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RESEARCH ARTICLE

The Effect of Some Vegetable Oils Added to Dairy Calf Rations on *In Vitro* Feed Value and Enteric Methane Production

Ali Kaya • Adem Kaya*

Atatürk University, Faculty of Agriculture, Department of Animal Science, Erzurum/Turkey

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ABSTRACT

The aim of this study was to determine the effects of the addition of Safflower, Sunflower and Corn vegetable oils to dairy cattle rations on *in vitro* gas and methane production, true dry matter (TDMD), organic matter (TOMD) and NDF (TNDFD) digestibilities values and microbial protein (MP) production. Dairy cattle TMR ration consisting of milk feed, corn silage, alfalfa hay and meadow hay was prepared as the control group, and the experimental groups were prepared with the addition of safflower, sunflower and corn vegetable oils at the level of 3% in each of the control groups, respectively. Vegetable oils added to the diet significantly affected *in vitro* gas production and organic matter digestibility (OMD). Methane (ml) production values in the experimental groups varied between 10.00 and 10.71 ml. The Metabolic energy (ME) and OMD values of the control and experimental groups varied between 7.00 and 7.29 MJ/kg DM and between 53.78 and 51.20. TDMD values of the rations were determined between 48.49 and 52.63%. While the control group had the highest TDMD value, the ration containing safflower oil had the lowest TDMD value. TNDFS values of the rations varied between 67.26 and 68.80%. As a result; Since the vegetable oils added to the ration increase the net energy lactation (NE_L) content of the ration, it can be said that it used to meet the energy needs of high milk yielding cattle in the lactation period, provided that they do not exceed the upper limits specified in the literature.

Please cite this paper as follows:

Kaya, A., & Kaya, A. (2021). The effect of some vegetable oils added to dairy calf rations on *in vitro* feed value and enteric methane production. *Journal of Agricultural Production*, 2(1), 1-6. <https://doi.org/10.29329/agripro.2021.344.1>

Introduction

Ruminants play a key role in sustainable agriculture by using the roughage and agricultural by-products that are not consumed by humans thanks to the microorganisms in the digestive system, and by converting them into high-quality foods such as meat and milk that can be consumed by human beings (Wright & Klieve, 2011; Gerber et al., 2013). In the rumen, volatile fatty acids are converted into H₂ and CO₂ as a result of fermentation of microorganisms (Hegarty & Gerdes, 1998). Archaea play an important role in rumen health and in producing and removing enteric methane from the rumen by hydrogenotrophic pathway by using H₂, which has toxic effects for certain microorganisms, as a substrate together with CO₂ (Hook et al., 2010). About 90% of the methane produced by archaea in ruminants is burped and the remainder is expelled from the rectum (Murray et al., 1976). It has been reported

that methane formed in ruminant animals causes energy loss due to the removal of metabolic hydrogen and carbon produced by fermentation (Martinez-Fernandez et al., 2014). They reported that the energy loss due to methane in ruminants is 2-12% of the digestible energy, and this rate rises to 15-18% in the case of feeding with low quality roughage (Kaya et al., 2012). On the other hand, it was stated that the greenhouse gas effect of methane released from ruminants as a result of fermentation is the effective gas after CO₂ gas (Sejian et al., 2011). For this reason, they stated that there are various management strategies in reducing the methane produced by ruminants, such as the quality of the roughage in the ration, organic acids, phenolic-containing plants and feed intake level, the speed of the feed passing through the digestive tract, the saturation level of the fat in the ration (Kaya et al., 2012; Gomaa et al., 2018). Martin et al. (2010) reported that the most effective method to reduce methane is

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to supplement the diets of ruminants with fat. Mathison (1997) stated that the addition of 4% canola oil to the ration containing 85% concentrated feed reduced enteric methane by 33%, in addition, coconut oil was the most suppressive of fiber digestibility in the rumen. The reduced methane production due to the addition of unsaturated fats to the diet has been attributed to the fact that fatty acids can serve as electron acceptors during biohydrogenation in the rumen (McAllister et al., 1996). If enteric CH₄ production is reduced, substrates (H and CO₂) can be included in the fermentation products, which will allow the animal to increase its energy use efficiency (Haisan et al., 2014). In this study, it was investigated whether the addition of 3% sunflower, safflower and corn oil to the diets of dairy cattle had an effect on gas production, methane production, microbial protein and *in vitro* true digestibilities in order to reduce methane released as a result of fermentation of feeds in ruminants.

Materials and Methods

Feed Material and Treatment Groups

In the study, TMR rations (Control) consisting of 25% milk feed, 25% corn silage, 15% alfalfa hay and 35% dry meadow grass given to dairy cattle in Atatürk University Research and Application Farm were used as feed material. Experimental groups were prepared by adding 3% safflower, sunflower and corn oils to TMR rations.

Chemical Analyzes

The samples belonging to the experimental groups were ground to pass through a 1 mm sieve for chemical analysis and dry matter, ether extract, crude fiber, crude protein, crude ash contents were determined according to the methods reported by AOAC (1998). The ADF, NDF and ADL contents of the ration content were determined with the ANKOM 2000 Fiber Analyzer device according to the method reported by Van Soest et al. (1991). The nutrient contents of the experimental groups are given in Table 1 on the basis of dry matter.

Table 1. Chemical compositions of the experimental groups

Ingredients (g/kg DM)	Control	Safflower Oil	Sunflower Oil	Corn Oil
Corn Silage	250	250	250	250
Alfalfa Hay	150	150	150	150
Dry Meadow Grass	350	350	350	350
Milk Feed (21% HP)	250	250	250	250
Safflower Oil	-	30	-	-
Sunflower Oil	-	-	30	-
Corn Oil	-	-	-	30
Chemical Composition (%)				
DM	92.23	92.53	92.53	92.53
CP	11.93	11.93	11.93	11.93
EE	4.52	7.52	7.52	7.52
NDF	59.12	59.12	59.12	59.12
ADF	30.78	30.78	30.78	30.78
ADL	14.06	14.06	14.06	14.06
CF	20.89	20.89	20.89	20.89
CA	7.73	7.73	7.73	7.73

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, CF: Crude fiber, CA: Crude ash

Determination of In Vitro Gas and Methane Production and Microbial Protein Amounts

The amount of gas released as a result of 24-hour incubation of the ration by method (GP) Menke et al. (1979) according to the technique reported. The methane contents produced at the end of the 24-hour incubation were determined using an Infrared methane analyzer (Goel et al., 2008). In order to determine the dry matter content of the ration content fermented with rumen fluid at the end of the incubation, the residue in glass syringes was boiled with NDF solution in a 250 ml glass beaker and filtered by passing through gooch por 1 crucibles (Blümmel et al., 1997). The ME,

NE_L and OMD values of the ration content were found with the following equations stated by Menke and Steingass (1988). CP, EE, CA data used in the equations are used as %.

$$ME = 2.2 + 0.1357 \times GP + 0.057 \times CP + 0.002859 \times EE$$

$$NE_L = 0.101 \times GP + 0.051 \times CP + 0.112 \times EE$$

$$OMD = 14.88 + 0.8893 \times GP + 0.448 \times CP + 0.651 \times CA$$

TDMD, PF, Microbial protein production (MP) and Microbial protein production efficiency (MPPE) values of ration content Blümmel et al. (1997) 's calculated using the formulas reported below.

TDMD = Dry matter incubated (mg) - Remaining dry matter (mg)

PF = (TDMD / Gas Production)

MP = (TDMD - (2.2 x Gas Production))

MPPE = ((TDMD - (2.2 x Gas Production)) / TDMD)

Determination of In Vitro True Dry Matter, NDF and Organic Matter Digestibilities

Ankom Daisy II incubator D 220 device was used to determine the *in vitro* true nutrient digestibility of the ration contents by the filter bag method (Van Soest et al., 1991). The rumen fluid used in the study was obtained from the rumen of 3 healthy head rams of Awassi breed, who were 2 years old and between 60-70 kg body weight, who received slaughter approval from the ethics committee of Erzurum Meat and Fish Institution, as reported by Kılıç and Abdiwali (2016). was brought to Atatürk University Faculty of Agriculture, Animal Science Department, Feed Analysis Laboratory in a screw cap bottle with a capped thermos container containing water at approximately 39°C. Rumen fluid was used for *in vitro* true nutrient digestibilities after filtering through four-layer cheesecloth by providing anaerobic environment under CO₂ gas. It was prepared to contain a total of 2 L of buffered rumen fluid, accompanied by the addition of CO₂ gas (1600 ml buffer solution + 400 ml rumen fluid) in each glass jar in the Daisy II incubator device. It was incubated in the Daisy II incubator for 48 hours. After 48 hours, all the bags were taken out of the glass jars and kept under tap water until clear water flowed, and after drying in the open, they were kept in the pre-prepared oven at 105 °C for 2-3 hours. After weighing the bags removed from the oven, the *In vitro* true dry matter digestibility (IVTDMD) was determined by applying the following formula. In order to determine the actual NDF content of the ration content, the bags, which were removed

and dried in an oven after 48 hours of incubation, were boiled in ANKOM 2000 Fiber Analyzer with NDF solution at 100°C for 75 minutes. After the process was finished, the bags were washed 2-3 times in water and dried in an oven at 105 °C for 2-4 hours. *In vitro* true NDF digestibilities (TNDFD) was calculated by substituting the dried bags in the formula. In order to determine the *in vitro* true OM digestibilities (TOMD) of the ration content, the bags were burned at 550 °C for 3-4 hours in the muffle furnace, and at the end of the incineration process, they were weighed on a precision scale and calculated with the following equation.

$$\%IVTDMD=100 - (((D3-D1)/(D2-D1))*100)$$

$$\%TNDFD=100 - (((D4-D1)/(D6-D7))*100)$$

$$\%TOMD=100 - (((D5-D2)/(D8-D7))*100)$$

D1: Tare of F57 bags, D2: Dry weight of ration content, D3: Amount of ration remaining after incubation, D4: Content of ration treated in NDF solution in Ankom 200/220 cellulose analyzer and dried in an oven, D5: Amount remaining after burning in a 550 °C ash oven, D6: % NDF content of the ration content, D7: DM content of the ration content, D8: % organic matter content of the ration content

Statistical Analysis

Statistical analyzes of the data obtained from the research were made using the SPSS 26.0 package program. Duncan multiple comparison test (Duncan, 1955) was used to compare the means of the groups.

Results and Discussion

Table 2 shows the 24-hour *in vitro* gas and methane production values and the mean values of ME and NE_L contents of the groups formed by adding 3% sunflower, safflower and corn vegetable oils to dairy cattle TMR rations.

Table 2. Average *in vitro* gas and methane production and ME and NEL values of the experimental groups

Groups	Gas (ml)	Methane (ml)	Methane (%)	ME (MJ/kgDM)	NE _L (MJ/kgDM)
Control	80.17 ^a	10.71	13.36	7.29 ^a	4.35 ^b
Safflower Oil	76.07 ^b	10.68	14.04	7.17 ^a	4.52 ^a
Sunflower Oil	72.93 ^c	10.00	13.72	7.00 ^b	4.4 ^b
Corn Oil	76.07 ^b	10.02	13.19	7.17 ^a	4.52 ^a
SEM	2.06	0.62	1.14	0.01	0.00
P	0.000	0.430	0.685	0.002	0.002

a,b,c: Means shown with different letters in the same column are significantly different (P<0.05). SEM: Standard error means.

Gas production values of the rations differed according to the added oils, and the lowest was observed in the group containing 3% sunflower oil (72.93 ml) and the highest in the control group (80.17 ml). Similar to the current study, it was stated that the addition of oil to the ration (Vargas et al., 2017, 2020) decreased the gas production values compared to the control ration. It was determined that there was no significant difference between the groups in terms of methane production (P>0.05). Compared to the *in vitro* methane (ml) content of the control group, it was determined that the *in*

vitro methane (ml) values produced in the rations containing sunflower and corn oil were numerically low. Compared to the % methane values obtained from the control group, a numerical decrease of 1.3% was observed in the corn oil-containing group. Since vegetable oils affect the amount of gas produced as a result of 24-hour incubation, differences in ME and NE_L values occurred. The highest ME value was determined in the control group, and the lowest value was determined in the group containing sunflower oil. The highest NE_L content was found in the rations containing safflower and corn oil, and

the lowest in the control group. The values determined for the NE_L contents of the rations were determined by Zhang et al. (2021) were found to be lower than the values reported. Among the groups prepared according to the classification made by Lopez et al. (2010), the groups with corn and sunflower added are partially antimethanogenic. It has been stated that the % methane content of feeds with low antimethanogenic potential should be between >11 and ≤14. It

is stated that the fact that the rations have antimethanogenic properties is important in terms of energy use efficiency and environment in animal nutrition (Navarro-Villa et al., 2013).

True digestive dry matter (TDDM), OMD, PF, MP and MPPE values of the experimental groups are given in Table 3. When Table 3 was examined, it was determined that the vegetable oils added to the ration affected the OMD values ($P < 0.05$).

Table 3. TDDM, PF, MP and MPPE values of the experimental groups

Groups	TDDM (mg)	PF (mg/ml)	MP (mg)	MPPE (%)	TDDM (%)
Control	319.96	3.86 ^b	137.36	42.92 ^b	53.78 ^a
Safflower Oil	315.36	4.01 ^{ab}	142.11	45.02 ^{ab}	52.32 ^b
Sunflower Oil	316.35	4.19 ^a	150.25	47.49 ^a	51.20 ^c
Corn Oil	317.04	4.03 ^{ab}	143.79	45.24 ^{ab}	52.32 ^b
SEM	9.76	0.18	10.58	2.42	0.26
P	0.937	0.042	0.419	0.047	0.000

a,b,c: Means shown with different letters in the same column are significantly different ($P < 0.05$). SEM: Standard error means. TDDM: True digestive dry matter (mg), PF: Scale factor, MP: Microbial protein production (mg), MPPE: Microbial protein synthesis efficiency (%).

The highest OMS value was observed in the control group (53.78%), while the lowest was observed in the group containing sunflower oil (51.20%). Addition of vegetable oil to dairy cattle TMR rations increased PF value ($P < 0.05$). The PF value was found to be the lowest with 3.86 mg/ml in the control group, and the highest value with 4.19 mg/ml in the group containing sunflower oil. It has been reported that the theoretical PF values of the feeds used in the nutrition of ruminant animals vary between 2.75 and 4.41 and these values should be taken into account in determining the synthesizing efficiency of microbial protein (Blümmel et al., 1997). It has been reported that the higher the PF value of a feed, the higher the microbial protein synthesis efficiency of that feed will be depending on it (Blümmel & Lebzién, 2001). It was determined that the TDDM values of the experimental groups varied between 315.36 and 319.96 mg, and the difference between the values was not significant ($P > 0.05$). The MP values of the control and treatment groups were found to be between 137.36 and 150.25, and the difference was found to be statistically insignificant ($P > 0.05$).

Average values obtained from control and treatment groups created by adding vegetable oil to dairy cattle TMR rations are given in Table 4.

Table 4. Effects of vegetable oils added to the ration on IVTDMD, TNDFD and TOMD

Groups	IVTDMD (%)	TNDFD (%)	TOMD (%)
Control	52.63	68.80	93.96
Safflower Oil	48.49	67.26	93.93
Sunflower Oil	48.56	67.74	94.01
Corn Oil	48.62	67.45	93.99
SEM	7.74	6.46	0.01
P	0.263	0.880	0.661

IVTDMD: *in vitro* true dry matter digestibility, TNDFD: *in vitro* true NDF digestibility, TOMD: *in vitro* true OM digestibility; SEM: Standard error means.

The differences between IVTDMD, TNDFD and TOMD values were not found significant *in vitro* conditions in the rations created with the addition of vegetable oil ($P > 0.05$). Unlike the present study, Vargas et al. (2017) reported in their study that there was a decrease in IVTDMD in the group containing sunflower oil compared to the control rations. It was observed that there was a numerical decrease in the TNDFD values in the rations containing vegetable oil compared to the control ration. This is due to the fact that the crude fat content of the ration increases due to the addition of vegetable oil, which negatively affects cellulose and fiber digestion (Ayaşan & Karakozak, 2011). Similarly, it has been reported that fiber and cellulose digestion is suppressed in diets created by adding fat to the diet (Bayat et al., 2017; Beck, 2017; Darabighane et al., 2021).

Conclusion

Vegetable oils added to dairy cattle TMR rations significantly affected gas production and OMS values ($P < 0.05$). The decrease in the *in vitro* NDF digestibility of the experimental groups is thought to be due to the decrease in gas production. As a result; It can be said that since the vegetable oils added to the ration increase the net energy lactation content of the ration, it can be used to provide the energy needs of high milk yielding cattle in the lactation period, provided that it does not exceed the upper limits specified in the literature, and by increasing the PF value, it will increase the microbial protein production and synthesis efficiency in the rumen.

Conflict of Interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

The Effects of Salicylic, Folic and Ascorbic Acid Treatment on Shelf Life Quality of Broccoli Florets

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ABSTRACT

This study aims to investigate the effect of Salicylic acid (2 mM), Folic acid (5 mg L⁻¹) and Ascorbic acid (2 mM) treatments on the shelf life and quality of 'Belstar F1' broccoli variety. Treated broccoli heads were stored at 21±2 °C for 4 days in plastic containers with lids. It is determined that at the end of the storage period, the lowest weight loss (2.74%), total soluble solids (8.07%), pH value (7.14) and the highest amount of titratable acidity (0.12%) were found in the group treated with ascorbic acid and the least change in color parameters (L*; 29.41, a*; -4.59, b*; 10.78) and the highest total chlorophyll content (0.32 mg/g) in the group treated with folic acid. It is thought that the effects of ascorbic acid, salicylic acid and folic acid treatment at postharvest storage period should be investigated in detail in molecular and biochemical studies for more concrete data.

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Introduction

Broccoli is a nutritionally important vegetable due to its high fiber content, ascorbic acid and antioxidants with low calorie (Büchert et al., 2011). Harvesting of broccoli is done before blooming and the sudden increase in energy during the harvest, the stress caused by nutrient and hormone deterioration activate the aging process in broccoli. This process manifests itself as chlorophyll breakdown (yellowing) and a decrease in nutritional value. As a result, these changes reduce the economic value of broccoli heads (Hasperué et al., 2013).

The loss of biochemical content in the post-harvest period in broccoli, which is considered to be the vegetable with the highest nutritional value per unit weight consumed, is of great importance. (Hasperué et al., 2016). Storage of broccoli flowers were studied with the increase in demand for minimally processed vegetables (Yuan et al., 2010). As with many other species of vegetables, broccoli also have a high respiratory rate and metabolic activity at post-harvest period. Due to rapid post-harvest spoilage and short post-harvest life,

the use of chemicals is limited to maintain product quality during storage and transportation, and alternative treatments must be tested for the use in post-harvest technology.

Storage temperature significantly affects the storage life of broccoli. There are many studies which investigated the effect of different temperatures postharvest quality of broccoli. While broccoli can be stored for 21-28 days at 0 °C, it can be stored for about 5 days at 10 °C (Cantwell & Suslow, 1997). Wang and Hruschka (1977) reported that when broccoli was stored at 0 °C and 95% relative humidity, its green colour was maintained for much longer than when it was not unrefrigerated. Broccoli is a highly perishable fruit, especially in shelf life conditions. Takeda et al. (1993) reported that chlorophyll levels in broccoli decreased during storage at 20 or 23 °C, while chlorophyll levels remained nearly constant at 2 °C. Shi et al. (2016) reported that broccoli showed rapid molecular and biochemical changes for 4 day- storage at 20 °C. Deschene et al. (1991) reported that broccoli florets rapidly senesced when stored in air at 23 or 10 °C and chlorophyll

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levels declined by 80-90% within 4 days at 23 °C and within 10 days at 10 °C.

Many treatments have been investigated to preserve the postharvest quality of broccoli. The treatments of some chemicals (Xu et al., 2016; Cai et al., 2019; Al Ubeed et al., 2017) such as preservation in modified atmosphere (Wang et al., 2019; Xu et al., 2006; Fernández-León et al., 2013), the use of LED rays (Hasperué et al., 2016; Jiang et al., 2019), UV-B (Topcu et al., 2015), UV-C (Dogan et al., 2018) radiation treatments, hormone treatments (Zheng et al., 2019), ethylene (Gong & Mattheis, 2003), 1-MCP (Gong & Mattheis, 2003; Cefola et al., 2010; Yuan et al., 2010), folic acid (Xu et al., 2021) were tested in broccoli preservation, and it was reported that the treatments preserve the visual quality of broccoli, prevent chlorophyll breakdown and delay senescences.

It is of great importance that the products applied externally are safe for consumption as well as extending the storage period for products with high postharvest losses and short-term storage period. In this study, the effects of salicylic, folic and ascorbic acid treatments, which are organic compounds, on storage of broccoli were investigated.

Ascorbic acid (AA) is a water-soluble vitamin that plays an important physiological role in suppressing reactive oxygen species (ROS) that occur in plants under stress conditions. In recent years, it has been noted that exogenous ascorbic acid treatments in the preservation of horticultural crops are a biologically safe molecule that can be used to maintain post-harvest quality (Alaey et al., 2011). Broccoli heads contain high levels of ascorbic acid, but it has been observed that this compound decreases rapidly in the postharvest period, especially during the shelf life (Nishikawa et al., 2003). During the aging process in broccoli, it has been observed that the accumulation of active oxygen species increases and therefore the content of antioxidants, especially ascorbic acid, decreases (Casajús et al., 2019).

Besides being a phenolic compound, salicylic acid is also defined as a plant growth regulator (Popova et al., 1997). It was observed that salicylic acid regulates different physiological and biochemical processes in plants. Some of those are plant growth (Tufail et al., 2013), stomatal conductivity (Hayat et al., 2010), photosynthesis (Khodary, 2004), seed germination (Babalar et al., 2007), disease resistance (Delaney et al., 1994; Dempsey et al., 1999), heavy metal, low temperature, high temperature and resistance to salinity stress (Hayat et al., 2008). It was also stated that salicylic acid, a natural compound, has a high potential in suppressing ethylene production and fungal rot in harvested fruits, prevents chlorophyll degradation and contributes to the preservation of color values (Leslie & Romani, 1988; Romani et al., 1989; Zhang et al., 2003; Babalar et al., 2007; Sayyari et al., 2009; Wei et al., 2011).

Folic acid (FA) is a water-soluble vitamin, also known as vitamin B9, which refers to tetrahydrofolate and its derivatives. Although the effect of folic acid on human health is known, its functions on plants have recently been discovered and little is known about the effect of exogenous folic

treatment on post-harvest physiology (Xu et al., 2021). It has been reported that folic acid regulates gene expression through the riboswitch mechanism and also plays a role in chlorophyll biosynthesis and oxidative stress tolerance (Al-Said & Kamal, 2008; Raeisi et al., 2017; Xu et al., 2021).

Xu et al. (2021) stated that different doses of folic acid treatments after harvest reduced the yellowing rate in broccoli, prevented weight loss and decreased respiratory rate and ethylene formation. In this study, the effective dose was determined as 5 mg L⁻¹ in broccoli. There is no literature investigating the effects of salicylic acid and ascorbic acid on broccoli storage.

In this study, the effects of folic, ascorbic and salicylic acid applications on the shelf life of broccoli flowers were investigated.

Materials and Methods

The variety "Belstar F₁" grown in Sarıcakaya region of Eskişehir province (40° 2' N, 30° 39' E; altitude: 520m) was used in the study. Broccoli was harvested during the commercial maturity stage (Average weight; 450-500 g, total soluble solids content; 7.7%, total titratable acidity; 0.14%). Homogeneously selected heads were treated salicylic acid (2 mM), ascorbic acid (2 mM) and folic acid (5 mg L⁻¹). 0.01% Tween 80 were added in prepared solution for better adhesive. The heads were dipped in prepared solutions for 10 minutes after dipping fruits waited one hour to dry. Control heads were treated with pure water. After all treatments, florets were kept in non-colour plastic containers (11x19x7cm) at 21±2 °C for 4 days.

Investigated Features

Broccoli florets were weighed at harvest and re-weighed in each storage periods and weight losses were calculated as percent loss of initial weight with following formula:

$$\text{Weight loss (\%)} = \left(\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right) \times 100$$

Color of broccoli florets was measured from three points of each floret using a digital chromameter (Konica Minolta, Japan).

Broccoli juice was obtained for total soluble solid (TSS), titratable acidity (TA) and pH analysis. TSS were measured with a refractometer (ATAGO Co. Ltd. Tokyo, Japan) and results were given as %. pH level was measured with pH meter (HI9321, Hanna, USA).

2 ml juice was used with 38 ml of distilled water for determined TA. The mixture was titrated with 0.1 N NaOH solution until the pH reaches 8.1. The amount of TA was calculated as % citric acid equivalents.

Total chlorophyll content of broccoli sample on a fresh weight (FW) basis was determined by using the colourimetric method of Yuan et al. (2010) using pure material obtained from ground broccoli florets and kept frozen at -18 °C. Frozen powdered samples of broccoli florets (2gr) were ground and extracted in 20 ml of 80% acetone, centrifuged at 5000 rpm for 5 min. The optical densities at 645 and 663 nm were recorded

for each sample and the total chlorophyll was estimated by the following formula.

$$\text{Total Chlorophyll(mg/g)} = \frac{(20.2 \times OD_{645 \text{ nm}} + 8.02 \times OD_{663 \text{ nm}} \times V)}{100 \times W}$$

where, OD is optical density, V is volume of the extract (ml) and W is weight of the sample (g).

Statistical Method

The research was carried out in 3 replications by designing random plots according to a 4x5 factorial trial pattern. In the study, the treatment method (Control, Folic Acid, Salicylic Acid and Ascorbic Acid) and shelf life (0 day, 1st day, 2nd day, 3rd day and 4th day) were taken in the statistical model, and it was investigated whether there was a statistically significant difference between the treatment and shelf life averages and the existence of interaction. In the statistical analysis of the study, two-way analysis of variance (Two-way ANOVA) was applied. In order to determine the effect of the treatment subjects on the properties examined in the experiment, the 'R studio program' was used in statistical analysis in terms of the property (R Core Team, 2020). In the research, Tukey HSD multiple comparison test at the 5% significance level revealed which treatment and shelf-life averages are statistically significant in terms of the existence of differences and interactions.

Results and Discussion

Weight Loss

Weight loss is a very important feature which is caused by the loss of water in stored products. Weight loss in stored products gives an idea about the storage life of the product. The averages of the weight loss of florets were examined and it was observed that there was a continuous increase in the weight loss (Table 1). When the treatment averages were examined, the average weight loss in the control group was 4.35%, the averages of ascorbic, folic and salicylic acid treatments were determined as 2.49%, 2.74% and 3.40%, respectively. When Table 1 is examined, it is seen that the difference between the treatment averages is statistically significant, and all the treatments compared to the control group have an effect on preventing weight loss. In addition, the existence of DayxTreatment interaction was determined ($p < 0.01$).

Similar to these findings, it was reported that folic acid treatment in broccoli florets (Xu et al., 2021); salicylic acid treatments in banana (Srivastava & Dwivedi, 2000), green onion (Freddo et al., 2013), strawberry (Shafiee et al., 2010) and peach (Khademi & Ershadi, 2013; Han et al., 2002) tomato (Kant et al., 2013); ascorbic acid treatment in lychee (Terdbaramee et al., 2006) and strawberry (Sogvar et al., 2016) have a preventive effect on weight loss compared to the control group.

It has been determined that folic acid treatment has a preventive effect on weight loss in broccoli. Although the findings are in agreement, Xu et al. (2021) stated that the weight loss of broccoli treated with folic acid was less than 1%

in 4 day storage at 20°C and there was no significant increase. In addition, at the 4th day, it was reported that 26 times more weight loss occurred in the control group compared to the group treated with folic acid. In the study, while the weight loss was 2.66% on the 1st day of storage period in broccoli treated with folic acid, this value increased to 5.16% on the 4th day. The difference between the findings is thought to be due to the use of broccoli florets as a material in this study, while Xu et al. (2021) used broccoli heads.

There was no statistically significant difference between the averages of the treatments (AA, SA and FA), the weight loss occurred in the florets treated with ascorbic acid at the lowest rate on the 1st, 2nd and 3rd days of the storage. On the 4th day of the storage period, it is seen that salicylic acid and ascorbic acid have similar values respectively %4.03 and %4.13. It was determined that the weight loss in the control group was 2 times more than ascorbic acid, which had the lowest average in terms of the properties examined. In this case, it can be said that ascorbic acid treatment is the most effective treatment in terms of preventing weight loss.

Color Parameters (L^* , a^* , b^*)

Changes in color values are one of the important factors affecting visual quality at the posharvest period. The L^* value indicates brightness, a^* value indicates green-red color tone, b^* value indicates blue-yellow tone.

Cantwell and Suslow (1997) have researched the color changes that occur in broccoli flowers during the storage period, they reported that as the storage period is extended, the yellowing was increased and the L (brightness) value was increased. Table 1 shows that the difference between all treatments (AA, FA and SA) compared with the control group is statistically significant ($p < 0.01$). Although there is no significant level between salicylic acid, ascorbic acid and folic acid treatment averages, the highest L^* value was determined in the folic acid treatment.

When the findings of a^* and b^* values of broccoli florets are examined, the difference between the treatment averages is statistically significant, and the existence of DayxTreatment interaction was detected (Table 1) ($p < 0.05$). A highest a^* value was determined in all treatments compared to the control group at the 4th day of storage period, control group a^* value average was -1.89, ascorbic, salicylic and folic acid average was -2.18, -2.47 and -2.76 respectively. The 1st, 2nd, 3rd and 4th day of the storage period has lowest a^* value averages at folic acid-treated florets.

Compared with the control group, all treatments (AA, SA and FA) prevent the increase in b^* value. In terms of the investigated feature, the presence of variety of treatment interaction was determined (Table 1) ($p < 0.01$). At the 4th day of storage period, control group b^* value average was 16.61, ascorbic, salicylic and folic acid average was 13.60, 13.18 and 12.65 respectively. At 1st 2nd 3rd and 4th day of the storage period, it was determined that the change in the b^* value occurred at the lowest level in the folic acid group. Considering these findings, it can be said that folic acid

treatment is the most effective treatment in order to prevent the change in a^* and b^* value during the 4-day storage period.

Similar to these findings, Xu et al. (2021), reported that folic acid treatment prevented the increase in L^* , a^* and b^* values during the storage period. In addition, there are also

studies reported to prevent the change in color values in different species; salicylic acid (Shafiee et al., 2010; Wei et al., 2011; Kant et al., 2013; Chavan & Sakhal, 2020) and ascorbic acid treatments (Gil et al., 1998; Terdbaree et al., 2006; Lin et al., 2007; Liu et al., 2014; Sikora & Świeca, 2018).

Table 1. Weight loss, Color parameters (L^* , a^* , b^* , h° and C^*) of 'Belstar F1' broccolis during shelf life at 20 °C

Investigated Features	Treatments	Storage Period (Day)					Mean ²
		0	1	2	3	4	
Weight Loss (%)	Control	-	3.63±0.04Ca	4.76±0.05Ba	6.55±0.22Aa	6.80±0.05Aa	4.35±0.66
	FA	-	2.66±0.13Db	4.37±0.06Ca	4.82±0.04Bb	5.16±0.27Ab	3.40±0.51
	SA	-	2.22±0.07Cc	3.73±0.07Bb	3.74±0.13Bc	4.03±0.02Ac	2.74±0.40
	AA	-	1.87±0.12Cd	3.18±0.03Bc	3.26±0.12Bd	4.13±0.01Ac	2.49±0.39
	Mean ¹	-	2.59±0.20	4.01±0.18	4.59±0.38	5.03±0.34	
L^*	Control	25.77±0.42	29.57±0.39	32.55±1.27	35.92±1.48	37.40±0.36	32.24±1.18W
	FA	26.61±1.09	29.24±1.97	29.63±1.28	30.49±2.00	31.10±0.16	29.41±0.69X
	SA	25.57±0.12	30.18±0.42	30.74±0.39	30.60±0.14	32.84±0.50	29.99±0.65X
	AA	25.85±0.79	29.12±0.57	29.12±0.90	32.28±0.17	34.38±2.47	30.15±0.92X
	Mean ¹	25.95±0.32z	29.53±0.47y	30.51±0.59xy	32.32±0.85wx	33.93±0.88w	
a^*	Control	-6.55±0.09Aa	-3.86±0.01Bb	-3.24±0.01Cc	-2.51±0.01Dc	-1.89±0.12Eb	-3.61±0.43
	FA	-6.42±0.09Aa	-4.99±0.51Ba	-5.32±0.01Ba	-3.45±0.02Ca	-2.76±0.05Da	-4.59±0.36
	SA	-6.53±0.04Aa	-4.84±0.36Ba	-3.67±0.09Cb	-2.93±0.09Db	-2.47±0.01Ea	-4.09±0.40
	AA	-6.28±0.23Aa	-4.86±0.02Ba	-3.32±0.00Cc	-2.61±0.01Db	-2.18±0.03Eb	-3.85±0.41
	Mean ¹	-6.44±0.06	-4.64±0.19	-3.89±0.25	-2.87±0.11	-2.32±0.10	
b^*	Control	6.95±0.03Bb	12.70±0.24Aa	16.41±0.84Aa	16.39±0.28Aa	16.61±0.29Aa	13.81±1.01
	FA	7.45±0.45Cb	9.65±1.08Bb	11.97±0.73Ab	12.18±0.36Ab	12.65±0.08Ab	10.78±0.58
	SA	8.56±0.44Ba	12.42±0.30Aa	12.95±0.43Ab	13.13±0.75Ab	13.18±0.66Ab	12.05±0.51
	AA	8.63±0.01Ca	11.80±0.45Bab	12.95±0.03ABb	12.88±0.89Bb	13.60±1.25Ab	11.97±0.54
	Mean ¹	7.90±0.26	11.64±0.45	13.57±0.57	13.64±0.56	14.01±0.56	

¹Storage time, ²Treatment, Different capital letters show significant differences for each treatment during the storage and the lowercase letters show significant differences among treatments for each sampling date. Means of treatments and storage time also show capital letter. Data are the mean ± SE of three replicates.

Total Soluble Solids (TSS) and pH Level

Sugar constitutes the majority of the TSS in the products. Sugar plays an important role in controlling the aging process as they are used as a carbon source to maintain increased respiratory activity in tissues during the post-harvest period. Some studies reported decrease in sugar levels and TSS amount in the post-harvest period in broccoli (Büchert et al., 2011; Topcu et al., 2015). In addition, there are studies reporting increase and decrease in fluctuations in TSS ratio (Düzen, 2019).

Kader (1992) stated that the increase in the amount of soluble solids in the stored products is due to water loss, the products that lose water become more concentrated and the TSS ratios increase. In the study, an increase was observed in the amount of TSS in broccoli florets during the storage period similar to Fernández-León et al. (2013) (Table 2). The differences between the treatments ($p < 0.05$) and the days of storage ($p < 0.01$) were statistically significant, and it was determined that the most effective treatment in terms of preventing the change in the amount of TSS were ascorbic acid

similar to the weight loss feature (8.07%) (Table 2). The 4th day of storage period, the mean amount of TSS in the control group increased up to 9.8%. In flowers treated with ascorbic, folic and salicylic acid, these values were determined as 8.50%, 8.67% and 8.67%, respectively. These findings show that all of the treatments (AA, SA, FA) prevented the change in the amount of TSS.

Similar to these findings, many researchers reported that salicylic acid treatment prevented the increase in the amount TSS compared to the control group; peach (Khademi & Ershadi, 2013), apple (Mo et al., 2008), apricot (Hajilou & Fakhimrezaei, 2013), tomato (Chavan & Sakhal, 2020), kiwifruit (Fatemi et al., 2013) and ascorbic acid treatment; pear (Lin et al., 2007), guava (Gill et al., 2014; Azam et al., 2020), strawberry (Sogvar et al., 2016) and lychee (Terdbaree et al., 2006).

PH value increased during storage period similar to Lebermann (1965) and Özer (1999). In addition, the existence of DayxTreatment interaction was determined in terms of the property examined ($p < 0.01$). It is seen that there is no

significant difference between the averages of treatment (AA, FA, and SA) on the 1st day of storage, however, ascorbic acid treatment has the lowest pH values on the 2nd, 3rd and 4th days of storage. At the 4th day of storage period, pH value averages control, AA, SA and FA treatment is respectively 7.7, 7.5, 7.3 and 7.2. These findings show that all treatments (AA, FA and SA) prevented changes in pH value compared to the control group. Similar to these findings, it has been reported that salicylic acid treatment has an inhibitory effect on the change in pH level in kiwifruit (Fatemi et al., 2013), and ascorbic acid treatment in guava (Gill et al., 2014; Azam et al., 2020).

Titrateable Acidity (TA)

A decrease in the amount of TA is observed with the use of organic acids as primary respiratory substrate. It was observed that, similar to the findings of Lebermann (1965), Topcu et al. (2015), there was decrease in TA values in broccoli during the storage period (Table 2). The existence of DayxTreatment interaction was determined in terms of this feature ($p < 0.01$). During the entire storage period, the lowest values in terms of

TA content were observed in the control group, and all the treatments performed prevented the losses in TA content (Table 2). At the 4th day, the titrateable acidity amount was 0.05% in the Control group; It is 0.09% (approximately 2 times more) in florets treated with ascorbic acid and salicylic acid, and 0.08% in florets treated with folic acid.

The florets treated with ascorbic acid during the entire storage period have the highest TA values. According to these findings, treatment of ascorbic acid is recommended to prevent postharvest TA loss in florets. Similar to these findings, Azam et al. (2020) reported that the treatment of ascorbic acid in guava preserved the amount of TA. When Table 2 was examined, higher TA averages were found in the florets treated with salicylic acid compared to the control group. Similar to these findings; Hajilou and Fakhimrezaei (2013) reported that ascorbic acid treatment preserved the amount of TA in apricot, Chavan and Sakhal (2020) reported that it preserved the amount of TA in tomato, Fatemi et al. (2013) reported it preserved TA in kiwi and Sogvar et al. (2016) stated that it preserved in strawberry.

Table 2. TSS, pH, TA and total chlorophyll content of 'Belstar F1' broccolis during shelf life at 20°C

Investigated Features	Treatments	Storage Period (Day)					Mean ²
		0	1	2	3	4	
TSS (%)	Control	7.67±0.33	7.83±0.17	8.50±0.29	9.17±0.17	9.83±0.44	8.60±0.24W
	FA	7.67±0.33	7.33±0.33	8.67±0.17	8.33±0.33	8.67±0.33	8.13±0.19WX
	SA	8.0±0.0	7.83±0.17	8.00±0.29	8.17±0.17	8.67±0.17	8.13±0.10WX
	AA	7.67±0.33	7.50±0.29	8.00±0.29	8.67±0.33	8.50±0.29	8.07±0.17X
	Mean ¹	7.75 ±0.13y	7.62±0.12y	8.29±0.14xy	8.58±0.16wx	8.92±0.21w	
pH	Control	6.81±0.07Da	7.32±0.03Ca	7.55±0.05BCa	7.66±0.05Ba	7.79±0.02Aa	7.43±0.09
	FA	6.75±0.01Da	7.19±0.05Cb	7.39±0.02Bb	7.41±0.03ABb	7.50±0.01Ab	7.25±0.07
	SA	6.81±0.06Ca	7.16±0.02Bb	7.24±0.01Bc	7.28±0.02ABc	7.33±0.01Ac	7.16±0.05
	AA	6.83±0.03Ba	7.20±0.03Ab	7.19±0.05Ac	7.21±0.03Ac	7.25±0.02Ac	7.14±0.04
	Mean ¹	6.80±0.02	7.22±0.02	7.34±0.04	7.39±0.05	7.469±0.063	
TA (%)	Control	0.14±0.00Aa	0.11±0.00Bc	0.09±0.00Cb	0.06±0.00Dc	0.05±0.00Ec	0.09±0.01
	FA	0.15±0.00Aa	0.13±0.00Bb	0.10±0.00Cb	0.08±0.00Db	0.08±0.00Db	0.11±0.01
	SA	0.14±0.01Aa	0.13±0.00Aa	0.11±0.00Bb	0.11±0.01Ba	0.09±0.00Ca	0.11±0.00
	AA	0.15±0.01Aa	0.14±0.00Aa	0.12±0.00Ba	0.12±0.00Ba	0.09±0.01Ca	0.12±0.01
	Mean ¹	0.14±0.00	0.13±0.00	0.10±0.00	0.09±0.01	0.08±0.00	
Total Chlorophyll Content (mg/g)	Control	0.41±0.01Aa	0.37±0.00Bb	0.22±0.01Cd	0.17±0.00Dd	0.08±0.00Ed	0.25±0.03
	FA	0.41±0.01Ba	0.44±0.01Aa	0.28±0.00Ca	0.26±0.00Da	0.20±0.00Ea	0.32±0.02
	SA	0.41±0.01Ba	0.44±0.01Aa	0.23±0.00Cc	0.21±0.01Dc	0.15±0.00Ec	0.29±0.03
	AA	0.40±0.00Bb	0.44±0.01Aa	0.25±0.01Cb	0.24±0.00Db	0.17±0.01Eb	0.30±0.03
	Mean ¹	0.41±0.00	0.42±0.01	0.24±0.01	0.22±0.01	0.15±0.01	

¹Storage time, ²Treatment, Different capital letters show significant differences for each treatment during the storage and the lowercase letters show significant differences among treatments for each sampling date. Means of treatments and storage time also show capital letter. Data are the mean ± SE of three replicates.

Total Chlorophyll Content

Chlorophyll degradation in the postharvest period has a significant impact on visual quality and biochemical contents. For this reason, it is of great importance to examine the changes in the chlorophyll content during storage. The changes

of the chlorophyll content of florets during storage are examined in Table 2, and it is seen that all treatments (AA, FA and SA) are effective in preventing chlorophyll degradation when compared to the control group. In addition, the presence of DayxTreatment interaction was determined ($p < 0.01$).

At the 2nd, 3rd and 4th days of storage, the highest total chlorophyll content was detected in folic acid-treated florets. On the 4th day of storage, the average of total chlorophyll content in the control group was 0.08mg/g, the amount of total chlorophyll content in the florets treated with folic acid was more than 2 times (0.20 mg/g). Similar to these findings, Xu et al. (2021) reported that folic acid treated broccoli had 2 times higher total chlorophyll content compared to control group.

Conclusion

It is of great importance to investigate practices for maintaining the shelf life quality of broccoli. In this context, this study was conducted to determine the effects of folic, ascorbic and salicylic acid treatments on the shelf life quality of broccoli florets.

In the study, it was determined that all treatments compared to the control group had a preventive effect on the change in all the investigated features. According to the findings, ascorbic acid treatment is recommended to researchers to prevent changes in weight loss, TSS, pH and TA amount, and folic acid treatment is recommended to prevent changes in color values and total chlorophyll amount.

During the shelf life conditions, ascorbic and folic acid was the most prominent treatment to maintain the quality of broccoli florets (cv. Belstar F1). However, it is thought that the effects of ascorbic acid, salicylic acid and folic acid should be investigated in detail in molecular and biochemical studies for more concrete data.

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Conflict of Interest

The author declares no competing interests.

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RESEARCH ARTICLE

Investigation of Seasonal Female and Child Labor Use in Cotton Agriculture: The Case of Mardin Province

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ABSTRACT

In the study, it has been tried to determine the dimensions of seasonal female and child labor employed in cotton agriculture in Mardin province and the factors that are effective in the seasonal agricultural workers working in cotton harvesting themselves and their children. For this purpose, the data obtained by face-to-face interviews with 150 seasonal female workers in the cotton-producing villages of Mardin province were determined by simple random sampling method. It was used in the analysis of the probit model with the LIMDEP statistical program. In the study, according to the results of the CART analysis, it was determined that the most important factor affecting the annual income level of female working in the cotton harvest is the annual working period and the education level of the children. In the probit model, which predicts whether female working in the cotton harvest business have children under the age of 18, the employee's nationality other than the Turkish Republic, the number of children under the age of 18 working in the cotton harvest business, and the probability of the children of those with social security other than SSI (green card, etc.) increases, the probability of being married decreases. It can be suggested that female working in the cotton harvest in Mardin and their children under the age of 18 can become SSI employees, raise their living standards, and improve social, accommodation and nutrition education conditions.

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Introduction

Agriculture; It is an indispensable field of activity that meets the most basic needs of people such as nutrition and clothing, which is defined as the production of plant and animal products, increasing their quality and efficiency, protecting, processing, evaluating and marketing under appropriate conditions. The agricultural sector, which has become one of the most important sectors in the world, is the sector with the highest employment after the service sector and is an important economic activity area especially in developing countries (Anonymous, 2010).

Today, a large part of the population lives in rural areas in almost all countries except developed countries. The fact that a significant part of the population is engaged in agriculture is certainly not a sign that those living in rural areas are fully employed or earn enough to earn their living. In addition, in many developing countries, the agricultural sector is a sector

where important social problems arise and grow gradually (Anonymous, 2010) Turkey is one of the most suitable countries for agricultural activities in terms of its climate zone and local structure. In our country, intensive and intensive expression in the stages of growing agricultural products. Families who do not have sufficient income level seek employment by migrating to regions where agricultural labor is needed in order to work during planting and harvesting times when agricultural work is intense (Bülbül, 1982).

The most obvious form of seasonal employment or unemployment is seen in agricultural areas where production is largely dependent on natural conditions. In the field of agriculture, which opens up a wide range of work opportunities at the time of sowing or during the harvest season, employment opportunities suddenly shrink and unemployment occurs outside of the planting and harvest seasons. In these periods, when seasonal activities are at their fastest, the

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agricultural workforce is not sufficient to carry out agricultural activities, while a significant part of the same workforce is unemployed during the dead season becomes (Ekin, 1971).

Seasonal agricultural production and the need for intensive labor in the production process require seasonal employment of agricultural workers. Seasonal agricultural workers, which are defined as workers who migrate from one place to another for agricultural production and return to their homes at the end of the season, are expressed as the heart of sustainable agricultural production. Almost half of the world's workforce (estimated 1.3 billion employees) is engaged in agricultural activities. According to the October - 2017 statistics of the Turkish Statistical Institute (TSI), 19.3% of the workforce employed as 28 million 645 thousand people is agricultural workforce. According to the Parliamentary Research Commission Report (2015), seasonal agricultural work in Turkey It is a very large sector, including female, children, and the elderly, which make up about half of the 6.3 million agricultural workforce (PRCR, 2015; TSI, 2016).

Seasonal agricultural workers are a poor group who do not have health insurance, whose monthly income is below the minimum wage, and who share these scarce resources with a large household. More than 60% of the world lives below the poverty line, at least 80% of them do not have social security and 70% of them live below the poverty line. It has been revealed in various studies that. Seasonal agricultural workers are living in the world where premature deaths and diseases are high due to unsuitable living conditions and housing conditions, lack of basic sanitation, inadequate unbalanced nutrition, accidents and injuries, pesticide cultivation, extreme heat and cold, lack of access to services, and those who are exposed to the worst conditions of working life considered as a group experiencing all dimensions of social exclusion. Working and living conditions increase the inequality in the working life of seasonal agricultural workers and significantly endanger their health and safety (Kümbetoğlu & Çağa, 1994).

Seasonal agricultural workers are one of the disadvantaged groups due to the difficult conditions in their working life. In our country, approximately 300 thousand people leave their homes and travel to places thousands of kilometers away with unsuitable vehicles, live in shelters that can be called primitive, are faced with malnutrition and work for very low wages. In addition, the lack of social security increases health problems and future anxiety. The education of children, on the other hand, is a problem that has never been addressed (Bora, 2012).

Seasonal agricultural workers in Turkey are workers who do not have the opportunity to work in the labor market and seasonally go to regions with more agricultural job opportunities because they cannot obtain sufficient income from their own agricultural enterprises. The fact that a significant part of the households in Turkey's agriculture is landless suggests that the number of these workers, who have no alternative but to sell their labor, is quite high. These people earn their living either as agricultural workers or by tenancy/partnership. In addition, households who cannot earn

enough income in the cities go to the regions where there are more agricultural job opportunities as seasonal agricultural workers in order to earn their livelihood. These workers work in physically and mentally challenging environments due to the unique characteristics of agricultural production and mobile work conditions. Seasonal workers are mostly unregistered and lack any form of social security. For employers, these workers are cheap, precarious, and replaceable, flexible workforces (Işık & Pınarcıoğlu, 2012).

Some of the seasonal agricultural workers come to a certain region and work there for a certain period of time and return to their hometowns, while others migrate from city to city according to the product throughout the year. Seasonal migration in Turkey is generally based on industrial crops such as cotton, tobacco and sugar beet is taking place. Hundreds of thousands of seasonal agricultural workers from Eastern and Southeastern Anatolian regions, together with households, migrate to Çukurova, Black Sea, Aegean and Central Anatolia regions, especially during hoeing, irrigation and harvesting of cotton, hazelnut, tobacco, tea, sugar beet and grape plants. Although the exact number is not known, it is estimated that there are about 1 million seasonal agricultural workers in Turkey. In recent years, it is seen that not only seasonal agricultural worker migration from the Eastern Regions to the West, but also a reverse migration movement has occurred. In fact, it is known that in this migration movement that took place, not only refugees from the West to the East, but also those who had to leave their countries such as Syria, who had the advantage of cheap labor and were trying to earn a certain income, migrated from their regions (Gülçubuk et al., 2003).

Almost all of the family members working in seasonal agricultural labor started with the participation of female in working life. Historically, female's participation in working life in the world has gone through long processes. When we look at it, in the mentality of people from the past to the present, the place of woman is at home, she is responsible for taking care of her family, husband and children, she lives under male domination, her understanding is dominant. The transition to a settled life with agriculture did not change the secondary status of female (Doğan & Kaya, 2013).

There are various economic and social reasons such as education, rising wages, technology and decreasing birth rates among the reasons for female's participation in the workforce at increasing rates throughout the historical process. The increase in the education level of female brings with it both the increase in the age of marriage and the fact that female begin to gain bargaining power at home. In addition, due to the improving working conditions and rising wages in the historical process, the job market has become more attractive to female; because the alternative cost of not working under these conditions is increasing. On the other hand, the increasing cost of living conditions, especially in urban areas, necessitates the participation of female in the labor market. In addition, with technological developments, female is able to both work and do housework, which is seen as their "homework", relatively more comfortably and with less time spent. Beyond that, the modernization that comes with the increase in the education level of both men and female has

begun to change the entrenched gender gap, albeit slowly, and the decrease in fertility rates, again with the effect of education, has enabled female to increase their presence in the labor market (Doğan & Kaya, 2013).

However, despite all the positive developments, the female workforce still lagged behind the male workforce and could not get rid of the secondary workforce status (Ecevit, 2010).

Since most rural female is often unable to attend school or not at all, there is no alternative employment alternative. Therefore, most of the female in rural area have started to work in seasonal agricultural labor in order to contribute to the household economy with their families. However, there are not only female but also children who have never started their education life or who have to work in seasonal agricultural labor with their families while continuing their education. Child labor, which is one of the most important problems of working life; It is defined as "work that often prevents children from living their childhood, diminishes their potential and dignity, and is harmful to their physical and mental development".

Agricultural work is among the "worst forms of work" for children. In addition to risks such as sun exposure, insect bites, contact with agricultural chemicals, bending over, carrying heavy loads, children as mobile seasonal agricultural workers face many risks such as living in nylon tents, not being able to eat enough, not being able to access clean water, not going to school. Another danger for children working in agricultural areas is the risk of contracting parasitic and other infectious diseases. One of the most important problems of agricultural worker children is long working hours. The working hours of children working in agriculture are above the average and can reach 60 hours per week.

According to the "Global Estimates of Child Labor Report" prepared by the International Labor Organization (ILO, 2004), it is noteworthy that there are still 152 million child workers worldwide, of which 73 million are in "dangerous" jobs. Agriculture has the highest share in the distribution of children working in Turkey by sectors. While 45% of child workers work in agriculture, 31% are employed in the services sector and 21% in industry. Especially, the rate of girls working in agriculture is 58%, which is above the general average. Children aged 6-14 years account for 66 percent of the increase in child employment in agriculture and 90% of the increase in unpaid family workers. It is this increase in the number of child workers aged 6-14 that causes the increase in child labor in total. A significant portion of children working in agriculture are seasonal migrant agricultural workers and children who have to migrate with them. From 2013 to June 2018, 168 of 319 child occupational homicides occurred in the agriculture-forest sector. Agricultural occupational homicides, which are 53 percent of the total occupational homicides, were mostly experienced due to traffic accidents, followed by drowning and poisoning. Children who are transported to the fields die by drowning or poisoning in car crates or in the water they enter to cool off. 3 out of every 5 children who died in the field of agriculture died while working as seasonal agricultural

workers or shepherds. The basis of labor resources in seasonal agricultural work is established through female and child labor. On the other hand, farm children also have completely unpaid family labor. Especially in this field, the working age has fallen below 10. Among the children who lost their lives, there are also children who are 5 years old (ISIG, 2018).

Considering the general situation of seasonal female's and child labor; In Turkey, it is seen that the segments that tend to get rid of poverty and meet family needs through seasonal agricultural work as a survival and subsistence strategy are especially concentrated in Eastern and Southeastern Anatolia Regions (Şen & Altın, 2018). As a matter of fact, these regions include the settlements where rural poverty is most severe in terms of small commodity producers and wage workers. Terrorism, crowded household population, high and chronic unemployment, low investment level, industry and service sectors not providing sufficient job opportunities, blood feuds etc. Regional characteristics, such as local characteristics, have severely limited the opportunities for work and livelihood in the place of residence. While the immigration resorted to feed the reserve army of wage workers settled in the cities, this segment has turned into an indispensable cheap labor army for domestic capital. While some of the workers work in the informal sector, which is insecure, low-paid and high-risk, in the centers or districts of large cities, another part of them has turned to seasonal agricultural work (Yıldırım, 2016).

In general, seasonal agricultural work includes a form of work and migrant labor, which is estimated to reach 3-4 million in Turkey and that irregular migrants and refugees (refugee families) who have recently come to our country, mainly from the Syrian region, also tend. The province of Mardin, on the other hand, is a province that both receives and sends immigrants in seasonal agricultural work. Mardin Province is located in the upper Mesopotamian Basin of the Southeastern Anatolia Region. It is a province bordering the Syrian State and covers 1.1% of the country's territory with an area of 8891 km². Vegetable and animal activities are very important in the economy of Mardin province. A large part of the total gross product is obtained from agriculture and animal husbandry. Grain production generally takes the first place in terms of increasing the diversity of the products produced. In order to increase agricultural production in the province, product change studies are continuing rapidly. With the introduction of the GAP (Southeast Anatolia Project) at the end of 2013, the irrigated agriculture will both disappear and the second crop has increased in planting throughout the province, and therefore production has increased in parallel. In this context, cotton production has become an indispensable product for the province in question. The province of Mardin is a place that migrates for agricultural labor and partially receives migration during the cotton harvest. Families living in Mardin districts and villages of the districts and especially refugee Syrian families from outside the province come to the cotton production enterprises of the district during the cotton harvest to work as seasonal workers. With the harvest starting from September-October to mid-November for the cotton harvest, the cotton harvest is an important source of income for the local and seasonal agricultural workers of Mardin. In this

context, the province of Mardin, where cotton farming is carried out intensively, was chosen as the working area and the factors that affect the seasonal female working in cotton farming and the female working with their children to do this job were tried to be determined. It is thought that the study will be beneficial in order to find solutions to the problems of female and child labor in the investments made in Turkey in general and to guide rational planning within the province.

Materials and Methods

The primary data sources of the research are survey data obtained by face-to-face interviews with 150 seasonal female workers, which were determined by simple random sampling method in Artuklu, Derik, Kızıltepe, Nusaybin and Savur counties where cotton is produced in Mardin province and their villages. Statistical data obtained from the Forestry Directorate consist of time series data obtained from institutions such as the Food and Agriculture Organization of The United Nations (United Nations Food and Agriculture Organization), Turkish Statistical Institute, and data obtained from national and international studies on the subject.

The main population of the research consists of seasonal female workers working in cotton farming in Mardin province and female workers working with their children under 18 years of age. This study was carried out to determine the factors that affect the cotton harvesting work of female and their children under the age of 18 to do this work.

In determining the number of people surveyed, Simple Random Sampling Method was used based on the number of enterprises engaged in cotton farming in line with the 2018 production data obtained from the Mardin Provincial Directorate of Agriculture and Forestry, and the number of surveys was determined. In determining the number of seasonal workers surveyed in the study, it was studied within 5% margin of error and 95% safety limits (Çiçek & Erkan, 1996). As a result of the calculations, the number of surveys to be made was determined by the formula below.

$$N = \frac{Nz^2\sigma^2}{d^2(N-1) + z^2\sigma^2}$$

In the formula;

n = sample volume,

N = Total number of units of the sampling frame

σ^2 = Population variance

d = Acceptable error ($\bar{x} \pm 0.05$)

z = It shows the Z value in the Standard Normal Distribution table according to the acceptable error rate

According to the sampling results, the total number of surveys conducted in the region was calculated as 137. However, considering the inaccuracy or missing data in the data to be obtained from the survey study, the sample volume was expanded by 10% to 150. It was carried out in 5 districts, namely Artuklu, Derik, Kızıltepe, Nusaybin, and Savur. The number of surveys conducted in these districts was determined by proportioning the cotton numbers in the districts.

In the study, the data obtained through the questionnaire were used in crosstab analysis in order to reveal the characteristics of the employees, in the CART analysis in the SPSS program to determine the factors that are effective in the work of female and child workers, and in the probit model analysis with the LIMDEP statistical program. Factors affecting female and female working with their children in doing this job were determined. Therefore, the model was estimated by the probit method used in the estimation of the limited dependent variable model. The functional state of the model is shown below.

$$Y = f(X_1 X_2 X_3 X_4 X_5 X_6 X_7)$$

Y: Those who work in cotton harvesting (Female: Children under 018 years: 1)

X1: Female characteristics

X2: Age of Employee

X3: Nationality (TC: 1, Others: 2)

X4: Marital Status (Married: 1, Single: 2, Widowed: 3)

X5: Days Worked per Year

X6: Social Security Status (Yes: 1, No: 0)

X7: Whether the Wage He Gets is Rewarded for His Labor (Yes: 1, No: 0)

Results

The survey study on the dimensions of seasonal female and child labor use in cotton farming in the province of Mardin was conducted by interviewing 150 seasonal female workers in 5 districts, namely Kızıltepe, Derik, Nusaybin, Midyat and Savur. The socio-economic characteristics of the employees participating in the survey are given in Table 1 It is seen that the group with the highest average age of the surveyed employees consists of the age group of 31-50 with 55.3% (83 employees).

27.3% of the female working in cotton harvesting are Turkish citizens and 8.0% are other nationalities. Considering the female working with their children, 50.0% are Turkish citizens and 14.7% are other nationalities. Looking at the general total, 77.3% of the employees are Turkish citizens.

22.0% of female working in cotton harvesting stay at home, 12.7% in tents and 50.7% in other places. Considering the female working with their children, 34.7% stay at home and 30.0% stay in a tent. In general, it has been determined that 56.7% of the employees stay at home.

4.7% of female working in cotton harvesting stated that they provide transportation by their own vehicles, 2.0% by bus and 28.7% by rented vehicle. Considering the female working with their children, 9.3% stated that they provide transportation by their own vehicles, 8.0% by bus and 47.3% by rented vehicle. Looking at the general total, it has been determined that the highest 76.0% of them travel with a rented vehicle.

While 28% of the female working in the cotton harvest business signed a contract, 7.3% did not. Considering the female working with their children, 53.3% signed a contract, while 11.3% did not. Overall, the highest 81.3% of the employees signed contracts.

While 0.7% of female working in cotton harvesting take occupational safety precautions, 34.7% do not take occupational safety precautions while working. When we look at the female working with their children, 14.0% of them take occupational safety precautions while 50.7% of them do not

take occupational safety precautions. In general, 85.3% of the employees do not take occupational safety precautions while working.

While 2.0% of the female working in the cotton harvest business have social security, 33.3% do not have any social security. When we look at the female working with their children, 4.0% of them have social security while 60.7% of them have no social security. In general, 94.0% of the employees do not have social security.

Table 1. Socio-economic characteristics of the employees participating in the survey

Variables	Groups	Female		Female working with her child		Total	
		N	%	N	%	N	%
Age groups	15-30	46	30.7	1	0.7	47	31.3
	31-50	7	4.7	76	50.7	83	55.3
	51-70	-	-	19	12.7	19	12.7
	71<	-	-	1	0.7	1	0.7
	Total	53	35.3	97	64.7	150	100.0
Nationality	TC	41	27.3	75	50.0	116	77.3
	Other	12	8.0	22	14.7	34	22.7
	Total	53	35.3	97	64.7	150	100.0
Place of stay	Home	33	22.0	52	34.7	85	56.7
	Tent	19	12.7	45	30.0	64	42.7
	Other	1	0.7	-	-	1	0.7
	Total	53	35.3	97	64.7	150	100.0
Transportation	Own tool	7	4.7	14	9.3	21	14.0
	Bus	3	2.0	12	8.0	15	10.0
	Rented vehicle	43	28.7	71	47.3	114	76.0
	Total	53	5.3	97	64.7	150	100.0
Contact signing status	Yes	42	28.0	80	53.3	122	81.3
	No	11	7.3	17	11.3	28	18.7
	Total	53	35.3	97	64.7	150	100.0
Status taking occupational safety precautions	Yes	1	0.7	21	14.0	22	14.7
	No	52	34.7	76	50.7	128	85.3
	Total	53	35.3	97	64.7	150	100.0
Social security status	Yes	3	2.0	6	4.0	9	6.0
	No	50	33.3	91	60.7	141	94.0
	Total	53	35.3	97	64.7	150	100.0

CART analysis results in the Figure 1; It has been determined that the most important factor affecting the annual income level of female working in the cotton harvest is the annual working period of the cotton harvest. The incomes of those who think that there are changes in meeting the needs of the house and children (who are considered important in the 2nd and 4th ranks) and in paying their debts have increased and their incomes are significantly affected by these factors.

CART analysis results in the Figure 2; It has been determined that the most important factor affecting the annual income level of female working with their children under the age of 18 in the cotton harvest is the education level of female. The fact that the fee is greater than 45 ₺ affects these factors significantly.

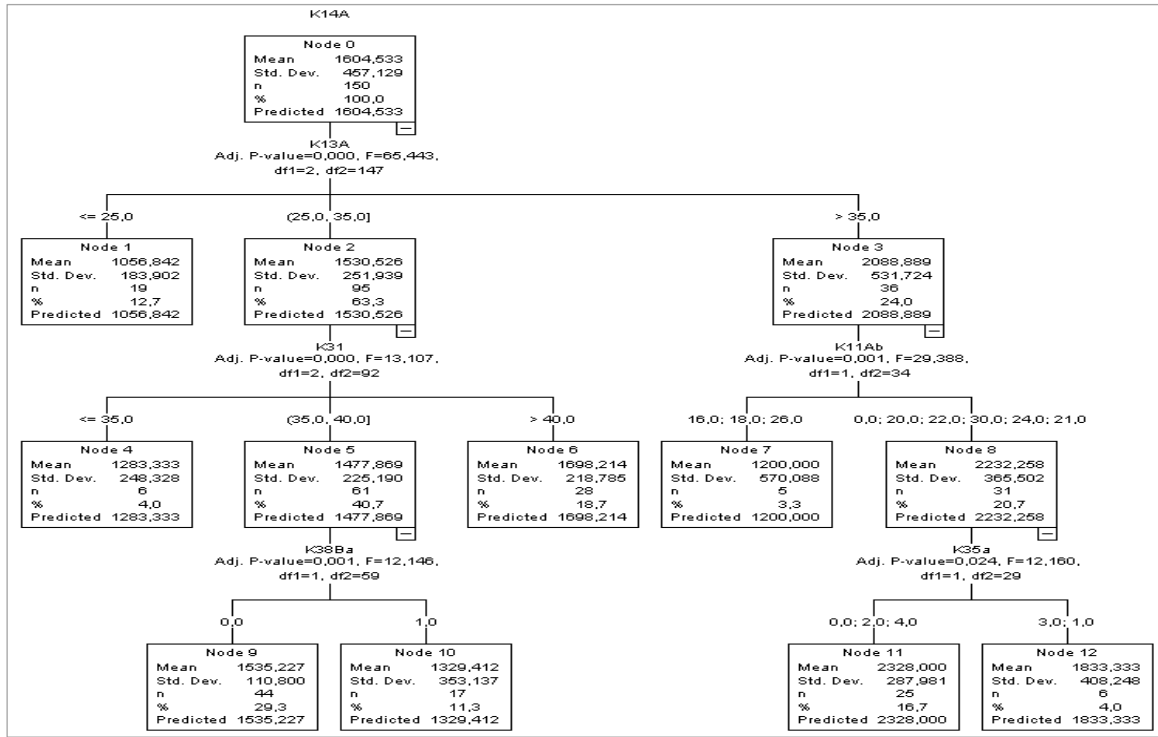


Figure 1. Factors affecting female's annual income level, CART model (Growing method: CART, Dependent variable: Factors affecting female's annual income level, K14A: Annual income from cotton harvesting [₺], K13A: Annual working time [months], K11Ab: Age of working child under 18, K35a: Whether there is a change in economic status [Yes: 1, No: 0], K31: Last year's daily fee [₺], K38Ba: Contract signing status with employer or ambassador [Yes: 1, No: 0]).

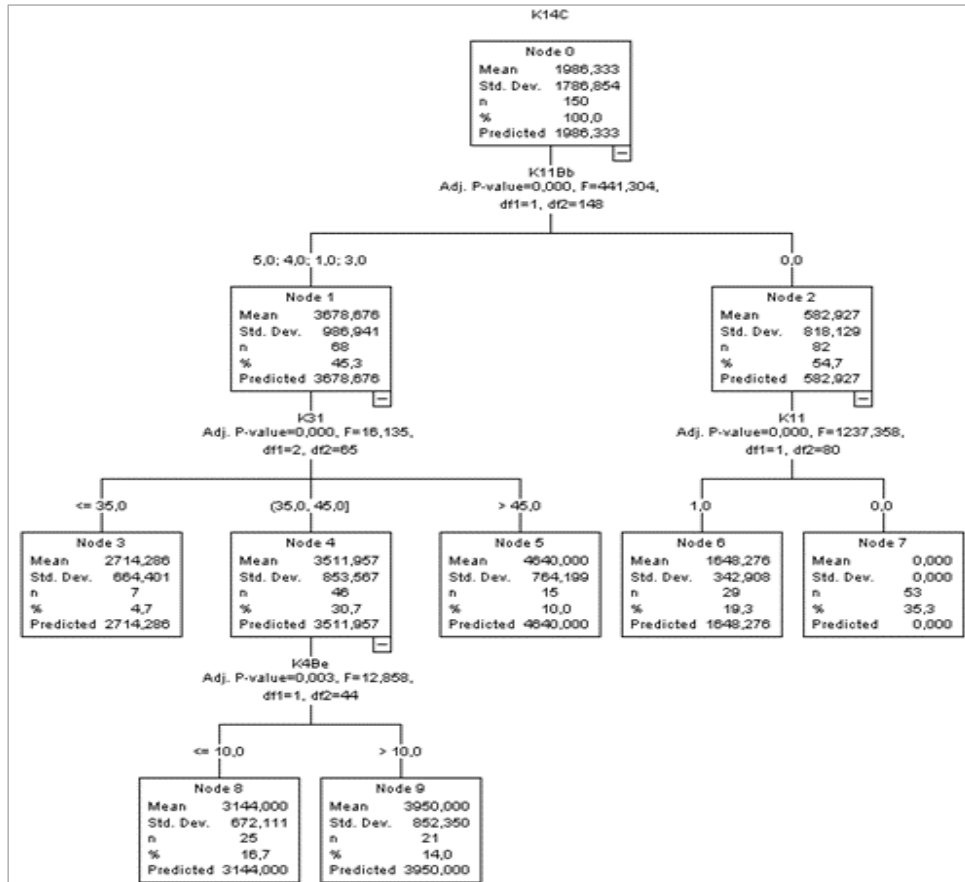


Figure 2. Factors affecting the annual income level of female working with their children, CART model (Growing method: CART, Dependent variable: Factors affecting annual income level of working female, K14C: Annual income from cotton harvesting [₺], K11Bb: Education level of female working with children under 18 [Illegal, primary, secondary, high school], K11: Satisfaction status of working female with children under 18 [Yes: 1, No: 0], K31: Last year's daily fee [₺], K4Be: Age of the employee's 2nd child, if any).

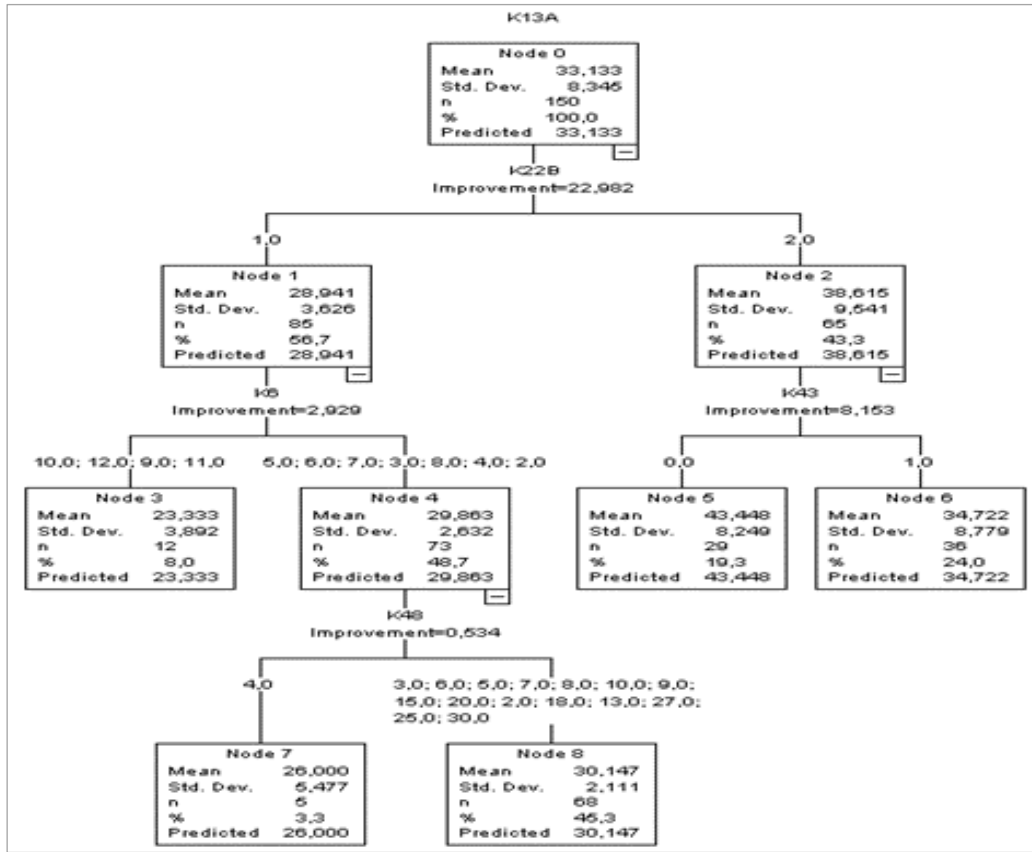


Figure 3. Factors affecting the annual working time of female, CART model (Growing method: CART, Dependent variable: Factors affecting female's annual working time, K13A: Annual working time [months], K22B: Working time in cotton harvest [months], K6: Total number of individuals in the family, K48: How many years do you consider working as a seasonal worker?, K43: Whether or not he has a house of his own [Yes: 1, No: 0]).

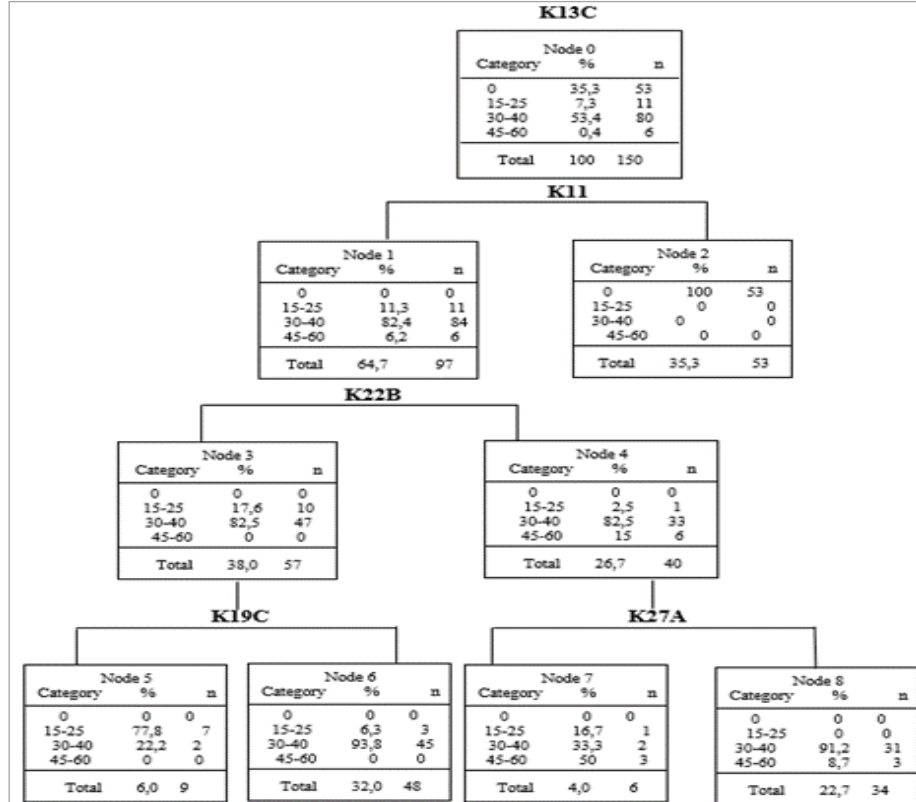


Figure 4. Factors affecting the annual working time of female working with their children, CART model (Growing method: CART, Dependent variable: Factors affecting annual working time of working female with children, K13C: Annual working time [months], K11: Satisfaction status of working female with children under 18 [Yes: 1, No: 0], K22B: Working time in cotton harvest [months], K19C: Seasonal withdrawal from work in case of another job opportunity [Yes: 1, No: 0], K27: Finding a cotton harvest job [own, agent-ambassador-sergeant, other]).

CART analysis results in the Figure 3; The most important factor affecting the annual working time of female working in the cotton harvest is how many days a year they work in the cotton harvest. It can be said that these variables are important in the number of days worked in the cotton harvest by female who work more than 2 months per month and do not have their own house.

CART analysis results in the Figure 4; It has been determined that the most important factor affecting the annual working time of female working with their children in the cotton harvest is that at least 1 child works in cotton harvesting. The number of days worked by female who work with their children is affected by the fact that at least 1 child works in cotton harvesting and spends time in this job for 1 month, and the number of days worked by female who consider longing for their relatives as 4th and 5th important.

In general, the probability of the dependent variable in probit models is explained by increasing the independent variables by one unit, or in other words, by marginal effects. The estimated model of the dependent variable in the study of whether there are female working with their children in the cotton harvesting business is given in Table 2. In the study, it was determined that while the age and marital status of the employee were important at the 1% significance level, variables such as the employee's nationality and social security status were important at the 5% significance level. In the estimation model, it is observed that there is a negative

relationship between the employment status and marital status of the female working with their children, and a positive relationship between the age, nationality and social security status of the female working in the cotton harvesting business. Having a nationality other than Turkish Republic increases the number of children under the age of 18 working in this job. This shows that mostly Syrian families work with their children. It increases the probability of working for children under the age of 18 of people with social security other than SSI (green card, etc.). However, being married reduces the possibility of a child under the age of 18 working. In probit models, we should look at the "marginal effects" of the variables to show how this change will have an effect on the probability of the dependent variable by increasing the independent variables by 1 unit. Marginal effects show us how the effect of this will appear on the dependent variable by increasing the independent variable by 1 unit. According to the chart, when the marginal effects of the data are taken into account, the age of the employees is one more year, the probability of working for children under the age of 18 working in the cotton harvest is 6%, the probability of having a child under the age of 18 is 0.29%, and the probability of having a child under the age of 18, etc. It has been observed that having social security, such as a child under the age of 18, increases the probability of working by 71%, while being married reduces the probability of working in the cotton harvest for a child under the age of 18 by 27%.

Table 2. Probit model estimation results of whether female work with their children in the cotton harvest business

Variables	Coefficient	Standard Error	Marginal Effect
Constant	-17.140	0.026	-4.510
Employee's Age	0.230***	0.010	0.060
Employee's Nationality	1.113**	0.490	0.293
Employee's Marital Status	-1.029***	0.041	-0.270
Employee's Social Security Status	2.716**	0.274	0.714
Annual Working Time	0.006	7.994	0.001
Daily wages	0.040	4.319	0.010
Status Situation of Remuneration	0.852	2.286	0.224

*** %1 significance level, ** 5% significance level

Discussion

Economic sectors are generally divided into three parts: agriculture, industry and services. Although the ratio of those working in the agricultural sector is gradually decreasing due to development; Agriculture still maintains its characteristic of being an important sector in meeting the basic needs of the population, especially in nutrition, and in rural development. The rate of workers in the agricultural sector in our country is around 20% (5.4 million) of the total employment in 2015 (TSI, 2016), and it is stated that approximately half of this is seasonal agricultural workers (PRCR, 2015). For seasonal migrant agricultural workers in our country, it is very difficult to give an exact number since they are generally unregistered. However, in some studies, figures such as 300 thousand (PRCR, 2015), 485 thousand (Selek Öz & Bulut, 2013) and 546 thousand

(Çelik et al., 2015) are expressed. When children are added to this, their number is estimated to be around 1 million (PRCR, 2015).

However, seasonal migrant agricultural work has become an important sector not only in our country but also in other countries of the world. As a matter of fact, in a study, it was stated that waged agricultural workers working in the agricultural sector worldwide constitute 40% of the total employment in the sector (Hurst et al., 2007). In a study based on official data from the USA, it was stated that 2.5 million people work in agriculture annually in the country, of which approximately 1.4 million are migratory and temporary seasonal agricultural workers, especially those coming from abroad (ILO, 2004; Donham & Thelin, 2016).

Seasonal agricultural labor is a form of seasonal labor to meet the labor shortage in agricultural production. Two different aspects of this craftsmanship, temporality and wandering, draw attention (Özbekmezci & Sahil, 2004). Accordingly, we come across two different terms: seasonal migrant agricultural worker and seasonal local agricultural worker. Seasonal migrant agricultural workers are the labor force group that moves mostly with their families from one region to another agricultural area, according to the crop pattern and worker demand, when agricultural work is intensified (Semerci et al., 2014).

Seasonal local agricultural workers, on the other hand, can be defined as workers who live in their own settlements and participate in agricultural activities for a short time, working in return for wages, salaries or daily wages (Beleli, 2013).

Based on the definitions mentioned above, seasonal migrant agricultural workers can be defined as real persons who come from outside the region where agricultural production is carried out and work at any stage of agricultural production, with or without a contract, in return for wages (Görücü & Akbıyık, 2010).

While some of the academic studies on seasonal migratory agricultural work are about seasonal agricultural workers coming to a certain area; some include their legal status (Selek Öz & Bulut, 2013), working and living conditions (Özbekmezci & Sahil, 2004; Görücü & Akbıyık, 2010; Şimşek, 2012; Kaya & Özgülner, 2015) female's life difficulties (Çelik et al., 2015); focused on a number of issues, such as child labor exploitation and education issues (Gülçubuk et al., 2003). Another part of the studies on this subject consists of studies in the form of reports prepared by various organizations. Although these are quite comprehensive and based on on-site observations, seasonal workers in the province of Mardin and the cotton harvest were excluded from the scope of these studies.

Conclusion

Since seasonal agricultural workers are unregistered employment, they could not find a place in the agenda of the legislation, regulations and relevant institutions. In today's conditions, where the number of seasonal agricultural workers is increasing, the agricultural policies that cause this number are the subjects that should be investigated primarily. After January 24, 1980, a policy change emerged that would make agricultural production subject to market conditions. The way for imports has been cleared, input subsidies have been removed, product price support has almost ended and its scope has been narrowed. Input costs in agriculture have increased. Due to the conflicts that have been going on for years, the state of emergency, the village evacuation, and the lack of land reform, few landed and landless peasants have been added to the city of Mardin. Each of these has brought about an increase in the number of seasonal agricultural workers. These reasons are increasing rather than disappearing. As a result, seasonal agricultural work has become a family business as the only way to live and work. Seasonal agricultural work has become a source of livelihood for poor families working in cotton farming in Mardin, by migrating to other seasonal

agricultural regions at certain times of the year, and by working with local products in their own country at certain times. Recently, with the increasing refugee Syrian family population in Turkey, there has been an increasing labor demand for agricultural products in the province of Mardin.

- As can be understood from the results of the analysis, the factors that affect the female working in the cotton harvest and their children under the age of 18 to do this work are explained. Employees are generally low-educational, uninsured and unregistered workers, trying to live in primitive places such as houses and tents, deprived of environmental cleanliness and hygienic conditions, and deprived of social and cultural life activities.

- For this purpose, suggestions and measures that can be taken to guide policy makers in order to improve the living conditions of seasonal workers working in the cotton harvest in Mardin province can be listed as follows.

- Arrangements should be made to enable seasonal agricultural workers to unionize and bargain collectively.

- It is necessary to improve the current situation of seasonal migrant agricultural workers in terms of social security, to make the employment contract in agriculture compulsory, to pay wages for business intermediaries and landowners/workers, and to benefit from universal health insurance.

- In order for the children of the compulsory education age to reach the education services, the children of the workers who are in the compulsory education age should be admitted to the Regional Primary Boarding Schools in their own regions or the places they go as guest students or in the places where seasonal agricultural works such as transported education are carried out, such as courses, schools, etc. Children are required to attend school by choosing the most appropriate opportunity.

- Measures should be taken for the preparation of exposure to pesticides and chemicals from agriculture, to be raised and to health services, free public health center, shelter and living will be provided to live in a humane way, bathrooms, toilets, etc. should be provided. ensuring that places are appropriately suited and you are informed about business development. "Consolidated Temporary Settlement Areas" can be created for sheltering in adequate, safe and healthy accommodation areas such as container houses instead of tents in primitive conditions.

- It can be ensured that the workers can travel safely and healthily during the migration season, and the road safety can be increased during the transportation of seasonal agricultural workers from their residences to the places where they will work and from the field to the accommodation area.

- Ensuring the safety of accommodation areas and seasonal agricultural workers, taking effective measures to prevent illegal agricultural work of foreign nationals, and security awareness raising activities can be carried out in Mardin, which is a place of immigration and immigration.

Measures should be taken for the socialization of seasonal migratory agricultural workers, awareness-raising activities and social cultural activities can be carried out in order to prevent all kinds of social exclusion and to ensure social cohesion.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Determination of Yield, and Cold Hardiness of Some Triticale (*xTriticosecale* Wittmack) Genotypes in Eastern Anatolia Region

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ABSTRACT

Triticale (*xTriticosecale* Wittmack) is a grain used in animal feed and is known for its high efficiency, high nutritional quality and resistance to stress factors. Triticale is an alternative plant used for the utilization of marginal areas due to these properties. This study was carried out at three different locations in Erzincan and Muş province and Pasinler districts of Erzurum province. Two candidate line and registered triticale varieties (Umranhanım) and 22 triticale lines in the advanced breeding stage were assessed comparatively in terms of efficiency yield and cold resistance parameters. According to the results of this study, Candidate-2 and Line (1, 6, 8, 11, 15, 17, and 18) genotypes were the prominent genotypes in terms of yield. In addition, low precipitation in May and June caused serious losses in yield. Because this period is the pollination period for grains in the Eastern Anatolia Region. Additional irrigation may be recommended in years when precipitation in this period is insufficient. In addition, it has been concluded that it is important to include cold test studies in breeding programs in regions where winter damage is experienced intensively as well as included in the selections.

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Introduction

Genetically, triticale (*xTriticosecale* Wittmack) is a cool climate cereal type obtained by hybridizing wheat and rye. Triticale can generate more yield than wheat, especially in barren regions where soil depth is not suitable for wheat cultivation and winters are severe (Küçüközdemir et al., 2019).

12.80 million tons were produced in an area of 3.81 million hectares in the world. The countries with the highest triticale production are respectively; France, Germany, Belarus, China, Lithuania, Hungary, Austria, Czech Republic, Romania and Serbia (FAOSTAT, 2019). In Turkey, a total of 215.1 tons of triticale was produced from an area of 64.1 thousand hectares, and the average yield is 336 kg/da (TSI, 2019).

In the evaluation of marginal areas, it is stated that Triticale is the priority plant that is capable to increase the

cultivation areas and production significantly with the development of new varieties (Müntzing, 1989; Mergoum et al., 1992; Kun, 1996).

Due to limitations in intensive agriculture and possible climatic changes, it will not be easy to increase the production to the extent that it will feed the growing world population. Therefore, the aim is to grow plant species which are more efficient in marginal soils. These plant species should be able to produce high yields with low inputs in marginal or low yield areas. Although, triticale is a newly cultivated plant species, it is rapidly spreading to various production systems (Pfeiffer, 1994).

Soil conditions such as drought, pH level, salinity, lack of trace elements, and toxicity are factors limiting grain yield. Triticale is an advantageous plant in such conditions compared

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to commonly grown cereals. Triticale is an alternative crop to other cereals, especially wheat and barley (Benbelkacem, 1998; Maças et al., 1998; Royo & Aragay, 1998).

Due to the above-mentioned characteristics, it is considered that triticale is an important alternative crop plant in Eastern Anatolia Region, especially in areas where wheat yield is low and unused barren land available for utilization. Therefore, it is important that triticale varieties which are suitable for the ecological conditions of the region and have high efficiency and yield stability are developed and offered to farmers. However, in addition to the genetic yield potential of a variety, the environmental conditions in which the plants are grown are also influence the yield. Under such circumstances, the stability of yield in various environmental conditions is of great importance. Therefore, it is necessary to determine that which genotypes have a stable yield under different environmental conditions. The aim of this study was to determine high efficiency, winter hardiness and high-quality genotypes that could be grown in Erzurum and similar ecological conditions.

Materials and Methods

The trial was carried out for one year during 2019-20 in the trial areas in Erzurum, Pasinler district and Erzincan and Muş province under dry conditions. Two candidate line, Umranhanım types and 22 triticale lines were used in the study. Umranhanım variety used in the study is a winter, medium early, medium-tall (100-120 cm) variety. It is cold and drought resistant.

The winter season trial was established in two different locations in the "Chance Connected Full Blocks" trial design with three replications (Yıldız & Bircan, 1991). Treatments were distributed to the parcels according to chance (Little & Hills, 1978; Yıldız & Bircan, 1991; Mead et al., 1994). Each parcel consisted of 6 plant rows of 6 m in length with 20 cm spacing, and the area of a parcel was 7.2 m² (6 m length x 1.2 m width).

Since there is no recommended date for planting triticale, the planting for the trial was carried out between the dates of September 1 and October 1 which is the most suitable date for planting winter wheat (Özcan & Acar, 1990). The seeds were sown with row spacing 20 cm apart at a depth of 4-6 cm and 475 seeds per m² with a seed drill. Ammonium nitrate (26%) was used as a nitrogen fertilizer source. Half of the nitrogen fertilizer was applied during sowing and the half during bolting at a rate of 6 kg N and 6 kg P₂O₅ per decare while the phosphor fertilizer was all applied with the planting (Kıral & Özcan, 1990; Akkaya, 1993). Weed control was carried out during the tillering period in rainless and windless weather using the 2,4-D herbicide at a rate of 200 cc/da (Özcan, 1994).

When the wheat reached at harvestable maturity, 50 cm was cut off from each parcel as edge effect and the remaining parts were harvested and blended with a parcel harvester. (Kıral & Özcan, 1990; Akkaya, 1993). Cold test studies were carried out according to the method used by Küçüközdemir et al. (2020). Plants were tested at three different cold temperatures (-17, -19, and -21). In this study, LD50, which is the degree to which more than 50% of a population died, was used to determine the degree of cold hardiness.

Statistical Analysis

The data were determined according to analysis of variance using SPSS 10.0 software package and when the medium was determined, Duncan's Multiple Range Test was used.

Results and Discussion

In the study conducted with 25 triticale genotypes, significant statistical differences were found among the yield of the examined genotypes in all locations and between all locations. The mean values of yield and the statistical groups of the factors according to these averages (P<0.01) was given in Table 1.

Table 1. Yield of location and genotypes (kg/da)

Genotypes	Erzincan**	Pasinler**	Mus**	Mean**
Candidate-1	217.0 a	395.0 ab	301.3 gh	304.4 D-G
Candidate-2	185.3 de	355.0 a-c	499.0 a-c	346.4 A-D
Umranhanım	217.0 a	398.3 ab	493.7 a-c	336.3 A-E
Line-1	194.3 c-e	414.7 ab	503.7 a	370.9 A
Line-2	159.0 g	374.3 a-c	392.0 c-g	308.4 C-G
Line-3	205.0 bc	352.3 a-c	399.0 a-g	318.8 B-G
Line-4	186.0 de	344.0 a-c	331.7 f-h	287.2 E-G
Line-5	130.7 h	347.3 a-c	395.0 b-g	291.0 E-G
Line-6	183.0 d-f	431.7 a	424.0 a-f	346.2 A-D
Line-7	177.0 ef	300.3 bc	418.7 a-f	298.7 D-G
Line-8	212.3 ab	363.0 a-c	502.0 ab	359.1 A-C
Line-9	183.0 d-f	272.3 c	466.7 a-d	307.3 C-G
Line-10	183.3 d-f	353.3 a-c	363.3 d-h	300.0 D-G

(continues on the next page)

Table 1 continued

Genotypes	Erzincan**	Pasinler**	Mus**	Mean**
Line-11	166.7 fg	399.7 ab	507.3 a	357.9 A-C
Line-12	125.7 h	355.7 a-c	447.0 a-d	309.4 C-G
Line-13	133.3 h	398.0 ab	328.3 f-h	286.6 E-G
Line-14	183.0 d-f	361.0 a-c	334.0 e-h	292.7 E-G
Line-15	190.0 c-e	371.0 a-c	439.7 a-e	333.6 A-E
Line-16	190.7 c-e	361.3 a-c	403.3 a-g	318.4 B-G
Line-17	224.3 a	361.7 a-c	506.0 a	364.0 AB
Line-18	155.0 g	369.3 a-c	446.0 a-d	323.4 A-F
Line-19	193.7 c-d	369.0 a-c	276.0 h	279.6 FG
Line-20	180.0 ef	301.3 a-c	328.3 f-h	269.9 G
Line-21	201.0 b-d	316.0 bc	411.7 a-f	309.6 C-G
Line-22	201.0 b-d	345.7 a-c	316.3 f-h	287.7 E-G
Mean	183.1 C	360.5 B	405.4 A	316.3

According to the Duncan test, the averages shown with the same letter are not important in their group ($p < 0.05$).

On the basis of locations, the highest yield was determined in Mus location (405.4 kg/da). This location was followed by Pasinler location (360.5 kg/da). The lowest yield was measured in Erzincan location (183.1 kg/da). The reason for the low yield in the Erzincan location is the low precipitation during the flowering period, which coincides with May and June (Figure 1). Low precipitation during the flowering period caused insufficient pollination. In other locations, precipitation and temperatures remained within seasonal normal. As a result, higher yields were obtained in Pasinler and Mus locations (Table 1).

The average of the genotypes in the study was determined as 316.3 kg/da. The highest average yield was determined in Line-1 (370.9 kg/da) genotype. Candidate-2, Umrhanım variety, Line-6, Line-8, Line-11, Line-15, Line-17, and Line-18 were statistically in the same group with the maximum group. On the contrary, the lowest average yield was measured as 269.9 kg/da in Line-20 genotype.

Yields in Erzincan location varied between 125-217 kg/da according to genotypes. The highest yields were measured in Umrhanım cultivar and Candidate-1 genotype, which are known to be drought tolerant. In this location, low precipitation in May and June caused yield loss (Figure 1). However, it was observed that genotypes that were more tolerant than other genotypes were relatively less affected by stress factors.

In Pasinler location, as the highest yield was measured 431.7 kg/da, the lowest yield was determined as 272.3 kg/da. Line-1, Line-11, Line-13, and Candidate-1 genotypes and Umrhanım cultivar are other prominent genotypes in this location (Table 1). Precipitation and temperatures at seasonal normal in this location positively affected plant growth (Figure 2). Especially the rains during the flowering period encouraged the formation of grains.

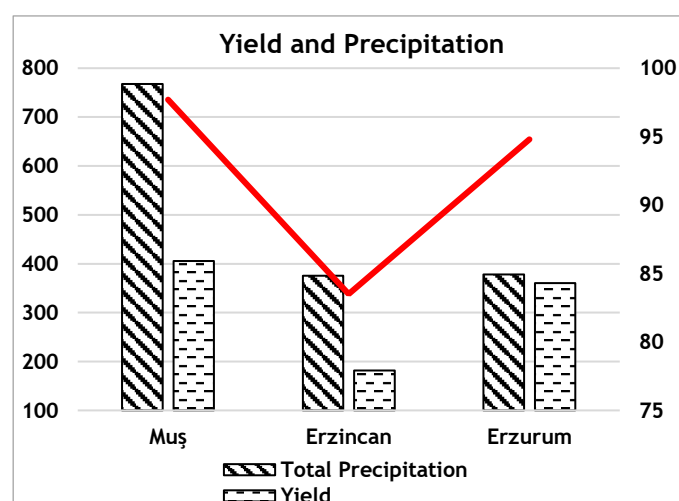


Figure 1. Yield and precipitation.

In the Mus location, where the highest yields were obtained in the study, the yields varied between 507.3-276 kg/da. Line-1, Line-11, and Line-17 were the genotypes with the highest yield in the study. Also, Umrhanım variety, Candidate-2, and Line 8 genotypes were other prominent genotypes (Table 1).

Similar to our work, Küçüközdemir (2016) carried out a study under Erzurum conditions for 10 years with 4 triticale and 3 wheat varieties to obtain the highest and stable grain yield of 418.9 kg/da with Umrhanım. Another study on triticale in Erzurum's arid conditions, the total yield was between 219.9-466.6 kg/da and the differences between the Triticale genotypes were considered significant (Küçüközdemir et al., 2018). In another study, the yield of triticale was determined as 137 kg/da in Erzincan location and 414 kg/da in Erzurum location (Küçüközdemir et al., 2019).

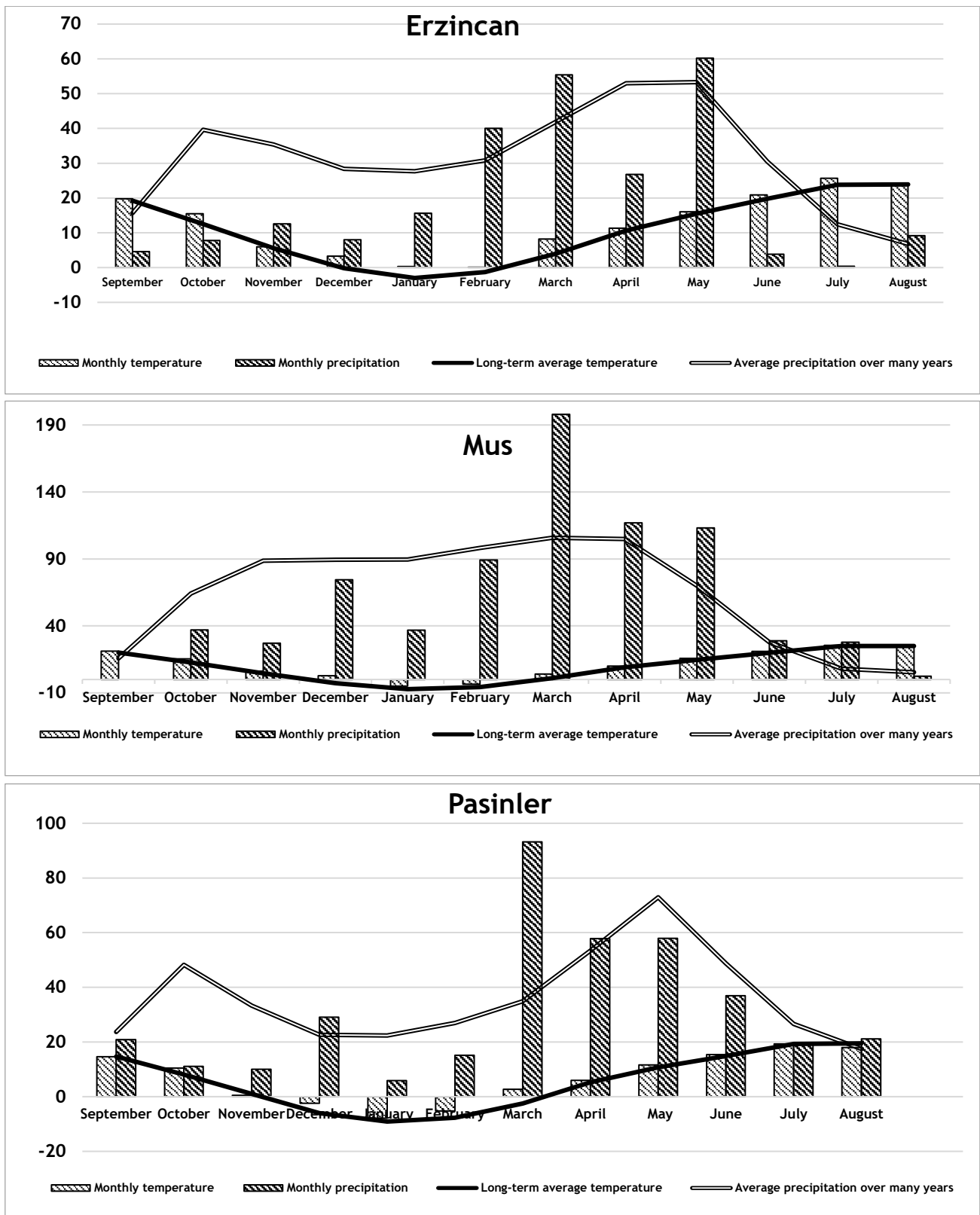


Figure 2. Climate data of locations.

Cold Hardiness

Plants do not only struggle with drought stress in the natural process. Especially in high altitude regions, cold damage causes serious yield losses. The most effective way to combat cold damage is to develop new varieties that are resistant to cold. In this study, cold resistance degrees of triticale genotypes were determined under controlled conditions. According to the test results, it was observed that the genotypes were viable 98.5% at minus 17 degrees, 94.1% at minus 19 degrees, and 75.7% at minus 21 degrees (Figure 3). The reason why viability values are so high is that these

genotypes have been selected for cold resistance for many years. In a study on triticale, winter varieties were found to be more than 85% cold hardiness in -21 degrees (Küçüközdemir et al., 2019). In another study on bread wheat (*Triticum aestivum* L.) that another type of grain known as the parent of triticale, it was determined that some bread wheat lines were more than 60% cold hardiness in -21 degrees (Küçüközdemir et al., 2020). In another cold resistance study on bread wheat, cold hardiness was found to be 67 percent in -21 degrees (Karagöz et al., 2020). This situation changed according to plant species and genotypes. Because triticale is the most resistant to cold among cereals (Küçüközdemir et al., 2019).

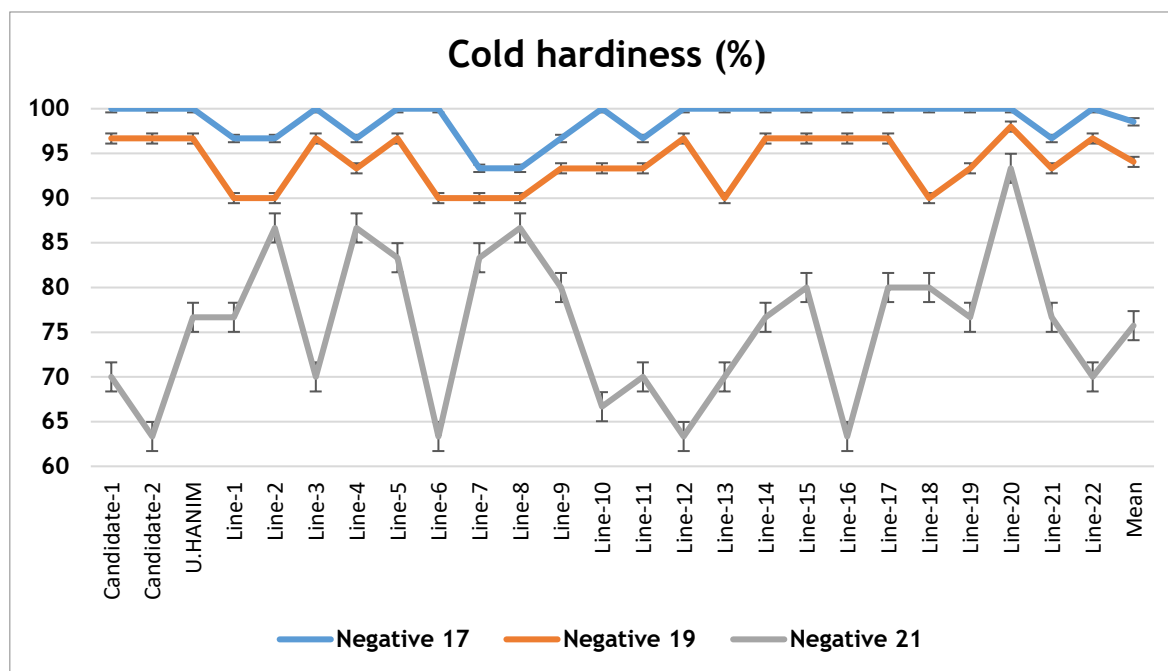


Figure 3. Cold hardiness test.

Conclusion

This study was conducted with three different locations and 25 different genotypes. According to the results of this study, Candidate-2 and Line (1, 6, 8, 11, 15, 17, and 18) genotypes were the prominent genotypes in terms of yield. In addition, low precipitation in May and June caused serious losses in yield. Because this period is the pollination period for grains in the Eastern Anatolia Region. Additional irrigation may be recommended in years when precipitation in this period is insufficient. In addition, it is a very important that cold resistance observations are included in the breeding programs in regions such as East Anatolia with severe winters and high risk of frost to avoid producers in the region from being affected by winter damage and have a more efficient production. Also, cold resistance studies should be a breeding selection criterion in cold climates such as the Eastern Anatolia region. It is very important to cold hardiness varieties in cold climates for a more stable production. The absolute way to achieve this is the need to develop cold-hardiness varieties.

Conflict of Interest

The authors declare that they have no conflict of interest.

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REVIEW ARTICLE

Use of Phytochemicals as Feed Supplements in Aquaculture: A Review on Their Effects on Growth, Immune Response, and Antioxidant Status of Finfish

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ABSTRACT

Aquaculture production is increasing day by day to meet the protein need of the global population. Various feed additives are used in aquaculture to enhance growth, stimulate immunity, prevent diseases, and strengthen the antioxidant status of fish. Phytochemicals attract attention among these feed additives. As phytochemicals are natural products, they are considered to be safe for fish, humans, and the environment. In this paper, we reviewed recent studies that utilize phytochemicals as feed additives in cultured fish species. In agreement with the available literature, we inferred that phytochemicals could be used in aquaculture. However, as some studies reported undesirable effects on growth, we believe that phytochemicals are more effective in immunostimulation and enhancing antioxidant status rather than growth-promoting. Possible reasons for growth retardation were emphasized. Although available evidence suggests that phytochemicals display beneficial effects, we discussed the possible use of phytochemical combinations to obtain even more desirable results. To conclude, we think that phytochemicals can exert synergistic effects, and this approach should be investigated in future studies.

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Introduction

Aquaculture has become an important industry and the world's fastest-growing agricultural business sector and is an important commercial activity in many countries (Kumari & Sahoo, 2006; Villa-Cruz et al., 2009; Karga et al., 2020). Global aquaculture production has increased vastly in the last years for the protein requirement of humans (Özçelik et al., 2020; Salem et al., 2021). Aquaculture practices have become more intense to supply the demand because the wild fish stocks are getting scarce as days pass. With this intensification, problems and threats started increasing. These are high production costs, diseases, stress, environmental impact, animal welfare issues, and organic production demand (Sönmez, Bilen, Alak, et al., 2015; Sönmez, 2017; Arslan et al., 2018; Elbesthi et al., 2020). For example, fish are exposed to several infectious

diseases, reducing the fish yield (Erguig et al., 2015; Syahidah et al., 2015). The use of antibiotics and chemotherapeutic agents for controlling diseases can reduce mortality and improve growth rates; however, they are often considered an expensive and unhealthy way to treat any disease (Ferguson et al., 2010). Moreover, the antibiotic and chemotherapeutic residues can remain in fish tissues, which may threaten the health of human consumers and cause pollution in the aquatic environment (Bulfon et al., 2017; Erguig et al., 2015; Syahidah et al., 2015). Another problem that arises in aquaculture is high production costs, mostly due to the feed expense. Feed is a limiting factor, particularly in carnivorous fish culture, because so far, the fish meal in the feed has not been successfully replaced with another substance. And since the fish meal also comes from the wild fish populations, the

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scarcity of the fish stocks also affects feed availability and therefore has a great impact on the sustainability of aquaculture. Studies in the last few decades have intensely focused on natural products to overcome these problems (Cavalcante et al., 2020; de Oliveira et al., 2020; Ganeva et al., 2020; Adeshina et al., 2021; Sangari et al., 2021). Among these natural products, phytochemicals attract attention due to their great health benefits. Phytochemicals are, in simple terms, bioactive compounds of plant origin (Lillehoj et al., 2018), which exhibit a wide array of beneficial effects such as antiviral, anticancer, growth-promoting, antibiotic, antioxidant, immune-stimulating, and anti-inflammatory effects (Lillehoj et al., 2018; Mani et al., 2020; Zheng et al., 2019; Choudhari et al., 2020, Terzi et al., 2021). In this paper, we reviewed recent studies that utilize phytochemicals in fish culture.

Health Benefits of Phytochemicals

Phytochemicals are non-nutritive compounds, and they often have a pharmacological effect (Leitzmann, 2016). Based on its definition, although phytochemicals may include various groups, Chakraborty et al. (2014) classified them as essential oils, steroids, terpenoids, phenolics, pigments, flavonoids, and alkaloids according to their chemical structures. For humans, Oomah (1999) reviewed certain phytochemicals' preventive or therapeutic health effects on cardiovascular diseases, diabetic neuropathy, gastrointestinal disorders, gynecological

disorders, neurological disorders, inflammation, immunological disorders, vision, cancer, urinary tract infection. Previous studies on fish have also reported beneficial effects of phytochemicals, including but not limited to antimicrobial properties, immunostimulation, appetite stimulation, growth promotion, and antistress (Citarasu, 2010; Chakraborty & Hancz, 2011). Phytochemicals have great potential to be used in animal diets as the main active substance and/or source for the new drugs (Suttili et al., 2017).

Phytochemicals as Growth Promoters

The cost of aquaculture has led business owners to desire to harvest the maximum yield per unit area, while the scarcity of natural resources has driven scientists to do the same. Both situations add up to the same objective: faster growth of the fish with fewer costs. Hence, growth promotion is among the most important purposes of recent studies (Sönmez, Bilen, Albayrak, et al., 2015). Phytochemicals are successfully demonstrated to promote growth in various fish species. However, some studies obtained adverse effects on growth, probably due to the presence of anti-nutritional factors (Glencross et al., 2006; Chakraborty et al., 2014). Nonetheless, these anti-nutritional factors may be partially eliminated through processing techniques (Chakraborty et al., 2014). Recent studies investigating the effects of dietary phytochemical supplementation on growth in fish culture are presented in Table 1.

Table 1. Effects of dietary phytochemical supplementation on growth in fish

Phytochemical	Fish species	Dose* and duration	Notable results**	References
Quercetin	Blunt snout bream (<i>Megalobrama amblycephala</i>)	0.4 and 0.8%, 56 days	↑ FW ↑ WG	Jia, Yan, et al. (2019)
	Grass carp (<i>Ctenopharyngodon idella</i>)	0.4 g kg ⁻¹ , 60 days	↑ FW ↑ WG ↓ FCR	Xu et al. (2019)
	Nile tilapia (<i>Oreochromis niloticus</i>)	0.2, 0.4, 0.8, and 1.6 g kg ⁻¹ , 49 days	↑ FW ↑ WG ↓ FCR ↑ CF	Zhai and Liu (2013)
Genistein	Nile tilapia (<i>O. niloticus</i>)	3 g kg ⁻¹ , 56 days	↓ FW ↓ SGR	Chen et al. (2015)
Ferulic acid	GIFT (<i>O. niloticus</i>)	0.52 nmol kg ⁻¹ , 56 days	↑ FW ↑ WG ↓ FCR	Yu et al. (2017)
Organic acid blend (Formic acid, lactic acid, malic acid, tartaric acid, and citric acid)	Red hybrid tilapia (<i>Oreochromis sp.</i>)	0.5 and 1%, 140 days	↔ Growth	Koh et al. (2016)
Sesamin	Atlantic salmon (<i>Salmo salar</i>)	5.8 g kg ⁻¹ , 120 days	↓ FW ↓ SGR	Schiller Vestergren et al. (2012)
Resveratrol	Blunt snout bream (<i>M. amblycephala</i>)	1%, 56 days	↓ FW ↓ WGR	Jia, Yan, et al. (2019)
Curcumin	Common carp (<i>Cyprinus carpio</i>)	5, 10, and 15 g kg ⁻¹ , 56 days	↑ FW ↑ WG ↓ FCR	Giri et al. (2019)
	Grass carp (<i>C. idella</i>)	393.67 mg kg ⁻¹ , 60 days	↑ FW ↑ WG ↑ SGR ↓ FCR	Ming et al. (2020)
	Nile tilapia (<i>O. niloticus</i>)	5 mg kg ⁻¹ , 112 days	↔ Growth	Mahfouz (2015)

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Table 1 continued

Phytochemical	Fish species	Dose* and duration	Notable results**	References
Curcumin	Nile tilapia (<i>O. niloticus</i>)	50, 100, 150, and 200 mg kg ⁻¹ , 84 days	↑ FW ↑ WG ↑ SGR ↓ FCR	Mahmoud et al. (2017)
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	1, 2, and 4%, 56 days	↑ FW ↑ WG ↑ SGR ↓ FCR	Yonar et al. (2019)
Carvacrol	Rainbow trout (<i>O. mykiss</i>)	1, 3, and 5 g kg ⁻¹ , 60 days	↔ Growth	Yilmaz et al. (2015)
Alkaloids (Koumine, gelsemine, gelsenicine)	Blunt snout bream (<i>M. amblycephala</i>)	40 mg kg ⁻¹ , 84 days	↑ FW ↑ WG ↑ SGR ↓ FCR	Ye et al. (2019)
Sparteine	Rainbow trout (<i>O. mykiss</i>)	0.25, 0.5, 1, 2.5, and 5 g kg ⁻¹ , 62 days	↓ Growth	Serrano et al. (2011)
Tannic acid	European seabass (<i>Dicentrarchus labrax</i>)	10, 20, and 30 g kg ⁻¹ , 35 days	↓ Growth	Omnès et al. (2017)
Tannins	Beluga sturgeon (<i>Huso huso</i>)	0.05 and 0.1%, 42 days	↑ FW ↑ WG ↑ SGR ↓ FCR	Safari et al. (2020)
Condensed tannins	Japanese seabass (<i>Lateolabrax japonicus</i>)	100, 200, and 400 mg kg ⁻¹ , 56 days	↔ Growth	Peng et al. (2020)
Saponins	Common carp (<i>C. carpio</i>)	150 mg kg ⁻¹ , 56 days	↑ WG	Francis et al. (2002)
	Olive flounder (<i>Paralichthys olivaceus</i>)	6.4 g kg ⁻¹ , 56 days	↓ Growth	Chen et al. (2011)

* Doses given in the table are those supplemented to the fish groups where the notable results were observed. Not every dose was given since otherwise, the presentation of the varying results would be complicated.

** Results of some studies, in which some of the doses displayed different results, were given based on the general conclusions of that particular study. The reader is referred to the relevant article in the references list for exact results.

Symbols: ↑ indicates increase, ↓ indicates decrease, ↔ indicates no change.

Abbreviations: CF Condition factor, FCR Feed conversion ratio, FW Final weight, GIFT Genetically improved farmed tilapia, SGR Specific growth rate, WG Weight gain.

Phytochemicals as Immunostimulators

The immune system consists of various humoral and cellular components that protect the body from extraneous substances (Biller-Takahashi & Urbinati, 2014). Immunostimulation is a phenomenon in which the immune response of an organism is stimulated beforehand so that when an extraneous substance enters the body, it must face a more strengthened immune system. Studies have demonstrated that a wide variety of products other than phytochemicals

successfully stimulate the immune response in finfish (Mohamed et al., 2018; Bilen et al., 2020; Makled et al., 2020). Phytochemicals are usually considered safe for fish, humans, and the environment (Chakraborty & Hancz, 2011). Therefore, immunostimulation with phytochemicals is particularly important due to its possibility to replace or minimize the use of antibiotics or chemicals that display undesirable effects. Table 2 shows the studies conducted on the immunity of cultured finfish with dietary administration of phytochemicals.

Table 2. Effects of dietary phytochemical supplementation on serum biochemistry and immunity in fish

Phytochemical	Fish species	Dose* and duration	Notable results**	References
Quercetin	Blunt snout bream (<i>Megalobrama amblycephala</i>)	0.4 and 0.8% alone or combined with 0.5 or 1% resveratrol, 56 days	• Reversing high-fat diet-induced depression of immunity	Jia, Yan, et al. (2019)
	Olive flounder (<i>Paralichthys olivaceus</i>)	0.5% combined with 6.8% spirulina, 70 days	↑ LYS	Kim et al. (2013)
	Olive flounder (<i>P. olivaceus</i>)	0.25 and 0.5%, 60 days	↑ LYS • Improved immunity against external stress	Shin et al. (2010a)
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	1%, 14 days	↑ LYS ↑ MPO ↑ Total protein ↑ Antiprotease activity ↑ Bactericidal activity	Awad et al. (2013)

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Table 2 continued

Phytochemical	Fish species	Dose* and duration	Notable results**	References
Daidzein	Turbot (<i>Scophthalmus maximus</i>)	40 and 400 mg kg ⁻¹ , 84 days	<ul style="list-style-type: none"> • Mitigating intestinal inflammation • Improving the tight junction barrier 	Ou et al. (2019)
Caffeic acid	Nile tilapia (<i>Oreochromis niloticus</i>)	5 g kg ⁻¹ , 60 days	<ul style="list-style-type: none"> ↑ Phagocytic index ↑ Potential killing activity ↑ Respiratory burst activity ↑ MPO ↑ IRGE • Resistance against <i>Aeromonas veronii</i> 	Yilmaz (2019)
Ferulic acid	Nile tilapia (<i>O. niloticus</i>)	80 mg kg ⁻¹ , 60 days	<ul style="list-style-type: none"> ↑ Phagocytic activity ↑ LYS ↑ IRGE 	Dawood et al. (2020)
	Common carp (<i>Cyprinus carpio</i>)	100 mg kg ⁻¹ , 56 days	<ul style="list-style-type: none"> ↑ Total IgM ↑ Respiratory burst activity ↑ LYS • Resistance against <i>Aeromonas hydrophila</i> 	Ahmadifar et al. (2019)
Trans-cinnamic acid	Rainbow trout (<i>O. mykiss</i>)	250 and 500 mg kg ⁻¹ , 60 days	<ul style="list-style-type: none"> ↑ Blood granulocyte percentage ↑ Total protein ↑ Globulin ↑ LYS ↑ Total Ig ↑ Phagocytic activity ↑ Respiratory burst activity ↑ Potential killing activity ↑ IRGE • Resistance against <i>Yersinia ruckeri</i> 	Yilmaz and Ergün (2018)
Resveratrol	GIFT (<i>O. niloticus</i>)	0.5 g kg ⁻¹ , 45 days	<ul style="list-style-type: none"> • Enhanced immunity 	Zheng et al. (2019)
	Turbot (<i>S. maximus</i>)	0.05%, 56 days	<ul style="list-style-type: none"> • Mitigating inflammatory response caused by soybean meal 	Tan et al. (2019)
Curcumin	Common carp (<i>C. carpio</i>)	10, and 15 g kg ⁻¹ , 56 days	<ul style="list-style-type: none"> ↑ LYS ↑ Total Ig ↑ Total protein ↑ ALP ↑ Protease activity ↑ Peroxidase activity • Resistance against <i>Aeromonas hydrophila</i> • Anti-inflammatory effect 	Giri et al. (2019)
	Grass carp (<i>Ctenopharyngodon idella</i>)	393.67 mg kg ⁻¹ , 60 days	<ul style="list-style-type: none"> ↑ LYS ↑ Acid phosphatase ↑ C3 and C4 ↓ ALT ↓ AST ↑ LYS, C3, and antimicrobial peptide gene expression levels • Anti-inflammatory effect 	Ming et al. (2020)
	Nile tilapia (<i>O. niloticus</i>)	200 mg kg ⁻¹ , 60 days	<ul style="list-style-type: none"> ↑ Total protein ↑ Globulin ↑ α globulin-1 ↑ α globulin-2 • Resistance against <i>Aeromonas hydrophila</i> 	Abd El-Hakim et al. (2020)
	Nile tilapia (<i>O. niloticus</i>)	50, 100, 150, and 200 mg kg ⁻¹ , 84 days	<ul style="list-style-type: none"> ↑ LYS ↑ IgG ↑ IgM • Antibacterial effect • Resistance against <i>Aeromonas hydrophila</i> 	Mahmoud et al. (2017)

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Table 2 continued

Phytochemical	Fish species	Dose* and duration	Notable results**	References
Curcumin	Rainbow trout (<i>O. mykiss</i>)	1, 2, and 4%, 56 days	<ul style="list-style-type: none"> ↑ WBC ↑ Oxidative Radical Production ↑ Phagocytic activity ↑ Phagocytic index ↑ Total protein ↑ IgM ↑ Bactericidal activity ↑ LYS ↑ MPO • Resistance against <i>Aeromonas salmonicida</i> subsp. <i>achromogenes</i> 	Yonar et al. (2019)
Alkaloids (Koumine, gelsemine, gelsenicine)	Blunt snout bream (<i>M. amblycephala</i>)	20 and 40 mg kg ⁻¹ , 84 days	<ul style="list-style-type: none"> ↑ C3 and C4 ↑ IgM • Resistance against <i>Aeromonas hydrophila</i> ↑ IRGE ↓ TGF-β and IL10 expression levels 	Ye et al. (2019)
Tannins	Beluga sturgeon (<i>Huso huso</i>)	0.05 and 0.1%, 42 days	<ul style="list-style-type: none"> ↑ LYS ↑ Peroxidase activity ↔ ALT ↔ AST ↔ ALP 	Safari et al. (2020)
Condensed tannins	Japanese seabass (<i>Lateolabrax japonicus</i>)	100, 200, and 400 mg kg ⁻¹ , 56 days	<ul style="list-style-type: none"> ↔ ALP ↔ LYS ↔ IgM ↓ TNF-α ↓ IL-6 ↔ IL-8 ↔ Serum biochemistry • Protection against hypoxia 	Peng et al. (2020)

* Doses given in the table are those supplemented to the fish groups where the notable results were observed. Not every dose was given since otherwise, the presentation of the varying results would be complicated.

** Results of some studies, in which some of the doses displayed different results, were given based on the general conclusions of that particular study. The reader is referred to the relevant article in the references list for exact results.

Symbols: ↑ indicates increase, ↓ indicates decrease, ↔ indicates no change.

Abbreviations: ALP Alkaline phosphatase, ALT Alanine aminotransferase, AST Aspartate aminotransferase, C3 Complement 3, C4 Complement 4, GIFT Genetically improved farmed tilapia, Ig Immunoglobulin, IgG Immunoglobulin G, IgM Immunoglobulin M, IRGE Immune-related gene expression, LYS Lysozyme, MPO Myeloperoxidase, WBC White blood cell.

Phytochemicals as Antistress Agents

The survival of an animal depends on its internal balance and its compatibility with the environment (Cengiz, 2001). When an animal's internal balance is stable and compatible with its environment, the very animal lives under normal conditions. Stress, on the other hand, is the response of an animal to an abnormal condition (Cengiz, 2001). Stress response in fish generates a variety of physiological changes in mechanisms such as metabolism, immunity, behavior, gene expression, protein synthesis, endocrine, et cetera (Tort, 2011). In aquaculture, stress can cause susceptibility to

diseases, growth retardation, and interference of reproduction (Pickering, 1993). Furthermore, fish may get stressed easily in farm conditions due to handling, transportation, high stocking density, and poor water quality (Bilen et al., 2013; Almabrok et al., 2018). Phytochemicals are good feed additives for fish in farm conditions to cope with stress because some phytochemicals may exert a direct antioxidant effect beyond supporting the antioxidant system of the fish (Yu et al., 2017; Ahmadifar et al., 2019; Bhattacharjee et al., 2020). Recent studies investigating the antioxidant potential of dietary phytochemical supplementation in finfish are presented in Table 3.

Table 3. Effects of dietary phytochemical supplementation on antioxidant status in fish

Phytochemical	Fish species	Dose* and duration	Stressor	Notable results**	References
Quercetin	Blunt snout bream (<i>Megalobrama amblycephala</i>)	0.4 and 0.8% alone or combined with 0.5 or 1% resveratrol, 56 days	High-fat diet	↑ SIRT1 ↑ Cu/Zn-SOD ↑ CAT ↑ GPx	Jia, Yan, et al. (2019)
	<i>Channa punctata</i>	0.14 g L ⁻¹ , 21 days	Deltamethrin	• Amelioration of oxidative stress and acetylcholinesterase inhibition • Recovery from nucleic acid impairment and alteration of blood parameters	Bhattacharjee et al. (2020) ^A
	Grass carp (<i>Ctenopharyngodon idella</i>)	0.4 and 0.6 g kg ⁻¹ , 60 days	-	↑ SOD	Xu et al. (2019)
	Olive flounder (<i>Paralichthys olivaceus</i>)	0.25 and 0.5%, 60 days	Hypo-osmotic conditions	↓ SOD ↓ CAT ↓ H ₂ O ₂ ↓ Cortisol • Protection against stress	Shin et al. (2010a)
	Olive flounder (<i>P. olivaceus</i>)	0.25 and 0.5%, 60 days	Cadmium	↓ SOD ↓ CAT ↓ H ₂ O ₂ ↓ MDA • Protection against Cd exposure	Shin et al. (2010b)
Rutin	Silver catfish (<i>Rhamdia quelen</i>)	1.5 g kg ⁻¹ , 21 days	OTC	↓ LPO ↑ SOD ↑ GST ↔ GPx ↔ GR	Pês et al. (2018)
	Silver catfish (<i>R. quelen</i>)	0.15 and 0.30%, 21 days	-	↓ Cortisol ↓ LPO • Increased antioxidant status in various tissues	Pês et al. (2016)
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	500, 1000, and 2000 ppm, 28 days	OTC	• Preventing OTC induced hepatic damage and oxidative stress	Nazeri et al. (2017)
Daidzein	Turbot (<i>Scophthalmus maximus</i>)	5, 10, and 20 mg kg ⁻¹ , 84 days	-	↑ SOD ↑ GPx ↓ MDA	Hu et al. (2014)
Ferulic acid	GIFT (<i>Oreochromis niloticus</i>)	1.04 and 2.08 nmol kg ⁻¹ , 56 days	-	↑ SOD ↑ CAT ↑ GPx ↓ MDA	Yu et al. (2017)
	Nile tilapia (<i>O. niloticus</i>)	80 mg kg ⁻¹ , 60 days	Heat stress	↑ SOD ↑ CAT ↑ GPx ↓ MDA • Mitigating the effects of heat stress	Dawood et al. (2020)
	Common carp (<i>Cyprinus carpio</i>)	100 mg kg ⁻¹ , 56 days	-	↔ SOD ↑ CAT ↑ GPx	Ahmadifar et al. (2019)
Sesamin	Common carp (<i>C. carpio</i>)	0.5 and 1 g kg ⁻¹ , 90 days	Fluoride	• Alleviating renal damage and apoptosis ↓ ROS • Reduction of oxidative stress	Cao et al. (2015)
Resveratrol	Nile tilapia (<i>O. niloticus</i>)	0.1, 0.3, and 0.6 g kg ⁻¹ , 60 days	Oxidative stress-induced liver damage by H ₂ O ₂ injection	• Amelioration of liver injury ↑ Antioxidant activity ↓ LPO	Jia, Li, et al. (2019)
Curcumin	<i>Anabas testudineus</i>	0.5 and 1%, 2 and 56 days	-	• 14 days ↓ MDA ↓ GSH • 56 days ↔ MDA ↑ GSH	Manju et al. (2012)
	Common carp (<i>C. carpio</i>)	10, and 15 g kg ⁻¹ , 56 days	-	↑ SOD ↑ CAT ↓ MDA	Giri et al. (2019)

(continues on the next page)

Table 3 continued

Phytochemical	Fish species	Dose* and duration	Stressor	Notable results**	References
	Grass carp (<i>C. idella</i>)	393.67 mg kg ⁻¹ , 60 days	-	↑ GSH ↑ SOD ↑ CAT ↑ GPx ↑ GST ↑ GR ↓ ROS ↓ MDA	Ming et al. (2020)
Curcumin	Nile tilapia (<i>O. niloticus</i>)	200 mg kg ⁻¹ , 60 days	Melamine	↑ GPx ↑ SOD ↓ MDA • Mitigating adverse effects of melamine	Abd El-Hakim et al. (2020)
	Nile tilapia (<i>O. niloticus</i>)	5 mg kg ⁻¹ , 112 days	Aflatoxin B1	• Amelioration of aflatoxin-induced down-regulation of antioxidant gene expression levels	Mahfouz (2015)
	Rainbow trout (<i>O. mykiss</i>)	1, 2, and 4%, 56 days	-	↑ SOD ↑ CAT ↑ GPx ↓ MDA	Yonar et al. (2019)
Tannins	Beluga sturgeon (<i>Huso huso</i>)	0.05 and 0.1%, 42 days	-	↑ SOD ↑ CAT	Safari et al. (2020)
Condensed tannins	Japanese seabass (<i>Lateolabrax japonicus</i>)	100, 200, and 400 mg kg ⁻¹ , 56 days	Hypoxic stress	↑ Total antioxidant capacity ↑ CAT	Peng et al. (2020)
				↑ GPx ↔ GST ↔ SOD ↓ MDA	

* Doses given in the table are those supplemented to the fish groups where the notable results were observed. Not every dose was given since otherwise, the presentation of the varying results would be complicated.

** Results of some studies, in which some of the doses displayed different results, were given based on the general conclusions of that particular study. The reader is referred to the relevant article in the references list for exact results.

^A This study, unlike others, administered the phytochemical via bathing.

Symbols: ↑ indicates increase, ↓ indicates decrease, ↔ indicates no change.

Abbreviations: CAT Catalase, Cu/Zn-SOD Copper zinc superoxide dismutase, GIFT Genetically improved farmed tilapia, GPx Glutathione peroxidase, GR Glutathione reductase, GSH Glutathione, GST Glutathione s-transferase, H₂O₂ Hydrogen peroxide, LPO Lipid peroxidation, MDA Malondialdehyde, OTC Oxytetracycline, ROS Reactive oxygen species, SIRT1 Sirtuin-1, SOD Superoxide dismutase.

Overview on the Use of Phytochemicals in Aquaculture

In the present paper, we observed that phytochemicals have great potential to be used in aquaculture as feed additives. According to studies reviewed (Tables 1-3), we can infer that phytochemicals are more effective in immunostimulation and improvement of antioxidant status in fish than enhancing growth because some studies reported growth retardation after phytochemical supplementation. The reason behind this inference is that some phytochemicals may exhibit inhibitory effects on digestive enzyme activities (Chen et al., 2015) or adversely affect feed palatability (Serrano et al., 2011; Omnes et al., 2017). The phenomenon regarding palatability is particularly important for carnivorous fish as normally their diets do not contain herbal compounds; thus, certain phytochemicals may reduce ingestion (Lall & Tibbetts, 2009). Moreover, growth retardation may also be attributed to the dosage of the phytochemical supplement. As can be seen in Table 1, studies reporting decreased growth performance usually administered relatively high levels of phytochemicals. Furthermore, one should not neglect that the palatability is also under the influence of several other factors such as the chemical nature of the substances in the feed, water pH, water temperature, genetic factors, the threshold of the substance for a particular species, et cetera (Kasumyan & Døving, 2003). To avoid such undesirable outcomes, further studies should

consider the possibilities mentioned above while selecting the phytochemical and the dose of administration.

In terms of immunostimulation and antioxidant status, none of the reviewed studies (Tables 2 and 3) reported adverse effects. It is clear from the presented tables that phytochemicals are potent antioxidant and immunostimulatory substances that can be used in aquaculture. However, we have observed that only a small fraction of the studies utilized more than one phytochemical, but we think that combinations of phytochemicals may exhibit synergistic effects that can possibly result in more beneficial results. For example, Eberhardt et al. (2000) reported that Vitamin C is responsible only for 0.4% of the total antioxidant activity of apples. Based on this, Liu (2003) proposed that the antioxidant potency of fruit and vegetables comes from the synergistic effects of phytochemicals rather than one particular compound. As recently reviewed by Zhang et al. (2019), synergistic effects of combined phytochemicals were studied in other animals or human cell lines. However, there is a lack of studies on fish.

Conclusion

To conclude, the use of phytochemicals as feed additives is currently a popular field in aquaculture, and it has been heavily investigated in recent years. There is an adequate quantity of evidence to conclude that dietary supplementation of phytochemicals improves growth, stimulates the immune response, and improves antioxidant status in finfish. However, we think that further studies should investigate the possible synergistic effects of combined phytochemicals. Moreover, more comprehensive research is needed to evaluate the industrial application of phytochemicals at a larger scale.

Conflict of Interest

The authors declare that they have no conflict of interest.

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