Şemdinli-Balova	Cucumber	40	33.21
Şemdinli-Şapatan	Grapevine	54	23.88

The data obtained showed that ToRSV incidence was highest in Şemdinli district (28.20%) and lowest in Çukurca district (10%). ToRSV was detected in the tested tomato, pepper, cucumber and grapevine samples. None of the samples collected from Center were found to be infected with the ToRSV (Table 4). The results showed that ToRSV can be found in various cultivation sites in Hakkari province, but the virus is not wide spread in Hakkari province.

Table 4- ToRSV infection rate in tomato, pepper, cucumber and grapevine samples collected from Hakkari province

		Collected Samples -ToRSV Infected Samples				*Avarage infection rate (%)*
Province	*District*	*Tomato*	*Pepper*	*Cucumber*	*Grapevine*	
	Center	9-0	6-0	4-0	2-0	0
Hakkari	Çukurca	8-0	8-1	4-1	0-0	10
	Şemdinli	8-7	11-2	5-1	15-1	28.2
Total		25-7	25-3	13-2	17-1	16.25

ToRSV is a virus with a very wide host range. The damage caused on plants by this virus has encouraged us to work on it. ToRSV spreading from North America to other parts of the world, is also reported from Netherlands, Chile, Australia, Iran (Samuitiene et al. 2003; Moini 2010; Sokhansanj et al. 2012; Rivera et al. 2016; Roberts et al. 2018).

Presence of ToRSV can be determined by biological indexing, serological and molecular methods. Mechanical inoculation to herbaceous plants is also applied and is known to be simple and reliable. On the other hand, biological indexing is a time-consuming method and it requires considerable experience, meaning that only a limited number of plants can be tested by use of this method. Enzyme Linked Immunosorbent Assay (ELISA) and Double-Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) can be used for serological diagnosis of ToRSV. Moini (2010) detected ToRSV by ELISA method in the leaf samples collected from apples in the north-east region of Iran. The genome of most plant viruses consists of RNA. Detection of the RNA sequence by PCR requires some changes. Prior to the application of PCR, RNA must have a reverse copy called cDNA. The RT-PCR is a very sensitive method and there may be inhibition problems in the samples. It should also be noted that the use of this method requires experienced researchers (OEPP/EPPO 2005). Detection of ToRSV by RT-PCR has been developed for multiple strains of ToRSV in both herbaceous and woody plants (Griesbach 1995). Msikita (2007) compared ELISA with RT-PCR methods for ToRSV detection and preferred RT-PCR with the identified appropriate primary sequence. Digiaro et al. (2007) studied the development of degenerate and specific primers for differential and simultaneous RT-PCR detection between subgroups A, B and C of grape infecting nepoviruses. They were designed

specifically for RNA-1 3'-UTR for grapevine and provided a source for studies on the determination of this factor in grapevine with obtained positive results. A real-time RT-PCR test has been developed for rapid and sensitive detection of ToRSV. Stewart et al. (2007) tested samples for ToRSV primarily by ELISA. Real-time RT-PCR detection of ToRSV was performed in host tissues and a comparison was made between real-time PCR and ELISA. It was concluded that the results obtained by real-time PCR were more sensitive than ELISA. It was also seen that the samples that did not show positive results by ELISA were positive when tested in much lower amounts by real-time RT-PCR. Osman et al. (2008) compared low-density sequences using real-time TaqMan PCR and RT-PCR in the detection of grapevine viruses and examined the reliability of the results for ToRSV. This was the first report on the use of low-density sequences in the detection of plant viruses. Tang et al. (2014) detected the presence of ToRSV on grapevine by targeting RNA-1 3'-UTR region by real-time Taqman RT-PCR. In terms of specificity, sensitivity and reliability in the detection of ToRSV, real-time TaqMan RT-PCR and other real-time RT-PCR methods were compared. The real-time TaqMan RT-PCR used in that study was designed for the highly conserved region of ToRSV 3'-UTR. The TaqMan real-time RT-PCR test showed that the method can be widely used in the overall detection of ToRSV over a wide range of hosts and it also served as a resource for the method used in our research.

4. Conclusions

The presence of ToRSV in Turkey has been reported in tomato, pepper, cucumber (Fidan 1995; Arlı-Sökmen & Şevik 2006), stone fruit (Azeri & Çiçek 1997), blackberry (Sertkaya 2010) and strawberry (Yeşilçöllü et al. 2011). The methods used in these studies were ELISA and RT-PCR. In the studies where the primary method was real-time RT-PCR, grapevine was preferred as a host for detection of ToRSV. In this study, we detected the presence of ToRSV in different hosts. Samples with CT values ≤38 were accepted infected with ToRSV. CT value of the positive control was found to be 15.6, but CT value of the other samples that were considered positive was higher. CT values increased as the density of the virus decreased in the samples. This may have stemmed from the evaluation of different hosts. In the survey, ToRSV was detected in tomato, pepper, cucumber and grapevine in Şemdinli district and in pepper and cucumber in Çukurca of Hakkari province. Of the 80 samples, 13 (16.25%) samples were found to be infected with ToRSV and it is good to note that areas infected with ToRSV are uncommon. ToRSV-infected plants are concentrated mostly in Şemdinli. It is noteworthy that the uncommon use of pesticides and the use of local seeds in the fields observed are widespread. ToRSV can be transmitted by mechanically, nematode vectors, seeds and pollen in some plants, therefore it will be appropriate to comply with the internal quarantine rules. Although there have been a number of attempts to identify the presence of ToRSV in Turkey, this study is the first report of molecular detection of ToRSV in different hosts by real-time TaqMan RT-PCR.

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Effect on Yield and Some Quality Characteristics of Seed Harvest at Different Stages of Maturity in *Nigella sativa* L.

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ABSTRACT

Nigella sativa L. is a significant medicinal and aromatic plant due to the usage of both its seed and fixed oil. The aim of the study is to investigate the effects on seed yield, various yield components, fixed and essential oil content, and chemical composition (2018 year) of black cumin when harvesting the seed at four different stages of maturity. These stages were 25% (SH_1), 50% (SH_2), 75% (SH_3) and 100% (SH_4) browning of capsules. Two-year field experiments (2017 and 2018) were set up according to a randomized complete block design with triplicate, using a black cumin population obtained from the Burdur province under Isparta ecological conditions.

Significant statistical differences were found among the harvest stages in regards to the seed yield, plant height and the thousand-seed weight, while no differences were found in the numbers of capsules and branches, essential oil and fixed oil contents in both experimental years. Seed yield and its components increased during the harvest stage up to SH_3, while essential oil and fixed oil contents decreased insignificantly from SH_1 to SH_4 in both years. According to the combined years; plant height, the number of capsules, the number of branches, the 1000 seed weight, seed yields, essential oil and fixed oil contents varied between 38.3-42.5 cm, 6.03-6.85 capsule plant^{-1}, 6.50-6.91 branches plant^{-1}, 2.30-2.57 g, 307.3-542.3 kg ha^{-1}, 0.087-0.101% and 31.14-32.69%, respectively.

The main components of black cumin essential oil were characterized by cymol (25.01-26.90%), thymoquinone (2.39-4.41%), carvacrol (10.12-10.41%), junipene (5.33-6.66%), **Δ**-3-carene (5.55-8.71%), β-pinene (2.98-3.65), trans-sabinene hydrate (8.02-11.93%) and α-thujene (7.82-9.42%) according to harvest stages in the 2018 season.

Considering the present results, SH_3 stage was advised because of its higher seed yield. The contents of essential oil composition of black cumin varied according to harvest stages.

Keywords: Harvest stages, Seed yield, Thymoquinone, Essential oil composition

1. Introduction

Black cumin is an annual herbaceous plant which is grown widely in Southwest Asia, Europe and North Africa, and the Afyon, Isparta, Burdur and Konya provinces in Turkey (Baytop 1984). The species of *Nigella sativa* L., *Nigella damascena* L. and *Nigella arvensis* L. are traded and cultivated in Turkey because they are widely used in folk medicine and as a culinary spice (Kar et al. 2007). Black cumin is used in the food industry as an ingredient in food products, herbal teas and bakery products, and it is also used in the pharmaceutical, cosmetic and dyeing sectors (Baytop 1984). Nigella seeds contain protein, alkaloid, saponin, fixed and essential oil (Işık et al. 2017), crude fiber, minerals and vitamins (Güler et al. 2006).

Generally, black cumin has been grown based on natural precipitation (without watering) in continental climatic conditions. A higher seed yield can also be obtained under irrigated conditions. However, farmers prefer higher yielding plants such as fruits, vegetables, corn, potato, sugar beet etc. in irrigated conditions. In order to compete with these crops, it is of great importance to investigate the factors affecting the yield and quality characteristics of black cumin, one of these being harvest time. An early harvest of black cumin may cause a loss of yield due to non-uniform ripening, and a late harvest may cause-the seed spilling as a result of capsule dehiscence. In addition to the amount of thymoquinone, which is one of the main active compounds of black cumin, essential oil can also be influenced by the maturity stages. There are a few studies in which a proper harvest stage was determined for high seed yield and quality in non-uniform ripening plants such as black cumin. Özel (2008) stated that the yield and quality of anise were affected by the harvest stage, and the highest seed yield, essential oil and anethole were obtained from the primary umbel completely mature stage. Similarly, Omidbaigi et al. (2003) determined that anise harvested in different levels of maturation caused changes in the yield and the essential oil composition of the crop. Kara (2017) reported that the highest grain yield of buckwheat occurred when 80% of the grains had turned brown. There are some research findings indicating that the chemical composition of black cumin is affected by both genetic and environmental factors (Arslan et al. 2012; Ertaş 2016).

The purpose of the present study was to investigate the effect of change on seed yield and yield components, fixed and essential oil contents and composition of black cumin when harvesting the seed at different stages of maturity.

2. Material and Methods

The experiment was carried out during the 2017 and 2018 years under the Isparta ecological conditions. From March to the end of August of 2017 and 2018, there was a total precipitation of 213.9 and 198.2 mm and an average temperature of 17.0 and 18.2 °C (Table 1).

Table 1- Temperature and precipitation data of the experiment area*

Climatic factors	*Years*	*Months*						*Total or Average*
		March	*April*	*May*	*June*	*July*	*August*	
Average Temperature (°C)	2017	7.3	10.6	14.9	20.1	25.1	24.0	17.0
	2018	9.2	14.8	16.8	20.3	24.3	24.1	18.2
	Long Years	9.3	10.8	15.6	20.1	23.4	25.8	17.5
Precipitation (mm)	2017	74.4	25.6	49.5	30.9	13.1	20.4	213.9
	2018	65.9	51.0	43.3	30.8	4.0	3.2	198.2
	Long Years	42.9	56.6	50.8	24.4	12.8	0.3	187.8

*; Data were taken from Isparta Meteorological Station

In the years 2017 and 2018, the experimental soil had a sandy-loamy, low organic matter (1.75% and 1.68%, respectively), slightly alkaline (pH 7.86 and 7.51, respectively) and limy (15.47% and 13.16% $CaCO_3$, respectively).

In this study, a local black cumin population, which is cultivated in the Burdur province, was used as genetic material. Harvest stages were arranged according to the randomized complete block design, with three replicates. Seeds were sown in 20 cm x 5 cm on row spacing. Each plot area was 5 m^2 (5.0 m x 1.0 m) and consisted of 5 rows. Seeds were sowed by hand at 1-2 cm depths using a dibbler in the first week of March in both experimental years. The total quantity of phosphorus with 46% P_2O_5 and half of ammonium sulphate with 21% N fertilizers were applied at the time of sowing at a rate of 60 kg ha^{-1} and 40 kg ha^{-1}, respectively, and the other half of ammonium sulphate was applied when the plant was at a height of 15-20 cm (Tunçtürk et al. 2012). The plants were non-irrigated at any growing periods, and all the cultural practices were applied in both years.

In order to determine the seed yield and quality properties of black cumin, the plants were harvested from 3 rows in the center of each plot at four different stages of seed maturity as follows; SH_1 (Seed harvest): browning of 25% of capsules, SH_2: browning of 50% of capsules, SH_3: browning of 75% of capsules and SH_4: browning of 100% of capsules. After the harvest, the capsules were dried for one week on wire racks and the seeds in the dry capsules were then manually blended. Seed yield (kg ha^{-1}), plant height (cm), the number of branches (branches $plant^{-1}$), the number of capsules (capsule $plant^{-1}$) and the 1000-seed weight (g) were determined as described by Telci (1995).

For each of the harvest stages, 100 g of powdered black cumin samples in 0.5 L of water were hydro-distilled using Clevenger apparatus for 3 hours according to the standard procedure described in the European Pharmacopoeia for determining the essential oil content (v/w, %). The fixed oil content (%) of back cumin seeds belonging to each harvest stage were determined using *NMR* (Nuclear Magnetic Resonance, Bruker mqone) apparatus. The measurement was conditioned for 30 minutes at 20 °C and 35 °C in NMR, and the results are presented as a percentage (%). The chemical composition of the essential oil was identified by GC-MS (Gas Chromatography-Mass Spectrometry, Shimadzu 2010 Plus GC).

All the data were evaluated with analysis of variance (ANOVA) using an SAS Statistics Package Program. Means were compared using the LSD (Least Significant Difference) test.

3. Results and Discussions

3.1. Seed yield and its components

Significant statistical differences were found among the harvest stages of black cumin in relation to seed yield, plant height and the thousand-seed weight. However, no differences were found in the number of capsules, the number of branches, or the essential oil and fixed oil contents in either year or combined years (Table 2). Significant statistical differences (except for essential and fixed oil content) were found between the mean of the years. The plant height, number of capsules, number of branches and seed yield were higher in the first year, while the thousand-seed weight was higher in the second year. Essential oil and fixed contents were not changed significantly according to the mean the years. Differences in the yield and some yield components between the years might be due to higher raining during the growing period in the second year (Table 1). Sufficient moisture in the soil promotes the nutrition uptake of the plants, so rainfall in the first year increased the seed yield. Similar results that were obtained in other studies have also reported that seed yield and other plant characteristics varied depending on the climatic conditions of the year (Sadeghi et al. 2009; Ghamarnia & Jalili, 2013; Kara et al. 2015).

According to the combined years, the highest plant height (43.1 cm), number of branches (6.91 branches plant^{-1}), number of capsules (6.85 capsule plant^{-1}), 1000 seed weight (2.57 g) and seed yield (542.3 kg ha^{-1}) were obtained from the SH_3 stage. The lowest values of these characteristics were determined from the SH_1 stage (Table 2). The plant height slightly increased by delaying the harvest stage, but there weren't significant statistical differences among SH_2, SH_3 and SH_4. Similarly, there weren't significant differences between all harvest stages in the number of branches and capsules per plant in both years. These results can be explained by the determinate growth of black cumin, due to the ceasing of growth after the flowering stage. The 1000 seed weight decreased at the last harvest stage (SH_4). This could be as a result of the respiration losses which occurred in the seed storage. The reason for this is that when the plant reaches harvest maturity, the photosynthesis decreases, but respiration continues (Bugbee & Salisbury 1988). The seed yield increased up to the SH_3 stage, and it decreased in following harvest stage (SH_4). This decrease may have occurred due to the decrease of the 1000 seed weight at the SH_4 (Table 2). Özgüven & Şekeroğlu (2007) and Sadeghi et al. (2009) informed that there was a positive relationship between seed yield and its such as the number of branches, number of capsules and the 1000 seed weight. In studies conducted on another plant by Özel (2008) and Kara (2017) they reported that seed yields of anise and buckwheat with non-uniform ripening increased up to a certain ripening stage, and then it decreased. In other studies that were conducted, black cumin seed yield varied between 367.8-527.3 kg ha^{-1} (Telci 1995), 166.7- 600.0 kg ha^{-1} (Arslan et al. 2012), 676.6-903.3 kg ha^{-1} (Kulan et al. 2012) and 325.9-416.3 kg ha^{-1} (Seyyedan et al. 2014). In comparison with the above studies, the differences in seed yield could be as a result of a variety of characteristics, maturity periods of genotypes, climatic factors and agricultural practices (Karim et al. 2017; Selicioğlu 2018; Sultana et al. 2018).

Table 2- Effect on yield, some yield characteristics, essential oil and fixed oil content of seed harvest at different stages of maturity in black cumin

Harvest stages	*Plant height (cm)*			*Number of branches (branches plant^{-1})*		
	2017	*2018*	*Combined years*	*2017*	*2018*	*Combined years*
SH_1	43.3 b	33.4 b	38.3 b	6.96	6.03	6.50
SH_2	48.1 a	33.5 b	40.8 ab	6.80	6.30	6.55
SH_3	48.4 a	34.8 a	41.6 a	7.26	6.56	6.91
SH_4	49.8 a	35.4 a	42.5 a	7.20	6.35	6.76
$LSD_{Harvet\ stages}$	4.63	1.20	2.65	-	-	-
F value	12.81**	9.11*	20.90**	0.52 ^{ns}	0.55 ^{ns}	0.88 ^{ns}
CV (%)	3.27	3.04	3.22	7.13	8.07	7.58
Years	47.4 A	34.2 B	LSD_{years}: 2.17**	7.05 A	6.30 B	LSD_{years}: 0.45*

Harvest stages	*Number of capsule (capsule plant^{-1})*			*1000 seed weight (g)*		
	2017	*2018*	*Combined years*	*2017*	*2018*	*Combined years*
SH_1	6.70	5.63	6.16	2.20 b	2.40 b	2.30 b
SH_2	6.80	6.46	6.63	2.30 ab	2.50 ab	2.40 b
SH_3	7.13	6.56	6.85	2.48 a	2.66 a	2.57 a
SH_4	6.70	5.36	6.03	2.43 ab	2.56 ab	2.45 ab
$LSD_{Harvet\ stages}$	-	-	0.55	0.261	0.252	0.158
F value	1.63 ^{ns}	3.50 ^{ns}	2.62 ^{ns}	6.56 *	5.56 *	7.87 *
CV (%)	4.07	7.21	6.82	3.81	3.32	3.56
Years	6.83 A	6.01 B	LSD_{years}: 0.39*	2.35 B	2.50 A	LSD_{years}:0.07*

Harvest stages	*Seed yields (kg ha^{-1})*			*Essential oil content (%)*		
	2017	*2018*	*Combined years*	*2017*	*2018*	*Combined years*
SH_1	311.7 c	303.2 c	307.3 d	0.099	0.103	0.101
SH_2	479.5 b	451.0 b	465.2 b	0.096	0.102	0.099
SH_3	564.9 a	519.8 a	542.3 a	0.088	0.096	0.092
SH_4	441.6 b	434.1 b	437.8 c	0.085	0.089	0.087
$LSD_{Harvet\ stages}$	39.50	23.8	13.5	-	-	-
F value	195.630**	397.45**	488.94**	3.29 ^{ns}	2.12 ^{ns}	6.41 ^{ns}
CV (%)	4.90	3.84	2.45	5.44	6.27	5.59
Years	449.4 A	426.7 B	LSD_{years}: 9.58*	0.092	0.097	LSD_{years}: ns

Harvest stages	*Fixed oil content (%)*		
	2017	*2018*	*Combined years*
SH_1	32.36	33.02	32.69
SH_2	31.38	33.71	32.54
SH_3	32.20	31.48	31.53
SH_4	30.81	30.87	31.14
$LSD_{Harvet\ stages}$	-	-	-
F value	2.67 ^{ns}	3.65 ^{ns}	2.32 ^{ns}
CV (%)	2.50	2.98	2.75
Years	31.68	32.27	LSD_{years}: ns

SH; Seed harvest, *, **; significant at P<0.05 and P<0.01 probability levels, respectively, ns; non significant

3.2. Essential oil and fixed oil content

Considering the combined years in the present study, the essential oil and fixed oil content varied between 0.087-0.101% and 31.14-32.69%, respectively. The essential oil and fixed oil content tended to decrease only slightly from SH_1 to SH_4 (Table 2). However, differences among in the harvest stages in both years weren't statistically significant.

El-Gamal & Ahmed (2017) reported that the essential oil content of fennel slowly decreased depending on delaying harvest time in the seed maturity period, while it was higher in early harvest stages. Telci et al. (2009) stated that the essential oil content of fennel was higher in the early stages of fruit development than the advanced stages. However, in our study, seeds were harvested at different stages of maturity, therefore, the rate of decrease in essential oil content was lower. In the plants harvested of seed, the synthesis of essential oil and fixed oil can be completed when it reaches the stage of maturity. In comparison to previous studies, the essential oil content of the present results were lower than the values reported: 0.5% (Bourgou et al. 2010), 0.5-1.6% (Ramadan 2007), 0.27-0.35% (Toncer & Kızıl, 2004), while showing parallels with the findings of Benkaci et al. (2013) and Kara et al. (2015). Kara et al. (2015), Mohammadi et al. (2016) and Mazaheri et al. (2019) reported that fixed oil content varied between 26.0-32.5%, 31.6-40.0% and 34.0-39.0%, respectively. These differences might have been due to air temperature, precipitation, soil fertility, agronomic conditions and harvest stages (Telci 1995; Kulan et al. 2012; Kara et al. 2015)

3.3. Chemical composition of essential oil

Essential oil compositions in black cumin oil were shown in Table 3. Cymol, thymoquinone, carvacrol, junipene, **Δ**-3-carene, β-pinene, trans-sabinene hydrate and α-thujene were determined as the main components in the essential oil of black cumin. According to the harvest stages, the cymol, thymoquinone, carvacrol, junipene, Δ-3-carene, β-pinene, trans-sabinene hydrate and α-thujene varied between 25.01-26.90%, 2.39-4.41%, 10.12-10.41%, 5.33-6.66%, 5.55-8.71%, 2.98-3.65, 8.02-11.93% and 7.82-9.42%, respectively. The rates of these components varied according to harvest stages. Generally, α-thujene, α-pinene, β-pinene, Δ-3-carene, cyclopropane, 4-terpineol and thymoquinone content decreased, while cymol, trans-sabinene hydrate, cyclohexen and carvacrol contents were increased by delaying harvest stages up to SH_4.

These changes could be as a result of climatic (especially temperature) differences between each harvest stage. The amount of temperature until the next harvest stage may affect the synthesis of essential oil composition. For example, there were about 26 days in the first year and 24 days in the second year between the first harvest and the last harvest. Synthesis of secondary metabolites in plants can be effected from biotic and abiotic factors as well as genetic traits (Telci et al. 2014). In comparison to previous studies, rates of main essential oil components varied: 42.4% thymoquinone and 10.3% carvacrol (Mahmoudvand et al. 2014), 67.7% thymoquinone, 8.4 % carvacrol, 4.8% junipene, 2.3% p-cymen and 1.9% 4-terpineol (Palabıyık & Aytaç 2018). These differences might have been due to variety, various ecological conditions including air temperature, radiation, precipitation and soil fertility affecting the production of secondary metabolites of black cumin (Benkaci et al. 2007; Mahmoudvand et al. 2014; Kara et al. 2015).

Table 3- Chemical composition contents in different harvest stage of black cumin

*Chemical components**	*RI*	*Harvest stages (%)*			
		SH1	*SH2*	*SH3*	*SH4*
α-Thujene	927	9.42	8.37	7.82	8.71
α-Pinene	933	3.37	2.73	3.58	2.09
3-Hexanol (CAS) Hexan-3-ol	948	0.03	0.03	1.68	0.13
2-Pentanol, 4-methyl- (CAS) 4-Methyl-2-pentanol	957	-	-	1.91	-
Sabinene	981	1.29	1.40	1.42	1.47
β-Phellandrene	991	-	-	1.69	1.55
β-Pinene	997	3.65	3.60	3.06	2.98
Pyridinepropanoic acid, α-methyl-.beta.-oxo-, thyl es	998	0.03	-	0.12	-
α-Terpinene	1001	0.07	0.42	0.52	0.65
Cymol	1025	25.62	25.01	26.90	26.70
Limonene	1031	1.20	1.49	2.41	1.73
Eucalyptol (1,8-Cineole)	1035	0.11	0.12	0.14	0.11
2-methyl-5-(1-methylethyl)-, (1.α-,2.al)	1041	0.06	1.36	-	-
γ-Terpinene	1053	2.16	1.41	1.16	2.69
Linalool	1062	0.58	-	-	-
Δ-3-Carene	1073	8.71	5.55	-	-
Trans-sabinene hydrate	1100	9.15	8.02	10.85	11.93
Cyclopropane, 1,1-dimethyl-2-(3-methyl-1)	1102	-	3.99	3.89	3.45
4-Terpineol	1190	3.93	3.06	-	2.19
Cyclohexen-1, 4-methyl-1-(1-methylethyl)- 4-Terp.	1194	-	-	3.98	5.11
Benzeneethanol, α-, α-dimethyl acetate	1198	0.12	0.13	-	-
Cis-p-Mentha-2,8-dien-1-ol	1209	2.21	2.66	-	1.04
β-Cyclocitral	1214	-	-	1.15	-
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)	1255	0.21	2.44	2.15	4.26
Thymoquinone	1261	4.41	2.70	2.82	2.39
Carvacrol	1313	10.12	10.13	10.41	10.43
(1S,4R)-p-Mentha-2,8-diene, 1-hydroperoxide	1326		3.17	-	-
α-Longipinene	1361	0.73	0.86	-	1.07
Junipene	1419	6.66	6.45	5.33	6.56
(-)-Caryophyllene oxide	1749		1.99	-	-
(-)-Caryophyllene oxide	1982	1.16	-	-	-
Dimethoxy-Cis-9-Octadecene	2113	-	-	2.37	-

SH; Seed harvest, RI; Retention Indice, *; Contents of chemical compounds belong to 2018

4. Conclusions

As a results of the research: i) The SH_3 harvest stage had the highest seed yield, with a mean of 542.3 kg h^{-1} so that in compared to the other harvest stages, it increased by 14.2-43.3%. The yield and its components increased by delaying the harvest stage up to SH_3, and decreased in the following harvest period (SH_4). Therefore, under Isparta's ecological conditions, black cumin should be harvested in the SH_3 stage due to the higher seed yield.

ii) Differences among the harvest stages in relation to essential and fixed oil content in both years weren't statistically significant.

iii) Cymol, thymoquinone, carvacrol, junipene, Δ-3-Carene, β-pinene, trans-sabinene hydrate and α-thujene were identified as major compounds of black cumin's essential oil, and rates of these components varied according to the harvest stages.

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Effect of Adding Different Boron Sources to Diets Containing Low Calcium and Phosphorus on Some Bone Parameters of Weaned Akkaraman Lambs

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ABSTRACT
The aim of this study was to investigate the effect of addition of different boron sources (ulexide, colemanite, etibor-48) to diet containing low Ca, P on bone parameters and bone strength in weaned lambs. 50 Akkaraman single male lambs weaned at 2.5 months of age were used. Lambs were divided into 5 groups. Groups were; positive control, negative control, supplementation with colemanite, supplementation with etibor-48, supplementation with ulexide. Roughage and concentrated feed was given twice in day. At the end of 90 days, 6 animals from each group were slaughtered. The weight, dry weight, length, width and ash levels of the femoral and tibial bones were significantly increased in added boron (B) sources groups compared to negative control. With the supplementation of the colemanite and ulexide to diets, the femoral and tibial Ca content was higher compared to negative control. The P content in the femoral bone increased in groups added of all boron sources than that in negative control, also in tibial P content in the colemanite and ulexide groups increased compared to negative control. The supplementation of all B sources had improved B levels and breaking strength of bones compared to controls.

Keywords: Bone Parameters, Colemanite, Etibor-48, Ulexide, Weaned Lambs

1. Introduction

There are wide variety mines in fertile land of Turkey. Turkey, with a total boron (B) reserves of 3.3 billion tons, with 73% share of the world's B reserves is in first place. The element B is present in various forms in environments such as soil, rock, ground, ocean water and atmosphere. There are about 230 kinds of B minerals in the world. More than 150 B minerals have been found in nature, including sodium, calcium and magnesium salts. At the present time, most of these varieties such as borax, tincal, colemanite, ulexite, probertite, pandermite, szyabelite, hydroboracite and kernite have commercial value. Tinkal ($Na_2B_4O_7.10H_2O$) and colemanite ($2CaO.3B_2O_3.5H_2O$) are the most available in terms of reserve and national economy in Turkey. Etibor-48 ($Na_2B_4O_7.5H_2O$) important deposits in Turkey Eskişehir in (24%), while the colemanite beds Emet (Kütahya) and Bigadiç (Balikesir) located in the vicinity (74%), ulexide ($NaCaB_5O_9.8H_2O$) is less, and if necessary, supplied as a by-product. (Anonymous 2019).

B is an essential element found in most tissues in trace amounts. It plays an important role in bone formation, vitamin D metabolism, prevention of Ca and Mg losses and formation of estrogen and steroid hormones (Newnham 1994), and also metabolic enzyme activity (Devirian & Volpe 2003). B is quickly absorbed by organisms, no stored in soft tissue, extracted by urine. Fat, muscle, heart, lung and intestine have lower levels of B but higher levels of bone (Moseman 1994). B plays a regulatory role in the metabolism of some minerals (Ca, P, Mg, Al and Mo). Bone development and strength are very important in breeding animals, especially long-walking animals. The bone length, diameter, weight and ash percent are very effective biometric measures for bone development. B has recently been used in rats and poultry feeds. Although B is one of the most important minerals for bone and joint health (Chapin et al. 1997; Schauss 2009), its deficiency is affected by the function and composition of some tissues such as skeleton, kidney and brain (Nielsen et al. 1991). Thus according to Hunt and Nielsen (1981) there is a relationship between bone structure and B, and calcification insufficiency in chicks leg deformation is improved by the addition of B. Similarly, Fry (2007) reported that positive relationship between boron and cholecalciferol. Some studies have shown that in chickens fed diets with insufficient B and cholecalciferol, dry weight of femoral and concentration of Ca, Cu, Zn are reduced (Bai & Hunt (1996), additionally, B deficiency increases bone abnormalities (Hunt & Nielsen 1981). Colemanite, ulexite and etibor-48 from B sources have not been studied in animal nutrition. B sources used in this experiment have never been tested in lambs before. Due to low B levels of grain cereals, the B contents of the concentrated feeds prepared with grain cereals are also low (WHO 1998).

The hypothesis of the study is use of some boron sources in young ruminant diets containing low Ca and P may have a positive effect on bone development, thus, it is thought that boron sources such as colemanite, ulexite and etibor-48 can be used as mineral additives in young ruminant diets and will contribute to the national economy. This study was conducted to determine the effects on some bone parameters and strength in weaned Akkaraman lambs of different boron sources supplementation to lamb ration including low level Ca and P and availability of this tree B sources in animal nutrition.

2. Material and Methods

2.1. Animals and experimental treatments

Femoral and tibial bones were obtained from slaughtered 50 head male Akkaraman lambs weaned at 2.5 monthly age, In the trial, concentrated feed including 17% CP and 2800 kcal kg^{-1} ME (Table 1) and dry meadow grass (10.46% CP and 1786 kcal kg^{-1} ME) were used. Colemanite (50.8%, $2CaO.3B_2O_3.5H_2O$), ulexide (43%, $NaCaB_50_9.8H_2O$) and etibor-48 (48%, $Na_2B_4O_7.5H_2O$), were provided from Eti Maden Operations General Directorate.

Table 1- Ingredients and chemical composition of feedstufts

Ingredients, %	*Positive control*	*Negative control*
Sunflower meal	36.3	36.3
Maize	32	32
Barley	30	30
Mineral mix. and Vit. (Premix)*	0.1	0.1
Limestone	1.2	-
Salt	0.4	0.4
Analysed nutrients		
CP,%	17.00	17.00
Ca,%	1.36	0.74
P,%	0.59	0.45
B,%	0.0053	0.0053
ME, kcal kg^{-1}	2800	2800

*; Primex, contained/kg Mn. 33 mg, Zn 25 mg, Fe 20 mg, Cu 6 mg, I 800 mg, Sel 66 mg and Co 160 mg; Vit-A 20.000 IU kg^{-1}, Vit D3 3.000 IU kg^{-1}, Vit E 25 mg kg^{-1}, K3 8.000 mg, B1 4.000 mg, B2 6.000 mg, B6 8.000mg, B112 20 mg, Niacine 20.000 mg, Pantothenate 12.000.mg, Colin cloride 15.000.mg

2.2. Determination of bone parameters

Procedures of this experiment were approved by the Animal Experiments Local Ethics Committee (Decision No: 2013-4-13) of Ankara University, Ankara/Turkey.

At the beginning of experiment, were formulated standard diet without 3 different B sources (colemanite, ulexite and etibor-48) and ration including low Ca and P (Table 1). Ca, P and B analyzes of the feeds used in the research (forage and feed stuffs in the structure of concentrate feeds) were made and considering B levels and purity degrees of B sources determined in feeds. Concentrates were prepared as the B levels 90 ppm kg^{-1} DM (NRC 2007).

Treatment groups included: Ca and P standard level, (positive control); including low Ca and P and B-free, (negative control); including low Ca and P and supplementing colemanite 90 ppm kg^{-1} DM; including low Ca and P and supplementing ulexide 90 ppm kg^{-1} DM and including low Ca and P and supplementing etibor-48 90 ppm kg^{-1} DM (NRC 2007).

Fifty weaned male Akkaraman lambs of equal weight and age were randomly assigned to five treatment groups and 10 lambs in each group. Forage/concentrate feed ratio was prepared as 60/40. Daily feed requirement of lambs was calculated according to NRC (2007) recommendations, the feed amount was adjusted as percentage of live weight by weekly. Forage was provided ad libitum, and concentrate feed was given separately in two meals, morning (8.00 am) and noon (14.00 pm) at 90th day of trial, six lambs from each groups were chosen/selected randomly and slaughtered. Right tibial and femoral bones were separated to determine some bone parameters of slaughtered lambs. Bones were soaked in hot water, and cleared from tissues, and then stored for 12 h at 4 ^oC, and analysed the following day. Bones were weighted on a sensitive balance (0.001mg) and then their length and diameters were measured with a digital caliper (Tresna, USA). After leaving from bones marrow and oil, they were dried at 105 ^oC for 24 h and re-weighed to determined dry weight. The bone breaking strength were determined by three point bending test with 500 kg load cell on (TA-HD Plus Texture Analyser, UK) and a probe with adjustable distance was used for the measurement. The bones were broken horizontally from their long axes. Dried fat-free bones were broken by a cutting tool after cleaning from interior tissues for ash analysis and ground, and after ashed at 610 ^oC for 24 h in a muffle furnace (Protherm, Turkey) and % ash were determined. Femoral and tibial ash samples were weighed (0.5 g) and transferred into porcelain crucible. After adding of nitric acid–hydrocloric acid, burned at 180 ^oC for 30 min. in microwave (Cem-Mars5) 175 PSI, and filtered through paper filters without ash into 100 mL plastic bottles for mineral analysis (Ca, P and B), completed to 100 mL with

distilled water. Then mineral matters in ash were read by ICP (Optima 2100 DV ICP / OES, PERKIN ELMER) at suitable wavelength (Boss & Fredeen 2004).

2.3. Statistical analysis

The data were analyzed by one-way analysis of variance. To evaluate the structure of the data Kolmogorov-Smirnov one sample test was used for normality assumption and Levene test was used to examine the homosceasticidy (Önder 2018), results showed that all traits had normal distribution and variances were equal (P>0.05). Duncan's multiple comparison test was used to compare the means with significance level of 0.05 (Genç & Soysal 2018). SPSS package program was used to analyse the data (SPSS 2002).

3. Results

3.1. Bones parameters

The effect of addition of different B sources on weight, dry weight, length, width and ash values of right tibial and femoral bones of weaned Akkaraman lambs is given in Table 2. As seen, the weight of right femoral bone of Akkaraman lambs was the highest in B groups compared to negative control (P<0.05). While there was no difference between B treatment groups, there were determined significant differences in between B groups and control groups (P<0.05).

Table 2- The effect on femoral and tibial bone parameters of different boron sources

Parameters	*Bone*	*Positive control*	*Negative control*	*Colemanite*	*Ulexide*	*Etibor-48*	*P*
Weight, g	Femoral	29.95±0.155^{a}	26.10±0.122^{c}	28.85±0.227^{b}	28.30±0.210^{b}	28.23±0.445^{b}	0.436
	Tibial	40.12± 0.164^{a}	37.58±0.262^{c}	39.78±0.105^{a}	39.82±0.238^{a}	38.73±0.155^{b}	0.292
Dry weight, g	Femoral	20.12±0.179^{a}	16.87±0.578 ^{c}	20.67±1.026^{a}	20.15±0.198^{a}	18.39±0.290^{b}	0.138
	Tibial	29.34±1.044^{a}	26.82± 0.021^{c}	29.48± 1.053^{a}	29.82± 1.011^{a}	27.10±0.027^{b}	0.177
Length, cm	Femoral	17.92±0.021^{a}	16.35±0.036^{b}	17.90±0.024^{a}	17.85±0.025^{a}	16.99±0.027^{ab}	0.165
	Tibial	19.43±0.441^{a}	18.10±0.031^{b}	19.27±0.096^{a}	18.98±0.102^{a}	19.33±0.228^{a}	0.184
Width cm	Femoral	2.39±0.423^{a}	1.96±0.521^{b}	2.32±0.233^{a}	2.38±0.425^{a}	2.28±0.327^{a}	0.457
	Tibial	2.27±0.623^{a}	1.97±0.447^{b}	2.13±0.282^{a}	2.22±0.326^{a}	2.11±0.135^{a}	0.419
ash,%	Femoral	57.91±0.389^{a}	54.88±0.270^{b}	57.05±0.228^{a}	56.87±0.393^{a}	56.99±0.340^{a}	0.315
	Tibial	63.07±0.309^{a}	58.76±0.614^{b}	61.76±0.836^{a}	63.12±0.495^{a}	62.62±0.518^{a}	0.386

a,b,c; Means with different superscript within same line significantly differ (P<0.05)

The tibial weight reported in this study ranged from 37.58 g to 40.12 g. The lowest tibial weight was determined in negative control (P<0.05), There were no significantly different between positive control with colemanite and ulexide. Also Etibor-48 was significantly lower than the positive control and the other B groups (P<0.05).

Dry weight of the femoral and tibial ranged from 16.87 to 20.67 g and 26.82 to 29.82 g respectively, in present study. Dry weight of femoral and tibial bones was significantly higher in boron groups according to negative control (P<0.05). The best results among B sources were found in colemanite and ulexide groups (P<0.05). There were no significantly different between positive control with colemanite and ulexide.

Length of femoral and tibial ranged from 16.35 to 17.92 cm and 18.10 to 19.43 cm respectively. There was no significant difference between positive control and B treatment groups in terms of tibial and femoral length, whereas the lowest length was determined in negative control with low Ca and P content (P<0.05).

The width and ash content of femoral and tibial bones increased in all B groups compared to negative control (P<0.05). There was no significant differences between B sources and positive control.

3.2. Bone mineral contents

The effect on bone mineral composition of different B sources addition to Akkaraman lambs diets is presented in Table 3. It can be seen that the dietary supplementation of colemanite and ulexide compared to negative control improved Ca level of femoral and tibial bones (P<0.05). There was no significant differences between positive control and colemanite group. Ca level was significantly higher in colemanite added group than other B groups (P<0.05). The results showed that B sources positively affected femoral P levels compared to negative control (P<0.05). There was no significant difference among B sources for P content. Colemanite and ulexide supplementation to diet increased P level of tibial bone compared to negative control. There was no difference between etibor-48 and negative control. On the other hand, the highest P level in femoral and tibial bones were found in positive control (P<0.05). Mg content of bones was not affected by the addition of the B sources. B content of femoral and tibial bones was significantly higher in treatment groups using B sources compared to the control groups (P<0.05), but no

significant difference was found between B sources. Supplementation of B sources has shown positive effect on B levels of femoral and tibial bones (P<0.05).

Table 3- The effect on mineral composition femoral and tibial bones of different B sources, DM%

Parameters		*Positive control*	*Negative control*	*Colemanite*	*Ulexide*	*Etibor-48*	*P*
Ca	Femoral	25.15± 1.079^{a}	13.01 ± 2.235^{c}	24.53 ± 1.241^{a}	20.606 ± 1.471^{b}	15.91± 1.868^{bc}	0.130
	Tibial	27.82± 0.287^{a}	23.23± 0.122^{c}	27.75 ± 0.715^{a}	25.10± 0.837^{b}	23.72± 0.019^{c}	0.195
P	Femoral	14.95± 0.700^{a}	7.24± 4.615^{c}	10.76± 8.129^{b}	11.08± 6.242^{b}	10.26± 9.246^{b}	0.136
	Tibial	19.29± 1.194^{a}	13.54± 1.361^{c}	15.08 ± 1.097^{b}	15.82± 1.858^{b}	14.82± 1.752^{bc}	0.431
Mg	Femoral	0.19 ± 1.086	0.18±1.574	0.17 ± 2.874	0.17± 2.158	0.18± 1.580	0.000
	Tibial	0.21± 1.315	0.22± 1.322	0.21± 1.523	0.22± 0.985	0.22± 0.948	0.000
B	Femoral	0.607± 0.090^{b}	0.543± 0.057^{c}	0.648± 0.380^{a}	0.644± 0.035^{a}	0.651± 0.046^{a}	0.336
	Tibial	0.721± 0.049^{b}	0.717± 0.015^{b}	0.762± 0.009^{a}	0.793± 0.027^{a}	0.807± 0.003^{a}	0.432

a,b,c; Means with different superscript within same line significantly differ (P<0.05)

3.3. Bone breaking strength

The effect of addition of different B sources on bone breaking strength of weaned Akkaraman lambs is given in Table 4. The addition of B sources to diet containing low Ca and P significantly increased the breaking strength of femoral bone of Akkaraman lambs compared to control groups (P<0.05) (Table 4). No difference in breaking strength was observed among ulexide and etibor-48 groups. The addition of colemanite to diets was lower than effect on breaking strength compare to other B treatment groups in femoral and tibial bones. Breaking strength in tibial bone increased by supplementation ulexide and etibor-48 (P<0.05). On the other hand, the lowest breaking strength was determined in negative control including low Ca and P for both bones. No significant difference in breaking strength was observed between colemanite and positive control.

Table 4- The effect of different boron sources on fracture strength of femoral and tibial bones

Parameters	*Positive control*	*Negative control*	*Colemanite*	*Ulexide*	*Etibor-48*	*P*
Femoral (N)	781.69± 14.725^{c}	720.34± 13.702^{d}	822.50± 11.366^{b}	937.15± 15.964^{a}	970.10± 17.542^{a}	0.156
Distance,cm	3.84±0.229	4.28±0.165	4.07± 0.278	5.75± 0.326	5.49±0.437	0.000
Tibial (N)	891.62± 12.606^{b}	743.24± 22.497^{c}	889.19± 12.658^{b}	997.47± 14.863^{a}	1001.01± 15.584^{a}	0.141
Distance,cm	7.58±0.326	8.03±0.330	8.00 ± 0.148	7.07± 0.747	8.29± 0.595	0.000

N; Newton, a,b,c; Means with different superscript within same line significantly differ (P<0.05), **; (P<0.05)

4. Discussions

4.1. Bone parameters

No studies were conducted on the use of colemanite, ulexide and etibor-48 in animal nutrition, especially in ruminant nutrition, whereas all animal nutrition studies were related to boric acid.

In this study, B sources had a positive effect on weight of femoral and tibial bones compared to negative control group including low Ca and P. Thus, the addition of B to the diet containing low Ca and P improved femoral weight. While no difference was found between the B sources in terms of femoral bone weight, colemanite and ulexite gave better results on tibial weight than etibor-48. Kurtoğlu et al. (2005) reported that the addition of 5 and 25 mg kg^{-1} DM B to the poultry diet with sufficient and inadequate vitamin D3 content did not affect tibial weight. In this study, the addition of B sources significantly increased dry weight of femoral and tibial bones compared to negative control. This increase is a result of compensating the mineral deficiency by adding B sources to diet since there was Ca and P low levels in negative control. The best results in terms of dry weight were determined in colemanite and ulexite groups. Bai & Hunt (1996), reported that femoral dry weight, Ca, Cu and Zn concentration in femoral decreased in chicks fed with rations insufficient B and cholecalcipherol. In this study, B sources increased the length of femoral and tibial bones compared to negative control. These findings are similar to findings of Devirian & Volpe (2003), indicating that addition of B in rats and chicks increases bone length. Also, Hunt et al. (1994) reported that B provides maturation in the developmental regions of long bones. In our study, there was no significant difference in bone length between B added groups and positive control including standard Ca and P. The addition of B sources to diet positively affected width of femoral and tibial bones compared to low Ca and P content group. No study was found to be compared with these results. The effect of B sources on ash level of femoral and tibial bones was positive compared to negative control. But, there was no difference between positive control and treatment groups. It was stated that addition of B to diet increased bone ash (Qin & Klandorf 1991; Wilson & Ruszler 1997), whereas did not affect (Fassani et al. 2004; Yıldız et al. 2011). Differences in research results may be

attributed to differences in B sources and animal species. The presence of Ca and Na minerals in the structure of B sources used in this study may be the cause of the increase in the rate of bone ash. On the other hand, Kheiri & Rahmani (2006) reported that tibial ash was affected by Ca and P change, and also according to Edwards (1987), supplementation of B to chick diet increased tibial bone ash percentage and reduced the incidence of tibial dyschondroplasia.

4.2. Bone mineral content

In femoral and tibial bones, the lowest Ca level was determined in negative control containing low Ca and P, but higher Ca content was observed in positive control. colemanite and ulexite from B sources significantly increased Ca levels compared to negative control group. The highest Ca content among the treatment groups was determined in colemanite added group in femoral and tibial. The presence of Ca in the structure of colemanite and Ca and Na in the structure of ulexite from treatment groups may be reason for the increase in bone Ca content. According to Brown et al. (1989), excessed B level in diet increases the amount of Ca retained in the body. Browning et al. (2012), stated that broiler chicks fed with low Ca / P diet had more Ca accumulation in their bones than those of fed with high Ca / P diet. Contrary to results of this study, Fassani et al. (2004) expressed that addition of B to poultry feed did not affect bone Ca levels. In this study, the addition of B sources had a positive effect on the P level of bones compared to negative control. These results were supported by Bozkurt et al. (2009) who reported that bone Ca and P content, with supplementation of boric acid 30 and 60 ppm to poultry diets including low Ca and P, raised to control group including standard Ca and P level. Ca and P are essential for skeletal structure and development in growing young ruminants. Ca and P are form of hydroxyapatite in bone tissue (Van den Top 2009). Ca and P deficiency can leads to rickets in young growing animals.

In this study, to give good results in diet including low Ca and P of B sources may be explained with resistance against to Ca and P deficiency. The addition of B sources has no positive effect on Mg content of femoral and tibial bone. Some studies has been reported that B has a regulatory effect on mineral metabolism, especially on Ca, P and Mg metabolism, and has a positive effect on bone development and mineralization (Nielsen et al. 1988; Chapin et al. 1998; Armstrong et al. 2000; Miyamoto et al. 2000). In our study, B content of femoral and tibial bones increased significantly in treatment groups compared to control groups. The increase in B level is a result of adding B sources to diet. In some studies in poultry, as the amount of B in feed increases, the amount of B in bones increases (Wilson & Ruszler 1997; Wilson & Ruszler 1998; Yıldız et al. 2011), supporting the findings of this study.

4.3. Bone breaking strength

In present study, addition of B sources to diets significantly increased breaking strength of femoral and tibial bones of Akkaraman lambs compared to control groups. The lowest breaking strength was determined in negative control. In this group, the Ca and P level in diet is lower than standard level, which may have caused a decrease in breaking strength. The findings of study are supported by Armstrong & Spears (2001). Who states that B together with Ca and P contributes to the maintenance of bone strength. Similarly, Schauss (2009) also states that B is one of the most important minerals in bone and joint health. Nielsen (2004) reported that bone strength is reduced in broilers and pigs fed with low B diet but high levels of B supplementation increase bone strength in chicks. Rossi et al. (1993), reported that 5 mg kg^{-1} B addition to diets, tibial fracture resistance in male broiler increases. Breaking strength of bones is very important for bone strength in young animals, especially in breeding animals and animals that have to walk long distance.

5. Conclusions

Adding different B sources to growing Akkaraman lamb diets positively affected weight, dry weight, length, width, ash and mineral content of femoral and tibial bone compared to negative control group. The addition of colemanite, ulexite and etibor-48 to diet positively affected breaking strength and B content of the femur and tibia bones compared to both the positive and negative control groups. It can be used as mineral additive colemanite, ulexite and etibor-48 in Akkaraman lambs diets especially including low Ca and P.

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Effect of Heritability, Genetic Advance and Correlation on Yield Contributing Traits in Upland Cotton

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ABSTRACT
This research was planned to study the heritability (broad sense), correlation, genetic advance and behavior of different characters in segregating population of upland cotton. The material consists of segregating population of fourteen crosses along with their seven parents. Parental varieties and segregating population show significant difference for all traits under the study. Plant height, ginning out turn (GOT), bolls per plant and yield per plant showed heritability ranging from 78.9 to 27.3. Significant genotypic correlation of yield with plant height was 0.698, bolls per plant was 0.930, GOT was 0.692, fiber strength was 0.548 and with fiber fineness was 0.435. Phenotypic correlation of yield per plant with plant height was 0.520, boll per plant was 0.894 and GOT was 0.476. It can be suggested that plant height, GOT and bolls per plant are important yield contributing traits as they are positively correlated with seed cotton yield per plant. High value for bolls per plant, GOT and yield per plant was recorded in BH-167 × V4 and CIM-534 × V4, which can be utilized in future breeding program.

Keywords: Ginning out turn, Broad sense, Gene action, *Gossypium hirsutum*, Segregating population

1. Introduction

Cotton is mainly cultivated for its fiber and seed oil (Masood et al. 2019). Worldwide, there are eighty countries which are cotton producers. Among them top six are China, India, USA, Pakistan, Brazil and Australia (Shuli et al. 2018). Cotton production plays a significant role in Pakistan's economy as its share in GDP is approximately 0.8% and value addition in agriculture is approximately 4.8%. In 2017-18, cotton production was 11.95 million bales and in 2018-19 production was 9.861 million bales, which was about 17.5% lower than the previous year (Pakistan Economic survey 2018-19). Major problems in yield reduction were mainly due to climatic change along with low genetic variability of cotton varieties, biotic and abiotic stresses (Panni et al. 2012). Developing a cultivar of upland cotton, having high yield, required genetic knowledge of yield contributing characters of cotton, which can be useful for breeder in the improvement of genetic structure of plant (Abbas et al. 2008). In yield improving program of cultivar through hybridization, success is based on heritable variability, selection and use of parental cultivars (Gul et al. 2014).

The study of genetic advance (GA) and broad sense heritability (H^2) will be helpful in identifying superior lines in segregating population. Those characters which have moderate to high value of GA and H^2 can be utilized in improvement of yield (Abbas et al. 2013). High to moderate value of H^2 was observed in number of seed per boll, boll weight and plant height (Raza et al. 2016), yield and boll per plant (Abbas et al. 2013).

In cotton, yield is influenced by different traits like boll weight, bolls number per plant and GOT (Ahmad et al. 2008). So, the indirect selection procedure for yield improvement was always preferred. Correlation study will be useful to understand the behavior of yield contributing characters (Alkuddsi et al. 2013). It can be helpful to understand the inter-relationships between different traits and in evolving selection principles (Kloth 1998). Different characters are either positively or negatively correlated with one another, so in case of positive correlation improvement in one trait may lead to an increase in the quality of other traits or vice versa (Desalegn et al. 2009). Yield was positively correlated with plant height (Salahuddin et al. 2010), boll weight, boll number per plant (Iqbal et al. 2006; Rasheed et al. 2009; Salahuddin et al. 2010) and monopodial branches (Iqbal et al. 2006). Thus, selection for these can be helpful in improving yield. For the improvement in yield, the behavior of yield

contributing traits will be helpful in selection. For this reason, current experiment was designed to understand the behavior of yield contributing traits and also to access that the variation present in population was either due to genetic or environment. Purpose of this research was to assess H^2, GA and correlation in fourteen crosses and that information will be helpful in selection of subsequent generations and in development of genotypes, which have enhanced yield.

2. Material and Methods

2.1. Experimental material

The current research was designed to study H^2, GA and correlation to assess the performance of fourteen crosses along with parentage in segregating population of upland cotton. The germplasm of seven parents and their fourteen crosses (Table 1) were provided by the department of Plant Breeding and Genetics, Faculty of Agricultural Sciences and Technology (FAST), Bahauddin Zakariya University (BZU), Multan. Theses crosses were developed during 2013-2014 (Munir et al. 2016). The selection of parents for hybridization process was based on different morphological, yield characters and fiber traits. The experiment was conducted in a RCBD (randomized complete block design) with three replications and each replication consisted of 30 plants of each cross along with parental lines, during 2015, at the experimental area of the department of Plant Breeding and Genetics, FAST, BZU, Multan (30° 15' 33.0" N, 71° 30' 57.5" E) on loamy soil. The climate of Multan is arid to semi-arid with annual rainfall of up to 175 mm and average temperature during the growing season ranged from 28 °C to 37 °C. Row to row distance was 75 cm and distance between plants was maintained at 30 cm. Agricultural practices were applied consistently from seedling stage to harvesting. Data was recorded at maturity for characters such as plant height, number of bolls per plant (B/P), node of first fruiting branch (NFB), monopodial branches, number of seeds per boll (S/B), boll weight, ginning out turn (GOT), fiber fineness (FF), staple length (SL), fiber strength (FS) and yield/plant.

Table 1- List of parents and F_2 population

Breeding material	*Genotypes*
Parents	BT CIM-599, CIM-573, MNH-786, CIM-554, BH-167, MNH-886, V4
Crosses	BT CIM-599 × MNH-886, CIM-573 × MNH-886, MNH-786 × MNH-886, CIM-554 × MNH-886, BH-167 × MNH-886, BT CIM-599 × V4, CIM-573 × V4, MNH-786 × V4, CIM-554 × V4, BH-167 × V4, BT CIM-599 × BH-167, CIM-573 × BH-167, MNH-786 × BH-167, CIM-554 × BH-167

2.2. Statistical analysis

Data of all above mentioned traits was subjected to analysis of variance (Thomas & Maurice 2008) to evaluate variation among genotypes. Genetic parameters, genotypic, environmental and phenotypic variance equations were given below (Equations 1, 2 and 3, respectively), H^2 and GA equations were also given (Equations 4 and 5, respectively) were determined through method of variance component (Breese 1972; Larik et al. 1980 & 1987).

$$Genetic\ Variance = Genotype\ MS - Error\ MS \quad (1)$$

$$Enviromental\ Variance = Error\ MS \quad (2)$$

$$Phenotypic\ Variance = Genotype\ variance + Error\ variance \quad (3)$$

$$Broad\ Sense\ Heritability\ (H^2) = \frac{Genotype\ variance}{Phenotypic\ variance} \quad (4)$$

$$Gentic\ advance\ (GA) = \sqrt{Phenotypic} variance \times H^2\ \times k \quad (5)$$

K is constant = 2.06 at 5% selection intensity.

H^2 was categorized in three groups: High value was > 60%, moderate value was 30-60% and low value was 0-30% (Robinson et al. 1966). Johnson et al. (1955) also classified GA into different categories: High value (> 20), moderate value (10-20), and low value (0-10). The correlation between yield and yield contributing traits was identified through phenotypic and genotypic correlation analysis. Correlation study was carried out using statistical method given by Kwon & Torrie (1964) and formulae for calculation of genotypic and phenotypic correlation are given below (Equations 6 and 7). The analysis was performed using R STAT software for analysis of ANOVA, H^2 and correlation.

$$rp = {Mij}\Big/{\sqrt{[Mii).(Mjj)]}} \quad (6)$$

$$rg = \text{Covgij} \Big/ \sqrt{[\text{Var gi}).(\text{Var gj})]} \quad (7)$$

3. Results and Discussion

According to ANOVA, highly significant variation was found for plant height, NFB, monopodial branches, B/P, S/B, boll weight, GOT, SL, FS, FF and yield/plant (Table 2). Mean performance of crosses and their genotypic, phenotypic and environmental variations is given in Table S1 and S2, respectively.

Table 2- Mean squares of parents and F_2 population in cotton genotypes

Trait	*DF*	*MS*
PH	20	264.766**
NFB	20	9.072**
B/P	20	118.044**
MB	20	0.296*
S/B	20	39.925**
BW	20	0.463**
GOT	20	27.191**
SL	20	8.725*
FS	20	13.789*
FF	20	1.39*
YP	20	2051.27**

*; Significant at 5%, **; Significant at 1%; PH, Plant height; NFB, Node of first fruiting branch, B/P; Number of bolls per plant, MB; Monopodial branches per plant, S/B; Number of seeds per boll, BW; boll weight, GOT; Ginning out turn, SL; Staple Length, FS; Fiber Strength, FF; Fiber Fineness, YP; Yield per plant

High value (>60) of H^2 was found for B/P (81.9), yield/plant (78.9), plant height (78.5), GOT (78.1) and boll weight (60.5). While, moderate H^2 (30-60) were observed for NSPB (58.0), SL (54.1) and low value (0-30) of H^2 was observed for FF (27.3). GA showed high value for yield/plant (45.84), plant height (16.42), B/P (11.28), GOT (5.24) and SL (2.28). Boll weight and FF showed low value of GA that was 0.57 and 0.09, respectively (Figure 1).

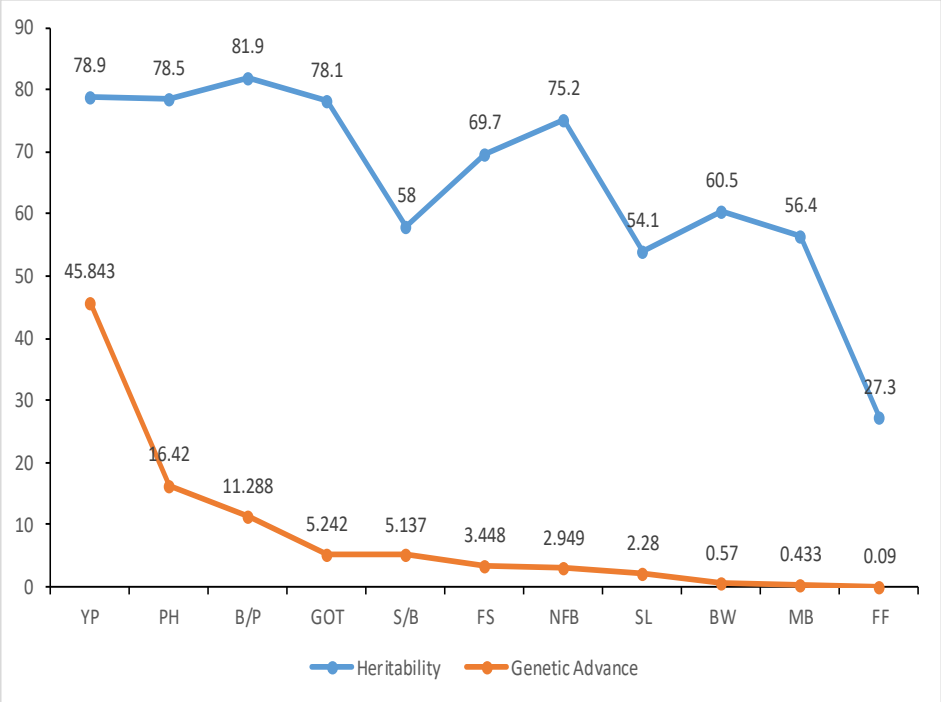

Figure 1- Estimation of heritability and genetic advance for seed cotton yield and its components (parents and F_2 population)

Genotypic relationship of yield/plant was found positive and statistically significant with plant height (0.689), B/P (0.93), GOT (0.692), FS (0.548), FF (0.435) and SL (0.43). Non-significant but favorable relationship was found among yield/plant

and boll weight (0.311). B/P correlation with GOT (0.655) and FS (0.579) was positive and significant. Whereas, GOT was negatively correlated with FF (-0.248). In order to improve yield/plant selection is supposed to be based on B/P and GOT (Table 3). Phenotypic association between yield and B/P (0.894) was highly significant and favorable in selection. Favorable and significant correlation was also found between yield and plant height (0.52) as shown in Table 3.

Table 3- Genotypic (upper diagonal) and phenotypic correlation (lower diagonal) in parents and F_2 population for yield and its components

Traits	*PH*	*NFB*	*B/P*	*MB*	*S/B*	*BW*	*GOT*	*SL*	*FS*	*FF*	*YP*
PH	1	0.404	0.592*	0.031	0.521*	0.238	0.298	0.301	0.255	-0.634*	0.689*
NFB	0.286	1	0.196	0.309	0.338	-0.29	0.007	-0.287	-0.087	-0.457*	0.1
NBP	0.423	0.151	1	0.14	0.223	-0.054	0.655*	0.206	0.579*	-0.433*	0.930**
MB	0.026	0.249	0.088	1	0.343	-0.187	0.027	-0.345	-0.201	-0.506*	0.069
NSPB	0.316	0.236	0.149	0.176	1	0.391	-0.203	0.208	-0.472****	-0.054	0.361
BW	0.224	-0.215	-0.093	-0.15	0.195	1	0.162	0.626	-0.093	0.068	0.311
GOT	0.24	0.051	0.462	0.082	-0.195	0.115	1	0.029	0.536	-0.248	0.692**
SL	0.289	-0.19	0.193	-0.248	0	0.366	-0.035	1	0.102	-0.353	0.430*
FS	0.157	-0.076	0.406	-0.164	-0.24	-0.101	0.396	0.04	1	-0.289	0.548*
FF	-0.297	-0.218	-0.28	-0.169	-0.061	0.119	-0.113	-0.201	-0.16	1	0.435*
YP	0.520*	0.063	0.894**	0.013	0.235	0.352	0.476*	0.34	0.359	0.22	1

*; Significant at 5%, **; Significant at 1%, PH, Plant height; NFB, Node of first fruiting branch, B/P; Number of bolls per plant, MB; Monopodial branches per plant, S/B; Number of seeds per boll, BW; boll weight, GOT; Ginning out turn, SL; Staple Length, FS; Fiber Strength, FF; Fiber Fineness, YP; Yield per plant

H^2 provide an idea of variation present in a population either due to the environment (non-heritable) or genetics (heritable variation). But GA provides information of genetic variation that is either fixable (additive) or non-fixable variation (dominance or epistasis). H^2 estimation along with GA provide reliable tool for selecting segregating population (Alkudsi et al. 2013). Yield/plant is a complex trait and controlled by several polygenic characters, such as B/P, boll weight, GOT, NSPB and plant height. In current experiment, high value of H^2 was found for B/P, boll weight, GOT and plant height except NSPB. GA value for B/P and plant height was moderate, while other traits showed low value. Results showed that those traits which have moderate value of GA along with high value of H^2 can be improved by simple selection procedure, because of the presence of additive type of gene action. In Pakistan, cotton is usually grown in wheat-cotton crop rotation, where early maturing cotton varieties are preferred. Various characters contributed in earliness of cotton plant (Shappley et al. 1998). Earliness in genotypes can be predicted based on NFB trait in cotton. High H^2 value and low value of GA exhibited that this trait was under the influence of dominant gene action. Due to presence of non-fixable gene action, selection should be made in later generations (F_5 generation) in order to develop earliness in cotton. Additionally, monopodial branches were less desirable to breeder because of high infestation rate of pest. Usually, lower number of monopodial branches is preferred due to this reason (Munir et al. 2018). High value of H^2 was detected but GA value was low, which meant that non-dominant type of gene action was present. Muhammad et al. (2016) also found high H^2 value and low GA value and recommended that selection should be delayed up to later generation because of existence of non-additive type of gene action. In addition, monopodial branches were influenced by dominant gene action due to non-fixable variation therefore, selection should be delayed (Abro 2003). In the weaving industry, fiber traits have considerable importance. So, FS, SL and FF are significant fiber characters. Good spinning required better SL that is mandatory because of upgrading of spinning methodology (Tabasum et al. 2012). In fiber traits FS, FF and SL exhibited high to low H^2 value with low GA value. Therefore, for the improvement of such traits, selection should be delayed to later generations (F_4 or F_5 generation). Yield/plant has considerable importance in breeding program. GA along with H^2 showed high valuefor yield/plant which showed the presence of additive gene action. Khan et al. (2010) also described high value of H^2 and GA for yield/plant and recommended that selection can be performed in early population because of the presence of additive type of gene action. High H^2 and GA values were observed for yield/plant, B/P and plant height (Soomro et al. 2010). However, high H^2 value and low value of GA was also detected for boll weight (Rasheed et al. 2009), GOT (Shahzad et al. 2015) and S/B (Soomro et al. 2010). High H^2 value and moderate GA value was observed for B/P (Rasheed et al. 2009) and plant height (Khan et al. 2010; Raza et al. 2016). Moderate to high value of H^2 was reported for SL, FF and FS with low value of GA (Shahzad et al. 2015). The results exhibited the presence of non-heritable variations and suggesting the selection of these traits in later generations (Desalegn et al. 2009; Shahzad et al. 2015).

In results, positive association was found between yield/plant and plant height, B/P, GOT, FF and SL. It showed that strong correlation was present between these traits. Selection criteria based on these characters will lead to enhancement in yield/plant. Plant height and B/P showed high H^2 value and moderate GA value indicating that selection based on traits like plant height and B/P will be helpful in improvement of yield. However, selection based on GOT, FF and SL will not be productive due to presence of non-fixable gene action. Those traits which show high H^2 and GA values are easily improved through selection process and for those that have a low H^2 value, selection must be delayed to later generation. B/P was positively associated with GOT and FS. Thus, by selecting plants which have high number of B/P will lead to improvement in GOT and SL. Selection based on plant height will ultimately result in the increase in B/P because the presence of positive linkage between these traits. Positive correlation was found between plant height and GOT and B/P, ultimately leading to enhancement in yield/plant (Joshi et al. 2006). GOT had positive association with B/P, plant height and NFB and with

yield/plant (Mustafa et al. 2007). For improvement in yield/plant, selection criteria depend on B/P and plant height because by improving yield/plant the following traits will also improve like GOT, FS and FF due to positive association of these traits with plant height and B/P. Significant and positive association was found between yield/plant and B/P (Iqbal et al. 2006; Rasheed et al. 2009) and plant height (Shahzad et al. 2015). Based on results and finding of other scientists, it can be suggested that for the improvement in yield/plant selection should be based on B/P.

4. Conclusions

A significant difference was shown by parental varieties and segregating population. Plant height, B/P, GOT and yield/plant show high H^2 value, but plant height and B/P showed moderate value of GA indicating that these traits were under the influence of additive type of gene action. It can be predicted that the traits which have a low H^2 value and also low GA value have non-fixable variation. Therefore, selection for such traits must be delayed to later generation. Plant height, B/P and GOT are important yield/plant contributing traits and are found to be positively correlated with yield/plant. Selection criteria based on plant height and B/P will lead to improvement in yield/plant. High number for B/P, GOT and yield/plant was recorded in BH-167 × V4 and CIM-534 × V4. This cross can be utilized in future breeding program and for yield improvement in cotton.

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Abbreviations and Symbols	
H^2	Broad sense Heritability
GA	Genetic advance
PH	Plant height
MB	Monopodial branches
BW	Boll weight
YP	Yield per plant
NFB	Node of first fruiting branch
B/P	No. of bolls per plant
S/B	No. of seeds per boll
GOT	Ginning out turn
FF	Fiber fineness
FS	Fiber Strength
SL	Staple length

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Supplementary data Table 1- Mean performance of parents and F_2 population

Genotypes	*PH (cm)*	*NFB*	*B/P*	*S/B*	*MB*	*BW (g)*	*GOT (%)*	*SL (mm)*	*FS (g/tex)*	*FF (µg/inch)*	*YP (g)*
BT CIM-599 x MNH-886	123.93	10.10	23.30	27.92	1.38	4.28	32.14	28.32	27.21	4.23	99.88
CIM-573 x MNH-886	108.92	9.83	15.95	26.16	2.00	4.27	33.18	22.89	25.20	4.32	68.09
MNH-786 x MNH-886	123.56	13.56	18.47	28.14	1.82	3.27	35.30	21.93	28.21	4.19	60.09
CIM-554 x MNH-886	110.91	9.81	21.70	26.19	2.00	3.50	29.61	24.91	30.32	4.16	75.22
BH-167 x MNH-886	101.99	9.80	29.65	22.03	2.00	3.08	37.48	23.46	29.18	4.13	91.19
BT CIM-599 x V4	124.55	11.54	29.47	27.48	1.60	3.36	35.43	24.47	26.90	4.28	99.10
CIM-573 x V4	137.38	10.20	33.03	28.74	1.36	4.39	37.84	26.13	30.43	4.08	145.15
MNH-786 x V4	116.91	11.22	31.71	22.60	1.76	3.63	38.44	24.47	29.37	4.26	114.84
CIM-554 x V4	122.13	12.32	24.27	30.67	1.75	4.43	38.05	25.33	27.98	4.22	107.85
BH-167 x V4	134.64	12.76	36.23	26.24	2.15	4.03	39.44	28.03	32.17	4.05	147.23
BT CIM-599 x BH-167	127.86	12.93	25.89	23.00	1.60	3.55	37.46	24.47	28.10	4.17	89.63
CIM-573 x BH-167	128.55	11.45	30.33	29.61	2.30	3.84	37.37	23.17	28.25	4.36	116.52
MNH-786 x BH-167	124.12	10.45	20.68	19.49	1.36	3.63	34.08	26.23	29.67	4.13	75.05
CIM-554 x BH-167	124.31	12.85	28.03	31.82	1.61	3.40	28.77	25.67	26.00	4.26	94.30
BT CIM-599	111.14	13.67	25.69	22.31	1.64	3.40	36.46	24.59	29.37	4.20	87.25
CIM-573	126.40	9.71	26.53	20.94	1.93	3.60	37.45	25.17	30.00	4.06	95.37
MNH-786	134.66	12.57	40.70	31.61	1.95	3.86	39.44	26.67	29.33	3.96	157.03
CIM-544	114.42	10.82	29.65	21.83	1.45	3.90	37.78	24.83	29.53	4.33	115.71
BH-167	123.25	13.95	21.20	29.15	2.47	3.81	32.74	25.78	22.43	3.98	80.87
MNH-886	112.85	11.95	18.70	24.31	1.49	4.15	34.23	26.05	28.05	4.24	77.46
V4	110.83	6.86	16.95	25.84	1.50	4.16	36.57	28.32	26.00	4.34	70.51

PH; Plant height, NFB; Node of first fruiting branch, B/P; Number of bolls per plant, MB; Monopodial branches per plant, S/B; Number of seeds per boll, BW; boll weight, GOT; Ginning out turn, SL; Staple Length, FS; Fiber Strength, FF; Fiber Fineness, YP; Yield per plant

Supplementary data Table 2- Estimation of genotypic, phenotypic and environmental variance of parents and F_2 population

Traits	*GV*	*PV*	*EV*	*Mean*
YP	627.76	795.75	167.98	98.49
PH	80.89	102.99	22.10	121.11
B/P	36.65	44.74	8.08	26.10
S/B	10.72	18.48	7.76	26.00
GOT	8.29	10.61	2.32	35.68
FS	4.02	5.76	1.74	28.27
NFB	2.73	3.62	0.89	11.35
SL	2.27	4.19	1.92	25.28
BW	0.13	0.21	0.08	3.79
MB	0.08	0.14	0.06	1.77
FF	0.01	0.03	0.01	4.19

GV; Genotypic Variation, PV; phenotypic Variation, EV; Environmental Variation, PH; Plant height, NFB; Node of first fruiting branch, B/P; Number of bolls per plant, MB; Monopodial branches per plant, S/B; Number of seeds per boll, BW; boll weight, GOT; Ginning out turn, SL; Staple Length, FS; Fiber Strength, FF; Fiber Fineness, YP; Yield per plant

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Production of Agricultural Biodegradable Mulch and Evaluation it through Heat and Moisture Distribution in Soil

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ABSTRACT
The need to increase agricultural production in proportion to population growth and water crisis management requires initiatives that can increase the quantity and quality of crops by using soil moisture storage methods while preserving the environment. According to problem presented, in order to simulate the temperature, humidity and radiation of the farm environment, a control system, along with laboratory facilities were designed and constructed. Then, the production and evaluation of different types of soil mulches from biodegradable and petroleum polymers were performed by aiming investigate the effect of these soil mulches on soil temperature and moisture at different depths. Produced mulches were placed in a laboratory soil bed. The average molecular weight, the gel content and the percentage of elongation at the breakpoint for biodegradable mulches were 4906.56 g mol^{-1}, 4.68% and 4.63%, respectively and the mean values of tensile strength and the percentage of elongation before the ultraviolet aging process were 13.41MPa and 396.71%, respectively. Acceptable values of statistical indicators were calculated with the response surface method. In conclusion of the soil temperature and humidity changes for different types of mulch, the velocity of temperature rise is reduced in deep levels due to the resistance made by soil moisture. The amount of moisture reduction for dark and uncoated mulch by moving from surface to depth was far more when compared to other mulches, and there was no significant change at the depth of transparent mulches.

Keywords: Response surface method, Soil moisture, Soil temperature

1. Introduction

With the reduction of fossil resources and increase of human-made and environmental hazards, researchers are now looking for alternatives to replace plastic products. Bioplastics, which are biodegradable and destructive, have the same properties as plastics. They also have additional properties, including carbon footprint and organic biomarkers.

Soil temperature is an important control parameter in agriculture, which is more important than environment temperature. (Pramanik et al. 2015). The use of mulch in agricultural fields is a technique for saving water and controlling temperature and garden pests (Schonbeck & Evanylo 1998). For better performance of agricultural products, the approximate temperature in soil was changed from 25 to 32 °C, and it is recommended to apply 8 and 15 °C for increasing the root thickness (Tindall et al. 1991). In addition, controlling the temperature of the plant root can prevent the emergence of plant diseases and lead to premature production (Díaz-Pérez et al. 2007). The required actual amount of heat at times varies with the physiological conditions of the plants. Heat requirement is often expressed in GDD or GDH. Therefore, with having sufficient information about minimum and maximum temperatures for different plants in different physiological conditions and with meeting their thermal requirements, it is possible to avoid loss of energy and plant degradation.

Considering the increasing environmental pollution and the depletion of fossil resources, a growing number studies over the past few years have attempted to replace synthetic plastics derived from petroleum compounds with plastics made from renewable sources. This product was developed in the early 1980`s with starch-based compounds and fat polyesters (such as poly hydroxy butyrate or polyacetate) (Zhang et al. 2008). Oxidation and extract water and carbon dioxide from the polymeric material resulted from breaking the molecular coarse chains into smaller components in these polymers (Fattahi 2015).

Tensile characteristics and functional characteristics, including water vapor evaporation and optical characteristics were studied while they were being used in farms. Despite the fact that the mechanical properties of the destructive mulch were reduced after only five months, they met the farms functional requirements and finally became known as a suitable substitute for polyethylene in grape fields (Touchaleaume et al. 2016).

The degree of degradability is another topic that has been studied in this domain of research (Kyrikou & Briassoulis 2007; Sivan 2011; Li et al. 2014). In order to determine the degradability, different types of biodegradable mulch were used and they were then compared with conventional types of polyethylene mulch. The effect of isopods showed that the disintegration rates of starch- and cellulose-based plastics increased (Wood & Zimmer 2014).

Another method in studying the effects of mulch on the growth process is radiation measuring solarization. Also, a comparison of transmission, reflection and absorption rays of the sun in short and long wavelengths was made (Papadakis et al. 2000; Scarascia-Mugnozza et al. 2004; Heißner et al. 2005). The results of this research showed that the biodegradable mulch used with the maximum transmission of the solar spectrum and the minimum infrared spectrum can increase the temperature of the soil bed. In addition, it may prevent the passage of the photosynthetic spectrum to reduce the growth of weed (Vox & Schettini 2007).

Two variables of reflectance and radiation transmission coefficients in the mulches were identified as important parameters in soil temperature identification. However, there was a significant difference between the measured and calculated values of temperature by model for the transparent film due to the air layer between the soil and the mulch (Ham et al. 1993; Ham & Kluitenberg 1994; Heißner et al. 2005).

Therefore, reliable results were obtained by designing an experimental set-up with a control over all parameters that will be measured in this study. In this research, by creating an appropriate experimental environment, the soil moisture and temperature changes under the mulch were investigated with a quasi-solar thermal source. Previous studies had relied on standard methods for degradability, which was also used in the present research (Mortazavi et al. 2013; Akrami et al. 2016). In this study, the verification tests of mulch degradability were also carried out.

2. Material and Methods

2.1. Preparation of mulch films

The mulch films were produced from the raw material of LDPE and color additives were produced in Imam Petrochemicals Port. For all types of Oxo-biodegradable, an additive was used that combines the salts of fatty acid (50 to 70%), rare compositions of earth (10 to 20%) and lubricants (10 to 20%) from P-Life Japan Inc.

The first stage of the production included mixing the granules with a ratio of 100 and 3 *phr* for LDPE and the additive, respectively. In the next step, the mixture was extruded. The extruder was set to gradually increase the initial temperature to 140, 150, 155, 160, and 165 °C and the rotational speed of the co-rotating twin screw was set to 150 *rpm* by the ZSK extruder. The thin films were prepared using film blowing machine (Coline BL 180/600) by adjusting the temperature of the device at 8 points: 140 to 175 at intervals of 5 °C and adjusting spiral of 25 to 30 *rpm*. The average film thickness was obtained according to the ISO 4593 standard at 10 points (ISO 1993).

2.2. *Degradability tests*

In this test, the samples were exposed to the UV-light type A (at a wavelength of 340 nm) with a radiation intensity of 0.89 ± 0.02 watts m^{-2} nm^{-1} in accordance with the ASTM D-5208 standard for 200 hours at 50 °C (ASTM 2014). The accuracy of mulch degradability is determined by examining the weighted average of Mw, gel content and elongation at the breakpoint of samples after the aging test.

The weighted average molecular mass was determined according to ASTM D6954 and the method of gel permeation chromatography. The apparatus used in this experiment was PL GPC 220 and TCB solvent was used at 160 °C. To prevent the degradation of the polymer during the test, antioxidant of BHT was added to the mixture. (ASTM 2004). The gel content was measured according to the ASTM D 2765-1 standard so as to determine the portion of the polymer that becomes crosslink in an abiotic destructive process, which is unsolvable (ASTM 2006). Initially, in this method, a sample with a specified mass of residual materials from the abiotic destructive test was immersed in a boiling solvent P-Xylene for 12 *hrs*, while it was inside the 120 steel wire mesh. During this period, the soluble part of the sample is dissolved in the solvent and only the insoluble portion (gel) remains inside the wire grid. The remaining mass of the sample is determined by weighing after removing the grid and drying it. Finally, the gel content of the sample is obtained according to the Equation (1):

$$\%gel = (W_2 / W_1) * 100 \quad (1)$$

W_1, is the initial sample weight and W_2, is the residual weight of the sample after being placed in the boiling solvent.

Test conditions for measuring the tensile properties of plastic films with thickness less than 1 mm has been determined according to the principles of ISO 572-3. The Tinius Olsen H5KS from England was used to carry out the tensile tests.

2.3. Experimental Setup

In order to simulate the test conditions on agricultural mulches and in accordance with open space and to create the same conditions for all experiments, an environment was created with the control of the temperature and intensity of electromagnetic radiation. To achieve this goal, an isolating chamber was used to adjust the temperature and ventilation and a test system was placed inside the chamber. The design and construction of all the above mentioned equipment was carried out at the laboratory of Department of Biosystems Engineering, at Ferdowsi University of Mashhad. The temperature of the isolation chamber was adjusted by a temperature controller of the Samwon SU-105 model and the cooling and heating system were connected to it. Therefore, the only heat source delivered to the surface of the soil was electromagnetic waves from the bulbs. The test system includes a soil bed with typical traits for the plant cultivation. The sidewall and floor of the soil bed were covered with thermal insulation from EPS of a thickness of 60 mm.

The distribution of the heat at various depths of the soil with the highest accuracy demands a lot of temperature sensors at certain intervals from each other and in different parts at each level from the depth. In this research, three temperature sensors of DS18B20 have been used for every depth level. The water-proof model of these sensors is placed at the desired depth to measure the temperature which has relatively high moisture content. Also, the four depths were defined between 0 and 40 cm (Figure 1).

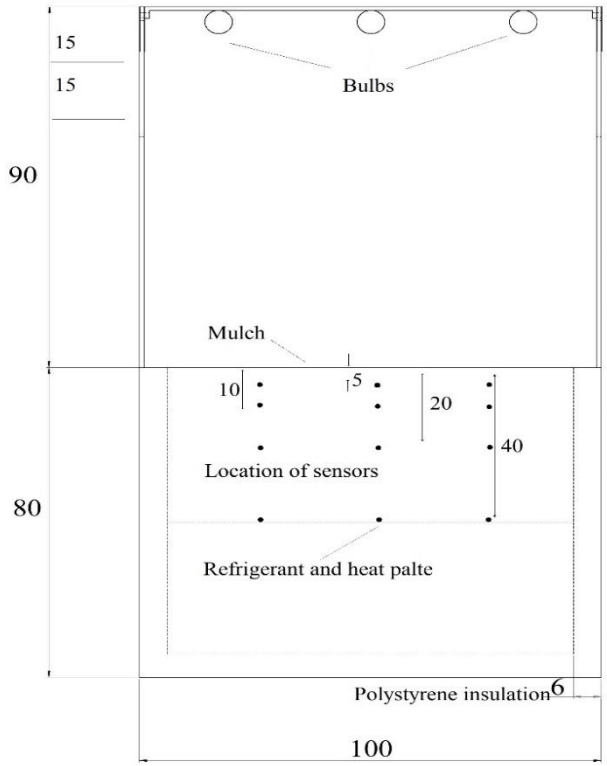

Figure 1- Designed system for evaluation of the mulch by examining the thermal distribution and soil moisture distribution (Measures in cm)

The YL-69 moisture sensor was used to measure moisture content at three depths. Because the AW in agricultural activities should be between FC and PWP, was tried to set the soil moisture content in this range at the start of the experiments. Each of FC and PWP values were calculated using PTF provided by Mohanty et al. with coefficients of 0.9 and 0.84, respectively. (Mohanty et al. 2015). Temperature and moisture changes in the soil were recorded at different depths during the experiment at specified intervals.

The high-pressure sodium lamp was used to simulate the solar radiation which perfectly match the photosynthetic range of solar radiation when compared with other lamps.

The Kipp & Zonen Pyranometer CMP3 model was used to determine the proper location of the bulbs so that the intensity of the radiation can be constant across all parts of the surface. Given the average sunlight during sun hours in the area under study (Khorasan-e Razavi: Iran: 250 to 350 W m^{-2}), the minimum, average and maximum values of the radiation intensity at 60, 75 and 90 cm heights of the bulbs were selected from the soil surface through measuring the amount of radiation from the bulbs at 3 points in the soil surface. This radiation range has also been used in previous studies (Braunack et al. 2015; Shakeri 2016).

The actual view of the isolation chamber and the equipment used in the test system are shown in Figure 2. At the start of experiment, the temperature and humidity of the whole soil bed are set at a specific point so that the changes in heat and moisture caused by the radiation can be analyzed. During the experiment, a refrigerant system was prepared and it was tried to maintain a constant temperature at a depth of 40 cm and to create boundary conditions at this depth. To achieve this, the cooling circuit was activated with water. Both the thermal and refrigerant systems were connected to a temperature controller of the Samwon SU-105 model and at the set point commands were issued to set up each of the thermal and refrigeration systems.

Figure 2- Representation of the equipment used in conducting experiments to record the changes in soil temperature and humidity. A: Temperature isolation chamber (A1: Air conditioning fan A2: Electronic board setting of temperature and startup of heating and freezing system). B: The space above the soil bed (B1: The location of the connection cables to the sensors B2: The entrance of refrigerant pipes to a depth of 40 cm). C: The system for installing a heating and freezing system at a depth of 40 cm (C1: PT100 temperature sensor, C2: key and connection to the coolant flow regulator pump, C3: key and connection to the thermal plate embedded at a depth of 40 cm). D: Electric circuit for HPS bulb startup: Capacitor, ballast and Igniter for each bulb

The produced mulch films spread over the surface of soil and the irradiation system and data recording was launched. The temperature of the chamber, that embraced the experimental system, was kept unchanged until the end of experiment so that only the HPS light bulbs can be affected and the effect of surrounding temperature was neutralized (25 °C). The experiment lasted until the different surface temperatures in the soil bed reached a fixed point, which occurred approximately after 48 hours.

2.4. Experimental design and statistical analysis

The experimental design used in this study was the Response Surface Method with the face composition central design (FCCD), which is widely used among the response surface methods (Bas & Boyaci 2007). Independent variables were coded at three levels of -1, 0, +1 and the experiments were performed at a central point with five replications (Table 1).

Table 1- Coded levels of values of independent variables

Independent variables	*symbol*	*-1*	*0*	*1*
Radiation Intensity (w/m^2)	A	250	300	350
Time (h)	B	12	30	48

In this research, equation (2) was used to determine the relationships between independent variables (numerical variables, including intensity of electromagnetic radiation in 250, 300 and 350 W m^{-2}, duration of radiation at 12, 24 and 48 hours, and nominal variables, such as the mulch type in four levels like transparent, dark, biodegradable and uncoated surfaces) with the predicted response (the soil temperature at depths of 0, 10, 20, and 40 cm):

$$Y = b_0 + \sum_{j=1}^{k} b_j x_j + \sum_{j=1}^{k} b_{jj} x_j^2 + \sum \sum_{i<j}^{k} b_{ij} x_i x_j \quad (2)$$

Where; y, is the predicted response; b_0, is the constant coefficient; k, is the number of independent variables; b_i, linear effects; b_{jj}, is the effect of squares; b_{ij}, is interaction effects, and x_i, x_j are independent encoded variables.

The fitting of response levels and the significance of model coefficients were determined by the analysis of variance per response in Design Expert 10 software. The validity of model was evaluated using R-squared, R-adj and CV.

To comparison of the mean of the different levels of each treatment was also made by Minitab 17 software so as to evaluate the moisture changes during the experiment as well as in different depths of the soil.

3. Results and Discussion

3.1. Molecular weight distribution

The results of variations in the concentration of test solutions during the retention time (exit from the device column) are represented in Figure 3. Since smaller particles of the solution are placed in the stationary phase cavities of the device and then they cross through the gaps, so time out of the device becomes longer. Therefore, the distribution of the solution concentration in the outlet solution can show the distribution of molecular mass of solutions very well. The mean number of *Mn* was used to determine the flexibility and viscosity. An accurate assessment for the relative amount of materials with higher molecular weight, which has a high contribution in the physical properties of polymer, was measured by the weighted average of *Mw*. Weighted average of *Mz* was used to detect the presence of materials with very high molecular weights. *Mv* and the average viscosity to molecular weight are correlated. The molecular weight distribution index of PDI indicates the heterogeneity of the sample and this ratio is close to one of homogeneous polymers (Equations. 3-7). The molecular weight was calculated and the percentage of the distribution below the distribution graph in each molecular weight range is presented in Table 2 and Table 3 respectively.

$$PDI = \frac{\bar{M}_w}{\bar{M}_n} \tag{3}$$

$$\bar{M}_v = \left[\frac{\sum_{i=1}^{N} H_i (M_i)^{\alpha}}{\sum_{i=1}^{N} H_i}\right]^{1/\alpha} \tag{4}$$

$$\bar{M}_z = \frac{\sum_{i=1}^{N} H_i (M_i)^2}{\sum_{i=1}^{N} (H_i M_i)} \tag{5}$$

$$\bar{M}_w = \frac{\sum_{i=1}^{N} (H_i M_i)}{\sum_{i=1}^{N} H_i} \tag{6}$$

$$\bar{M}_n = \frac{\sum_{i=1}^{N} H_i}{\sum_{i=1}^{N} (H_i / M_i)} \tag{7}$$

Where; H_i, is the height of the GPC curve and *Mi*, is the molecular weight of substance washed in i-th magnitude of the inhibitory volume and α is the Mark-Houwink coefficient.

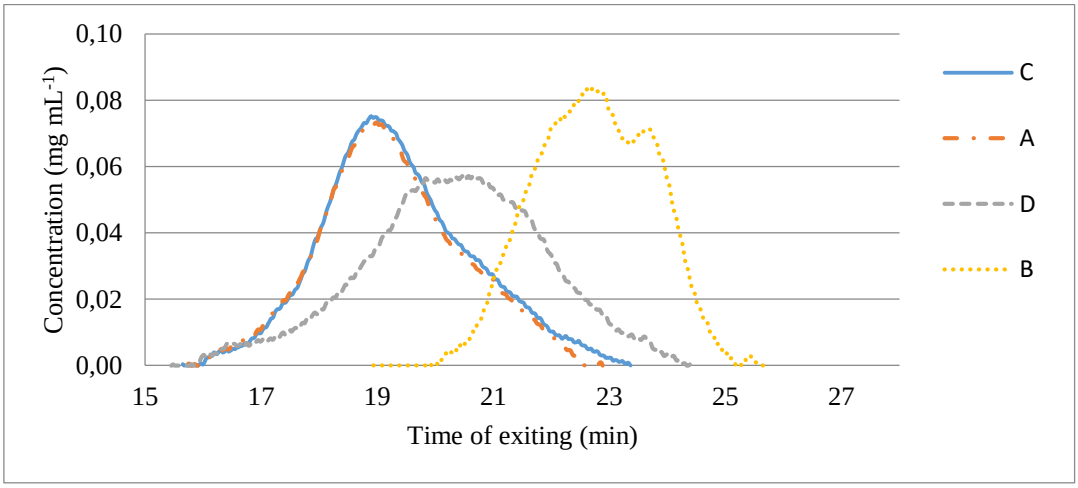

Figure 3- Changes in the concentration of solutions during exiting from the GPC device. A: with biodegradable additive and no UV aging, B: with biodegradable additive and UV aging, C: no biodegradable additive and no UV aging, D: no biodegradable additive and with UV aging

Table 2- The values of the average molecular weight calculated on the basis of g.mol^{-1} and the distribution index of molecular weight and the Mark-Houwink coefficient of GPC detector

Mulches	*Mn*	*Mw*	*Mz*	*Mv*	*PDI*	*α*
No additive	23696.6	142174	487420	93850.1	5.99979	0.4457
With additive	26499.4	169563	671216	96978.4	6.39875	0.3453
No additive and with UV aging	8482.23	119533	937725	51743.4	14.0922	0.4143
With additive and UV aging	1375.58	4906.56	13445	3147.69	3.5669	0.3021

Table 3- Percentage range for distribution of average molecular weight (A: with biodegradable additive and no UV aging, B: with biodegradable additive and UV aging, C: no biodegradable additive and no UV aging, D: no biodegradable additive and with UV aging)

Mw (g mol^{-1})		*Sample value in range (%)*			
Lower range	*Upper range*	*A*	*B*	*C*	*D*
1E+6	5E+6	2.61	0	1.522	2.63
1E+5	1E+6	39.65	0	38.62	19.13
1E+4	1E+5	47.83	13.65	49.56	51.17
1000	1E+4	9.88	62.42	10.27	25.25
72	1000	0	23.91	0.009	1.8

The advantage of using the GPC method with triple detectors (light scattering, differential refractive index, and viscometer), in comparison with similar methods, such as static light scattering and osmometry, lies in the fact that not only does this method determine the average value of molecular weight, it also facilitates the full distribution of the material. Given that each part of the distribution graph of concentration represent a specific feature of solution, the physical and mechanical properties of the solutions can be obtained by calculating each the weighted average parameters. As already mentioned in the existing literature, the use of the GPC method is necessary, compared to other chromatographic methods. This is due to the existence of tight and long chains in LDPE polymers. The values obtained for the mean of molecular weight and Mark-Houwink coefficients with different solvents (TCB and DBM) are in line with those of previous studies (Coto et al. 2007; Boborodea et al. 2015). According to ASTM D6954 for thin films, when passed the UV aging period, biodegradable mulches must gain average weight-average molecular weight (MW) of 5000 g mol^{-1} or less. According to Figure 3, since solutions "B" and "D" stayed inside the device a long time, they will have smaller particles and will definitely have a lower average molecular mass. Also, about 63% of the particles of "B" sample were in the range of 1,000 to 4,000 and the rest was less than 5000 g mol^{-1}. Therefore, one of the conditions for bio-degradability was met in the mulch type produced in this experiment. In this type of LDPE mulch about 23% of the particles were higher than 5000 g mol^{-1}.

3.2. Gel content

Gel formation is a frequent side reaction of the oxidative degradation in polymers, especially polyolefin. Gels are cross-linked structures resulting from the free radical nature of oxidative degradation. They are insoluble in nonreactive solvents, which do not break additional bonds. Normally, gels are not available to biodegradation. Some gels dissolve into further oxidative degradation and become available for ultimate biodegradation. However, the pro-oxidant (catalyst) may be excluded from the gel structure because of solubility changes in the gel phase. In this case, the gel would become a non-degradable or very slow-degradable new fraction within the polymer. It is important to establish the extent of gel and its nature or permanence in the polymer residue (ASTM 2004). The comparison of four different groups in samples which were produced with three replicates per group is presented in Figure 4. This is for determining the residual content of the insoluble matter, which results from the gel content test. The results showed that the percentage of residual samples which are not under ultraviolet aging and lack biodegradable additives is more than other samples. The functional properties of polymer obtained from cross linking, based on the determination of gel content, can be specified and reviewed. Therefore, with the increase of the additive of Dicumyl peroxide as a cross linking agent, the percentage of gel content increases. There is a probability of degradation in the sample with less than 5% of non-solvents and with an additive after being exposed to ultraviolet aging.

Gels are cross-linked polymer structures, which are insoluble in solvents, and they do not break the primary or cross-linking bonds in the polymer. Cross-links were created during Oxo-biodegradation of polymers. Chemical bonds, especially carbon-carbon bonds are created by the degradation process, and consequently, they extremely resistant to solvent degradation. The results of this study indicated that the used additive could not create a crosslinking among the polymeric chains of the production. Therefore, the percentage of residual non-additive samples was even higher than the biodegradable samples given the aging condition. The reduction of gel content suggests that additive molecules have created covalent bonding among the base form of polymer molecules. These materials lead to the loosening of polymeric chain, and consequently, the residual percentage of the sample is reduced when it is placed in a hydrocarbon solvent solution. This indicates the natural behavior of linear viscoelasticity at the end points of the chains (Zhang et al. 2012).

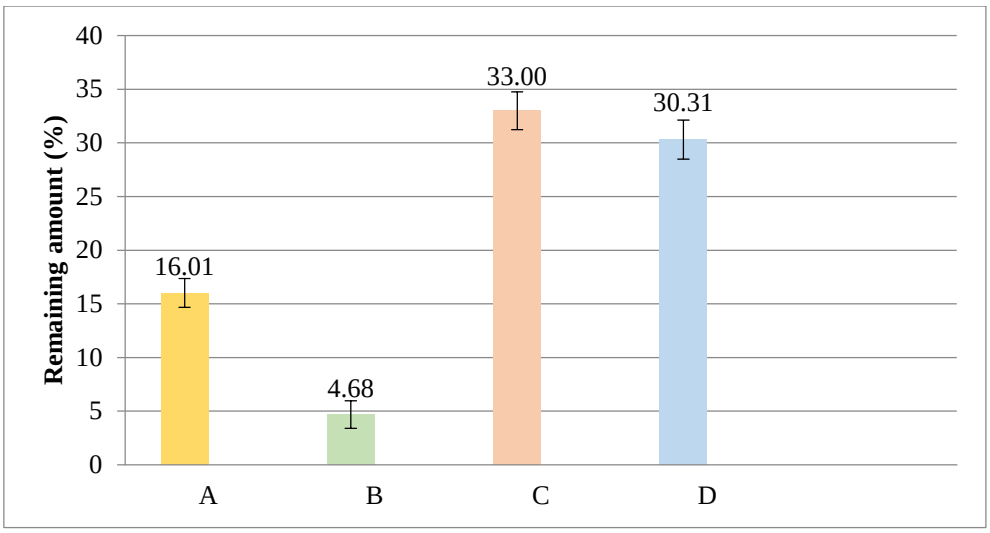

Figure 4- Results of testing the gel content of the produced samples (A: with biodegradable additive and no UV aging, B: with biodegradable additive and UV aging, C: no biodegradable additive and no UV aging, D: no biodegradable additive and with UV aging)

3.3. Tensile test

According to the European Standard EN 13655, in order to maintain mulch integrity in the field and to deal with environmental damage (resulting from wind, rain or animals) a minimum value of resistance was defined i.e., tensile strength and elongation at breakpoints of 12 *MPa* and 200%, respectively (Takahashi 2007). This should be noted that this is a prerequisite for the use of agricultural mulches. If biodegradable mulch is used, the results gained from the ultraviolet aging test should also be taken into account. The elongation at breakpoint concerning at least 75% of tested specimens in the initial strain was expressed in a standard below 5 percent (Wu et al. 2004). The results of the tests are shown in Figure 5 at the moving speed of 50 mm.min^{-1} with the moving upper jaw and the constant lower jaw of the strength measuring device.

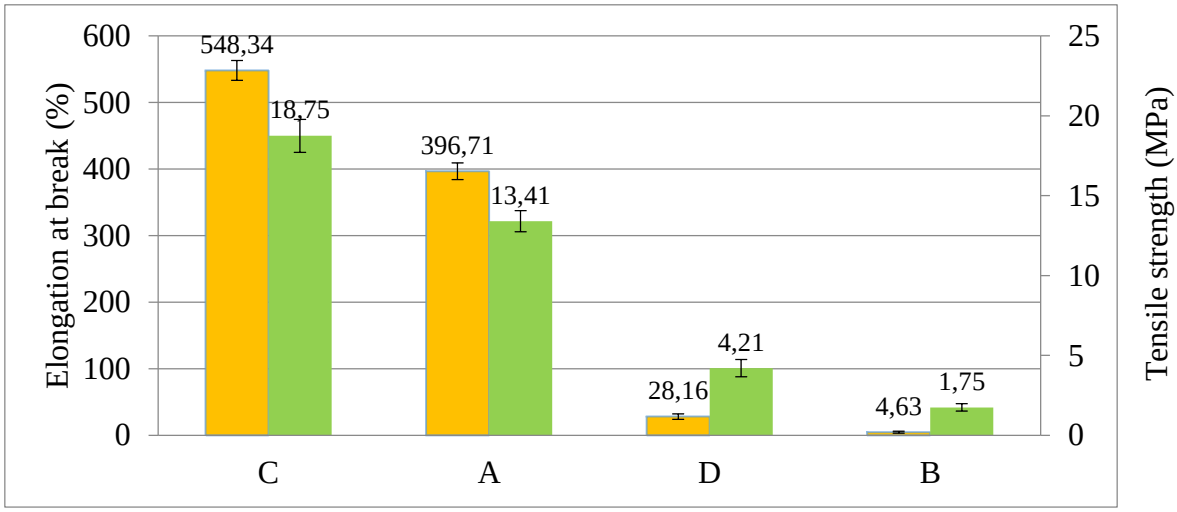

Figure 5- Chart of variation percentage increase in length and tensile strength at break point in pre and post ultraviolet aging conditions

The addition of the biodegradable additives decreased the strength of the samples. Incorporating these compounds into strong hydrogen bonding in polyolefin compounds can decrease the strength of polyethylene chains and affect the mechanical properties (ASTM 2018). However, it should be noted that with the addition of these additives, a special feature is gained in the mulch, which is far more important than the amount of reduced strength. In fact, as can be seen in the results, the mechanical properties obtained from the additive samples are higher than the minimum requirements of EN 13655 and there will be no problem in using these products. The biodegradable product in the present study has a higher mechanical strength, as opposed to other products presented in previous similar reports (Briassoulis 2004, 2006). Sample test results conform to the ASTM D6954 standard when they are aged under the conditions of biodegradability of the samples (ASTM 2004). Despite the fact that the tensile strength of the LDPE mulch has reached the specified range for biodegradability after UV aging (4.21 *MPa*), but the elongation at the breakpoint failed to reach the required range.

3.4. Heat and moisture changes in the soil bed

According to the results of variance analysis of temperature and humidity at different levels of soil by the response surface method, a significant difference was observed at 5% level for the tested mulches (Figure 6, Figure 7, Figure 8). The comparison of temperature variations at different levels of soil indicated that with the increase of soil depth, the amount of heat penetration decreases as Figure 8 and Figure 9 illustrate. Acceptable values for the statistical indices were calculated through the response surface method. The aim was to predict temperature variations at different soil levels for different mulch types. This method was effective in terms of reducing the number of experiments and saving time (Table 4 and Table 9).

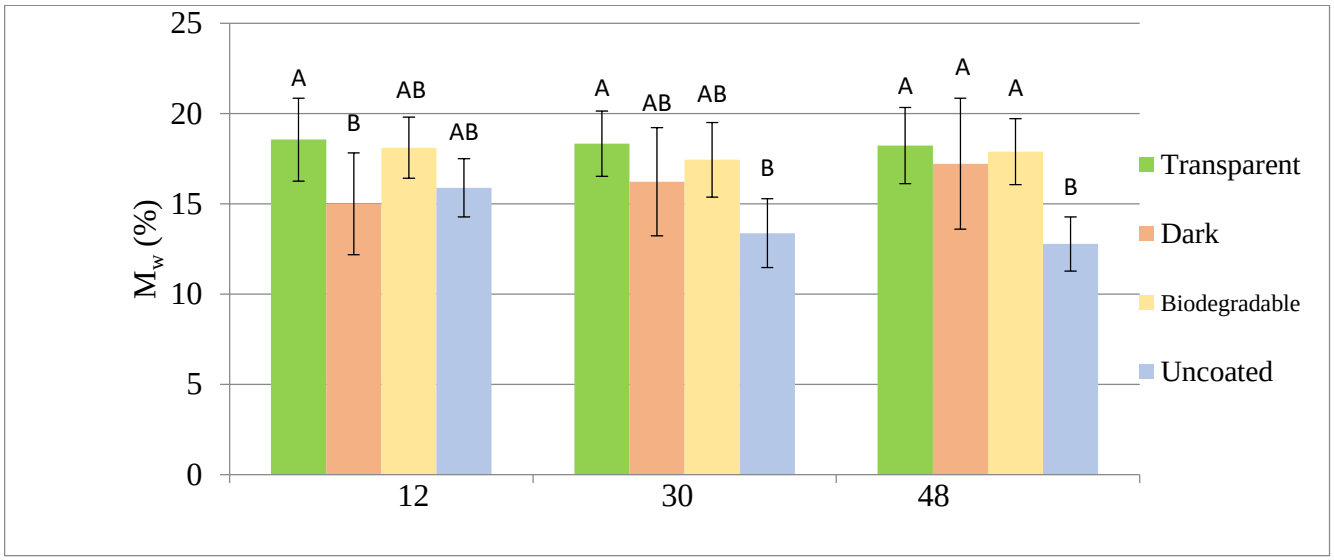

Figure 6- Moisture changes during the test run time for different mulches

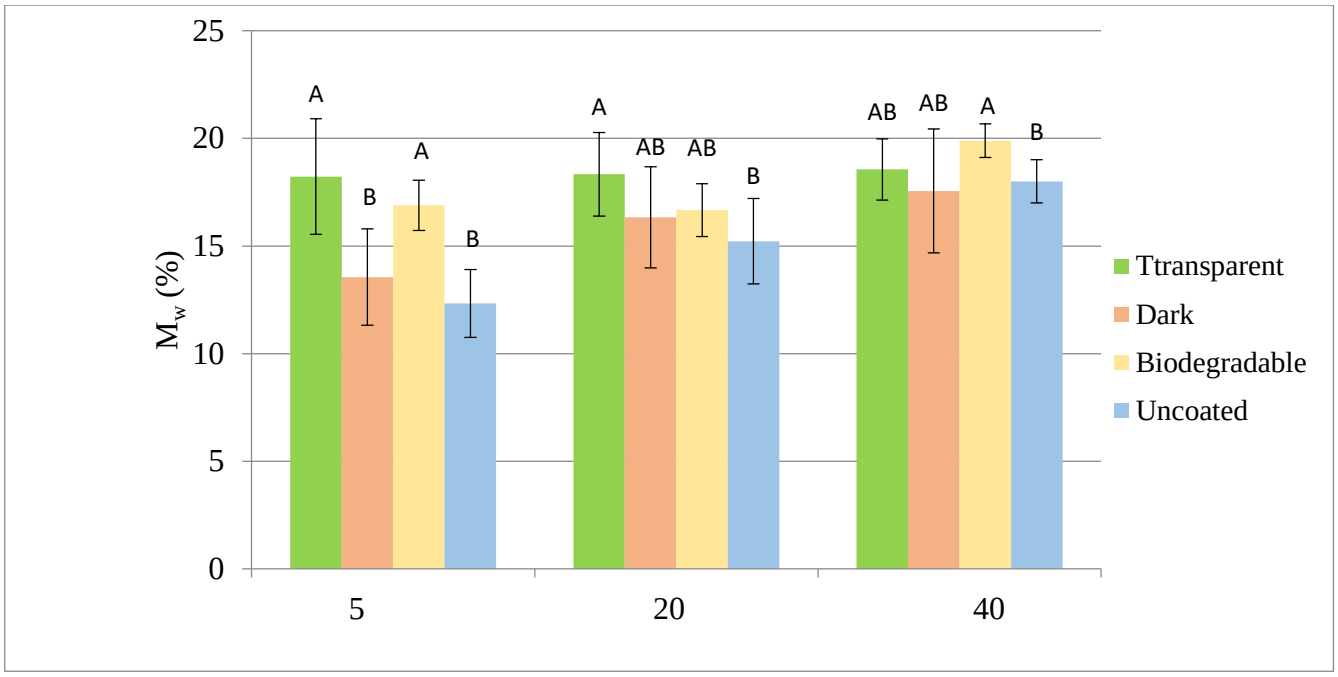

Figure 7- Moisture changes during the experiment at different depths of soil in different mulches

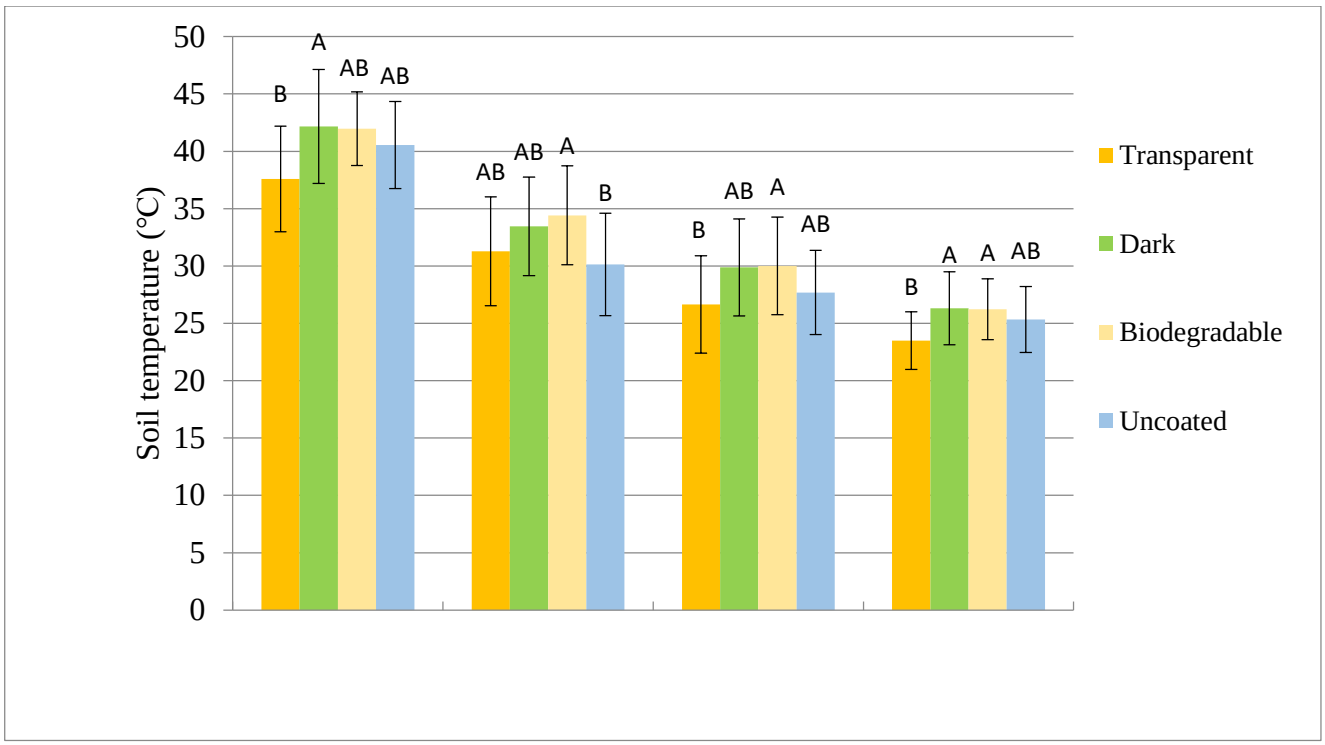

Figure 8- Temperature variations at different depths for different mulches

Table 4- Extraction results based on the response level for the statistical index and predicted equations for temperature in different depth of soil. A: intensity of radiation (w m^{-2}). B: Radiation duration (hr)

		Depth (cm)			
		0	*10*	*20*	*40*
Statistical indexes	*Mean*	40.57	32.32	28.56	25.34
	Std. Dev.	1.03	0.66	0.73	1.11
	R-Squard	0.9678	0.9877	0.9819	0.9224
	Adj-R-Squ	0.9470	0.9798	0.9703	0.8587
	C.V. %	2.54	2.04	2.56	4.40
Final Equation for every mulch	*Black*	T0=-199.04+1.48*A+0.51*B -1.87E-04*A*B-2.33E -03*A^2-4.67E-03*B^2	T10=55.54+0.49*A+0.53*B +4.920E-04*A*B-7.89E-04*A^2-6.47E-03* B^2	T20=-86.29+0.7*A+0.28*B +7.72E-04*A*B-1.17E-03*A^2-3.89E-03*B^2	T40=-59.75+0.58*A-0.17*B +6.6E-04*A*B-1.02E-03*A^2+2.81E-03*B^2
	Transparent	T0=221.8-1.38*A +0.68*B-1.87E-04*A*B +2.4E-03*A^2-6.495E-03*B^2	T10=107.950.627*A+0.48*B +4.92E-04*A*B+1.07E-03*A^2-4.85E-03*B^2	T20=134.46-0.77*A+0.1*B +7.72E-04*A*B+1.26E-03*A^2-6.24E-04*B^2	T40 =78.76-0.35*A-0.08*B +3.34E-04*A*B+5.42E-04*A^2+2.56E-03*B^2
	Bio degradable	T0=-92.05+0.83*A+0.36*B-1.87E-04*A*B-1.36E-03*A^2-2.42E-03*B^2	T10 =-25.8+0.31*A+0.64*B +4.92E-04*A*B-5.53E-04*A^2-8.11E-03*B^2	T20=-18.83+0.28*A+0.4*B +7.72E-04*A*B-5.42E-04*A^2-5.47E-03*B^2	T40=+.31+0.16*A+0.15*B-1.56E-04*A*B-3.21E-04*A^2+1.25E-03*B^2
	No mulch	T0=82.72-0.39*A+0.48*B-1.87E-04*A*B+7.43E-04*A^2-2.84E-03*B^2	T10=6.62+0.05*A+0.49*B +4.92E-04*A*B-8.31E-05*A^2-5.14E-03*B^2	T20=39.45-0.15*A+0.14*B+7.72E-04*A*B+2.65E-04*A^2-1.75E-03*B^2	T40=55.05-0.24*A-0.1*B +6.01E-04*A*B+4.2E-04*A^2+2.07E-03* B^2

The highest and lowest heat transfer rates were observed in the transparent and biodegradable mulch, respectively. So, the lowest soil temperature was recorded in transparent mulch at levels of 0 to 10 *cm*, which continues to the lower levels in the same way. The radiation energy input from the soil surface, after passing through the mulch on the pathway to the soil, is used to evaporate the moisture existing in the bed. The energy used for the evaporation increases and the velocity of temperature rise in the depth decreases.

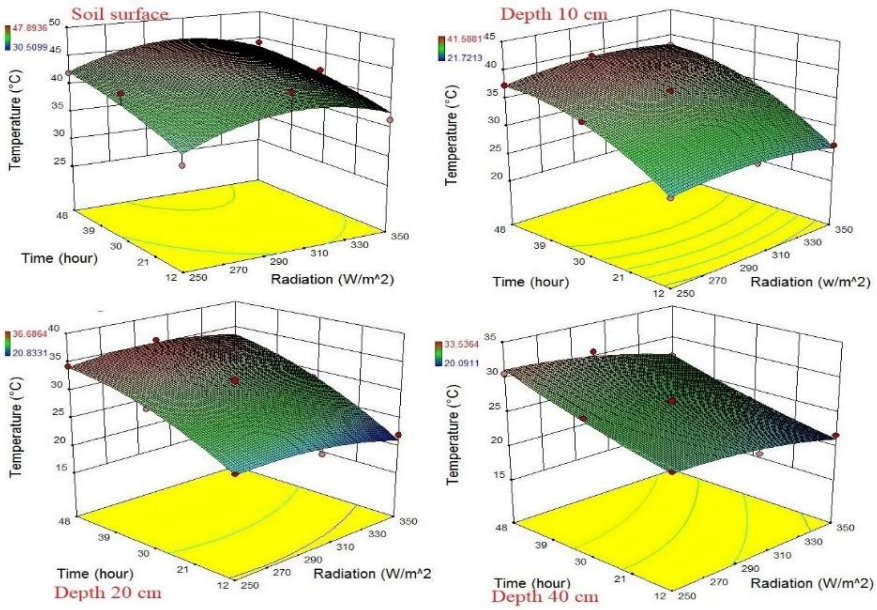

Figure 9- Soil temperature changes at different depths in biodegradable mulch

The study shows that there is a close relationship between temperature and soil moisture. Moisture can directly affect heat transfer from the surface to the depth of the soil and can change the thermal conductivity coefficient (Scarascia-Mugnozza et al. 2006). In this research, the highest temperature was observed for the dark mulch. It was assumed that the absence of covering at higher levels could increase the soil temperature more than other treatments, yet the results did not confirm this assumption. Because most of the consumed energy was dedicated to the evaporation and transfer of moisture from lower levels to the surface of the soil. Mulching decreased the moisture transfer rate and consequently the energy consumption (Yin et al. 2016). In the study by Xiukang et al. (2015), the temperature difference at high levels between coated and uncoated soils was considered by the absorption and reflection of solar electromagnetic waves (Xiukang et al. 2015). The reason for the difference in temperature between the mulches can be attributed to the optical properties, which include the coefficients of reflection, absorption and transmission of electromagnetic waves in each of them (Kasirajan & Ngouajio 2012). As the previous research has pointed out, the conservation of thermal energy within the soil bed increases with increasing the transmissivity coefficient in long wave (middle infrared) in the mulch; a feature being visible in biodegradable mulch (Vox & Schettini 2007). Perhaps this can justify the temperature of the uncoated soil. The temperature cannot be maintained due to the absence of these waves and the temperature of uncoated soil was observed less than the other ones. On the other hand, passing short waves (near infrared) from the mulch will direct heat energy into soil (Touchaleaume et al. 2016). The passage of these waves in the mulch without biodegradable additive was more than that of other types; therefore, the thermal energy transmission in this type of mulch increased, leading to an increase in temperature and use of energy to evaporate the process. This is evident in the dark mulch, which can be due to an increase in the rate of evaporation, and consequently, accelerating the decrease in moisture content (Moreno et al. 2016).

By inserting the carboxylic acid group used in the biodegradable additive into the polyethylene structure, the polymeric chains pore is filled, which reduces the penetration rate of water vapor and helps maintain moisture (Han & Krochta 1999). Specific heat capacity for soil remains stable when moisture in soils, which use biodegradable mulches, is maintained. As a result, more energy is needed to increase its temperature. For this reason, the increase of temperature in soils with lower moisture content will be higher than other soils. This is because the specific heat capacity decreases (Zhang et al. 2008). The presence of more pores in the molecular structure of polyethylene, compared with the biodegradable structure, increases the permeability of water vapor in this type of coating. The loss of moisture in the soil bed accelerated the temperature rise because of its reduced specific heat capacity. As a result, higher temperatures were attainable in the soil bed with dark polyethylene coatings. It should also be noted that percentage of waves passing the near infrared and visible region of dark mulches is higher than other (Moreno et al. 2016). To have the temperature rise and degradability simultaneously, the dark color additives are suggested to be used in the compounds of biodegradable mulches.

Based on the results obtained and comparing them with the results of previous studies, it can be concluded that the use of mulch can regulate the vertical distribution of soil moisture. Moisture can be maintained because it is not evaporated on the soil surface due to the lack of porosity changes. As the high temperature of the soil accelerates and improves in the plant growth process, excessive increase in some plants, for example in corn, can bring about some problems, including leaf shrinkage and may also reduce seed filling. Also, the improvement of microbial activity in the soil and the increased rate of degradation of soil organic matter are other consequences of excessive increase of soil temperature (Zhou et al. 2012; Yin et al. 2016). Besides, soil placement at a temperature of close to 50 °C near the surface (0 to 10 cm depth) for 3 to 4 weeks in soil solarization is recommended as it reduces and manages plant diseases, and controls pathogens of the soil and weeds (Luvisi et al. 2015, 2016).

Therefore, this research shows that the stated goal can be achieved provided that the designed system is put into practice. The increase of soil temperature and improvement of moisture conditions can lead to a better selection of the best depth of cultivation for farmers, when compared with the non-use of mulches (Subrahmaniyan & Zhou 2008; Liu et al. 2009; Xiukang et al. 2015).

4. Conclusions

Considering that the evaluation of the efficiency of soil mulches in open fields requires a great deal of time, the method presented in this research and the design of the laboratory environment and the response surface method can significantly reduce the time and energy demanded to achieve the results. Also, the quality and production capabilities of the product were assured through a careful-examination of the validation test results for biodegradable mulches in accordance with the standards. Temperature and moisture content in different soil depths at the time applying the mulches significantly changed during the experiment process while this was not the case in non-mulched soil. This difference was more tangible at higher levels (0 to 10 *cm*). Nevertheless, the amount of moisture change for transparent and degradable mulches was not significant during the experiments. The sharp temperature increase from higher levels to the lower depths of dark mulch and no coating was observed. While the temperature in the depths of the soil increased with a mild slope for transparent and biodegradable mulch. According to the results of this research and the investigations carried out in this area, given the importance of preserving the environment, the production of biodegradable mulches can facilitate the usage of the evaluation method applied in the study for a wide range of applications.

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Abbreviations and Symbols	
AW	Available water
BHT	Butylated hydroxytoluene
CV	Coefficient of variation
DBM	Dibenzoylmethane
EPS	Expanded polystyrene
FC	Field capacity
FCCD	Face composition central design
GDD	Growth degree days
GDH	Growth degree hours
GPC	Gel permeation chromatography
HPS	High pressure sodium
LDPE	Low density polyethylene
Mn	The Number Average Molecular Weight
Mv	Viscosity Average Molecular Weight
Mw	The Weight Average Molecular Weight
Mz	Z-average molecular weight
PBAT	Polybutylene adipate-co-terephthalate
PDI	Polydispersity index
PHB	Polyhydroxybutyrate
PL GPC	Polymer laboratory gel permeation chromatography
PTF	Pedotransfer functions
PWP	Permanent wilting point
TCB	Trichlorobenzene
UV	Ultra violet

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