SEPTEMBER
2019

INTERNATIONAL JOURNAL OF AGRICULTURE, ENVIRONMENT AND FOOD SCIENCES

YEAR : 2019
VOLUME : 3
ISSUE : 3

www.jaefs.com
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editor@jaefs.com

JAEFS

e-ISSN : 2618-5946
doi : 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

Int J Agric Environ Food Sci

e-ISSN : 2618-5946
DOI: 10.31015/jaefs

www.jaefs.com

September

Volume : 3 Issue : 3 Year : 2019

JAEFS

International Journal of Agriculture, Environment and Food Sciences

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Int J Agric Environ Food Sci 3 (3) September 2019

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (3) June 2019

DOI: 10.31015/jaefs

Production Information

Journal Name	International Journal of Agriculture, Environment and Food Sciences
Abbreviation	Int J Agric Environ Food Sci
Subjects	Agriculture, Environment and Food Sciences
e-ISSN	2618-5946
Publisher	Gultekin Ozdemir
Owner	Gultekin Ozdemir
Language	English
Frequency	Quarterly (March, June, September, December)
Type of Publication	International, Scientific, Open Access Double-blinded peer review Widely distributed periodical
Manuscript Submission and Tracking System	JAEFS uses the submission system of TUBITAK-ULAKBIM JournalPark Open Journal Systems - http://dergipark.gov.tr/jaefs
License	Journal is licensed under a Creative Commons Attribution 4.0 International License
Legal Responsibility	Authors are responsible for content of articles that were published in Journal.
Indexed and Abstracted in	TÜBİTAK ULAKBİM TR Dizin, Crossref, Directory of Open Access Journal (DOAJ), AGORA (Access to Global Online Research in Agriculture), AGRIS (Agricultural Science and Technology Information), WorldCat, Google Scholar, SOBIAD, Scilit, ROAD (Directory of Open Access Scholarly Resources), Neliti, International Citation Index, ROOT Indexing, ResearchBib, Index Copernicus International, ESJI, JournalTOCs, TEELS, ResearchGate, Microsoft Academic
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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (3) September 2019

DOI: 10.31015/jaefs

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JAEFS Journal is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles.
Journal publishes Quarterly (March, June, September, December).
The publication language of the journal is English and continues publication since December 2017.
A Digital Object Identifier (DOI) number has been assigned for each article accepted to be published in JAEFS, starting from December 2017.
Journal of JAEFS welcomes article submissions and does not charge any article submission or processing charges.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (3) September 2019

DOI: 10.31015/jaefs

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.1 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):112-120 (2019)

Improving grain yield, protein ratio and nitrogen use efficiency of durum wheat (*Triticum Durum* Desf.) hybrids using spad meter as a selection criterion

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Abstract

Chlorophyll content can serve as a guide for nitrogen management in agricultural systems. Hence, the investigating leaf chlorophyll in crops could be of benefit to boost production. The present study evaluated 15 different hybrids of durum wheat (*Triticum durum* Desf.) combinations in F_3, F_4 and F_5 generations for nitrogen use efficiency (NUE), grain yield and protein content using chlorophyll meter index (CCI) under three different nitrogen levels (0, 120 and 240 kg N/ha). The results showed that N levels significantly influenced the grain yield and quality traits of durum wheat genotypes, and accordingly, SPAD readings could be used as an indirect selection criterion in durum wheat breeding to achieve the desired production targets. Genetic correlations among grain yield, CCI, grain nitrogen yield (GNY) and protein were high in F_3 generation under high nitrogen regimes. It was also observed that all the generations of Zenit × Menceki, Mersiniye × Menceki, Zenit × Mersiniye, Mersiniye × Spagetti and Spagetti × Menceki crosses have high yield potential and yield stability. It was concluded that the evaluation of the segregation populations at different generations in the same year and selection in the later generations might make a significant contribution to reduce the costs.

Keywords: Breeding, Durum wheat, Hybrid, Nitrogen use efficiency, SPAD-Chlorophyll

Received: 03 June 2019 Accepted: 06 August 2019 Published: 05 September 2019

Introduction

Wheat production occupies an important place for ensuring human nutrition and there is great varietal potential to increase its productivity as the area under crops cannot be increased in several regions. It is wider adaptability as well as the quality of nutritive values than other cereals (Yassin et al., 2019). Hence, it has frequently emphasized the development of appropriate breeding techniques, especially the improvement of new, high yielding quality varieties which are indispensable for vertical expunction i.e., increase productivity (Pena et al., 2002; Tester and Langridge, 2010; Kizilgeci et al., 2019a). It is widely grown food cereal worldwide, due to its wider adaptability as well as the quality of nutritional values than other cereals. Furthermore, it is as a strategic crop that plays a key role in the national economy for several countries (Yildirim et al., 2018; Kizilgeci et al., 2019b). Its demand is increasing day by day to meet the food security of an increasing population (Otu Borlu et al., 2018; Khaled et al., 2018).

Nitrogen requirement of wheat is higher owing to its role in vegetative growth and generative development (Van Keulen and Seligman, 1987; Frederick and Camberato, 1995; Kizilgeci et al., 2016). It has been reported that there is a significant association between nitrogen rates and yield components as well as the yield of wheat (Colkesen et al., 1993) and it is observed that the yield was increased in wheat more than 50% due to nitrogen application (Karaca et al., 1993). This effect is determined by the stay-green period of the spike and flag leaf (Quanyi et al., 2007). Especially in arid climates, photosynthesis in the spike provides important aids to the dry matter which contributes filling of grain (Tambussi et al., 2007). Although the yield and quality traits of wheat largely depend on the optimum nitrogen level (Dogan et al., 2008; Iqbal et al., 2012; Aydogan Cifci and Dogan, 2013) and genotypic variation (Barbottin et al., 2005).

The significant in genetic improvement of nitrogen use efficiency was considered in different studies at various N levels. Ortiz-Monasterio et al. (1997) noted nitrogen use efficiency (NUE) genetic progression of 0.4-1.1 % per year depending on the N levels in spring CIMMYT wheat varieties between 1962 and 1985. Cormier et al. (2014) estimated genetic progress of 0.30-0.37% per year between 1985 and 2010 using 195 European elite winter varieties at optimal and suboptimal N levels. Due to the law of diminishing marginal utility, the nitrogen rates beyond a threshold does not provide improves in yield but increases the production costs and leads to environmental pollution. For that reason, the development of wheat varieties with high NUE considered an essential target for the researchers.

Using SPAD readings to evaluate leaf chlorophyll

Cite this article as:
Kizilgeci, F., Akinci, C., Yildirim, M. (2019). Improving grain yield, protein ratio and nitrogen use efficiency of durum wheat (*Triticum Durum* Desf.) hybrids using spad meter as a selection criterion. Int. J. Agric. Environ. Food Sci., 3(3), 112-120.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 112-120
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1

content has become common and an understanding of the association between these parameters is essential (Markwell et al., 1995). These findings suggest that the association between SPAD readings and chlorophyll concentration per leaf area remains to be established (Xiong et al., 2015). The main objective of plant breeding is to reach the targeted in a short time of frame requires starting with early generations as soon as possible in the selection of promising lines. Early generation testing is based on single-crop yields in F_2, F_3 and F_4 generations. Despite the high environmental impact in single crop selection in early generations, it has been suggested that the use of F_3 generations in early selection will be successful. Significant genetic gains have been achieved so far without the aid of physiological selection tools by conducting wheat breeding programs global.

Recently, it is necessary to improve the methods and management conditions that will improve the effectiveness of existing breeding methods. The proficiency of selection could be improved if specific physiological and/or morphological properties related to yield under specific conditions can be classified and used as selection criteria to plant breeding (Acevedo, 1991; Barutcular et al., 2016; Barutcular et al., 2017). Total chlorophyll content per leaf area can be evaluated quickly, single, and non-destructively using a portable chlorophyll meter such as SPAD-502 (Minolta, Osaka, Japan). SPAD-502 indirectly measures leaf chlorophyll and nitrogen (N) content. Over the past few years, the chlorophyll meter index (CCI) has been widely used to control nitrogen nutrition in cereals. For this purpose the research using SPAD meters was focused on optimizing N application time in durum wheat, maize and rice (Shukla et al., 2004; Vetsch and Randall, 2004; Debaeke et al., 2006; Kizilgeci et al., 2016), to regulate nitrogen supplies in wheat and barley (Peltonen et al., 1995; Spaner et al., 2005), to assess nitrogen status of plant (Tosti and Guiducci, 2010; Xiong et al., 2015), and to use as a reference indicator of N deficiency (Cartelat et al., 2005).

In winter wheat, significant and positive associations were found between the chlorophyll concentration index (CCI) and the yield at the heading stage of wheat (Bavec and Bavec, 2001) and the grain filling stage (Jiang et al., 2004). Yildirim et al. (2011) and Barutcular et al. (2016) noted that CCI can be used as selection criteria to identify high production and quality durum wheat genotypes at rain-fed and irrigated environments. Genetic relationships between grain yield and CCI among F_2 durum wheat progenies was reported at low nitrogen conditions (Kizilgeci et al., 2017). Several researchers have pointed out that grain yield could be predicted by CCI in rice (Kailou et al., 2011), bread wheat (Yildirim et al., 2013) and bread wheat under stress and non-stressed conditions (Barutcular et al., 2016; Jahan et al., 2019). Liu et al. (2017) reported that it is possible to derivate the lines having higher CCI than their parents at heading and later growing stages CCI measurements. However, the present use of SPAD meter is limited in large or small-scale breeding populations and its introgression into elite backgrounds. The stabilization of SPAD meter, as an indirect method for plant selection, provides clarification and direction for breeders to identify and combine this device into new cultivars with high photosynthesis that work synergistically to enhance grain yield. The aim of this study was to assess the grain yield, protein ratio and NUE of hybrid durum wheat populations grown under different nitrogen levels and to investigate the possibility of using CCI as an indirect selection criterion for these traits.

Materials and Methods

This study was carried at Dicle University Research Station, Diyarbakir, Turkey during 2011-2012, to evaluate 15 hybrids of durum wheat combinations in F3, F4 and F5 generations for nitrogen use efficiency (NUE), grain yield and protein content using SPAD chlorophyll meter index (CCI) under three nitrogen levels. The soil of the experimental soil had pH between 7.5 and 7.6 indicating slightly alkaline in nature. The soils were classified as clay loam and salinity was low. Organic matter and phosphorus contents were very low while, potassium content was very high. The soils contain lime between 10.0-11.0% at the depth of 0-60 cm. Total precipitation was 550 mm, and 66 mm more than long-term averages. Precipitation during the critical wheat development stages (from stem elongation to heading and grain filling) was 335 mm. The temperature was 1-2°C higher than the long-term average for the March-May period in Diyarbakir.

Three durum wheat landraces viz. Misiri (1), Mersiniye (3) and Menceki (5) and three commercial durum wheat cultivars viz. Zenit (2), Spagetti (4) and Levante (6) were used as seed material. Crosses were made among the genotypes in a 6 x 6 half diallel design to obtain 15 different cross combinations. Generation advance of F_3, F_4 and F_5 populations from the cross combinations were obtained during 2008, 2009 and 2011 years. The populations were assessed under three nitrogen rates (0, 120 and 240 kg N/ha) in split plots with three replicates. The nitrogen levels were arranged as the main factor while the genotypes were placed a sub-factor. Nitrogen fertilizer was applied in splits at sowing time and tillering stage, while, phosphorus at the rate of 60 kg/ha as P2O5 was applied to all plots as a basal dose. The seeds were planted in 2-meter-long rows in each plot with 20 cm distance between the rows and 10 cm seed spacing. The experiment was conducted under rain-fed conditions without irrigation.

Investigated Traits

Chlorophyll content index was measured using the Chlorophyll meter (SPAD-502; Minolta, Osaka, Japan), which indirectly calculates the amount of total chlorophyll, called "chlorophyll concentration index" (CCI; ranging from 0 to 99.9). Measurements were made in the open air and at 12-14 hours of the day during the heading stage (ZGS 55) using flag leaf of all plants in the plot.

Plant grain yield (g/plant) was determined by dividing the value obtained after threshing all the plants in the plot by the number of plants. Protein content (%) was measured using the NIT System Infratec 1241 Grain Analyzer (Foss, Hillerod, Denmark) without grinding the grain.

Grain nitrogen yield (GNY) (mg/plant), grain yield NUE (NUEgy) and grain nitrogen yield NUE (NUEgny) were determined according to Yildirim et al. (2007).

Statistical Analysis

Data were analyzed using split-plot ANOVA, and the differences between the genotypes were analyzed by using the least significant difference test to detect the differences between the genotypes at 5% significance (SAS, 1998). Biplots analyses were realized using the software GenStat 12th (Genstat, 2009) package program.

Results

The research findings revealed that the nitrogen rates were significant for CCI, grain yield, protein content, GNY, NUEgy and NUEgny traits in all segregation populations of

F_3, F_4, and F_5 (Table 1). The differences between genotypes were significant in all traits except CCI was found to be not significant. Considering the Genotypes × N interactions were significant in F_4 for grain yield, in F_3 for protein content, in all generations for GNY, in F_5 for NUEgy and in F_3 and F_5 for NUEgny.

The range of CCI for the generations of F_3, F_4, and F_5 under different nitrogen rates were 35.6-40.4, 34.4-38.5, and 35.6-39.5 at N_0 level, 41.4-47.7, 45.3-49.3, and 41.4-47.4 at N_1 conditions, 45.1-49.3, 45.5-50.1, and 45.3-49.6 at N_2 conditions, respectively (Table 2). While, the genotypic differences for the three generations were not significant at N_0 and N_1 rates for CCI, differences among the genotypes at the N_2 rate were significant. Hybrid means of CCI produced an increase with the increase of nitrogen rate. The difference was higher between N_0 and N_1 than between N_1 and N_2 (Figure 1). The highest CCI was found in the 1×3 hybrid combination of F_4 generation at the N_2 nitrogen rate, while the lowest value was determined in 5×6 hybrid combination of F_4 at the N_0 condition. When the hybrid means of the segregation generations were examined under different nitrogen rates, the 2×6 hybrid combination achieved the highest values in the N_0 and N_1 rates for F_4 generation and in the N_1 condition for F_5 generation.

Average single plant grain yield values for the F_3, F_4, and F_5 generations under different nitrogen levels were 5.44, 5.91, and 5.26 g/plant at N_0, 7.57, 6.45, and 5.82 g/plant at N_1 and 7.15, 7.00, and 6.59 g/plant at N_2, respectively (Table 2). The results showed that genotypic differences were significant in N_1 for all three generations and in N_0 only on F_5 generation (Table 2). These differences continued following generations for the increase in grain yield due to the increase in nitrogen rates (Figure 1). The yield of the F_5 generation remained lower under all nitrogen rates compared to F_3 and F_4 generations. The maximum grain yield was produced from the 1×5 hybrid combination of F_3 and F_4 in the N_1 nitrogen dose and the lowest value was produced in the 5×6 hybrid combination of the N_1 nitrogen rate in the F_5 generation among all segregation generations. The maximum yield values were in 2×5 hybrid combinations in all rates and generations and were found to be higher than the general average except for F_4 and F_5 generations at N_2 nitrogen rate.

The protein content values for the F_3, F_4, and F_5 generations was ranged 14.7-16.2, 14.2-16.5, and 14.9-16.1% at N_0, 16.1-17.6, 16.9-17.9, and 16.2-17.7% at N_1 and 17.4-18.4 19.0, and 16.8-18.3% at N_2 (Table 3). As it is seen Figure 2, the genotypic differences were important in F_3 generation of nitrogen rates of N_0 and N_1, N_0 in F_4 generation, and N_1 and N_2 rates in F_5 generation. Protein content increased with increasing N_0 to N_2. The maximum protein content was achieved by N_2 nitrogen rate for the F_4 generation of the 3×6 hybrid combination, while the minimum value was produced by 1×2 hybrid combination at the N_0 rate of the F_4 generation. Among the hybrid combinations, the protein content of the 3×5, and 3×6 hybrid combinations were achieved the higher values than the average of the hybrid and nitrogen rates.

GNY values of F_3, F_4 and F_5 generations were varied from 107.4 to174.5, 112.7 to 180.8, and 95.5 to 175.3 mg/plant at N_0, from 149.3 to 258.9, 134.3 to 250.9, and 92.4 to 197.2 mg/plant at N_1, from 173.2 to 249.2, 152.0 to 246.2, and 154.9 to 236.3 mg/plant at N_2 (Table 4). It was observed GNY was too low at N_0 (Figure 2). The optimum value for GNY was produced by 1×4 hybrid combination of F_5 generation at N_2 level, while the minimum value was produced by 3× 6 hybrid combination of F_5 generation at N_0. The GNY values of the 1× 5, and 2×5 hybrid combinations were achieved higher value than the other hybrid averages.

The highest NUEgy value was found in the 1×2 hybrid combination at N_0 nitrogen rate in the F_5 generation, while the lowest was obtained from the 3×5 hybrid combination in the F_5 generation at N_2 nitrogen dose (Table 4). NUEgy and NUEgny significantly decreased at N_1 and N_2 respect to N_0 (Figure 3) The NUEgy and NUEgny values of 1×5, 2×3, and 2×5 hybrid combinations were found to be higher than the average of hybrids and nitrogen levels.

The results pertaining to GNY, protein and CCI had a significant positive relationship with grain yield estimated at F_3, F_4, F_5 segregations and N rates (Figures 4 and 5), while NUEgy and NUEgny did not show significant relationships with grain yield and CCI. In contrast, protein and CCI did not show a correlation with grain yield on hybrid means based on evaluation under investigation (Figure 6). The CCI had a stronger positive relationship with the grain yield, GNY and protein content at F_3 generation and N_2 rate, while they did not correlate to NUE traits.

Table 1. Mean squares for variables recorded on 15 hybrid populations evaluated at three nitrogen rates at F_3, F_4 and F_5 generation

	Mean Squares																		
		CCI			Grain Yield			Protein content			GNY			NUEgy			NUEgn		
Source of Variation	d.f.	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
Nitrogen (N)	2	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Replication	6	***	**	***	**	***	***	**	**	***	***	***	***	***	***	***	***	***	***
Genotype (G	14	ns	ns	ns	**	*	**	*	**	***	***	*	***	**	*	***	**	*	***
G×N	28	ns	ns	ns	ns	*	ns	**	ns	ns	*	*	**	ns	ns	***	**	ns	***
Error	84	5.31	5.08	5.22	1.49	1.29	1.53	3.33	0.38	0.22	653.95	1030.63	645.11	70.80	102.67	51.96	0.04	0.07	0.04
C.V.%		5.26	5.09	5.27	18.44	17.58	20.97	0.32	3.64	2.78	14.00	18.24	16.15	18.96	22.48	17.85	18.19	23.35	18.25

*,**, ***, indicates data significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. ns: non-significant

 DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1

Table 2. Average values of CCI and grain yields of three segregation level at different nitrogen rates.

Hybrid	CCI									Grain yields (g/plant)								
	N0			N1			N2			N0			N1			N2		
	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
(1x2)	37.8	37.2	38.2	43.3	45.3	44.4	46.8	50.0	45.7	5.592	5.150	7.214	7.105	6.634	7.468	6.932	6.645	6.658
(1x3)	37.5	37.5	37.9	46.3	47.6	46.6	48.6	50.1	49.2	5.348	5.900	6.145	8.078	5.891	6.217	6.440	8.213	5.695
(1x4)	36.7	37.8	37.7	47.7	46.3	43.5	45.1	49.4	48.8	4.623	5.720	5.793	7.027	6.747	6.749	6.015	8.005	6.513
(1x5)	39.4	38.1	36.8	46.0	46.7	42.4	46.2	46.9	46.5	5.201	6.654	4.692	9.211	9.286	6.550	8.176	6.678	6.526
(1x6)	35.6	37.1	35.6	44.5	46.5	44.5	48.6	48.8	48.4	4.263	4.735	4.923	7.983	7.272	5.756	7.175	5.227	6.451
(2x3)	39.3	38.0	39.3	44.8	46.9	43.6	49.2	48.6	46.6	5.704	6.390	6.890	7.800	6.087	5.813	6.577	7.036	6.803
(2x4)	40.5	38.2	39.3	45.8	46.4	44.4	48.3	49.7	45.3	4.900	6.526	4.902	6.219	5.427	5.605	6.834	7.164	7.347
(2x5)	36.0	36.1	38.4	46.2	48.1	46.5	49.0	48.9	45.7	6.306	6.548	5.980	8.568	8.095	7.064	7.234	6.626	6.330
(2x6)	37.6	38.5	38.1	46.1	49.3	47.4	47.4	47.3	49.1	4.494	5.841	5.248	6.846	6.352	6.227	7.116	7.138	6.648
(3x4)	40.4	38.2	38.9	46.4	49.1	45.8	46.7	46.9	46.9	5.662	6.913	5.337	8.544	6.370	4.411	8.575	6.875	6.351
(3x5)	38.1	38.5	39.0	45.2	46.6	44.8	49.2	46.8	46.9	6.026	7.164	4.800	8.361	5.812	5.388	7.371	8.401	5.508
(3x6)	37.5	36.1	38.4	45.9	46.5	45.4	46.4	45.9	49.6	6.160	5.129	4.048	5.390	5.655	5.263	7.691	7.214	6.670
(4x5)	38.7	36.9	37.3	44.0	47.6	44.8	49.3	47.2	46.8	6.956	5.673	4.307	6.872	6.290	6.595	6.707	5.552	8.236
(4x6)	39.5	36.8	39.5	41.4	48.9	41.4	48.6	49.7	47.9	4.577	4.957	4.711	6.737	4.807	4.747	6.119	7.034	6.346
(5x6)	37.4	34.4	36.0	45.8	46.6	45.8	48.6	48.6	47.8	5.821	5.375	3.884	8.748	5.980	3.401	8.210	7.192	5.843
Hybrid mean	38.1	37.3	38.0	45.3	47.2	44.8	47.9	48.3	47.4	5.442	5.912	5.258	7.566	6.447	5.817	7.145	7.000	6.528
LSD $_{0.05}$ (G)	ns	ns	ns	ns	ns	ns	1.871	2.007	1.775	ns	ns	1.269	1.608	1.925	1.644	ns	ns	ns
				F_3	F_4	F_5							F_3	F_4	F_5			
LSD $_{0.05}$ (N)				0.97	0.94	0.96							0.400	0.475	0.395			
Dose mean	37.8			45.8			47.9			5.537			6.610			6.891		

ns: non-significant

Table 3. Average values of protein content and grain nitrogen yield (GNY) of three segregation level at different nitrogen levels.

Hybrid	Protein content (%)									GNY (mg/plant)								
	N0			N1			N2			N0			N1			N2		
	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5	F3	F4	F5
(1x2)	16.1	14.2	14.9	16.7	17.0	16.5	17.9	18.0	16.8	144.7	116.9	173.4	189.1	180.9	197.2	199.4	191.9	177.3
(1x3)	17.1	15.2	16.0	16.8	17.7	17.0	18.4	18.5	17.7	145.3	143.5	157.1	217.1	167.0	167.9	190.1	242.5	161.6
(1x4)	14.7	16.4	15.9	16.7	17.5	17.1	18.0	18.3	18.3	108.7	150.5	147.4	186.8	187.2	184.6	173.2	234.7	189.7
(1x5)	16.1	14.8	16.0	17.5	16.9	17.0	17.4	18.4	18.0	133.9	157.6	120.6	258.9	250.9	178.8	227.4	196.2	188.2
(1x6)	15.9	14.8	15.0	16.1	17.2	17.1	17.6	18.1	18.1	108.2	112.7	117.9	205.7	199.8	157.7	201.2	152.0	186.9
(2x3)	15.2	15.4	15.9	16.9	17.4	16.9	17.6	17.9	17.6	138.7	156.3	175.3	209.7	168.7	156.2	184.8	200.8	191.5
(2x4)	15.6	15.1	15.0	17.0	17.5	16.2	18.2	17.9	17.1	122.6	156.4	118.1	168.6	151.8	144.0	199.3	205.2	201.6
(2x5)	15.6	16.1	15.6	17.6	17.4	16.3	18.2	18.6	17.5	156.7	167.9	149.4	241.4	225.6	183.8	210.4	196.9	177.5
(2x6)	14.7	15.1	15.4	17.4	17.4	17.7	18.2	17.9	18.3	107.4	140.6	128.8	189.8	177.1	175.3	207.4	205.0	194.6
(3x4)	15.7	15.9	15.1	17.5	17.0	16.6	18.2	18.0	17.7	142.5	175.1	129.4	238.6	173.2	116.1	249.2	197.7	179.7
(3x5)	16.2	15.8	15.1	17.3	17.5	16.9	18.0	18.3	17.6	157.6	180.8	116.6	231.2	162.2	145.4	212.3	246.2	154.9
(3x6)	14.9	16.0	15.7	17.3	17.9	17.0	18.3	19.0	17.8	137.6	129.0	95.5	149.3	160.9	140.8	225.6	219.3	190
(4x5)	15.8	15.9	16.1	17.1	17.8	17.4	18.0	18.0	17.9	174.5	143.9	110.4	188.1	179.0	182.6	193.6	160.2	236.3
(4x6)	15.2	14.6	15.2	17.0	17.6	17.1	17.9	17.5	17.3	112.4	117.4	114.6	183.3	134.3	130.7	176.4	196.6	175
(5x6)	15.7	16.5	15.9	16.9	17.3	17.0	18.2	17.6	17.6	145.1	141.0	98.3	236.6	164.9	92.4	238.5	203.1	164
Hybrid mean	15.6	15.5	15.5	17.1	17.4	16.9	18.0	18.1	17.7	135.7	146.0	130.2	206.3	178.9	156.9	205.9	203.2	184.6
LSD $_{0.05}$ (G)	1.223	1.174	ns	0.778	ns	0.755	ns	ns	0.580	37.94	ns	33.23	42.08	54.68	44.77	47.73	ns	ns
				F3	F4	F5							F3	F4	F5			
LSD $_{0.05}$ (N)				0.235	0.259	0.194							10.7	13.5	10.7			
Dose mean	15.5			17.1			17.9			137.3			180.7			197.9		

ns: non-significant

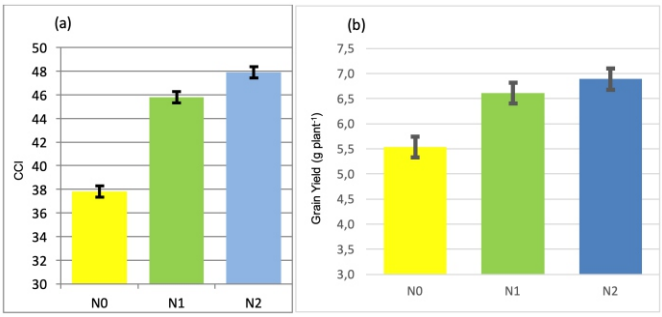

Figure 1. (a) Chlorophyll content (CCI) and (b) grain yield of all hybrids over all segregations. Values of LSD for CCI: 0.96 and plant grain yield: 0.42

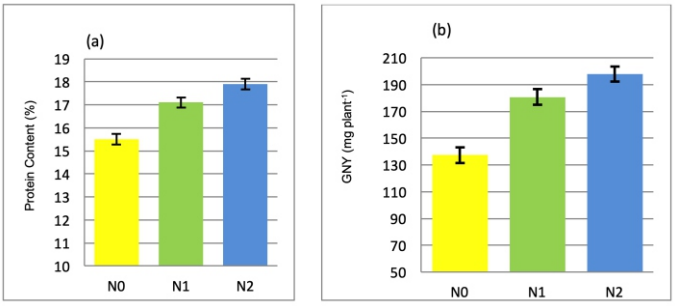

Figure 2. (a) Protein content and (b) GNY of all hybrids over all segregations. Values of LSD for Protein content: 0.23 and GNY: 11.6

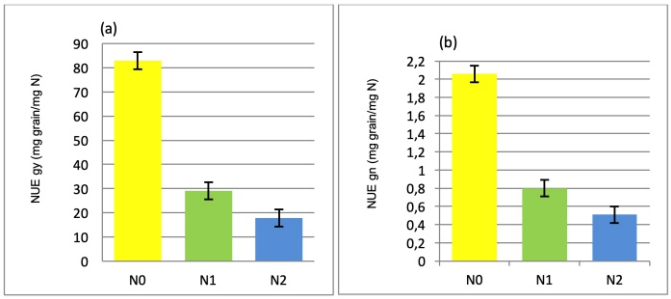

Figure 3. (a) NUEgy and (b) NUEgn of all hybrids over all segregations. Values of LSD for NUEgy: 3.56 and NUEgn: 0.095

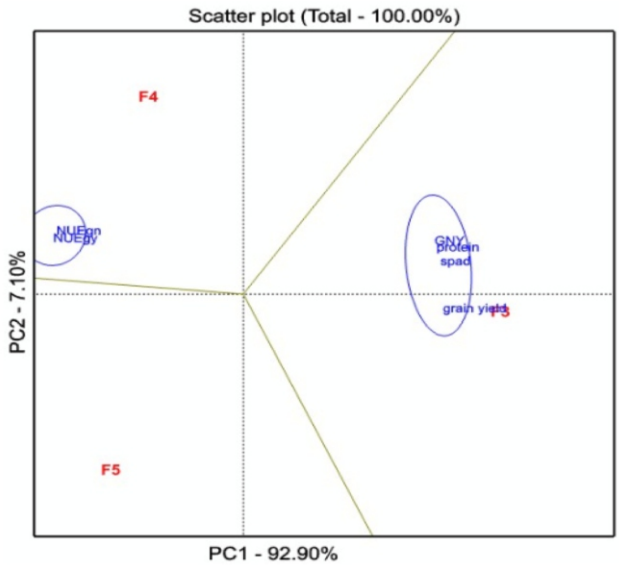

Figure 4. Biplot of traits by segregation population interaction evaluated over all hybrids

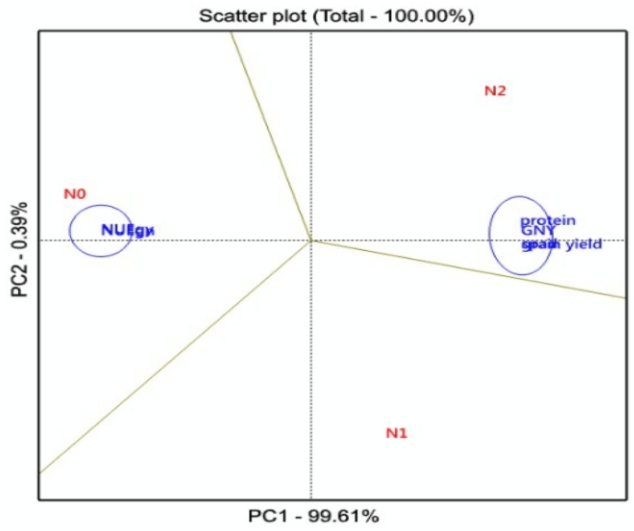

Figure 5. Biplot of traits by N dose interaction evaluated over all hybrids

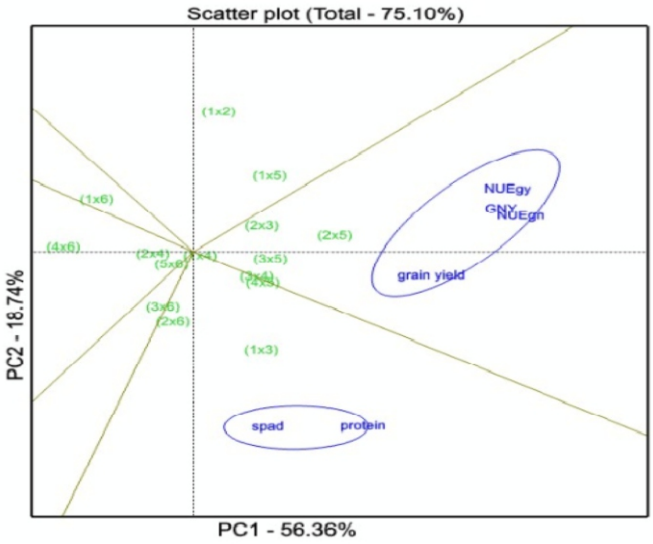

Figure 6. Biplot of traits by genotype interaction over all segregation level and N rates

Discussion

In this study, in spite of 15 different hybrids of durum wheat combinations at F_3, F_4 and F_5 generations were evaluated in terms of grain yield, protein content and NUE at three different nitrogen rates. The use of the chlorophyll meter (Minolta 502-SPAD) as a selection criterion was investigated for the traits under study. Meanwhile, the increasing amounts of nitrogen (N) fertilizers have been used for expanding to increase of CCI in hybrids. According to Bavec and Bavec (2001), Lopez-Bellido et al. (2004), Ziadi et al. (2008), Lin et al. (2010) there was a positive relationship between CCI and nitrogen concentration in the leaf. According to findings, genotypic differences for flag leaf chlorophyll content (CCI) in all generations (F_3, F_4, F_5) appeared only in high nitrogen conditions. Therefore, as in harmony with an earlier study (Kizilgeci et al., 2017), selection can be suggested at high levels of nitrogen to identify differences among the lines in breeding programs.

The results of the study indicated there was a significant relationship among the grain yield of hybrids and nitrogen rates application, which was in agreement with the

DOI: https://dx.doi.org/10.31015/jaefs.2019.3.1

results of A Ortiz-Monasterio et al. (1997), Le Gouis et al. (2000) and Van Ginkel et al. (2001), Bonfil et al. (2004), Guarda et al. (2004), on the other hand, some researchers reported that, fertilization over a certain nitrogen rate leading to reduction in grain yield, with consider the effect of genotype and environmental conditions (Ellmer et al., 2001;Cossey et al., 2002).

Several studies detected significant G×N interactions for agronomic performances (Barraclough et al., 2010; Cormier et al., 2014), meaning that the genetic values of varieties differ among the N levels. Significance of G×N interactions directly affects the correlations of genetic values between N levels, and hence, the best varieties at high N may not be the best at low N. In our study G×N interaction of the traits changed depending on F stage and traits (Table 1). Non-significant G×N interactions for CCI indicates that it is a stable trait as an indirect selection criterion, when it would be significantly associated with desired traits, like yield, protein content etc. (Figures 4-5). Given that we observed relatively high heritability prediction (71.5 h^2) of CCI (Tahmasebi et al., 2014) also supported our suggestion.

The significant differences among the genotypes for grain yield in the segregation populations were observed under varying levels of nitrogen. Although, yield increment was recorded with increasing nitrogen rate compared to moderate nitrogen, the difference between genotypes was rather narrow. Therefore, the chances of success for the selection at the optimum level of nitrogen fertilization for plant breeding could be increased. These results indicate that selection can be made independently on segregation stages.

As pointed out, in wheat, different studies indicated that indirect selection at high N can be an effective strategy to breed for low N conditions (Brancourt-Hulmel et al., 2005; Laperche et al., 2006). According to this scenario, if the genetic correlation is high then the selection is made on the basis of yield or other agronomic characteristics in high N rates. It was observed that the genotypic correlation of all other properties was low except CCI under high N rates. So, the selection should be made under normal nitrogen conditions, except for CCI, to increase the efficiency of selection according to the current study.

Although protein was positively correlated with nitrogen application, the protein is a nitrogenous compound, it can be said that the increase of the protein content in the plant organs with the increase in nitrogen rates, it is an expected result as reported in previous studies (Delogu et al., 1998; Ottman et al., 2000; Sade and Soylu, 2001; Woolfolk et al., 2002).

Grain protein change was effective due to environmental factors such as temperature, light intensity and soil moisture (Gauer et al., 1992; Sajo et al., 1992; DuPont and Altenbach, 2003), and the agronomic management factors like the soil tillage (De Vita et al., 2007); Zebarth and Sheard (1992), nitrogen application rates, application time, and application form as well as genotypes. Nitrogen rates and segregation level were not predictive of genotypic differences in protein proportion. Since genotype x nitrogen rate interactions for the protein content was generally unimportant, successive selection in breeding can be done at different nitrogen rates.

The genotypic differences in varied segregation populations for GNY, NUEgy and NUEgny were observed to be low in high nitrogen rates (Table 2). With low and moderate nitrogen rates, the response of the genotypes changed depending on the segregation level, and genotypic differences were higher in moderate nitrogen rate than in low nitrogen rate. The findings provided the success of the selection process for NUE. Protein content and GNY indicated that genetic responses varied according to nitrogen rates and level of segregation and that both properties had to be assessed separately in breeding.

In general, the chlorophyll content was observed high in the hybrid combination of Misiri genotype used as a parent. Spagetti and Menceki varieties used in the hybrids achieved the maximum yield potential. It was observed that protein ratio was high for each segregation (F_{3-5}) and different nitrogen rates in the hybrids in which the Zenit variety was used as a parent. It appears that hybrids with higher nitrogen rates were different from hybrids that stand out in terms of protein content. As a parent variety Spaghetti produced an enhancing effect on GNY, NUEgy and NUEngy in all its hybrid combinations. On the other hand, Mersiniye × Spagetti hybrid achieved high combination ability especially in early segregation levels. It could be acknowledged that the use of landraces in breeding programs would allow a positive contribution to the success of durum wheat breeding and enhance the narrow range variation. The landraces that used in the current study had considerable enhancing effects on the grain yield and GNY, NUEgy and NUEgny in the hybrid combinations.

There is a growing interest for using chlorophyll meter in durum wheat breeding might enable to breeders for evaluating the current variability more effectively, both by reducing labour and by allowing the more single plant to be screened and development of kind of tools is important to continue progress through plant breeding. It is possible to reach the favourite yield and quality results by using SPAD meter as an indirect selection criterion in durum wheat breeding, these results were supported by the findings of Yildirim et al. (2009) which indicated that CCI could be used as a selection criterion to classify the high yielding durum wheat breeding lines at early segregation of progenies.

The results showed that the genotypes that were examined under different nitrogen rates in wheat breeding would be crucial to reveal the genotypic effect or inactive genes of a genotype. In the present study, there were no genotypic variations in grain yield in several combinations at low and high nitrogen rates. A similar situation had been observed at low and medium nitrogen rates for CCI, at high nitrogen rates for protein content GNY, NUEgy and NUEgny traits. The optimum of environmental factors such as heat and drought stress, management techniques and soil factors, as well as the nitrogen content of the soil, in order to reveal genotypic effects at the highest level, would be leading to the existing genetic variability to be used more effectively in durum wheat breeding.

One of the most significant practical outputs of this research, a low budget and a limited number of hybrid populations, this method is based on the fact that 3-4 generations from F_3 to F_6 growing in the same year and at different nitrogen rates as a result of the production of their seeds without being subjected to selection in different segregation levels, as the hybrid populations in this investigation. According to this method, the selection at the last segregation combination with the highest value for investigated traits for all generations and more than one nitrogen rate will allow both high genetic progress and high stability to be achieved. All these facilities will be provided with low input, time and labour.

Conclusion

Testing the genotypes at different nitrogen rates in wheat breeding is crucial to reveal the genotypic effect or inactive genes of a genotype. In this study there were no genotypic differences in grain yield in many combinations at low and high nitrogen rates. Similar findings were also observed at low and medium nitrogen rates for CCI, at high nitrogen rates for protein content GNY, NUEgy and NUEngy traits.

This it is suggested that in addition to environmental factors such as heat and drought stress, management techniques and soil factors, the nitrogen content of the soil, has the potential to achieve the varietal potential of wheat genotypes. This method is based on the fact that 3-4 generations from F_3 to F_6 growing in the same year and at different nitrogen rates as a result of the production of their seeds without being subjected to selection in different segregation levels, as the hybrid populations in this study. According to this method, the selection at the last segregation combination with the highest value for investigated traits for all generations and more than one nitrogen rate will allow both high genetic progress and high stability to be achieved. All these benefits will be attained with low input, time and labour.

Acknowledgement

This work was supported by Dicle University research supporting foundation (DUBAP-11-ZF-13).

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.2 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):121-126 (2019)

Second Crop Potential of Soybean Lines for Diyarbakır Location on the Yield and Quality

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Abstract

The importance of vegetable oils and oilseeds, which have an important role in human nutrition and many branches of industry, is increasing day by day. Soybean is one of the most important industrial plants in the world. Despite having the most suitable agricultural land to cultivate soybean, Turkey meets through imports almost all of its soybean needs. The aim of this study is to determine the suitability of soybean genotypes as second crop production for Diyarbakır conditions. In this study, KA-04.03.07, KA-04.06.01, KKMA-118, KSA-26, S-02.14.11, S-03.03.7, Sa-01.08.15 advance lines and Arısoy, Blaze, SA-88 Bravo, Ataem-7, Umut-2002 and GAPSOY-16 varieties were used as materials. According to the results of the research, average grain yield of genotypes was 2,37 t/ha and highest grain yield was obtained from GAPSOY-16 (4,00 t/ha) variety, followed by S-03.03.7 line (3.66 t/ha). The lowest grain yield value was obtained from Umut-2002 (1,33 t/ha) variety. As a result of the correlation analysis, a positive and significant relationship was found between grain yield and thousand grain weight. It is concluded that GAPSOY-16 variety and S-03.03.7 line can be successfully grown as second crop in Diyarbakır province.

Keywords: Soybean, Yield, Diyarbakır, Genotype, Quality, Second Crop

Received: 03 June 2019 Accepted: 06 August 2019 Published: 11 September 2019

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most important legume plants in the world due to its high protein content. The gene center of soybeans is northeastern China (Popovic, 2010). In Turkey, the cultivation of soybeans started in the Black Sea Region in the 1930s and today the production of soybeans as the main crop is in the Thrace, Marmara, Black Sea and Mediterranean Regions, and the second crop is in irrigated agricultural areas of the Aegean, Southeastern Anatolia and Mediterranean regions. Depending on the variety and growing conditions, soybean contains approximately 35-45% protein content and 19-22% oil content. Soybean is the main nutrient for millions of people worldwide due to its high protein and oil content (Popovic et al., 2011, 2013). Also, legumes are superior previous crops, compared to non-leguminous crops, because they fix atmospheric N (Vyn et al., 2000; Gül et al, 2008).

Soybean diversity is of great importance for growers to achieve high and stable yields. Main objective of modern agriculture is to achieve sustainability through high-yield varieties and hybrids that are resistant to diseases, pests and other adverse environmental conditions. These varieties were obtained using plant breeding methods based on selection, hybridization, and gene recombination in the appropriate genotypes. The main target in soybean breeding is yield increase and stability. Novel varieties must adapt to different growing conditions.

Soybean needs 550-600 mm water during the growing period. For this reason, it should be remembered that soybean farming could be done only by irrigation at the low rainfall areas. Low air humidity, especially in extremely hot areas, also adversely affects the filling of the bean grains, reducing yield. The necessary moisture must be provided with irrigation in dry weather conditions. Flowering and pod formation are most water needed period among developmental stages of soybeans.

In soybean breeding, special attention is paid to the development of varieties containing high amounts of protein and oil content besides high and stable yield (Hollung et al., 2005; Vidic et al., 2010).

When the usage areas of soybeans are examined, it will be possible to see the wide range of soybeans. Some of them are dough products, baby foods, confectionery products, non-allergenic milk and milk products, special dietary products. Soybeans are also industrial plants in terms of both human health and industrial products. Due to the high (18-26%) oil content of soybean seeds, it is classified in oilseed plants. (Kolsarıcı et al., 2005). After the oil of soybean is separated from the seeds, the residuum obtained is one of the important raw protein sources for feeding animals (Yılmaz and Efe, 1998).

Cite this article as:
Ozkan, R., Bayhan, M., Albayrak, O., Karaaslan, D., Yildirim, M. (2019). Second crop potential of soybean lines for Diyarbakır location on the yield and quality. Int. J. Agric. Environ. Food Sci., 3(3), 121-126. DOI: https://dx.doi.org/10.31015/jaefs.2019.3.2
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 121-126
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

 DOI: https://dx.doi.org/10.31015/jaefs.2019.3.2

In 2016, 314 million tons of soybean production was produced in the world. Soybean imports in the world are 133.3 million tons; soybean exports were 132.5 million tons. According to TUIK (2017) data, Turkey's 2015/16 soybean production and marketing season were realized 2.3 million tons of soybean imports and 117 thousand tons exports.

280 tons of soybeans were produced in 84.1 ha areas at Diyarbakır in 2018 (TUİK, 2019). With the development of irrigation opportunities in the Southeast Region of Turkey, cotton, corn and fruit entered the region and the soybean plant, which is an important oil and protein store, has important production potential and advantage in the region. Soybean will eventually become an alternative to cotton and corn with government subsidies and the farmer's recognition. Some soybean genotypes (Ataem-7, Batem-201, Batem-219, Batem-220, Batem-223, Ata-135, Ata-137 and 581) have used previously conducted researchs in target region. Soybean genotypes gave high yields in Diyarbakır second crop conditions (Karaaslan, 2011). In the study carried out by Erbil and Gür (2017) in order to determine the performance of some advanced soybean lines in Şanlıurfa second crop conditions by using physiological and morphological parameters, suitable genotypes were determined.

In this study, the adaptation ability and production suitability of some soybean genotypes on Diyarbakır conditions as the second crop were investigated.

Materials and Methods

This study was conducted at Diyarbakır in 2017 and designed of completely randomized block experimental design with three replicates. Each experimental plot consisted of four rows with 5.0-m length 0.70 m/internal. In the research; KA-04.03.07, KA-04.06.01, KKMA-118, KSA-26, S-02.14.11, S-03.03.7, Sa-01.08.15 advanced lines and Arısoy, Blaze, SA-88, Bravo, Ataem-7, Umut 2002 and GAPSOY-16 soybean varieties were used. The lines are included in the middle-early group of soybean breeding activities carried out by the Black Sea Agricultural Research Institute and the GAP Agricultural Research Institute.

The results of some physical and chemical analysis of the soil of the experiment area at Faculty of Agriculture of Dicle University Experiment Station where the experiment was conducted are given in Table 1. The clay content of the soils is high and heavy. In addition, there are no significant problems with pH, salinity and groundwater. The soil of the trial area is low in organic matter, alkaline (pH 7.9) and clayey.

The genotypes were sown on 25 June 2017 with pneumatic sowing machine. Prior to sowing, pure 5 kg N per da and 5 kg P2O5 per da were given. As top fertilizer Ammonium nitrate in the form of pure 5 kg N per da was applied. Irrigation was carried out during the trial as necessary by drip irrigation. During the growing period, 8 irrigation (738 mm in total) was performed according to the needs of the plant and drip irrigation method was applied. The harvesting process was carried out on 18 October 2017. The plants harvested manually from the middle two rows of each plot were passed through the threshing machine. After the seeds were dried and cleaned, seed yield per hectare was calculated.

The province of Diyarbakır has a continental climate and has very hot and dry summers and cold and rainy winters. Climate data for the trial year are given in Figure 1. In 2017, the maximum rainfall in Diyarbakır occurred in November, December and January; The minimum rainfall occurred in June, July and August. In addition, in 2017, the maximum temperature in Diyarbakır was realized in June, July and August; The minimum temperature was in December and January.

In this research, plant height, first pod height, 1000 grain weight, grain yield, oil content, protein content and irrigation based water use efficiency (WUEIr) were investigated.

Irrigation Based Water Use Efficiency (WUE_{Ir}) (kg / ton): Irrigation based water usage efficiency (WUE_{Ir}), expressed as the ratio of total grain yield to the amount of irrigation water supplied, was calculated by the following formula.

(WUE_{Ir}) = TGY / TAIW + TAR

(WUE_{Ir}): Irrigation based water use efficiency (kg / ton)

TGY: Total grain yield (kg / m2)

TAIW: Total amount of irrigation water (ton / m2)

TAR: Total amount of rain (ton / m2)

Analysis of variance, correlation and regression analysis) was performed in the JUMP 13.0 package program.

Results and Discussion

In the second crop soybean cultivation, variance analysis values of some characteristics of different soybean genotypes are given in Table 2 and the resulting groups are given in Table 3. It can be observed that there was significant difference among genotypes for the characteristics of grain yield, plant height, firs pod height and WUE_{Ir}. Thus, these results show that there is wide variability between the cultivars (Table 2).

Plant Height (cm): Highest plant length (110.40 cm) was obtained from Sa-88 soybean line and the lowest plant length (76.02 cm) was obtained from Blaze variety. Our results were lower than the findings of Karaaslan (2011) and Yetgin and Arıoğlu (2009) reported 105- 138.8 cm and 91.67-122.2 cm plant height, respectively. The differences in plant height value of the varieties may be due to the differences in their genetic structure. Arslan and Isler (2002) in their study sowing frequency, sowing time, soil moisture and plant nutrients have stated that this character is effective. Findings obtained in the study, soybean plant height value of 50.5-75.0 cm Tayyar and Gül (2007) and 66.2-83.2 cm Karasu et al. (2002) and reported that it was 71.3-107.9 cm Tanriverdi et al. (2000) is similar to the findings. As a matter of fact, Arıoğlu (2007) stated that plant height may be between 30-150 cm depending on the differences in plant growing techniques and sowing time.Genotypes with plant height above 65 cm and first pod height above 10 cm should be selected for machine harvest (Yetgin and Arıoğlu, 2009).

Table 1. Soil analysis results of the experiment area

Depth of soil	pH	P_2O_5 (%)	Organic matter (%)	Lime (%)	EC (dS/m)	Soil structure				Field Capacity (g/10 g)	Fading point (g/100 g)	Volume weight (g/cm³)	Inf. Speed (mm/h)
						Sand (%)	Silt (%)	Clay (%)	Class Structure				
0-30	7.7	0.42	1.67	7.8	0.48	10	24	66	C	35.5	25.5	1.19	8
30-60	7.9	--	1.67	7.8	0.37	12	22	66	C	35.2	25.3	1.25	
60-90	7.8	--	--	8.7	0.42	12	21	67	C	36.4	27.0	1.27	

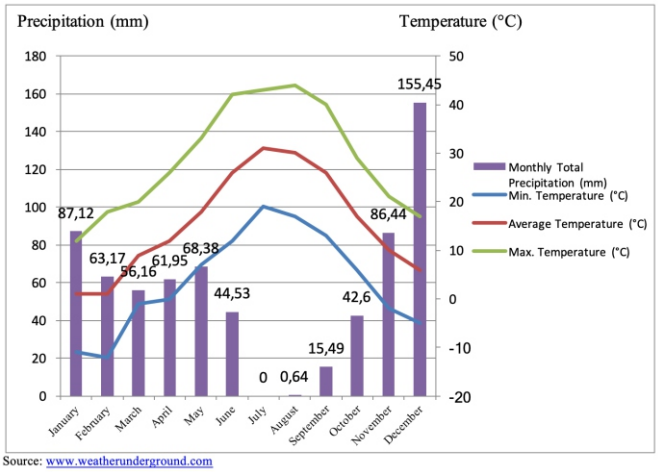

Figure 1. Some climatic data of Diyarbakır for 2017

growing techniques and sowing time. Genotypes with plant height above 65 cm and first pod height above 10 cm should be selected for machine harvest (Yetgin and Arıoğlu, 2009).

First Pod Height (cm): Umut-2002 variety had highest first pod height with 12.09 cm among different soybean varieties used in the research, while the lowest first pod height was obtained with KA-04.03.07 with 4.29 cm. In soybean agriculture, the first pod height is required to be high in order to minimize harvest losses (Yetgin and Arıoğlu, 2009). It was noted that there were significant differences between varieties in terms of first pod height in different studies conducted in different regions of Turkey. In the similar adaptation studies, the first pod height was changed between 9.2-15.4 cm (Karaaslan, 2011), 13.1-20.6 cm (Tayyar and Gül, 2007), 8.2-13.8 cm (Tanrıverdi et al., 2000) and 6.4-12.3 cm (Caliskan and Arioglu 2004). The findings of the researchers are a little bit higher than the values obtained in our study. In order to minimize harvest losses, first pod height is an important feature that should be considered.

Thousand Grain Weight (g): The highest value was obtained from KA-04.06.01 line with 164.68 g and the lowest value was followed by Bravo variety with 98.33 g. thousand grain weight is an important feature that affects the yield and the genetic structure of varieties and is formed by the effect of environmental conditions. Our findings were similar to the dates of Karaaslan, (2011); Yetgin and Arıoglu, (2009); Yaver and Pasha (2009); Aktaş et al., (2015); Söğüt et al. (2001); Söğüt et al. (2005) in terms of a thousand grain weight.

Grain Yield (t/ha): Statistically significant difference was found among different soybean genotypes for seed yield per hectare (Table 3). The highest yield was obtained from GAPSOY-16 (4,00 t/ha) variety, it is followed by S-03.03.7 line (3,66 t/ha). The lowest yield value (1,33 t/ha) was obtained from Umut-2002 variety. Our results were higher than the finding of Karaaslan (2011) who was working in second crop conditions of Diyarbakir condition. The average seed yield of varieties at same study was changed from 1,87 t/ha (cv. Batem-204) to 2,87 t/ha (cv. Ataem-7). Aktaş (2015) who worked with different soybean genotypes at same location had the highest grain yield from Blaze genotypes with 3,31 t/ha while lowest values belonged to Erensoy variety with 2,57 t/ha Average soybean seed yield was reported as 3,07 t/ha under the conditions of Sanliurfa (Erbil and Gür, 2017). Acar (2014), in order to determine the performances of some soybean varieties as the main and second crop in Kahramanmaraş ecological conditions, used 15 varieties. Seed yield in terms of the main product ERENSOY (3,96 t / ha), BLAZE (3,89 t / ha), CINSOY (3,72 t / ha); In the second product SA88 (3,19 t / ha), ATAEM-7 (2,64 t / ha), MAY 5312 (2,55 t / ha) determined that the high value varieties. Karakus et al. (2011), Harran ecological conditions as the main product and second crop as some soybean lines and varieties have made a study to determine the yield and yield components. At the end of the study, it was determined that seed yield was between 2.71-3.62 t / ha in the main crop trial and seed yield was between 2.38 - 3.95 t / ha in the second crop trial.

Oil Content (%): The highest oil content was obtained from Sa-88 with 25.00% and the lowest oil content was obtained from Blaze variety with 22.63% (Table 3). Karaaslan (2011), reported that the average oil content of the varieties ranged between 17.4% (Ata-140) and 20.0% (Batem-223) and oil content was influenced from genotpe x environment interactions. Aktaş et al. (2015) obtained the lowest value in Erensoy variety (17.4%) , while the highest oil content was obtained from Ataem-7 variety (20.5%). Yetgin and Arıoğlu (2009) reported that the highest oil content was obtained from S – 4240 with 22.40% and the lowest oil content was obtained from HA 16–21 line with 18.10%. Kan et al. (2011), in their study to determine the yield and quality characteristics of some soybean genotypes in Central Anatolia Region ecological conditions, in terms of oil content BDS-4 and ATAKİŞİ (19%) varieties have determined that they have high value.

Protein Content (%): The highest protein content among soybean genotypes was obtained from S-02.14.11 line with 42.82%, followed by KKMA-118 soybean line with 39.81% (Table 3). The lowest protein content was obtained from Sa-88 variety with 34.81%. The difference between the varieties for protein content must be affected by genotypic differences. Our findings are consistent with protein content

Table 2. Analysis of variance in the traits evaluated in field

SV	DF	Plant Height (cm)	Pod Height (cm)	Thousand Grain Weight (cm)	Grain Yield (t/ha)	Oil Content (%)	Protein Content (%)	Irrigation Based Water Use Efficiency (WUE_{Ir}) (kg / ton)
Genotype	13	373,84**	12,47**	843,34**	17725,90**	1,40**	10,52**	0.025 **
Replicate	2	308,95	1,73	1609,06	1151,30	0,11	0,86	0.002
Error	26	67,49	2,29	159,83	2722,30	0,24	2,17	0.004
C. Total	41	176,40	5,49	447,25	7402,90	0,60	4,76	0.01
CV (%)	-	9,23	21,75	9,89	21,96	2,07	3,83	21,43

**Significant at 1% of probability, *significant at 5% of probability by the F-test. SV: source of variation. d.f.: degrees of freedom

 DOI: https://dx.doi.org/10.31015/jaefs.2019.3.2

Table 3. Mean values of some characteristics of different soybean genotypes

Genotypes	Plant Height (cm)	First Pod Height (cm)	Thousand Grain Weight (g)	Grain Yield (t/ha)	Oil Content (%)	Protein Content (%)	Irrigation Based Water Use Efficiency (WUE_{Ir}) (kg / ton):
Arısoy	88,8 cd	5,63 d-g	125,45 cd	1,96 d-f	23,59 b-f	39,30 bc	0.23 d-f
Ataem-7	88,57 cd	7,38 b-f	112,55 c-e	1,74 ef	23,64 b-e	38,28 b-d	0.21 ef
Blaze	76,02 d	6,48 c-g	130,18 c	1,97 d-f	22,63 g	39,00 b-d	0.23 d-f
Bravo	78,73 d	5,59 d-g	98,33 e	1,61 ef	22,91 e-g	39,48 bc	0.19 ef
GAPSOY-16	94,00 bc	6,47 c-g	152,78 ab	4,01 a	22,92 e-g	38,6 b-d	0.48 a
KA-04.03.07	77,04 d	4,29 g	131,26 c	2,25 c-e	23,82 b-d	37,97 b-d	0.27 c-e
KA-04.06.01	89,60 cd	7,53 b-e	164,68 a	2,85 bc	22,79 fg	37,27 c-e	0.34 bc
KKMA-118	93,63 cd	8,02 b-d	122,48 cd	2,72 cd	23,26 c-g	39,81 b	0.32 cd
KSA-26	103,99 ab	8,38 bc	129,50 c	2,29 c-e	24,02 b-d	37,06 c-e	0.27 c-e
S-02.14.11	76,89 d	5,30 e-g	131,62 c	2,46 c-e	24,40 ab	42,82 a	0.29 c-e
S-03.03.7	80,62 cd	4,87 fg	129,12 cd	3,66 ab	23,23 d-g	38,35 b-d	0.44 ab
Sa-01.08.15	82,83 cd	9,16 b	133,23 bc	1,67 ef	24,08 bc	36,75 de	0.20 ef
Sa-88	110,40 a	6,03 c-g	107,95 de	2,68 cd	25,00 a	34,85 e	0.32 cd
Umut-2002	103,78 ab	12,09 a	119,11 c-e	1,33 f	24,07 bc	36,59 de	0.16 f
Means	88,92	6,94	127,73	2,37	23,59	38,29	0,28
LSD	13,73	2,52	21,15	0,87	0,82	2,46	0,10

findings of Karaaslan (2011), Aktaş et al., (2015), Yetgin and Arıoğlu (2009). Kan et al. (2011), in their study to determine the yield and quality characteristics of some soybean genotypes in Central Anatolia Region ecological conditions, in terms of crude protein ratio ARISOY, NOVA and ÜSTÜN (39%) varieties have determined that they have high value.

Irrigation Based Water Use Efficiency (WUE_{Ir}) (kg / ton): On the basis of genotypes, the highest WUEIr values were GAPSOY-16 (0.48 kg / ton), S-03.03.7 (0.44 kg / ton), while the lowest WUEIr values were Umut-2002 (0.16 kg / ton) (Table 3). In field conditions, differences in water use activities of plants are due to different environmental factors such as weather conditions, sowing dates, genetic characteristics, surface flow, drainage and different amounts of irrigation (Greenwood vd., 2005).

Correlation coefficients were calculated between the measured characters of soybean genotypes and the results were given in Table 4.

Table 4. Correlation analysis among investigated traits

	Plant Height (cm)	First Pod Height (cm)	Thousand Grain Weight (g)	Grain Yield (t/ha)	Oil Content (%)
First Pod Height (cm)	0.47**				
Thousand Grain Weight (g)	0.13	0.05			
Grain Yield (t/ha)	0.21	-0.26	0.44**		
Oil Content (%)	0.26	0.08	-0.27	-0.19	
Protein Content (%)	-0.42**	-0.37*	0.06	0.04	-0.36*

When the correlation relationships among soybean genotypes were examined, positive and significant relation were found between first pod height and plant height (r = 0.47 **), grain yield and thousand seed weight (0.44 **). Protein content was negatively correlated with plant height, oil content and first pod height. Söğüt et al. (2001) were found significant and positive relationships between yield and harvest index, plant height and height of first pods, as a result of the correlation analysis between the characteristics examined in their study in the Çukurova region. Isler and Caliskan (1998) found a significant and positive relationship between grain yield per decare and seed yield per plant at a level of 5% in a study conducted in the GAP region. In addition, they observed significant relationships between plant height and first pod height, first pod height and number of branches, plant yield and 100-seed weight; while correlations were negative between harvest index and first pods height and number of branches.

The results of the regression analysis of the grain weight - seed yield and plant height - protein content are given in Figure 2. and Figure 3. respectively.

Grain yield is a linear increase depending on the weight of a thousand grain weight (R = 0.28 y = -74.75475 + 2.4426672x) (Figure 2.); protein content is in a linear decrease depending on plant height (R = 0.40 y = 47.776376 - 0.1066142x) (Figure 3.) Therefore, genotypes with high grain weight and medium plant height should be considered in the selection of varieties.

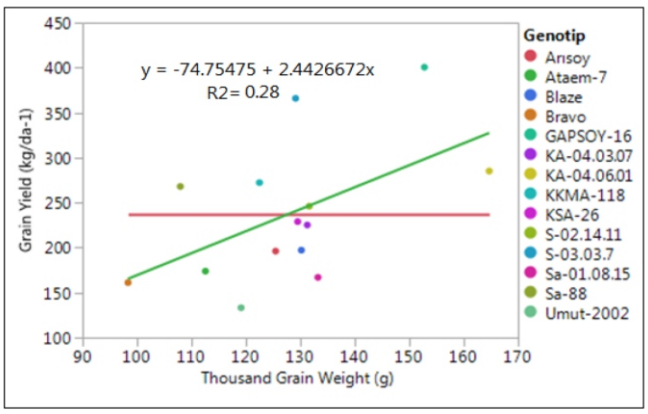

Figure 2. Grain yield (t/ha) - thousand grain weight (g) regression relationships

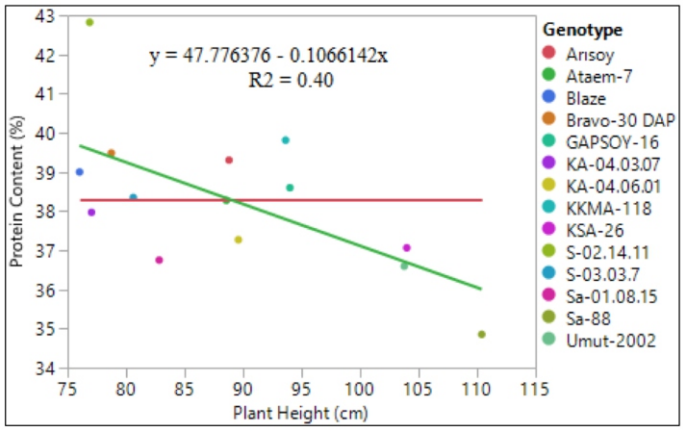

Figure 3. Protein Content (%) - plant height (cm) regression relationships

Conclusion

As a result; GAPSOY-16 variety and S-03.03.7 line will be suitable at the basis of yield in second crop soybean cultivation at Diyarbakır location. These genotypes also have satisfactory oil and protein content. It is foreseen that the Sa-88 genotype having the highest oil content can be used to increase the oil content in breeding studies with its grain yield capacity being above average. According to results, satisfactory income for farmers can be obtained under the second crop conditions of Diyarbakır by using appropriate variety. Considering that the seed yields will be increased by bacterial inoculation and nitrogen will be stored in the soil, the grain yields obtained from this study show that soybean cultivation could be applied profitably.

In order to fully demonstrate the advantages of second crop soybean production, it would be useful to carry out studies comparing economic analysis with other field crops.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.3 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):127-136 (2019)

Estimating post-harvest loss at the farm level to enhance food security: A case of Nepal

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Abstract

Food security is a major concern of the world in the context of increasing population, changing climate and declining scarce natural resources. Reducing food loss is equally important as increasing food production and productivity to feed the world, where 9.7 billion inhabitants were expected by 2050. Food loss can occur at several points along the food chain, however, harvest loss at the farm level is often overlooked which is directly impacting on sustainability. The paper attempts to estimate harvest loss at the farm level. A household survey was executed in 300 households from ten sample districts across Nepal. The percentage of harvest loss at the farm level was calculated for each crop grown as per - the season, plot and priority. Likewise, the multiple regression was executed to determine the level of influence of the socio-economic factors on the post-harvest loss at the farm level for the major crops. The mean harvest loss at the farm level found around 5 percent for the reported crops. The multiple regression model demonstrated that at the farm level, socio-economic factors might have a smaller influence on harvest loss as compared to physical and biological contributing factors. Nevertheless, reducing the post-harvest loss will increase food availability and thus the food security.

Keywords: Food loss, Postharvest loss, Food Security, Sustainability, Nepal

Introduction

Food production is a significant resource consuming function (Wohner, Pauer, Heinrich, & Tacker, 2019). Consequently, to feed the ever-increasing population, the reduction of food loss is as equally important as increase production and productivity. Approximately 3.3 billion metric tons of carbon dioxide, 250 cubic kilometers of freshwater and 1.4 billion hectare land are few resources among several other used to produce food, which is never consumed (Wohner, Pauer, Heinrich, & Tacker, 2019), and it has environmental and socio-economic impacts (Vilarino, Franco, & Quarrington, 2017).

Food loss is getting the heart of discussion around the world. However, a general consensus has not been achieved while defining food loss (Vilarino, Franco, & Quarrington, 2017). It is also differently referred to in the literature – "post-harvest food losses", "post harvest food losses", "food loss and waste", or "postharvest food losses" (Global Strategy, 2015). It is estimated that one-third of produced food never been consumed while around one billion people are hungry at the global level (FAO, 2019). Food loss occurs at several points (Chen, Wu, Shan, & Zang, 2018) along the long food chain – in the farmers' field, in the processing industry, in the distribution channel and the consumer homes (Borma, 2017). However, on the one hand, farm-level losses are often overlooked (Johnson, et al., 2018). On the other hand, the world is committed to ending hunger by 2030 under the Sustainable Development Goals (SDGs) (UN, 2018). More importantly, reducing food loss and waste is crucial to meet SDG 2 (End-

Cite this article as:
GC, A., Ghimire, K. (2019). Estimating post-harvest loss at the farm level to enhance food security: A case of Nepal. Int. J. Agric. Environ. Food Sci., 3(3), 127-136.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.3
Received: 17 June 2019 **Accepted:** 12 August 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 127-136
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

ing Hunger) and SDG 12 (Ensuring sustainable consumption and production patterns) (FAO, 2019).

The food system is under pressure of rapid population growth, decline productivity, increasing scarcity of natural resources and increasing diversion of agriculture products to the production of biofuel (McKenzie, Singh-Peterson, & Underhill, 2017). The world is anticipating 9.7 billion inhabitants by 2050 (UN, 2015). Moreover, developing countries have greater challenges in reducing food loss before reaching consumers (Wohner, Pauer, Heinrich, & Tacker, 2019). Post-harvest losses are the main causes of food insecurity in developing countries (Manandhar, Milindi, & Shah, 2018). A consensus can be observed that the reduction of food loss greatly contributes to enhancing food security, strengthening the sustainability of the food system and lowering economic costs (Vilarino, Franco, & Quarrington, 2017).

Spoilage of food may occur due to technical, managerial and/or financial constraints (Manandhar, Milindi, & Shah, 2018) in production, processing, distributing and storing functions. Food loss can be very specific for each product and supply chain but also get influenced by several other factors (Verma, Plaisier, van Wagenberg, & Achterbosch, 2019). It is affected by several factors – physical factors (temperature, moisture and oxygen), biological factors (insect, rodent and mold) and socioeconomic factors (farmers' family size, landholding size, grain storage duration, off-farm income, road accessibility, market price of grain and grain safety during storage) (Manandhar, Milindi, & Shah, 2018). Further, it can be expanded to socioeconomic, biological and/or microbiological, chemical or biochemical, mechanical and environmental factors (Global Strategy, 2015).

Estimation of food loss has many uncertainties (Vilarino, Franco, & Quarrington, 2017). However, it is estimated that around 30 percent of global food loss occurs at the production stage, 20 percent at the post-harvest stage and 35 percent at the consumption stage (Vilarino, Franco, & Quarrington, 2017). From the caloric perspective, loss in cereal has the top position (53%) followed by roots, tuber (14%), fruits, and vegetables (13%), however, fruits and vegetables are at the top (44%) considering the rate of food loss (Vilarino, Franco, & Quarrington, 2017).

The SDG Indicator 12.3.1 (Global food losses) has two components – (a) Food Loss Index and (b) Food Waste Index, and is a Tier II, which means it still demands more work and adoption by member countries (FAO, 2019b). Food loss is the loss along the field to the plate of consumers and food waste is the loss from the consumers' plate.

Reducing food loss is of special importance for developing countries like Nepal due to several reasons. Around two-third population are engaging in agriculture which produces 27.2 percent of the Gross Domestic Product (GDP) (MoF, 2018) and prevalence of the multidimensional poverty is 28.6% in Nepal (NPC, 2018).

The paper attempts to document and to estimate food loss in Nepal. Researches on food loss are very limited in Nepal. Few works have been done on selected commodities at a specific locality. Majority of researches are focused on either specific disease-pest or types of the storage system (Bhandari, Achhami, Karki, Bhandari, & Bhandari, 2015; Manandhar & Mainali, 2000; Paneru, Duwadi, Khanal, & Bhandari, 1996; Paneru, Poudel, & Thapa, 2018).

Meterials and Methods

Food loss has been generally categorized into two types – qualitative and quantitative. The qualitative food loss refers to the degradation of food qualities – taste, appearance and nutritional value (Global Strategy, 2015). On the other hand, quantitative food loss refers to a decline in food volume or quantity. It can be studied by different methods. They are – (a) general baseline survey, (b) probability sample survey, (c) experimental designs – field trails, and (d) multivariate linear regression fitting (Global Strategy, 2015).

This study focuses on quantitative food loss and food loss is considered as all kind of losses at farm level after harvesting – that is neither consumed nor sold from the farm. Purposive sampling of districts was done to make the sample as representative as possible considering resource constraint. Nepal is geographically diverse and thus has greater agro-biodiversity and farming systems (GC & Ghimire, 2018; GC & Yeo, 2019). Nepal produces several kinds of cereals, fruits, and vegetables. However, rice, wheat, and maize are the most consumed staple cereal in the world (Manandhar, Milindi, & Shah, 2018) and also in Nepal. Therefore, instead of crop-specific survey, a general survey has been carried out from randomly selected 300 households from 10 sampling districts in 2015.

The farming practices were recorded using a structured questionnaire. Food loss was taken as quantity loss in the past year based on respondent's reporting instead of physical measurement. The percentage of food loss was calculated from the quantity of post-harvest loss and quantity harvested. The equation (1) was used to calculate percentage food loss for an individual farming household.

$$\text{Percentage Food Loss (FLP)} = \sum_{i}^{n} \frac{QLa}{QHa} \times 100 \quad \ldots\ldots\ldots (1)$$

Where, QL = Quantity food lost, QH = Quantity harvested, and the subscript "a" = crop.

The percentage of Food Loss (FLP) was calculated for each season (winter and summer), each plot (single largest plot and additional plots) and each priority of crop (primary crop and secondary crop).

The mean harvest loss was estimated a confidence interval by using STATA command "mean".

Regression analysis was executed for the major crops to determine the socio-economic factors affecting food loss. The generalized equation for regression analysis is given in equation (2).

$$PC\_FoodLoss = \alpha + \beta Xi + \varepsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, PC_FoodLoss = Percentage of Food Loss on a crop reported from individual farming household

α = intercept of the equation

β = a matrix of coefficient for each independent variables

Xi = matrix of independent variables

Ɛ = error term

Selection of independent variable is crucial for any regression model. Food loss is affected by several factors – physical factor (temperature, moisture and oxygen), biological factor (insect, rodent and mold) and socioeconomic factor (farmers' family size, land holding size, grain storage duration, off-farm income, road accessibility, market price of grain and grain safety during storage) (Manandhar, Milindi, & Shah, 2018). However, for the study, we have considered only socio-economic characteristics of farming households. Paneru, et al (2018) also used several socio-economic variables including farming area, education, occupation and household size to estimate food loss. The description of variables used for the regression analysis has been presented in Table 1.

The data analysis was carried out using STATA software. To select the appropriate variables, the stepwise command was employed and the final model was obtained.

Results and Discussion

The food loss on the major crops the farm level was calculated for each season (winter and summer), each plot (single largest plot and additional plots) and each priority of crop (primary crop and secondary crop).

Postharvest loss of major crops

Rice, maize, wheat, potato, mustard, cabbage and lentils were found the major crop growing by the farmers in the surveyed districts of Nepal. For convenience, the annualized mean percentage of the harvest loss for those crops were calculated. The highest post-harvest food loss at the farm level was found for mustard and lentil. However, the lowest was found for potato. The mean harvest loss at the farm level for rice, wheat, maize, potato, mustard, cabbage and lentil was found 3.24±0.44, 4.88±1.11, 4.00±1.18, 3.01±0.87, 5.18±1.52, 4.76±1.55 and 5.18±1.19 percent respectively. The detail with observation has been presented in Table 2.

Moreover, the average weighted mean of harvest loss at the farm level for cereal was found 3.94 percent. Under the cereal group, rice, wheat and maize – both summer and winter were categorized. The graphical presentation of annual mean postharvest loss at the farm level was presented in Figure 1.

Percentage of food loss for winter season (S1) crops

Wheat, potato, mustard and maize were found the major winter season crop in Nepal. Table 3 provides the detail of the food loss at the farm level for winter crops from the single largest plot as the first priority crop. The average percentage harvest loss on wheat, potato, mustard and maize were found 5.40, 2.00, 3.38 and 2.99 respectively. However, the median harvest loss was found 0.60, 0.00, 0.00, and 0.98 percent for wheat, potato, mustard and maize respectively. Among these crops, wheat was found the most commonly grown crop, which was produced by 105 households as a primary crop in the single largest plot.

Potato, lentil, cabbage and mustard were found the popular crop, which was grown in the winter season in the single largest plot as the second choice after the crops described in Table 3. The highest loss was reported for cabbage and the lowest was reported for potato. Average percentage harvest loss at the farm level for potato, lentil, cabbage and mustard was found 4.23, 5.78, 7.03 and 5.71 respectively. However, the median value was found 0.00 percent for all crops under this category. Table 4 presents the detail under this condition.

Wheat, potato, mustard and lentil were the top choice of farmers in the winter season for additional plots. Table 5 presents the detail on harvest loss on the crops from the additional plots as the primary crop. The highest post-harvest loss was reported for mustard and the lowest post-harvest loss was reported for potato. The average percentage of harvest loss on wheat, potato, mustard and lentil was found 3.46, 2.84, 9.13 and 3.90 respectively. However, the median values were observed at 0.00 percent except for Mustard (1.52 percent).

Lentil, potato and mustard were found popular second choices for the winter crop in additional plots. The highest percentage of post-harvest loss was reported for lentil and the lowest post-harvest loss was reported for potato. The average percentage of harvest loss on lentil, potato and mustard was found 5.73, 1.86 and 5.66 respectively. However, the median value was found 0.00 percent for lentil and potato and 1.14 percent for mustard. The detail has been presented in Table 6.

Percentage of food loss for summer season (S2) crops

Rice (or paddy), maize and cabbage were found the most dominating crops in the summer season in the single largest plot. Around 60 percent farmers were found planting rice in the summer season as the first choice crop in their single largest plot. The highest percentage of post-harvest loss was reported for maize and the lowest percentage of post-harvest loss was reported for cabbage. The average percentage of the harvest loss in rice, maize and cabbage was found 3.26, 2.88 and 0.94 respectively. However, the median value was observed 0.00 except for rice (1.04 percent). The detail has been presented in Table 7.

Except for rice and maize, other crops were not found significantly growing during the summer season in the additional plots as a primary choice. The post-harvest loss for rice and maize were reported almost similar. The average percentage loss of the harvest at farm level was found 2.38 and 1.59 for rice and maize respectively. The median value was found 0.00 percent for both rice and maize under this condition. The detail has been presented in Table 8.

Estimation of harvest loss by Multiple Regression Analysis

To determine the determinants of the post-harvest loss of the crops, a multiple regression analysis was executed. The multiple regression analysis will enable to quantify the effect of several variables on the post-harvest loss.

Table 9 presents the result of the multiple regression model for post-harvest loss for rice, wheat, maize, potato, lentils and cabbage. The highest value of R-square was observed 0.81 for lentil and the lowest value was found 0.094 for maize. Furthermore, the majority of dependent variables have produced mix results for different crops. Nevertheless, use of past

weather information, having access to the internet, a percentage of total income coming from the farm, number of extension workers' visit, gender and household size produced positive results in all models. It means they are a positive contributor to the postharvest loss in all crops.

Moreover, the use of weather information announced, the number of male working in the farm, having access to the extension service, total area and having exclusively rainfed farming has yielded negative coefficients. It implies that they are positive contributors to reduce postharvest loss in those crops.

Table 1. Description of variables

Variable	Description	Observation	Mean	Std. Dev.
WethAnnounce	Use of weather information (Yes =1; No=0)	253	0.40	0.49
WethUsePast	Use of past weather experience (Yes=1; No=0)	284	0.33	0.47
FarmExp	Years of farming experience	300	24.83	14.18
FarmDec	Farming decision taken by HoH (Yes=1; No=0)	300	0.83	0.38
Rainfed	Having exclusively rainfed farming (Yes=1; No=0)	297	0.39	0.49
C_totalarea	Total cropping area in hector	293	0.91	0.86
Primaryocchoh	Agriculture as a primary occupation (Yes = 1; No=0)	300	0.79	0.41
Secondaryocchoh	Agriculture as a secondary occupation (Yes = 1; No=0)	300	0.68	0.47
Internet	Having internet access (Yes=1; No=0)	300	0.21	0.41
Electricity	Having electricity access (Yes=1; No=0)	300	0.92	0.28
Farmadultmales	Number of adult males working in the farm	300	2.09	1.40
Farmadultfemales	Number of adult females working in the farm	300	1.50	1.07
Educhoh	Years of schooling of HoH	300	6.27	4.62
Genderhoh	Gender of HoH (Male =1; Female =0)	300	0.85	0.35
Agehoh	Age of HoH in year	283	50.90	12.44
Hhsize	Household size	300	6.50	3.64
farmarea_numplots	Number of land parcel	300	1.70	0.65
farm_selldist	Distance to the nearest market	298	2.57	3.76
farm_selltime	Time to the nearest market	299	0.49	0.69
Outputcoop	Having membership of output selling cooperative	300	0.58	0.49
Inputcoop	Having membership of input selling cooperative	300	0.61	0.49
Getext	Having access to the extension service (Yes=1; No=0)	300	0.72	0.45
Extvisits	Number of extension visit per year	300	3.56	3.80
totalincome	Total income in NRs 10,000	296	34.32	46.00
pctonfarmincome	% of total income coming from farming	299	66.50	37.27
borrowedyn	Having credit access (Yes=1; No=0)	300	0.40	0.49
PC_Rice	% of postharvest loss on Rice	212	3.24	6.47
Rice_havest	amount of rice harvest in 1,000 kg	212	4.53	17.32
PC_Wheat	% of postharvest loss on wheat	149	4.88	13.58
Wheat_havest	amount of wheat harvest in 1,000 kg	149	1.80	7.21
Maize_havest	amount of maize harvest in 1,000 kg	116	0.74	0.98
PC_Maize	% of postharvest loss on maize	116	4.00	12.66
Potato_havest	amount of potato harvest in 1,000 kg	117	2.69	5.69
PC_Potato	% of postharvest loss on potato	117	3.01	9.46
Mustard_harvest	amount of mustard harvest in 1,000 kg	61	0.22	0.46
PC_Mustard	% of postharvest loss on mustard	61	5.18	11.90
Lentil_havest	amount of lentil harvest in 1,000 kg	45	0.76	3.70
PC_Lentil	% of postharvest loss on lentil	45	5.18	8.00
Cabbage_harvest	amount of cabbage harvest in 1,000 kg	50	9.01	16.48
PC_Cabbage	% of postharvest loss on cabbage	50	4.76	10.99

Table 2. Annual mean percentage of harvest loss of different crops

Crop	Mean	Std. Err.	[95% Confidence Interval]		Observations
Rice	3.24	0.44	2.37	4.12	212
Wheat	4.88	1.11	2.68	7.08	149
Maize	4.00	1.18	1.67	6.33	116
Potato	3.01	0.87	1.27	4.74	117
Mustard	5.18	1.52	2.13	8.23	61
Cabbage	4.76	1.55	1.63	7.88	50
Lentil	5.18	1.19	2.78	7.59	45

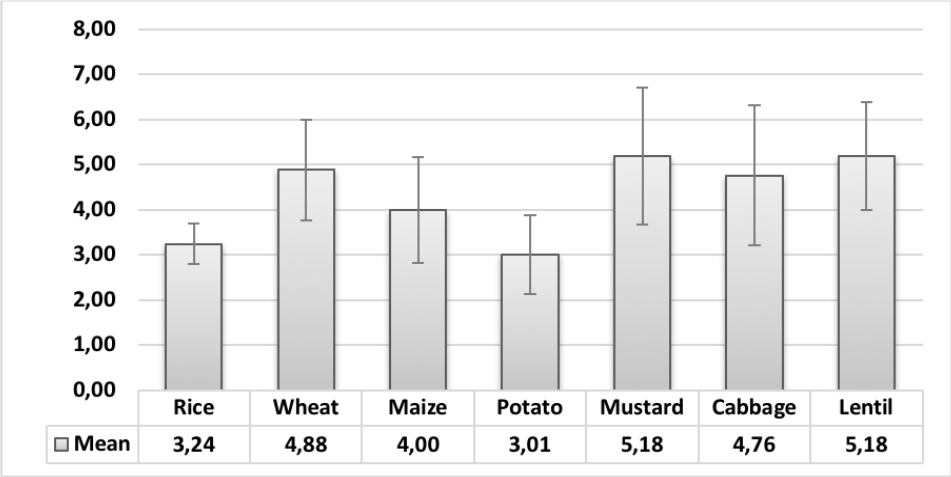

Figure 1. Percentage Food Loss for various crops at Farm Level

Table 3. Harvest Loss in S1P1C1 (in Percentage)

Percentiles	Wheat	Potato	Mustard	Maize
25%	0.00	0.00	0.00	0.00
50%	0.60	0.00	0.00	0.98
75%	5.88	2.44	5.00	6.25
90%	10.00	5.00	10.00	8.96
95%	21.88	11.11	12.50	9.76
99%	50.00	16.67	25.00	9.76
Maximum	100.00	16.67	25.00	9.76
Observation Number	105	42	25	17
Mean	5.40	2.00	3.38	2.99
Standard Deviation	13.10	3.85	5.92	3.82

Table 4. Harvest Loss in S1P1C2 (in Percentage)

Percentiles	Potato	Lentil	Cabbage	Mustard
50%	0.00	0.00	0.00	0.00
75%	0.00	11.11	5.88	0.00
90%	11.67	16.67	19.35	20.00
95%	13.33	33.33	58.33	50.00
99%	88.00	33.33	58.33	50.00
Maximum	88.00	33.33	58.33	50.00
Observation Number	29	14	14	14
Mean	4.23	5.78	7.03	5.71
Standard Deviation	16.44	10.06	15.77	13.99

Table 5. Harvest Loss in S1P2C1 (in Percentage)

Percentiles	Wheat	Potato	Mustard	Lentil
25%	0.00	0.00	0.00	0.00
50%	0.00	0.00	1.52	0.00
75%	4.78	4.84	9.09	10.00
90%	9.09	13.51	20.00	13.50
95%	10.00	14.29	70.00	13.66
99%	50.00	15.89	70.00	13.66
Maximum	50.00	15.89	70.00	13.66
Observation Number	72	24	14	10
Mean	3.46	2.84	9.13	3.90
Standard Deviation	8.03	5.00	18.70	5.93

Table 6. Harvest Loss in S1P2C2 (in Percentage)

Percentiles	Lentil	Potato	Mustard
25%	0.00	0.00	0.00
50%	0.00	0.00	1.14
75%	12.50	2.14	14.29
90%	16.67	9.09	18.33
95%	25.00	10.00	20.00
99%	25.00	10.00	20.00
Maximum	25.00	10.00	20.00
Observation Number	18	18	10
Mean	5.73	1.86	5.66
Standard Deviation	8.02	3.24	8.02

Table 7. Harvest Loss in S2P1C1

Percentiles	Rice	Maize	Cabbage
25%	0.00	0.00	0.00
50%	1.04	0.00	0.00
75%	4.00	0.00	1.39
90%	9.09	12.50	3.57
95%	14.29	20.00	4.67
99%	25.00	25.00	4.67
Maximum	53.57	25.00	4.67
Observation Number	170	45	12
Mean	3.26	2.88	0.94
Standard Deviation	5.89	6.40	1.59

Table 8. Harvest Loss in S2P2C1

Percentiles	Rice	Maize	Cabbage
25%	0.00	0.00	0.00
50%	1.04	0.00	0.00
75%	4.00	0.00	1.39
90%	9.09	12.50	3.57
95%	14.29	20.00	4.67
99%	25.00	25.00	4.67
Maximum	53.57	25.00	4.67
Observation Number	170	45	12
Mean	3.26	2.88	0.94
Standard Deviation	5.89	6.40	1.59

Table 9. Estimation of postharvest loss at the farm level for various crops by regression model

Variables	Rice	Wheat	Maize	Potato	Mustard	Lentil	Cabbage
Rice_havest	0.08						
	(0.07)						
Totalincome	0.01	-0.10**			-0.03	0.06	0.05**
	(0.01)	(0.05)			(0.03)	(0.05)	(0.02)
WethUsePast	2.07*	6.32*	3.62				
	(1.20)	(3.64)	(3.14)				
FarmExp	0.08*	0.07		0.16	-0.12*	-0.32**	0.37**
	(0.04)	(0.09)		(0.12)	(0.07)	(0.13)	(0.17)
FarmDec	0.84	-8.72		-2.66		11.77***	-10.96**
	(1.05)	(7.36)		(2.86)		(4.08)	(4.54)
Rainfed	-1.50*		-4.61	-2.70			-5.80*
	(0.80)		(2.99)	(2.13)			(3.23)
C_totalarea	-2.51***	-1.14	-2.44	1.17			-14.06***
	(0.75)	(0.89)	(2.38)	(1.26)			(4.67)
Primaryocchoh	-2.45**	2.93	-8.07	1.81	-2.76	8.07**	
	(1.22)	(3.85)	(5.63)	(1.50)	(2.41)	(2.85)	
Secondaryocchoh	-2.09*				2.58*		
	(1.10)				(1.51)		
Internet	1.90	-4.50	-3.83	2.94	6.57**		3.11
	(1.46)	(3.12)	(3.01)	(1.93)	(2.82)		(3.06)
Electricity	-9.79	3.52	-5.31		-6.37		
	(8.50)	(2.31)	(3.97)		(4.20)		
Borrowedyn	-1.58		7.82*				-5.61**
	(1.06)		(4.66)				(2.54)
Pctonfarmincome	0.03*						0.13**
	(0.02)						(0.06)
Extvisits	-0.09				0.26	0.99**	0.25
	(0.11)				(0.30)	(0.35)	(0.31)
Inputcoop	2.59**			-1.29		-5.02*	
	(1.24)			(1.47)		(2.82)	
Agehoh	-0.06	-0.28	0.22*	-0.09	0.22**	-0.19**	-0.44*
	(0.04)	(0.20)	(0.12)	(0.08)	(0.09)	(0.06)	(0.24)
Hhsize	0.33**	-0.56				-0.25	3.16***
	(0.16)	(0.55)				(0.33)	(1.04)
farmarea_numplots	1.76**	1.75		-2.07	1.79	-8.58**	3.53
	(0.87)	(2.23)		(2.58)	(1.13)	(3.55)	(2.25)
farm_selldist	-0.11	0.44	-2.68**		-0.62**	0.90***	
	(0.09)	(0.38)	(1.08)		(0.26)	(0.29)	
Getext	-2.25*					-6.18**	
	(1.24)					(2.77)	
Outputcoop	0.82			2.44			
	(1.06)			(3.03)			
farm_selltime		-4.01	10.79**	2.34	4.69**	-5.90***	
		(2.49)	(4.99)	(2.05)	(1.94)	(1.93)	
Farmadultmales		2.34		-0.96	-1.18*		-3.61**
		(1.70)		(0.78)	(0.62)		(1.53)
Educhoh		0.55*	0.99*		0.82***	-0.47***	-0.76
		(0.30)	(0.55)		(0.24)	(0.16)	(0.51)
Genderhoh		3.86	-5.74			14.89***	
		(2.87)	(4.48)			(3.32)	
Maize_havest			-2.29				
			(1.68)				
WethAnnounce			4.00		-2.61	-4.79**	-6.64**
			(2.91)		(1.54)	(1.95)	(3.09)
Potato_havest				-0.15			
				(0.11)			
Farmadultfemales				-0.92	1.09	3.21**	-3.12**
				(0.79)	(0.85)	(1.26)	(1.35)
Constant	11.16	9.58	5.98	9.73	-5.75	13.01*	25.53
	(9.50)	(15.26)	(8.60)	(6.49)	(7.58)	(6.37)	(15.90)
Observations	168	110	90	99	45	37	44
R-squared	0.32	0.20	0.31	0.09	0.62	0.81	0.54

Robust standard errors in parentheses. *** p<0.01, ** p<0.05, * p<0.1

Discussion

Rice, wheat and maize were found the major cereal crops. Besides cereals, potato, lentils, mustards and cabbages were found the most commonly grown crops. MoALD (2018) listed rice, wheat, maize, millet, barley and buckwheat as the major cereal crops (MoALD, 2016/17).

Joshi et al (2011) and Poudel and Shaw (2016) considered rice, wheat, maize, millet and barley as the major cereal crops (Joshi, Maharjan, & Piya, 2011; Poudel & Shaw, 2016). Regarding potato, MoALD categories it into cash crops, however, Joshi et al (2011) grouped it into cereal basket. Likewise, Maharjan and Khatri-Chhetri (2006) found rice, maize, wheat, millet, barley and pulses as a major crop growing by Nepali farmers (Maharjan & Khatri-Chettri, 2006). This study revealed that for the winter season, wheat, potato and mustard were found the major crops and for the summer season, rice and maize were found the major crop-growing by the Nepali farmers. However, maize and cabbage were found growing in both summer and winter season.

The postharvest loss of rice at the farm level was found 3.24±0.44 percent. In line with this FAO has reported the postharvest loss of 4-22 % in rice (FAO, 1998). Likewise, Boxall and Gillet (1984) found rice 3.3±2.2 percent postharvest loss on (Boxall & Gillett, 1984). Another study found the postharvest losses of rice in China was 7-13 percent (Kumar & Kalita, 2016). However, K.C. (1992) confirmed that the post-harvest loss in cereals was 15-20% in Nepal (KC, 1992). Pradhan and Manandhar (1992) reported the post-harvest loss of 8%, 7.4% and 13% for Mountain, Hills and Terai of Nepal respectively (Pradhan & Manandhar, 1992). Boxall and Gillett (1984) also confirmed altitudinal variation on the post-harvest loss.

The postharvest loss on wheat was found 4.88±1.11 percent. Boxall and Gillett (1984) found a postharvest loss on wheat of 2.4±1.9% in eastern Nepal. Furthermore, the quality of stored wheat was found lower in the hills of Nepal as compared to the plain area (Devkota, Devkota, Acharya, Shrestha, & McDonald, 2018). In Pakistan, the average postharvest loss on wheat is 3.5 percent (Baloch, 1999).

After harvesting, the loss on maize was found 4.00±1.18 percent. However, Paneru et al (1993) reported storage losses of up to 32 percent due to maize weevil (Paneru, Duwadi, Khanal, & Bhandari, 1996). On the other hand, some studies reported 30-35 percent loss on the grain in those ears which are stored in Kuniyo. Likewise, Manandhar and Mainali (2000) reported 7.44 percent losses in maize storage (Manandhar & Mainali, 2000). Another study found a post-harvest loss on maize up to 19.5±12.5 percent due to pests (Paneru, Poudel, & Thapa, 2018). Manandhar et al (2001) reported 40-50 percent losses in maize storage (Manandhar, Ransom, & Rajbhandari, 2001). Bhandari et, al (2015) reported a 10-15 percent loss in maize storage (Bhandari, Achhami, Karki, Bhandari, & Bhandari, 2015).

Postharvest loss on cabbage at farm level was found 4.76±1.55 percent, however, another study found 9 percent on cabbage (Kader & Davis, 2009; Udas, Rai, Khatiwada, Gurung, & Thapa, 2005).

The multiple regression model suggests that socio-economic variables have a smaller influence on the postharvest loss. The R-square value for all cereals was found less than 0.35. For potato, it was just 0.09. This implies that biological, climatic and other factors may have a stronger influence on the postharvest loss. Some other socio-economic variables may have a stronger effect; however, the study has covered all socioeconomic variables generally used for the regression analysis. Nevertheless, the R-square value for crops other than cereals has demonstrated relatively higher values – up to 0.81 for lentil. Furthermore, in the multiple regression model for lentil, cabbage and rice, 14, 12 and 11 explanatory variables were found significant respectively. On the other hand, in the regression model for potato, none of the independent variables was found significant.

The majority of the variables did not produce a consistent relationship with the postharvest loss on various crops. In one crop, the same variable was found contributor for loss and in another crop, the same variable was found as a contributor to the reduction of postharvest loss. Nevertheless, some of the explanatory variables have produced a consistent result for all crops. The use of weather information announced, number of a male family member in the farming, having access to extension services, total area and having exclusively rainfed farming were found a strong contributor to the reduction of postharvest loss in all selected crops.

Conclusion

Agriculture is facing an unprecedented challenge to feed peoples inhabiting the world. More importantly, the pressure on agriculture is ever increasing. At current statistics, around one billion people are in hunger and by 2050 the population in the world is expected to become 9.7 billion. Furthermore, climate change is exerting pressure on agriculture – mainly due to change in precipitation pattern, temperature, drought and other climate-induced disasters. However, production alone might not a single issue because it is estimated that around one-third of all food produced globally is never consumed which is a misuse of the scarce natural resources along with the labour. Developing countries have more issue on the post-harvest loss. However, an appropriate level of attention was not found regarding food loss and waste. Therefore, postharvest loss at the farm level has been estimated for rice, wheat, maize, potato, mustard, cabbage and lentils, which was found the major crops grown by the farmers. The average postharvest loss for those crops were found around 5 percent. Furthermore, multiple regression model suggested that the postharvest loss for those crops can be explained by various socio-economic variables. However, the level of explanation was found widely different from one crop to another. Therefore, careful selection of variables is very important. Moreover, food loss should be addressed from technological, cultural and behavioral and policy solutions which make the agriculture system more sustainable and improve the food security situation. The study concluded that existing postharvest loss can be reduced through effective agriculture extension service and by providing timely and reliable weather informa-

tion to the farmers. Moreover, increasing farm size may also reduce postharvest loss, which could be achieved through land consolidation.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.4 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):137-143 (2019)

Cancer preventive and neuroprotective potentials of red hulls, kernels and oleo-gum resins from Pistachio

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Abstract

This research was performed to assess cancer prevention and neuroprotective capacities of different parts of Pistachio (*Pistachio vera* L.). Red hulls, kernels and oleo-gum resins of Pistachio were extracted with methanol-MeOH and distilled water-dH_2O, and subjected to *in vitro* biological assays varying from 100 to 1000 µg mL^{-1} concentrations. Their anticancer activities were evaluated against A549, MCF-7, and HeLa human cancer cells. Neuroprotective activities of the extracts were tested through enzyme inhibition on AChE, BChE, and TYR, which are closely related to pathogenesis of neurobiological disorders, particularly Alzheimer's and Parkinson's diseases. Due to cancer and neurodegenerative diseases are associated with oxidative damage, the extracts were analyzed for their antioxidant activities. With respect to free radical scavenging activities of the extracts, red hull extracts were found as the most potent ones both DPPH (67.95±1.13 to 80.55±0.12%) and ABTS (86.92±0.10 to 92.04±1.06%) radicals. The highest anticancer activity were determined in MeOH and dH_2O extracts obtained from oleo-gum resin against HeLa cells (IC_{50} = 18.50±0.85 and 28.97±0.08 µg mL^{-1}, $p< 0.01$, respectively), whilst dH_2O-kernel extract was found to have the weakest anticancer activity towards A549 cells (IC_{50} = 268.66±1.02 µg mL^{-1}, $p< 0.01$). Neuroprotective potentials on AChE and BChE enzymes were resulted in the superiority of dH_2O-red hull extract was exerted the highest inhibition on AChE and BChE enzymes with 81.50±0.08 and 62.96±1.01% inhibition, respectively. However, dH_2O extract from oleo-gum resin showed the highest inhibitory effect on TYR enzyme (58.16±0.18% inhibition). *P. vera* is of valuable nutritional source for human diet. Other than kernel parts used as food, waste parts like red hulls and oleo-gum resins have been proven as a potential pharmacological source. Consequently, this study reveals that non-food parts of Pistachio could be valuable source for pharmaceutical industry.

Keywords: Pistachio, Cancer Prevention, Neuroprotective, Antioxidant, Enzyme Inhibition

Introduction

Cancer and neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), prion and motor neuron diseases etc. are of the most common diseases and disorders that cause a growing health problem worldwide. According to World Health Statistics reports, these diseases and disorders affect millions of people globally, and their incidence rates are expected to continue to increase rapidly for the following years. Currently, no effective therapy has still been revealed to fight cancer and neurodegenerative diseases, and thus dietary plants and their natural bioactive compounds offer extremely great opportunities for development effective treatment strategies (Newman and Cragg, 2016; Gezici, 2019a; Gezici and Sekeroglu, 2019a; WHO, 2019). Dietary medicinal plants (fruits, vegetables, spices, cereals, and edible tubers/roots) containing natural bioactive compounds such as phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, resveratrol, lycopene,

Cite this article as:
Gezici, S. (2019). Cancer preventive and neuroprotective potentials of red hulls, kernels and oleo-gum resins from Pistachio. Int. J. Agric. Environ. Food Sci., 3(3), 137-143.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.4
Received: 03 June 2019 **Accepted:** 13 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 137-143
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

carotenoids, quercetin, catechin, naringenin, organosulfur, curcumin, genistein, isothiocyanates, capsaicin, gingerol, anthocyanins, coumarins, lignans, quinones, and others have been demonstrated to possess valuable health benefits beside basic nutrition (Das and Gezici, 2018; Guizani et al., 2018; Roy et al., 2018,).

Recently, dietary medicinal plants have been gained a great interest to reduce Reactive Oxygen Species (ROS) including hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and superoxide ion (O_2) formation, occurring naturally in the human body. In living organisms, preventing the side effects of ROS is one of the most effective management strategy for oxidative-stress related diseases including cancer, cardiovascular disease, chronic kidney diseases, aging, diabetes, rheumatoid arthritis atherosclerosis, and neurodegenerative diseases (Reddy et al., 2003; Schieber and Chandel, 2014; Farzaei et al., 2018).

In the last few decades, natural antioxidants obtaining by dietary intake have a widespread interest instead of synthetic ones amongst the people. Previous studies have determined that a numerous natural herbal products and formulations obtained from dietary medicinal plants as natural antioxidant agents with powerful antioxidant capacities for reducing free radicals, metal chelators and singlet oxygen species (Reddy et al., 2003; Tabatabaei-Malazy et al., 2013; Guizani et al., 2018; Gezici and Sekeroglu, 2019b).

Among the dietary medicinal plants, Pistachio (*Pistacia vera* L.), belonging to the Anacardiaceae family, is native to Asia and distributed throughout the Mediterranean region (Bozorg et al., 2013). This plant has recently been ranked rich sources of antioxidants, and investigated for various pharmacological activities such as anti-inflammatory, antioxidant and antimicrobial activities, because of its wide range of secondary metabolites such as α-pinene, limonene, terpinolene, β-ocimene, camphene, resveratrol, carvacrol, abietadiene, gallic acid, catechin, eriodictyol, naringenin, genistein, apigenin, kaempferol, luteolin, cyanidin-3-galactoside (Rajaei et al., 2010; Bozorg et al., 2013).

Recent studies showed that Pistachio with whole parts including fruit, leave, gum, hull, oil, and seed possess potential usage for pharmacological purposes in traditional medicine, due to their comprehensive biological properties. In addition to their pharmacological usage, fruits of Pistachio have been commonly consumed as snack food and food additive (Rajaei et al., 2010; Fathalizadeh et al., 2015; Seifaddinipour et al., 2018).

By now, anticancer, antiproliferative, anticholinesterase, antityrosinase, antioxidant, and other biological activities of numerous medicinal and aromatic plants (MAPs) and secondary metabolites isolated from MAPs were analysed in our laboratory (Akgunlu et al., 2016; Sekeroglu et al., 2017; Gezici et al., 2017; Belkhodja et al., 2017; Karik et al., 2018; Gundogdu et al., 2018; Senol et al., 2018; Gezici, 2018; Sekeroglu et al., 2018; Das et al., 2019; Shida et al., 2019; Gezici and Sekeroglu, 2019a; Gezici and Sekeroglu, 2019b; Gezici, 2019a; Gezici 2019b; Sekeroglu and Gezici, 2019, Sekeroglu et al., 2019 *in press*). Take into consideration our ongoing projects, evaluation cancer protective potentials against human cancer cells, investigating neuroprotective activities through enzyme inhibitions on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and TYR (tyrosinase) enzymes, along with determination of antioxidant capacities of extracts obtained from red hulls, kernels and oleo-gum resins of Pistachio (*P. vera* L.) are the main objectives of the presented research.

Materials and Methods

Collection of Plant Material

Red hulls, kernels, and oleo-gum resins of Pistachio (*Pistachio vera* L.) used herein were collected from Gaziantep province of Turkey during the months of August - September 2018. The red hulls were separated from the kernels, and the hulls and kernels were dried in the laboratory conditions. The herbarium voucher of plant samples was kept at the Department of Biology, Kilis 7 Aralık University, Turkey.

Extraction of Plant Parts

P. vera L. parts including red hulls (PVRH) and kernels (PVK) were dried under the shade at laboratory conditions. The oleo-gum resins of pistachio (PVOR) was directly subjected to extraction after collection from the plant stem. Each plant part (50g) was powdered individually, and extracted with methanol (MeOH) and distilled water (dH_2O) by the method of maceration as described in our previous publication (Gezici and Sekeroglu, 2019b; Gezici, 2019a), and then the extracts were stored at -20°C until further analysis.

Free Radical Scavenging Activity

Antioxidant activities of the extracts were determined using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods (Gezici et al., 2017; Sekeroglu et al., 2017; Gezici and Sekeroglu, 2019b). Ascorbic acid was used a commercial standard for DPPH assay, whilst Trolox was used a commercial standard for ABTS assay. The extracts and commercial antioxidant standards were dissolved in DMSO at different concentrations (100 to 1000 μg mL^{-1}) for the assays.

Human Cancer Cells and Anticancer Activity

A549 (lung carcinoma), MCF-7 (breast adenocarcinoma), and HeLa (cervical cancer) human cancer cells and non-tumorous HUVECs (human umbilical vein endothelial cells), obtained from the American Type Culture Collection (ATCC, USA) were used to evaluate the potential anticancer and cytotoxic activities of PVRH, PVK, and PVOR extracts from Pistachio. The A549 and HeLa cancer cells were cultured on Roswell Park Memorial Institute Medium (RPMI, ThermoFisher Scientific), and the other cells were grown in Dulbecco's modified Eagle medium (DMEM): Ham's F12 nutrient medium (1:1) (ThermoFisher Scientific) in the flasks at 37°C in a humidified CO_2 (5%) incubator. The cell growing conditions and supplements were used as same described in the previous publications (Gezici, 2018; Gezici, 2019a). In order to determine anticancer activities of the Pistachio extracts, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as described by Mosmann (1983) with minor modifications (Gezici, 2019a). The absorbance was measured at 570 nm with a Thermo Lab systems

408 Multiskan multiplate spectrophotometer, and the dose response curve was used to generate the IC_{50} (µg mL^{-1}) values for each cells.

Neuroprotective Activity

In the presented research, neuroprotective activities of Pistachio extracts were tested through enzyme inhibition against AChE (acetylcholinesterase), BChE (butyrylcholinesterase), and TYR (tyrosinase) enzymes. The assays were conducted in 96-well microplate using ELISA microplate reader (Thermo Lab systems 408 Multiskan). Galanthamine hydrobromide (Sigma, St. Louis, MO, USA) was employed as the reference for AChE and BChE, while α-Kojic acid (Sigma, St. Louis, MO, USA) was used as the reference for TYR. The extracts and reference standards were dissolved in DMSO at different concentrations, and the final concentration of the extracts and reference standards were adjusted to 1000 µg mL^{-1} and 100 µg mL^{-1}, respectively.

AChE and BChE inhibitory activity of the samples was measured by slightly modified spectrophotometric method of Ellman et al. (1961). All reagents, conditions and calculations were same as described in the previous publications (Senol et al., 2018; Gezici and Sekeroglu, 2019b). Briefly, electric eel AChE (EC 3.1.1.Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1. Sigma, St. Louis, 7 MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5´-Dithio- bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. For determination the inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma), the modified dopachrome method with L-DOPA as substrate was used as described Sekeroglu et al. (2012) previously.

Statistical Analyses

The data, obtained from the assays, were expressed as mean and standard deviation of mean (mean±SD). The percentage of enzyme inhibition on AChE, BChE and TYR was calculated as $[(Abs_{control} - Abs_{sample})/Abs_{control} \times 100]$, where $Abs_{control}$ value is the absorbance of the control solvent (blank), where Abs_{sample} is the absorbance of the tested sample (plant extract or positive control in the solvent) in the presence of enzyme. The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. P value of <0.05 was considered to be statistically significant, $p < 0.01$ and $p < 0.001$ were considered to be very significant.

Results and Discussion

Free Radical Scavenging Activity

Free radical scavenging activities of Pistachio extracts were determined against DPPH and ABTS radicals at various concentrations. Red hulls and kernels extracts of *P. vera* showed remarkable free radical scavenging activity, but oleo-gum resins extracts demonstrated moderate activity, comparing the standard antioxidants. The results were presented in Table 1 as (%) inhibition percentage at 1000 µg mL^{-1} concentration (Table 1).

Table 1. Free Radical Scavenging Activities of *P. vera* L. Extracts at 1000 µg mL^{-1}

Plant part	Extract type	DPPH[a]	ABTS[a]
Red hulls (PVRH)	Methanol	80.55±0.12***	86.92±0.10**
	Water	67.95±1.13**	92.04±1.06***
Kernels (PVK)	Methanol	56.08±0.52***	61.92±0.05**
	Water	44.61±0.49**	73.80±0.48***
Oleo-gum resins (PVOR)	Methanol	29.15±0.50***	40.18±1.07**
	Water	35.08±1.06**	47.21±0.55**
Ascorbic acid[b]		74.02±0.14	---
Trolox[c]		---	78.50±0.36

[a] The values were expressed as inhibition (%) ± standard deviation.
[b] Ascorbic acid; a commercial standard for DPPH assay.
[c] Trolox; a commercial standard for ABTS assay.
**p value of < 0.01; ***p value of < 0.001

As can be seen in the Table 1, all the Pistachio extracts displayed higher ABTS radical scavenging effects as compared to those of DPPH scavenging capacity at the tested concentrations. In both cases, PVRH extracts exerted the highest scavenging activity on DPPH and ABTS radicals, whilst PVOR extracts demonstrated the lowest ones with the inhibition percentage values ranged between 29.15±0.50 to 47.21±0.55. The highest DPPH scavenging activity was determined in the PVRH-MeOH extract (80.55±1.12% inhibition, $p < 0.001$), when the highest ABTS scavenging activity was found to belong to the PVRH-dH_2O extract with the 92.04±1.06% inhibition ($p< 0.001$), which was closely followed by the PVRH-MeOH extract (86.92±0.10% inhibition, $p< 0.01$).

DPPH and ABTS assays have been commonly used to determine the free radical scavenging activity of plant extracts and their pure compounds (Reddy et al., 2003; Farzaei et al., 2018). Based on the free radical scavenging results, red hulls of *P. vera* L. were found to have the most significant antioxidant potentials than the other parts of the plant, which may be due to the fact that its rich secondary metabolites components such as epicatechin, quercetin, naringenin, luteolin, kaempferol,cyanidin-3-O-galactoside and cyanidin-3-O-glucoside (Bozorgi et al., 2013; Seifaddinipour et al., 2018). The obtained results were consistent with previous works carried out to determine antioxidant potentials of different Pistachio species such as *P. atlantica, P. terebinthus, P. khinjuk,* and *P. lentiscus* (Rajaei et al., 2010; Hosseinzadeh et al., 2012; Bozorgi et al., 2013). Accordingly, the hull of Pistachio can provide significant benefits to cope with the oxidative-stress related diseases.

Anticancer Activity

Cancer prevention potentials of *P. vera* L. parts were assessed against A-549, MCF-7, and HeLa human cancer cells, compared to HUVEC control cells. The Pistachio extracts exhibited noteworthy cytotoxic potentials towards the tested cancer cells in a dose and time dependently; however, the IC_{50}

values were varied depending on the cancer cells. The results of anticancer activity were given in Table 2, regarding as IC_{50} (µg mL^{-1}) values after 72 hours at 200 µg mL^{-1} concentration.

In this assay, the parts of Pistachio known as waste parts (PVRH and PVOR) were found to possess higher cancer prevention potentials, compared to those of the part consumed as food (PVK). As summarized in the Table 2, all the tested parts of *P. vera* L. caused much more cytotoxicity on HeLa cells, following by MCF-7 cancer cells. Methanol extracts of *P. vera* L. were found higher anticancer activity than those of the water extracts against all the cells. The methanol extract of PVOR exerted the highest anticancer activity towards HeLa cells (IC_{50} = 18.50±0.85 µg mL^{-1}, p< 0.01), when PVK-dH_2O extract was found to have the weakest anticancer activity against A549 human cancer cells (IC_{50} = 268.66±1.02 µg mL^{-1}, p< 0.01).

Table 2. Anticancer activities of *P. vera* L. extracts against A549, MCF-7 ve HeLa human cancer cells

Human cancer cells	Plant Part		IC_{50} values[a] (µg mL^{-1})
A549	Red hulls	Methanol	191.04±0.18*
		Water	232.75±0.49**
	Kernels	Methanol	240.23±0.64*
		Water	268.66±1.02**
	Oleo-gum resins	Methanol	164.50±2.01**
		Water	187.28±0.16**
MCF-7	Red hulls	Methanol	90.16±0.38*
		Water	105.02±0.86*
	Kernels	Methanol	122.40±0.21**
		Water	130.01±0.60*
	Oleo-gum resins	Methanol	80.36±0.77**
		Water	88.92±1.14**
HeLa	Red hulls	Methanol	40.15±0.98**
		Water	34.20±0.20**
	Kernels	Methanol	46.48±0.55*
		Water	52.69±0.46*
	Oleo-gum resins	Methanol	18.50±0.85**
		Water	28.97±0.08**
Doxorubicin [b]			8.15±0.02
DMSO (dimethyl sulfoxide) [c]			0

[a] Values were expressed as IC_{50} ± SD from three independent experiment (n=3).
[b] Doxorubicin, positive control.
[c] DMSO; dimethyl sulfoxide, negative control.
*p value of < 0.05; **p value of < 0.01

In previous studies conducted with the other Pistachio species revealed anticancer properties of the Pistachio extracts against cancer cells. According to Rezaei et al. (2012), *P. atlantica* fruit extract were analysed for its anticancer activity on human colon carcinoma cells (HT29) and the extract were showed powerful growth inhibition in cancer cells, as compliant with the results obtained from the presented research (Rezaei et al., 2012). Dimas et al. (2009) revealed antitumor activities of the gum extracts obtained from *P. lentiscus* var. *chia* in colorectal cancer developed mice, the extracts also induced suppression of growth of human colorectal tumor xenografts (Dimas et al., 2009). In another research performed with oleoresin obtained from *P. vera* L. were tested against hepatocellular carcinoma, cervical cancer, and melanocyte cells and determined significant cytotoxic potential on the tested cells, which is more similar to the current results (Almehdar et al., 2012).

As previously reported, high antioxidant activity and rich polyphenolic content of the herbal extracts are known to be closely related to inhibit cancer and neurodegenerative diseases efficiently (Reddy et al., 2003; Tabatabaei-Malazy et al., 2013; Newman and Cragg, 2016; Roy et al., 2018; Gezici, 2019a; Gezici and Sekeroglu, 2019a). Terpenes and phenolic components are the main bioactive phytochemicals found in different parts of *P. vera* L. These components have been known to possess significant antioxidant and anti-inflammatory effects, and so they are probably responsible for preventing cancer, as demonstrated by previous researches (Rajaei et al., 2010; Bozorgi et al., 2013; Fathalizadeh et al., 2015; Das and Gezici, 2018; Seifaddinipour et al., 2018).

Neuroprotective Activity

Neuroprotective activity of the PVRH, PVK, and PVOR extracts obtained from *P. vera* L. were assessed through enzyme inhibition assays towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and tyrosinase (TYR) enzymes at 100, 200, 400, 800, and 1000 µg mL^{-1} concentrations. As given in Figure 1, the dH_2O extracts of Pistachio exerted higher enzyme inhibitory effect against the tested enzymes than those of the MeOH extracts. Enzyme inhibitory potentials of the Pistachio parts on cholinesterase enzymes were resulted in the superiority of PVRH-dH_2O extract 81.50±0.08% inhibition on AChE, 62.96±1.01% inhibition on BChE, respectively (Figure 1).

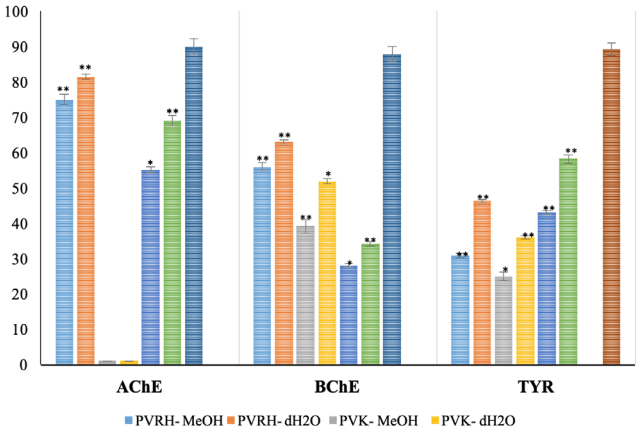

Figure 1. Enzyme Inhibition Capacities of P. vera L. Extracts against AChE, BChE and TYR

[PVRH: *P. vera* red hulls; PVK: *P. vera* kernels; PVOR: *P. vera* oleo-gum resins]
The values were presented as inhibition (%) ± standard deviation
*p value of < 0.05; **p value of < 0.01

In the contrary the inhibition on cholinesterase enzymes, PVOR-dH_2O extract was found to have the highest inhibitory effect on TYR enzyme (58.16±0.18%). PVK extracts inhibited BChE and TYR enzymes from moderate level to weak level, in which the higher cholinesterase inhibitory activity was observed against BChE (% inhibition from 39.10±0.12 to 51.94±0.68), compared to TYR (% inhibition from 25.03±1.01 to 36.04±0.65), whilst they demonstrated no inhibition against AChE enzyme even at the highest concentration (Table 3, Fig 1).

However, there have been a few studies focused on revealing anti-cholinesterase activity of the other Pistachio species, no study have been performed to screen anticholinesterase and anti-tyrosinase activities of different part of *P. vera* L. up to now. On the other hand, this is the first research that screened neuroprotective potentials of the extracts obtained from red hulls, kernels, and oleo-gum resins of *P. vera* L. In a previous work, aqueous extracts from *P. atlantica* and *P. lentiscus* leaves were determined regarding of their acetylcholinesterase inhibitory effects, and found as relatively weak AChE inhibitory activity (Benamar et. al., 2010). In another research on enzyme inhibitory effects of Pistachio species were performed using ethyl acetate and methanol extracts of the *P. terebinthus* kernels that showed no inhibitory activity against AChE and TYR, when they demonstrated inhibition on BChE at moderate levels (Orhan et al., 2012). These findings are consistent with the presented data for the kernel extracts.

On the basis of the findings obtained from the current work, the red hull part of the plant is seem to be a valuable agent for inhibition on AChE and BChE enzymes, while oleo-gum resin of Pistachio is a good candidate for inhibition against TYR enzyme. In fact, rich polyphenolic contents of *P. vera* L. are likely contribute to its remarkable neuroprotective capacity as reported previously (Rajaei et al., 2010; Hosseinzadeh et al., 2012; Fathalizadeh et al., 2015; Seifaddinipour et al., 2018).

Table 3. Neuroprotective Potentials of *P. vera* L. Extracts at 1000 µg mL^{-1}

Plant part	Extract type	% Inhibition ± SD [a]		
		AChE	BChE	TYR
Red hulls	Methanol	75.06±0.22**	56.01±0.90**	30.84±1.10**
	Water	81.50±0.08**	62.96±1.01**	46.32±0.08**
Kernels	Methanol	---[d]	39.10±0.12 **	25.03±1.01*
	Water	---[d]	51.94±0.68*	36.04±0.65**
Oleo-gum resins	Methanol	55.28±0.77*	28.13±0.98*	42.98±0.15**
	Water	69.12±0.94**	34.21±0.55**	58.16±0.18**
Galantamine [b]		90.04±0.86	87.94±0.20	---
α-Kojic acid [c]		---	---	89.35±0.18

[a] The values were given as inhibition (%) ± standard deviation (n=3).
[b] Galantamine; a commercial standard for AChE and BChE enzymes
[c] α-Kojic acid; a commercial standard for TYR enzyme.
[d] No inhibitory activity.
*p value of < 0.05; **p value of < 0.01

Conclusion

In the current research, anticancer, antioxidant and neuroprotective potentials of red hulls, kernels and oleo-gum resins obtained from Pistachio (*P. vera* L.) were analysed through *in vitro* test systems. Overall, the results obtained from this work showed that different parts of Pistachio could be a good candidate for cancer prevention and inhibition of the enzymes associated with pathogenesis of neurodegenerative diseases. As far as the literature survey, no study has been performed to examine anticancer and neuroprotective activities of the extracts obtained from different parts of Pistachio. Thus, this data could be the first report for the literature. The author suggest that Pistachio with whole part is a valuable natural source for curative purposes and further *in vivo* studies and clinical trials should be conducted to ascertain its bioactivity.

Acknowledgments

The author would like to thank Advanced Technology Application and Research Center (ATARC), Kilis 7 Aralik University for their technical support.

Conflict of interests

No conflict of interest with the contents of this article.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.5 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):144-149 (2019)

Combined use of mowing and chemical control for the efficient control of the noxious invasive species *Typha* spp.

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Abstract

Changes in weed communities due to changes of climate and agricultural practices have already been indicated. Cattail (*Typha* spp.) is considered as a noxious invasive species with an increasing dispersal in Greece and other countries, where it starts to become a serious problem particularly in wet areas and especially for perennial crops. Pot and field experiments were conducted in order to evaluate the efficacy of several herbicides and mowing against cattail. The results of the present study revealed the low efficacy of several herbicides (like imazamox, 2,4-D and MCPA) against *Typha* spp. plants. On the other hand, pot experiments showed that especially glufosinate and glyphosate (in high rate) killed the majority of cattail plants grown by rhizomes. Moreover, they key result of our field experiment is the strong indication of a synergistic action of mowing and chemical control, especially in the case of glufosinate and glyphosate. The case of *Typha* spp. confirms that the integration of several control methods and agronomic practices may ensure an efficient, long-term management of noxious and invasive weeds.

Keywords: *Typha* spp., Invasive, Management, Mowing, Herbicides

Introduction

Plant invasions have received global attention due to the environmental problems and economic costs that may cause (Richardson et al., 2000). These plants have often the potential to become competititve and noxious weeds and pose long-term problems for agriculture and natural environments (Westbrooks, 1991). Most invasive species are characterized by high growth rates, rapid and massive reproduction, high dispersal ability, phenotypic plasticity, high competitiveness and adaptability and tolerance of a wide range of environmental conditions (Bazzaz et al., 1986; Brunel, 2005). Early detection and rapid response to invasive species is viewed as the most economically efficient and ecologically effective approach (Hobbs and Humphries, 1995).

Typha is a genus with more than 30 species and hybrids, belonging to the family Typhaceae. Cattail species are aquatic or semi-aquatic herbaceous, rhizomatous, perennial plants which can be found as dominant competitors in wetland ecosystems in various environments both in Northern and Southern hemisphere (Ciotir et al., 2017). They are obligate wetland indicator plant species, which tolerate perennial flooding and moderate salinity. These characteristics, plus the rhizomatous expansion, are also responsible for species high capacity for colonization and formation of dense patches, resulting to monospecific dominance events (Bansal et al., 2019). Among the most common cattail species are *Typha latifolia* (broadleaf cattail) and *T. angustifolia* (narrow-leaf cattail); while lately *Typha x glauca* is also widely spread in North America (Ciotir et al., 2017; Pieper et al., 2018).

In general, *Typha* spp. is not a major weed problem in large scale agricultural systems (Bansal et al., 2019). However, maintenance and interspersion of *Typha* plants can lead to economic losses and reduced yield in several crops, both annual and perennial. The biological characteristics of *Typha* is the cause of this result, because this species forms dense patches in the invaded areas and acts as roost site for some

Cite this article as:
Kanatas, P. (2019). Combined use of mowing and chemical control for the efficient control of the noxious invasive species Typha spp. Int. J. Agric. Environ. Food Sci., 3(3), 144-149.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.5
Received: 21 June 2019 **Accepted:** 16 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 144-149
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

animals and birds. Linz and Homan (2011) reviewed the effects to sunflower production by the presence of blackbirds in North & South Dakota, U.S, in hybrid cattail (*T. x glauca*) dominated wetlands. Another study from Leitch et al. (1997) showed that farmers may observe >15% losses in the presence of birds and roost zones of *Typha* just 3 km away from their fields.

During the last years, there have been complaints from several regions of Greece for reduced efficacy of herbicides or increased competitiveness of many recently problematic species (Travlos and Chachalis, 2010; Travlos 2013). *Typha* spp. is one of these species, which has an ongoing spread in Greece and especially during the last decade it tends to be an agronomic problem for several perennial crops (olives, citrus etc), usually forming dense monocultures. The management of *Typha* species is considered to be very difficult (Bonanno and Cirelli, 2017). The individual characteristics of cattail spreading by rhizomes makes the management with mechanical methods of this species expensive and usually insufficient, while herbicides often fail to control this weed (Kanatas, unpublished data). The present study was conducted because of the increasing dispersal and the many reports in Greece of *Typha* spp. becoming increasingly difficult to control with mechanical or chemical methods. The main objectives of the present study were to evaluate the efficacy of several herbicides and to study the effect of mowing against cattail.

Materials and Methods

Pot experiments

Pot experiment was conducted at the Agricultural University of Athens (Greece) (Latitude: 37° 59' 1.70" N, Longitude: 23° 42' 7.04" E, Altitude: 30 m) during May-August 2019. Rootstocks and creeping rhizomes of *Typha sp.* from species dense stands were collected in mid-May 2018 from an olive orchard in western Greece (Fig 1). The propagating material was transferred to the laboratory of Agronomy in the Agricultural University of Athens, where a pot experiment was conducted. Plastic pots of 25 cm width and 25 cm depth were used. The pots were filled with clay loam soil from the specific olive orchard (Table 1). In each pot, two rhizomes were planted. The plant material was uniformly well-watered for a short period of 3 weeks, in order to have a vigorous and uniform growth of *Typha* spp.

At the height of 50-60 cm, the plants were sprayed with the herbicides described in Table 2 using a custom-built, compressed-air, low-pressure, flat-fan nozzle experimental sprayer, calibrated to deliver 300 L ha^{-1} at 250 kPa. The treatments were 6 (glyphosate at the recommended and double the recommended rate, imazamox, glufosinate, 2,4-D and MCPA) plus untreated control. Five replicates (pots) were used for each treatment. Survival of *Typha sp.* plants was recorded and dry biomass of the above-ground plant part was also measured (plants were oven-dried for 48 h at 70 °C) at 7, 21 and 35 days after treatment (DAT).

Field trial

In the olive orchard mentioned above a preliminary mowing experiment during summer of 2018 revealed the high regrowth capacity of *Typha* spp. after mowing (data not shown). Therefore, a field trial was conducted during summer of 2019 in order to evaluate the efficacy of the same herbicides tested in the pot experiments with or without a previous mowing treatment. Field experiment was arranged in a randomized block design with two factors, mowing (2 levels) and chemical control (7 levels, including the untreated control) and 3 replicates. The dimensions of each plot were 4 m x 1.5 m. Herbicides were applied with the same equipment as above and survival was recorded at 21 DAT.

Statistical analysis

For all the data, ANOVA was performed with Statistica 9.0 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK74104, USA). Mean comparison was performed using Student's least signification difference (LSD) test at $P < 0.05$, while all data were tested for normality and variance before further analyses.

Results and Discussion

In the pot experiment, survival and dry weight of cattail were measured at 7, 21 and 35 days after treatment (DAT). The results in Table 3 show that only glufosinate had a high efficacy even at 7 DAT, while glyphosate resulted in a survival reduction by 60 to 95% for the recommended and double the recommended rate, respectively. On the contrary, imazamox, 2,4-D and MCPA resulted in low efficacy against *Typha* spp. (survival ranged from 70 to 100% compared with the untreated control), while in some cases regrowth was obvious at 35 DAT (Table 3). In a previous study, Rodgers and Black (2012) showed that applications of imazamox at intermediate rates provided moderate control of cattail. Effects of the several herbicides on the biomass of cattail was also in full agreement with the previously mentioned effects on survival. Glufosinate and glyphosate in the high rate significantly reduced dry weight of sprayed *Typha* spp. plants (Table 4). This finding is in full accordance with previous studies, showing that chemical treatment with glyphosate is considered to be efficient, mostly in late summer when a carbohydrates storage in rhizomes occurs (Linz and Homan, 2011; Wilcox et al., 2018). Significant differences in the efficacy between several herbicides have been also reported by Enloe and Netherland (2017), who evaluated the efficacy of the herbicides clethodim, sethoxydim and fluazifop-P-butyl against aquatic native grasses, including broad-leaf cattail (*T. latifolia*). The biomass reduction of common cattail at 8 weeks after treatment with glyphosate (at a rate of 4.2 kg a.e. ha^{-1}) and imazapyr (at a rate of 1.4 kg a.i. ha^{-1}) was higher than 90% in both cases, in comparison with the three selective herbicides which did not provide sufficient control.

Field trial revealed the crucial role of the combined use of mowing accompanied by chemical weed control with specific herbicides, while other herbicides remained rather ineffective in both cases. In particular, none herbicide succeeded to adequately control *Typha* spp. without previous mowing. On the other hand, glufosinate and glyphosate applied during summer at 2-3 weeks after mowing resulted in efficacy up to 90% (Table 5). This finding reveals the important role of the age of

leaves on the efficacy of herbicides against cattail (Lishawa et al., 2017) and it can plausibly attributed to the significantly lower absorption of the herbicides in the surface of the older and harded *Typha* spp. leaves. Lishawa et al. (2017) where among the first who attempted to address the control of *Typha x glauca*, when plants are in young age, in contrast to most techniques which aim to mature plants when the species is already dominant.

The findings of the present study are also in agreement with the study conducted by Wilcox et al. (2018), suggesting that cattail will resprout even after cutting, so combined treatments with cutting and secondary applications could suppress regrowth. In particular, they found that wicking in late summer of cattail ramets with glyphosate was the most effective treatment to reduce *Typha* cover. Therefore, the authors suggest mid-summer cutting of *Typha ramets* and after that late-summer herbicide wicking of resprouted stems. This is applicable in small scale, but other options which are not labor-intensive and cost-ineffective should also integrated. Among them, as our findings revealed, mowing followed by chemical control is a very good option for larger areas. Another beneficial effect of mowing and cutting is that they ensure the removal of aboveground biomass of cattail and the reduction of degradation process of *Typha* spp. plant tissues. It is noticeable that previous studies have shown that removal of *Typha* spp. aboveground biomass is recommended in highly productive areas or in constructed wetlands, in order to avoid the accumulation of organic matter after decomposition (Álvarez and Bécares, 2006; Vroom et al., 2018). Elgersma et al. (2017) results showed that in high-nutrient wetlands the most effective combination to manage cattail hybrid invasion was herbicide with burning, proposing that nutrient inputs in wetland ecosystems can improve management efforts. Johnson et al. (2019) reported that one year after mechanical harvesting of dominant clonal cattail, the light transmission increased due to the aboveground biomass removal, while a submerged harvest lead to soil nutrients increasing availability. Another study showed that aboveground harvesting of *T. x glauca* stems and litter lead to reduced litter 4 years after the removal (Keyport et al., 2019). Lawrence et al. (2016) reported that herbicide application of cattail with glyphosate significantly affected wetland dynamics, by reducing *Typha* biomass and abundance, enriched nutrient pool (NH^{4+}, N, P, K) and increased light penetration one year after treatment. Nevertheless, the chemical treatment lead to significant degradation of native plant richness.

It has to be noted that the excessive use of herbicides results in selection pressure over time which eliminates susceptible weeds and biotypes and thus causes evolutionary changes in weed communities. Such a typical example is the one of *Conyza* spp. which found "empty space" due to the high efficacy of glyphosate on the majority of weeds, progressively developed resistance to glyphosate, invaded orchards and vineyards and now is one of the most serious weed problems of perennial crops throughout the Mediterranean area (Travlos and Chachalis, 2010). Consequently, sometimes they key in the management of *Typha* spp. could be the utilization of this plant. For instance, Lawrence et al. (2016) suggested that combined treatment of biomass harvest and disking can add to cattail control and could be a positive economical perspective for farmers due to cattail biomass utilization as biofuel, while at the same time the native plant community will be boosted. Lishawa et al. (2019) suggested that restoration techniques of cattail dominant areas should focus on periodic treatments including biomass removal, in order to preserve the native biodiversity. Vroom et al. (2018) proposed that *T. latifolia* can be utilized for the restoration of peatlands after their rewetting, because of the species capacity to control nitrogen and phosphorus concentrations from surface and pore water, as well as regulate CH_4 and N_2O emissions. Nevertheless, the selection of cattail species for potential phytoremediation must be based upon the species invasiveness risk (Bonanno and Cirelli, 2017; Gikas et al., 2018). Comprehensive studies regarding utilization of *Typha* biofuel production after following harvesting of plants biomass have been extensively reviewed by Bansal et al. (2019).

Fig 1. Olive orchard with a heavy Typha spp. infestation in western Greece.

Fig 2. Efficacy of several herbicides against Typha spp. at 35 DAT.

Table 1. Soil texture and chemical components of used soil (in both pot and field experiments)

Parameter	Value
Clay (%)	31.1
Loam (%)	35.3
Sand (%)	33.6
pH (1:1 H_2O)	7.09
NO_3 (mg kg^{-1})	11.4
P (mg kg^{-1})	14.4
K (mg kg^{-1})	187
$CaCO_3$ (%)	14.61
Organic matter (%)	1.77

Table 2. Herbicide treatments and application rates

Active ingredient	Rate (kg a.i. or a.e. ha^{-1})
glyphosate (x)	3.24
glyphosate (2x)	6.48
imazamox	0.05
glufosinate	0.75
2,4-D	0.9
MCPA	1.6

Table 3. Survival of Typha sp. plants at 7, 21 and 35 days after treatment (DAT) in comparison to the untreated control

Treatment	Survival (% of the untreated control)		
	7 DAT	21 DAT	35 DAT
glyphosate (x)	40^{ab}	20^{b}	30^{b}
glyphosate (2x)	20^{b}	10^{b}	5^{b}
imazamox	80^{a}	80^{a}	100^{a}
glufosinate	0^{b}	0^{b}	10^{b}
2,4-D	100^{a}	80^{a}	100^{a}
MCPA	90^{a}	70^{a}	100^{a}

Values followed by different letter in the same column indicate significant differences (P<0.05) based on Student's test

Table 4. Dry weight (DW) of Typha sp. plants at 7, 21 and 35 days after treatment (DAT) in comparison to the untreated control

Treatment	DW (% of the untreated control)		
	7 DAT	21 DAT	35 DAT
glyphosate (x)	70^{ab}	40^{b}	50^{b}
glyphosate (2x)	40^{bc}	30^{b}	20^{c}
imazamox	70^{ab}	70^{a}	100^{a}
glufosinate	30^{c}	30^{b}	20^{c}
2,4-D	100^{a}	90^{a}	100^{a}
MCPA	80^{a}	80^{a}	100^{a}

Values followed by different letter in the same column indicate significant differences (P<0.05) based on Student's test

Table 5. Survival of Typha sp. plants at 21 days after treatment (DAT) in comparison to the untreated control with and without previous mowing

Treatment	Survival (% of the untreated control)	
	Without previous mowing	With previous mowing
glyphosate (x)	70[b]	30[b]
glyphosate (2x)	50[c]	10[b]
imazamox	90[a]	70[a]
glufosinate	50[c]	10[b]
2,4-D	100[a]	80[a]
MCPA	100[a]	70[a]

Values followed by different letter in the same column indicate significant differences (P<0.05) based on Student's test

Conclusions

The results of the present study revealed the low efficacy of several herbicides (like imazamox, 2,4-D and MCPA) against *Typha* spp. plants. On the other hand, our pot experiments showed that especially glufosinate and glyphosate (in high rate) killed the majority of cattail plants grown by rhizomes. Moreover, they key result of our field experiment is the strong indication of a synergistic action of mowing and chemical control, especially in the case of glufosinate and glyphosate. The case of *Typha* spp. confirms that the adaptation of an integrated approach that employs chemical methods along with other agronomic practices may ensure a long-term invasive weed management. Consequently, integrated management strategies are essential in order to prevent the introduction and further dispersal of noxious invasive weeds.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.6 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):150-154 (2019)

Assessment of contribution of cabbage in rural livelihood and constraints of production in Dhankuta, Nepal

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Abstract

Cabbage production is an im¬portant farm enterprise for descent socioeconomic status and nutritional security of rural farmers in eastern hill of Nepal. The study was conducted to determine contribution of cabbage in livelihood of rural farmers and constraints in commercial cabbage production in Chhathar-Jorpati Rural Munic¬ipality, Dhankuta district of Nepal. A total of 60 cabbage producing households were randomly selected. Primary data were collected through 60 questionnaire survey. The data obtained were analyzed using MS-Excel and SPSS. Average land holding of farmers was 1.45 hectare. Cabbage occupied 67.99% of total land area under vegetables production with 60.06% contribution on annual income from vegetables and 23.69% contribution on total annual household income. Gross return and total variable cost per hectare were NRs. 195424.66 and NRs. 117566.70 respectively. The average gross margin per hectare from cabbage production was calculated Rs.77857.96; benefit cost ratio was estimated 1.66. Among total variable cost, labor cost (47.52%) was highest followed by nutrient (46.97%), seed (5.31%) and pesticides (0.21%) cost. Productivity of cabbage was 26.66 Mt per hectare. There is a huge potential for improvement of yield and benefit of crop, but the government should support farmers with subsidy in fertilizers and disease management programs.

Keywords: Cabbage, Economics, Farm Gate Price, Extension, Price Regulation

Introduction

Cabbage (*Brassica oleracea* var capitata) is one of the most important vegetable crops grown all over the world. It belongs to family- Brassicaceae. It is mainly consumed as raw as well as cooked vegetable, being rich source of vitamins (A, C and K), fibre, proteins and also anti-cancer property due to the presence of "Inole-3-carbinol" (Singh, Sharma, & Singh, 2009). Cabbage accounts the third position in terms of area planted out of 55 vegetables cultivated in Nepal Cabbage is being emerging profitable farm enterprise of farmers of Nepal, especially of eastern hill. Its many varieties are now grown in different parts of the country. In Nepal, Cabbage occupies 28071.4 hectares area, with total production of 484036.8 Metric ton and productivity is 17.2 Mt ha^{-1} (ABPSD, 2016). Dhankuta is most potential district for cabbage production and export, having highest productivity (26.2 Mt ha^{-1}) among other districts of Eastern region of Nepal with 1460 ha cropped area and 38234 Mt annual productions (ABPSD, 2016). Cabbage occupies largest area among vegetables area in Dhankuta (ABPSD, 2016). In 2015, vegetabels worth of NRs. 370 millions were sold from from Sidhuwa Agriculture Store, Chhathar-Jorpati Rural Municipality, Dhankuta. Out of the vegetables sold, cabbage had largest amount of 2500 tonns. According to United States Department of Agriulture (USDA) cabbage is the second most economical cooked vegetable in terms of price per edible cup. This relatively low cost of cabbage in comparision with most other vegetable and its unique antioxidant properties makes this crucifer vegetable a nutrition bargain.

Cite this article as:
Gahatraj, S., Rai, H.H., Uprety, R. (2019). Assessment of contribution of cabbage in rural livelihood and constraints of production in Dhankuta, Nepal. Int. J. Agric. Environ. Food Sci., 3(3), 150-154.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.6
Received: 10 February 2019 **Accepted:** 16 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 150-154
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Materials and Methods

Study Area and Sampling Design

The research was conducted in Chhathar-Jorpati Rural Municipality of Dhankuta district of Province 1 of Nepal. The site locates at 27^0 06' N latitude and $87^0$40' E longitude with an elevation of 2150 masl. A total of 60 cabbage producing households were randomly selected among 250 recorded households in Prime Minister Agriculture Modernization Project at time of study.

Data Collection and Analysis

Primary data were collected through face to face interview by use of pre-tested semi-structured interview schedule in July of 2018. Key Informants Interview (KII) was carried out to cross check and supplement collected information. Collected data were analyzed using SPSS and MS Excel software.

Cost and Return Analysis

The total variable costs of cabbage production was calculated by considering all variable inputs such as seed cost, organic manures cost, chemical fertilizers cost, pesticides cost and labor cost at their current market prices. All the cost were taken in NRs./ hectare.

Total Variable Cost = $C_{seed} + C_{organic\ manure} + C_{fertilizer} + C_{pesticide} + C_{labor}$

Where, C_{seed} = total cost on seed (NRs./ha), $C_{organic\ manure}$ = cost on organic manures (NRs./ha), $C_{fertilizer}$ = cost on fertilizer (NRs./ha), $C_{pesticide}$ = cost on pesticides (NRs./ha) and C_{labor} = cost on human labor (NRs./ha).

Gross Margin Analysis

Gross Margin = Gross Returns – Total Variable Cost

Where, Gross Returns = average price of cabbage × total cabbage production

Benefit Cost Analysis

It was calculated by using following formula:

$$\text{B/C Ratio} = \frac{\text{Gross Return}}{\text{Total Variable cost}}$$

Problems ranking

Preferential ranking for production problems and diseases was done by indexing. Knowledge scoring was done to assess the knowledge level of farmers according to the results of the questionnaire survey.

- Indexing was computed using following formula.
  I imp=$\sum$ (si×fi/N)
  Where, I imp=Index of importance
  $\sum$= Summation
  si =Scale value
  fi =Frequency of importance given by respondent
  N=Total number of respondents

Knowledge scoring to assess knowledge level was done as follow

- Each respondent were asked 17 questions regarding clubroot identification, spread and management.
- Each response was recorded as 'Yes' or 'No'
- One score was given if the response was 'Yes'
- Score below and equal to mean was categorized as low knowledge and score above mean categorised as high knowledge

Results and Discussion

Status of cabbage production

Vegetable is a major contribution on economy and nutritional health of Nepalese farmers. Temperate vegetables such as cabbage, cauliflower, garden pea, broad leaf mustard were major vegetable grown at study area. (Figure 1). According to villagers and agriculture stakeholders, income from cabbage was a major and dependable source of income of farmers in Dhankuta district of Nepal. This survey study revealed that income from cabbage production contributes a major part of total income from vegetable in Dhankuta district, Nepal. Income from cabbage had highest contribution (60%) on total income from vegetables (Figure 1).

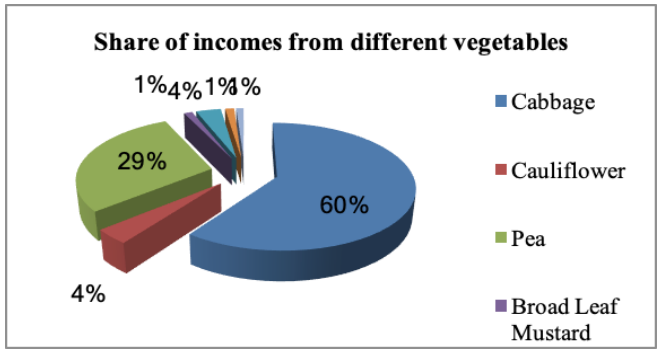

Figure 1. Share of cabbage on total income from vegetable

Chhathar-Jorpati Rural Municipality has been considered as vegetable hub of eastern region of Nepal. About 91.19% of cultivated land was under vegetable cultivation. Cabbage had highest cropped area among the vegetables grown over there. Cabbage production has attracted the youth of that locality preventing their unwanted migration to terai or foreign countries for earning. Cabbage is the major agriculture product exported to India through Kakarbhitta customs, Jhapa, a major customs of Eastern region of Nepal. Similarly, off season cabbage is the major fresh vegetable exported to India through Pashupatinagar customs, Ilam (Ojha, 2016). Dhankuta is most potential district for cabbage production and export, having highest productivity (26.2 Mg ha^{-1}) among other districts of Eastern region of Nepal with 1460 ha cropped area and 38234 Mg production (MoAD, 2016). Cabbage occupies largest area among vegetables area in Dhankuta (MoAD, 2016).

Figure 2. (a) Harvesting of cabbage and packaging; (b) Transportation upto main road on back by farmers (Photo credit: Sachin Gahatraj, Agriculture and Forestry University, Rampur, Chitwan, Nepal)

Major problems in production

Disease was the major problem in cabbage production in the study area with highest index value of 5.45. Marketing was the second important problem with index value 4.9 followed by Insect with index value 4.42, Irrigation with index value 2.5 and Input unavailability with index value 1.76.

Clubroot is a major soil-borne disease of Brassica crops having high economic impact worldwide (Dixon, 2009)the pathogen probably spread worldwide as a result of transfer on and in fodder taken by colonists as livestock feed. It is a moot point, however, whether there was much earlier spread by P. brassicae into China and subsequently Japan as Brassica rapa (Chinese cabbage and many variants. Severe and widespread epidemic of clubroot have been recorde in Kathmandu valley and Makawanpur of Nepal (Timila *et al*., 2008). Few year ago, it was recorded in cabbage growing fields of Dhankuta and spreading rapidly in the vacinity. Most of the commercial cabbage growing areas of Chhathar-Jorpati rural municipality have been already infested by clubroot pathogen. The yield of major Brassica crops at this locality such as; cabbage, cauliflower, broad leaf mustard is less than national average. Furthermore, clubroot has exacerbated the situation. Productivity has been drastically reduced since few years after field got infested with pathogen. In addition to productivity, cropping area under Brassica crops is also shrinking as farmers have started to seek alternative crops like maize, potato although these crops are not economically beneficial as brassica crops (cabbage, cauliflower and broad leaf mustard) at this locality. Clubroot has a cosmopolitan distribution and is responsible for up to 50-100% yield loss of cabbage (Karling, 1968). It is considered most destructive disease of Brassica crops not only because of extent of yield loss it cause but also because of difficulties in elimination from the area once get infested (Gahatraj *et al.*, 2019).

Cost of production

The largest proportion of the cost of cabbage production was found to be occupied by labor cost (47.52%) and plant nutrient cost (46.97%). Pesticide cost (243.82 NRs/ha) was very low applied on cabbage production was very low (Table 1). This implies that used of pesticide has been reduced in recent few years. It may be due to the reason that cabbage expoerted to India and Bangladesh from this district had to be assured as free from pesticide residue by rapid bioassay for pesticide residue (RBPR) before passed through quarentine at custom office. Nutrient cost was also a major cost on cabbage production, which was almost equal to labor cost. (Table 1). Cabbage is heavy feeder that is why it needs to be applied with ample amount of balanced macro and micronutrients.

Return from cabbage production

Productivity of cabbage was 26.66 Mt/ha at study area, which is quite equal to cabbage productivity (26.2 Mt/ha) of Dhankuta district and higher than national average productivity of 17.2 Mt/ha (AICC, 2017). The overall benefit cost ratio of cabbage production -considering total variable cost- was estimated to be 1.66. This means with one unit investment, 1.66 unit returns we get. Farm gate price was found to be far lower than wholesale price (NRs. 25-27 per Kg) at same time, at Kalimati Fruits and Vegetable Market, Kathmandu, Nepal (KFVWMDB, 2018).

Conclusion

In study area, cabbage is grown under largest area among vegetables. Vegetable had significant contribution on total income of farmers while cabbage was a major contributor. Although cabbage production was found to be quite profitable farm enterprise, price spread was found to be a major impeding factor of higher benefit of cabbage. There is high potential of increasing benefit of crop, but intervention of government is needed. In addition, government should also intervene on market regulation and on price policy to increase farm gate price. Diseases were found to be major hindering factor of production of cabbage. Among diseases, clubroot was major problem.

Table 1. Ranking of problem in cabbage production in Dhankuta, 2018

Problems	Level						Weight	Index	Rank
	6	5	4	3	2	1			
Disease	43	5	8	4	0	0	327	5.45	I
Insect	0	29	27	4	0	0	265	4.42	III
Inputs	0	0	0	15	16	29	106	1.76	V
Irrigation	0	3	9	16	18	14	149	2.5	IV
Marketing	17	24	16	3	0	0	295	4.9	II

Source: Field Survey, 2018

Table 2. Ranking of diseases hindering cabbage production in Dhankuta, 2018

Diseases	Level						Weight	Index	Rank
	6	5	4	3	2	1			
Clubroot	47	0	0	0	0	8	290	4.8	I
Sclerotinia rot	6	16	10	10	7	1	201	3.35	IV
Black rot	0	0	0	0	13	47	73	1.2	V
Damping off	2	18	26	14	0	0	248	4.13	III
Blight	9	23	15	13	0	0	268	4.46	II

Source: Field Survey, 2018

Table 3. Total variable cost of cabbage production (NRs/ha)

Inputs	Means	Percentage
Seed Cost	6240.75	5.31
Nutrient Cost	55219.3	46.97
Pesticide Cost	243.82	0.21
Labour Cost	55862.83	47.52
Total Variable Cost	117566.7	100

Source: Field Survey, 2018

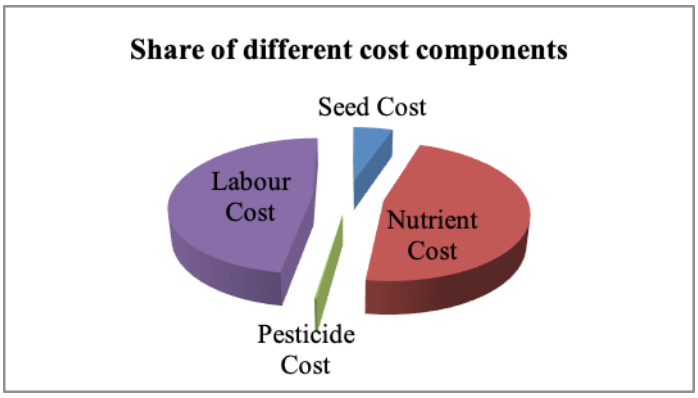

Figure 3. Share of different cost componets of cabbage production

Table 4. Economic statement of cabbage production in Dhankuta, 2018

Measuring Criteria	Average Value
Area	0.58
Productivity	26.66
Average Revenue	7.61
Gross Return	195424.66
Total Variable Cost	117566.7
Gross Margin	77857.96
Benefit Cost Ratio	1.66

Source: Field Survey, 2018

Acknowledgement

We are heartily thankful to Prime Minister Agriculture Modernization Project, Project Implementation Unit, Vegetable Zone, Dhankuta, Nepal for unstinting technical and financial support during survey. We are also indebted to Faculty of Agriculture, Agriculture and Forestry University, Chitwan, Nepal.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.7 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):155-161 (2019)

Twofold excessive utilization rate yields high financial equivalent but seriously threatens public rangelands in Turkey

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Abstract

The aim of this study was to estimate the utilization rate and financial equivalent of the utilized rangeland forage to quantify the extent of grazing pressure on the semiarid Turkish rangelands, and to attract the public attention to the importance of rangelands in national economy. The study was conducted in Erzurum province of Turkey. In permanent 12 representative sites in each of village rangelands, cages of 1 m height and 1 m × 1 m floor area, were placed and forage under cages was clipped to the ground at the end of the grazing seasons in 2007 and 2008. Simultaneously, the forage outside the cages was sampled with random quadrats. Financial equivalent of the utilized rangeland forage was estimated using surrogate market valuation method. In data analysis were employed descriptive statistical methods and one-way ANOVA test. According to the results, the average rangeland dry forage yield was 1012 kg.ha^{-1} and rangeland utilization rate was 69 per cent, roughly two-fold higher than suggested rates. Under the prevailing conditions, the financial equivalent of the utilized rangeland forage is about 526 TRY or 92 USD per hectare. It was concluded that utilization rate or grazing period should be deflated by 50% for sustainable resource use by allowing rangeland plants to regenerate.

Keywords: Rangeland forage, Semiarid rangelands, Financial equivalent, Surrogate market valuation, Eastern Anatolia

Introduction

Rangelands constitute the most important diversity and repository of the genetic resources. They contribute greatly to the ecosystem and enhance values of the farm products and promote rural tourism (Hopkins and Holz, 2006). They preserve soil and water (Altın et al., 2005) and release fresh water and oxygen. Rangelands are shelter and home of a variety of animals and plants, most of which are used for hunting or gathering by rural populations either for direct consumption or to be sold in markets. Rangelands also support honeybee farming as an important source of pollen and nectar. They provide free forage for domestic animals. Rangelands, owing to the above-mentioned benefits, have an important place in the livelihood of the rural populations and they greatly contribute to the national economy.

However, decades-long untimely and heavy grazing caused degradation and losses in functionality of the rangelands worldwide. It is thankfully that an awareness has been developed in Turkey and after the enactment of Pasture Law (Law No: 4342) in 1998, the National Rangeland Improvement and Management Scheme was put into action in the same year by the former Ministry of Agriculture and Rural Affairs in order to increase and maintain the productivity of degraded rangelands. Since then, governments have started to transfer substantial amount of funds to restore and rehabilitate the degraded rangelands. Nevertheless, for the sustainability of fund allocation, it is of great importance to keep rangelands in the agenda. Yet, scarcity of capital makes investment decisions one of the most important challenges that managers, donors or policy makers face across various options. More importantly, financial considerations have the crucial role in prioritization or assessing the capital investment opportunities, even some non-financial factors may also have to be regarded. So, in order to keep the rangelands in top of the

Cite this article as:
Kara, A., Dumlu, E.S., Uzun, M., Cakal, S. (2019). Twofold excessive utilization rate yields high financial equivalent but seriously threatens public rangelands in Turkey. Int. J. Agric. Environ. Food Sci., 3(3), 155-161.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.7
Received: 21 June 2019 **Accepted:** 19 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 155-161
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

investment opportunities list, it is of importance to quantify the contribution of rangelands to the economy to convince the policy makers and the donors on the profitability of investing in rangelands. Yet, this is a difficult task and most of the above-mentioned benefits obtained from the rangelands cannot be monetized and their actual economic and social values either are underestimated or not considered adequately (Cousins, 1999). Nevertheless, estimation of the dry forage yields and its utilized percentage may help quantify the financial equivalent of the rangeland forage and so their annual contribution to the economy, which ultimately show the importance of rangelands even when only forage production is considered, and other utilities are disregarded.

Among all the seven geographical regions, Eastern Anatolia seems to have very favorable conditions for animal production owing to its vast meadow and rangeland asset. Rangeland dependent extensive animal production has been a way of livelihood generation in the region for centuries. In Turkey, rangelands are commonly used vegetation covers, whose rights are left to the legal entity of each village with certain demarcation by the laws. Village flocks and herds graze separately under the supervision of herders or shepherds with daily excursions starting with sunrise and ending with the sunset (Kara et al., 2014).

A grazed, trampled or destroyed part of rangeland forage has been reported to be a measure of utilization for given rangeland, and its share in total production is described as rangeland utilization rate. In proper rangeland utilization, about 50% of utilization rate is recommended as normal suggesting that the rest should be left to allow rangeland to regenerate (Gökkuş and Koç, 2001). However, such a generalization may not be valid for all rangeland types and that utilization rate may vary according to the type of vegetation cover. For example, utilization rates of 20–30% for alpine tundra, 35–45% for western mountainous rangelands, 40–50% for short grass prairies, 45–60% for tallgrass prairies, and 45–55% for cool season grasslands have been recommended (Vallentine, 1990, cited in Gökkuş and Koç, 2001). Similarly, it has been reported that much less of rangeland forage should be grazed when rangeland condition is poor. Accordingly, 25–30% and 30–40% of utilization rates were suggested for poor and moderate condition rangelands and 50–55% of utilization rate was recommended for very good condition rangelands (Gökkuş and Koç, 2001).

In the rangeland related studies hitherto conducted in Turkey, mainly botanical composition was examined and the studies on forage yield, animal grazing and utilization are scarce and not addressed adequately. More importantly, previously conducted studies to determine the dry forage yield of the rangelands were limited with small-scale trial plots in protected, non-grazed areas, and determination of the utilization rate was out of their scope. Since there is little or no information on the degree of grazing and utilization rate of Turkish rangelands, generally approximate values have been used in rangeland rehabilitation studies in Turkey. Differing from the previous ones, the present study was conducted in a considerably wider area covering continuously grazed rangelands in 11 villages of 5 districts. It is expected that study findings will provide valuable information to be needed in future rangeland and animal related studies, and also be beneficial in sustainable fund allocation for the rangeland restoration and rehabilitation investments, not only in Turkey but also in countries sharing similar agroecological conditions, cultural and historical backgrounds of rangeland use pattern.

Materials and Methods

Material

The primary material of this study was obtained from the forage harvested from cages and random quadrats at the permanent 12 sites in the rangelands of 11 villages in Erzurum province, Turkey. In addition, the records of the official institutions in obtaining the relevant information related to the study subject were used as secondary material.

Study Area

The study area covers Erzurum province that reflects the main characteristics of the Eastern Anatolia region of Turkey regarding geography, climate, production type, and pattern (Figure 1). This region is known for its suitability for livestock production due to its one-third share in total rangeland asset of Turkey.

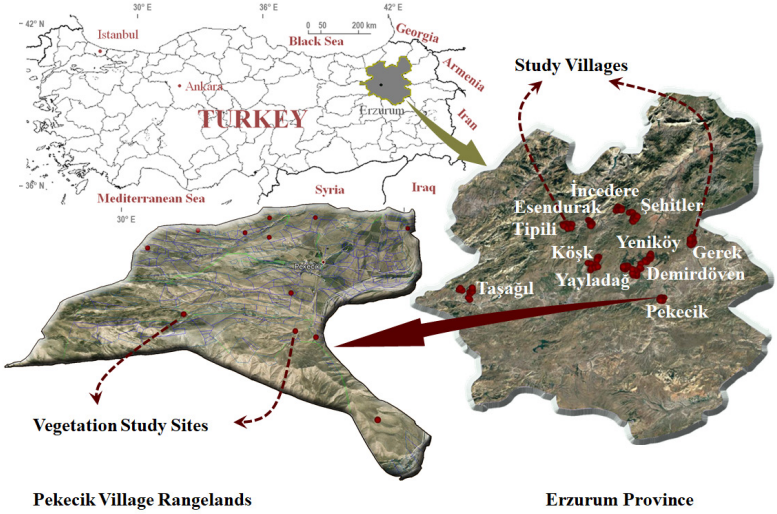

Figure 1. The study area in Turkey

High amount of rangeland asset and unfavorable climatic conditions, which limit crop production, have determined the way of livelihood generation, and so rangeland dependent livestock production system has prevailed for centuries in the region.

Erzurum has very rugged geography and very harsh terrestrial climate and is located within the 39 54' 31'' northern latitudes and 41 16' 37'' eastern longitudes. Altitude is ranging from 2000 m asl in plateaus to 3000 m asl and higher in the mountains and can be as low as 1000-1100 m asl in valley floors and 1500 – 1800 m asl in plains. Despite the existence of plain areas, the topography is fragmented in general and the dominant vegetation is steppe grasses (60%) as woodland is scarce (%6). Winters are long and harsh, and summers are short and hot. In a long term (1975 to 2006), the average number of frozen days and the days with snow cover are 154 and 113 days, respectively, while annual average temperature and total precipitation are 5.5 °C and 453.3 mm in respective order (TÜMAS, 2013). The annual and grazing season precipitations during 2007 and 2008 are presented in Table 1. According to the meteorological data, the year 2008 was distinctively draught compared to the previous year.

Table 1. Annual and seasonal precipitation during the years 2007 and 2008 (mm)

Period	Study Years		Difference	%
	2007	2008		
April-October	308.5	234.1	–74.4	24.1
Year Round	436.6	317.8	–118.8	27.2

Source: TÜMAS (2013)

Selection of the villages

In selection of the study villages, special emphasis was given on their representative ability over the surrounding area. Villages from different districts were purposively selected among those free from nomadic movements and boundary problems, and for which rangeland demarcation and allocation studies have been completed. Thus, from Aşkale, Narman, Pasinler, Köprüköy, Horasan, and Tortum districts in a total of 11 villages were selected for the study. In the study area, the altitude of grazing sites varied between 1593 m and 2847 m asl.

Study villages are apart from each other from a minimum of 7.9 km to a maximum of 126.5 km. Although sharing similar production patterns, they differ from each other regarding the acreage of rangelands and the fluctuating total animal asset (Table 2). Because the livestock is not of the same size or weight and weight variations require adjustments, the animal asset of the villages was expressed in animal unit equivalent (1 AU = 500 kg live weight). In animal unit conversions, the rates suggested in Turkey's Pasture Regulation (MBS, 1998) were used.

Calculation of the rangeland utilization rate

Rangeland utilization rate describes the percentage of forage that is grazed or removed by animals from the total forage amount produced by rangeland which should satisfy the condition not to cause rangeland degradation (Gökkuş and Koç, 2001). In order to determine the dry forage yield of the rangelands, cages with 1 m height and 1 m × 1 m floor area were placed in each of the 12 representative permanent sites in the rangelands of every village before the grazing seasons of 2007 and 2008, and the forage under the cages was clipped to the ground at the end of the grazing season, corresponding to the seasonal yield.

At the end of the grazing season of the year 2007, it was observed that 31 and 12 out of 132 cages were lost and disassembled, respectively. In the following year before the grazing season, the lost and disassembled cages were fixed and completed to 132. Again, at the end of the grazing season of the second year, 28 cages were lost or unsuitable for data collection. Because of the lost or disassembled cages, unavailable observations were treated as missing data and the observations from 89 cages in the first year and 104 cages in the second year were used in forage yield and utilization rate calculations.

In order to determine the dry forage amount removed from the rangelands during the grazing period, we considered the dry stubble yield at the end of the grazing season (Gökkuş and Koç, 2001). Thus, the rangeland stubble was sampled through four random quadrats in surrounding areas of each cage at the end of the grazing season. The rangeland stubble in the four random quadrats, equivalent to cage floor area (4 quadrats = 1.0 m^2), was clipped to the ground. The harvest weights of the forage and the stubble, and their dry weights after dehydration at 70 °C for 48 h in an oven were recorded.

Thus, average dry forage yields and four quadrat yields were obtained for each of the permanent sites in the village rangelands. Subsequently, the utilized or removed amount of rangeland forage was calculated by subtracting quadrat stubble yield from the cage forage yield and converted to per hectare yield. Finally, the utilization rate was calculated by dividing the utilized dry forage by the dry forage yield (Gökkuş and Koç, 2001).

Table 2. Rangeland and animal asset (in animal units) of the study villages

Villages in the Study Area	Rangeland Asset[1] (ha) (a)	Animal Asset (AU)[2]	
		2007 (b)	2008 (c)
Köşk	7349	1160	1418
Taşağıl	1177	518	600
Yeniköy	576	674	606
Yayladağ	452	538	510
Demirdöven	430	1159	832
Pekecik	217	111	239
Gerek	2138	734	941
Şehitler	883	716	718
Esendurak	191	79	140
Tipili	1548	330	442
İncedere	595	245	327
Total	15556	6264	6772

[1]Official records obtained from the directorates of agriculture operating in the study area

[2]Calculated on the basis of the official records

Calculation of the financial equivalent of utilized rangeland forage

We used surrogate market (also called substitute good) valuation method to estimate the value of the rangeland forage. The concept of the surrogate market or substitute good is used when one cannot directly estimate the market prices for certain environmental or non-market goods. In this case, valuation is made through the price of another similar good or service (proxy) to be substituted for the non-market good or service of interest (NRC, 1999; Cousins, 1999; Rehber, 1999; Torrell et al., 2014). Thus, dried meadow hay was considered to be the substitute of rangeland forage and the dried meadow hay prices available at Erzurum Commodity Exchange were used as a financial proxy to value the rangeland forage.

Data analysis

One of the important preconditions for the parametric statistical methods is the assumption of normal distribution for the variables under consideration, and it was tested using Skewness and Curtosis test. Yet, this test revealed that normality assumption was not satisfied, even after data transformation attempts. However, theoretical justification for the normality assumption is the central limit theorem which states that when sample size has 100 or more observations, violation of the normality is not a major issue (Gujarati, 1995). Following this theorem, we employed descriptive statistical methods and one-way variance analysis test (ANOVA), along with the non-parametric Kruskal-Wallis test as a robust alternative to one-way ANOVA (Sokal and Rohlf, 1995; Zar, 1999). Statistical analysis was performed using SPSS version 23.0 for Windows (IBM Corp. 2015).

Results

Rangeland dry forage yield

In the first year of the study, the highest dry forage yield was obtained from the Pekecik village rangelands of Köprüköy district (195.8 g.m^{-2}) and the lowest yield (60.9 g.m^{-2}) was from Yayladağ village rangelands of Pasinler district. In the second year of the study, on the other hand, the highest dry forage yield was obtained from the Tipili village rangelands of Tortum district (117 g.m^{-2}), while the lowest dry forage yields were from Yeniköy village rangelands of Pasinler district (43.7 g.m^{-2}). Again, the average dry forage yield of the whole study area was realized to be 126.6 g.m^{-2} and 80.5 g.m^{-2} for the first and second years, respectively. According to the results, the villages did not differ significantly ($p>0.05$) regarding the dry forage yield but the difference between the study years was very significant ($p<0.01$). The average dry forage yield of the cages for all villages and both years was 101.2 g.m^{-2} and the same per hectare was 1012 kg (Table 3).

Rangeland utilization rate

The rangeland utilization factor or rate was calculated as 66.1 and 71.6 per cent for the years 2007 and 2008, respectively, making an average of 69.1 per cent over two years (Table 3). The difference between the years was significant ($p<0.05$). Again, there were significant utilization rate differences among the villages ($p<0.01$). The lowest utilization rate was calculated for Köşk village (53.2% while the highest utilization rates were recorded for Yayladağ and Yeniköy villages (80.2%).

Estimation of the optimum length of the grazing period

For sustainable use, the rangeland utilization rate should satisfy not to cause rangeland degradation (Duan et al., 2017). For that reason, it should be arranged according to the rangeland condition in order to allow rangeland plants to regenerate. As stated earlier, 25–30% and 30–40% of utilization rates were suggested for poor and moderate condition rangelands (Gökkuş and Koç, 2001), although Teague et al. (2009) advised even much less utilization levels (20–25%) to ensure maintenance of rangeland in an excellent condition. Based on the collected data and the suggested utilization rates, we could estimate the optimum length of the grazing period for the study area to ensure regeneration of the rangeland plants at the existing stocking rates. So, following Gökkuş and Koç (2001), we considered two utilization rate scenarios in calculating the optimum length of grazing period in Table 4. According to Table 4, optimum length of grazing period varies between 60 and 80 days making a difference of 20 days between the two scenarios considered. The maximum length of the grazing period was calculated to be 80 days according to the second and the most optimistic scenario, in which 40% of utilization rate was taken into account.

The financial contribution of the rangelands to the national economy as a forage source

In the calculation of the financial contribution to the national economy, rangelands were taken into account only as a source of forage and all other possible utilities were ignored. The financial contribution of one hectare of rangeland area to

Table 3. The rangeland dry forage yield, stubble yield, utilized dry forage and utilization rate by the study years

Study Years	N	Dry Forage Yield (kg.ha^{-1}) (a)		Dry Stubble Yield (kg.ha^{-1}) (b)		Utilized Dry Forage (kg.ha^{-1}) (c = a – b)		Utilization Rate (%) (d = c × a^{-1})	
		$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$
2007	89	1255.7	99.6	396.4	32.7	859.3	88.0	66.1	2.2
2008	104	804.0	63.7	237.5	28.4	566.5	47.7	71.6	1.8
Total	193	1012.33	59.5	310.8	22.2	701.6	49.0	69.1	1.4

Table 4. The optimum length of grazing period at the existing number of grazing animals in the study area based on the suggested utilization rates

Items	Explanations	Scenarios for the optimum length of grazing periods	
		The First scenario	The Second Scenario
Suggested utilization rate (%)[3w]	(a)	30	40
Rangeland dry forage yield per hectare (kg×ha^{-1})[4]	(b)	1012	1012
Rangeland dry forage quantity to be grazed per hectare (kg×ha^{-1})	(c=a×b×100^{-1})	303.6	404.8
Herbage allowance for one AU (500 kg live weight) per day (kg×day^{-1})[3]	(d)	12.5	12.5
The number of animals to be allowed to graze per hectare per day (AU×day^{-1})	(e=c×d $^{-1}$)	24.3	32.4
Total rangeland acreage in the study area (ha)[5]	(f)	15556	15556
Total number of animals to be allowed to graze in one day (AU×day^{-1})	(g=e×f)	378011	504014
The number of total grazing animals in the study area (AU)[5]	(h)	6264	6264
The total length of the grazing period to be considered in the study area (day)	(j=g×h^{-1})	60.3	80.5

[3]Gökkuş and Koç, (2001) [4]Present study results (Table 3) [5]Table 2

Table 5. The contribution of the poor to moderate condition rangelands to the economy in Erzurum

Items	Value
Rangeland dry forage yield (kg×ha^{-1})[6]	1012
Utilized dry forage (kg×ha^{-1})[6]	701
Dry meadow hay price for the year 2019 (TRY×kg^{-1})[7]	0.75
Financial equivalent of the rangeland forage (TRY×ha^{-1})	525.8
Financial equivalent of the rangeland forage (1 USD = 5.715 TRY) (USD×ha^{-1})	92.0

[6]Table 3; [7]ETB (2019)

the economy was calculated using the dry meadow hay prices for the year 2019, obtained from the Erzurum Commodity Exchange, and were used as a financial proxy to determine the value of the rangeland forage (Table 5).

According to the calculations presented in Table 5, it can be said that study area rangelands make an annual financial contribution of 525.8 TRY or 92.0 USD (1 TRY = 0.175 USD) per hectare at the present utilization rates.

Discussion

The main focus of this study was the rangeland utilization and their financial contribution to the economy. This was challenged by estimating the dry forage yield and its utilized portion. Of course, type, depth and nutrient content of soils, sloping degree, prevailing wind directions, evapotranspiration are all important factors affecting rangeland biomass. However, for the ease and simplicity of the study these factors were not handled, and they were kept beyond the scope of this study and have been left as the subjects for further studies. Moreover, the findings related to rangeland vegetation and condition were not touched in this study because a number of previously conducted studies in the region revealed more or less similar patterns (Erkovan et al., 2003; Dumlu et al., 2011; Avağ et al., 2012; Çakal, 2016).

Regarding the financial contribution of rangelands to economy, of course, we admit that it certainly would be illogical to limit it only to source of herbage. However, what we would like to do in this paper is to emphasize the importance of rangelands even when only forage production is considered, and other utilities of rangelands are disregarded.

The average rangeland dry forage yield reported in this study is important in terms of giving an idea on dry forage yield of the rangelands sharing similar ecological conditions. We have estimated not only the dry forage yield but also determined how much of it is consumed or utilized by grazing animals.

Regarding the rangeland dry forage yields, the villages did not differ significantly ($p>0.05$) from each other but a significant yield difference was detected between the study years ($p<0.01$). The reason for this is most likely precipitation. In the areas with less than 600 mm annual precipitation, moisture played a key role in the composition, structure, and density of the plant communities (Kutiel and Lavee, 1999, cited in Maren et al., 2015) and so rangeland forage production is fluctuated and mainly determined by rainfall (Duan et al., 2017). Thus, about 36% of the yield gap in the second year was likely due to the low precipitation in the year; 27% and 24% less precipitation was realized for all year round and for the vegetation period from April to October, respectively (Table 1). In line with our findings, a significant effect of the precipitation has also been reported by O'Connor and Rouxt (1995), Khumalo and Holechek (2005), Browning et

al. (2012).

Although significantly differed among the villages, most likely due to the stocking rate differences, the rangeland utilization factor or rate was 66.1 and 71.6 per cent for the years 2007 and 2008, respectively. Low dry forage yields in the second year of the study brought about relatively higher utilization rates. Thus, heavy grazing problem significantly worsens ($p<0.05$) in the years of low forage production (Table 3). An average of 69.1 per cent utilization rate over two years is twofold higher than the suggested value (Gökkuş and Koç, 2001; Teague et al., 2009), and indicates a heavy grazing pressure on rangelands in particular for the studied area, and in general for the eastern Anatolia.

At this excessively high utilization rates, we calculated the financial equivalent of utilized rangeland forage to be 92.0 USD (1 TRY = 0.175 USD) per hectare in the present study. Rangeland condition of the studied rangeland sites was previously presented elsewhere that it varied from poor to moderate condition (Kara et al., 2015; Kara, 2019). Again, Avağ et al., (2012) reported that the majority of rangeland asset in Erzurum and eastern Anatolia, were in a moderate condition i.e. 63 and 60 percent. That is, about 2,519 thousand ha (60%) out of total 4,198,046 ha of the rangeland assets of eastern Anatolia (GTHB, 2018) are in a moderate condition. Thus, even when considering only the moderate condition rangelands, we could infer that the annual contribution of rangelands of eastern Anatolia to Turkish economy is about 232 million (2519 thousand ha × 92.0 $.ha^{-1}) US dollars. However, this high financial equivalent has been accomplished at the expense of rapid rangeland degradation, which means killing the goose that lays golden egg. In order to achieve sustainability either the length of the grazing period should be shortened, or existing stocking rates should be deflated by 50% to alleviate the heavy grazing pressure so that rangeland plants can regenerate.

Because village rangelands in Turkey are in common use, management of the grazing according to the herbage production, or deflating the existing stocking rates by 50 percent is an extremely difficult task since it requires halving the existing number of grazing animals. In a private farm with a private rangeland property, farmer can decide on the optimum stocking rate for better use of his or her rangeland. In common use, however, every farmer tries to use it as much as he or she can, in an opportunistic manner, ignoring the capacity of the rangeland. For that reason, instead of deflating stocking rates, shortening the grazing period may be easier.

As illustrated in Table 4, considering the suggested utilization rates by Gökkuş and Koç (2001), the maximum length of the grazing period was calculated 80 days (roughly three months) according to the most optimistic scenario (Table 4). As a matter of fact, roughly this length of grazing period could be achieved by shrinking the present utilization rate by 50% at the existing stocking rate without halving the number of grazing animals since the actual grazing period is for about six months in the study area (Kara et al., 2009).

Shortening the grazing period is also important for the profitability of the rangeland restoration investments since rehabilitated rangeland parts will soon be back to the previous condition in a few years without paying back the investment or harvesting the targeted results at the actual utilization level (Kara et al., 2014).

Conclusion

Although overgrazing is a well-known fact for Turkish rangelands, this study quantified the extent and severity of this problem. Again, this study also showed the huge amount of financial contribution of the rangelands even when considering their forage production for domestic animals. However, it is an inevitable truth that such a financial contribution is not sustainable at the existing utilization rates and achieved at the expense of rapid rangeland degradation. For this reason, it would not be meaningful to invest in rehabilitation of the rangelands unless effective and practical measures are taken.

For a sustainable economic contribution, urgent and immediate measures should necessarily be taken toward bringing down the high utilization rate to reasonable levels through setting fair stocking rate or grazing periods, i.e. utilization rate or grazing period should be halved to allow rangeland plants to gather strength and regenerate. Therefore, we suggest three months of grazing period starting from early June to late August.

Although present study findings represent the rangelands in Erzurum province of Turkey, we can make inferences and generalize the results for the rangelands sharing similar agro-ecological conditions, i.e. from poor to moderate condition rangelands in similar geographic and climatic conditions in eastern Anatolia, considering the wider study area, which covers 11 villages in five districts, making a total of 15556-hectare rangelands. We expect that the findings of this study will contribute positively to future studies in this regard, and that results could be used in the management of the rangelands, particularly in the improvement and rehabilitation practices not only in Turkey but also in countries sharing similar agro-ecological conditions, cultural and historical backgrounds of rangeland use patterns.

Acknowledgements

This paper has been produced from the research project, numbered TAGEM/HAYSÜD/05/01/01/01 and entitled "Analysis of the Factors Affecting Milk Production and Live Weight Gain in the Cattle Farms Dependent on Natural Grazing Lands in Erzurum" financially supported by the former Ministry of Agriculture and Rural Affairs, Turkey.

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.8 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):162-170 (2019)

Influence of varying preservation methods on the shelf life and proximate composition of *Pleurotus plumonarius* (Fr) Quel cultivated on *Andropogon gayanus* substrate

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Abstract

The objective of the work was to evaluate the influence of preservation methods on the storability and proximate composition of *Pleurotus pulmonarius*. The experiment was laid out in a completely randomized design (CRD) with three replicates. Freshly harvested mushrooms grown on *Andropogon gayanus* substrate and analyzed for its proximate composition were divided into four portions: sun dried (SD), oven dried (OV), blanched and unblanched stored directly in citric acid solution at varying concentrations (conc.) (0.0%, 0.1%, 0.3% and 0.5%). The microbial load count of the preserved samples and sensory evaluation carried out were significant ($P<0.05$) with sun and oven dried samples most preserved followed by blanched samples stored in citric acid solutions, with 0.5% conc. The proximate results showed that the protein, fat, and ash contents of mushroom preserved in citric acid solution decreased while sun and oven dried samples increased significantly with increase in fibre and carbohydrate. The result of the sensory evaluation on colour, texture and flavour showed that blanched sample stored in 0.5% citric acid solution was most preferred for colour, odour and texture.

Keywords: Citric acid solution, Mushroom, Proximate composition, Storability

Introduction

Some mushrooms have been found to provide a rich addition to the diet of man in the form of protein, carbohydrate, mineral, vitamins and enzymes (Okwulehie and Nosike, 2015) and are widely appreciated for their unique taste and flavour. Their medicinal (Ferreira et al., 2010) properties, such as: anti-inflammatory, anti-diabetic, anti-bacterial and anti-tumor which is attributed to the presence of bioactive metabolites (e.g. phenolic compounds, terpenes, steroids and polysaccharides) have also been observed. In particular, edible mushrooms can be a source of nutraceuticals with important antioxidant properties, which can positively influence the oxidative stress in cells and related diseases (Ferreira et at., 2009). Mushroom proteins are comparatively rich in the amino acids aspartic acid (9.10%–12.1%), arginine (3.70%–13.9%) and glutamic acid (12.6%–24.0%) but deficient in sulfur-containing amino acids, such as: cysteine and methionine (Cheung, 2010). Studies on edible mushroom have resurfaced as world nutrition is observed as shifting from processed to natural foods. This has almost led to warning for people to stay away from non fresh foods (Ihediohanma et al., 2014) due to uncertainty surrounding their safety. Mushroom production is a lucrative and profitable business especially for low income rural households and this industry is providing full or part time employment to both rural and urban poor and marginal farmers in many developing countries (Ferchak and

Cite this article as:
Ogwo, N., Ene, C.O., Ahaiwe, M.C., Chukwudi, U.P. (2019). Influence of varying preservation methods on the shelf life and proximate composition of *Pleurotus plumonarius* (Fr) Quel cultivated on *Andropogon gayanus* substrate. Int. J. Agric. Environ. Food Sci., 3(3), 162-170.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.8
Received: 29 March 2019 **Accepted:** 30 August 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 162-170
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Croucher, 2001). Currently, mushroom cultivation has been observed in more than 100 countries of the world with estimated total production of over 12 million metric tons (Suman and Sharma, 2007). It is considered one of the most important food items since ancient time and its consumption is increasing over the period for its significant role in human health, nutrition and disease management (Uddin, et al., 2011). The market value of dietary supplements from mushrooms is rapidly growing and estimated over U.S. $15 billion (Wasser, 2012; Panagiota and Philippoussis, 2015).

Shelf life is the length of time during which all of a commodity essential properties still remain acceptable for use, consumption or sale (Akram and Kwon, 2010). Mushrooms at ambient temperatures (< 22°C) have a short shelf-life of 1–3 days (Burton and Twyning, 1989), at 15°C their shelf-life is 2–3 days (Gormley 1981), whereas in the tropics they count only 24 hours (Wakchaure 2011) being one of the most perishable food products and tend to lose quality immediately after harvest. The shelf life is reduced due to post-harvest changes such as: browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture damage, related to their high respiration rate and moisture, relatively high protein content, and lack of physical protection to avoid water loss or microbial attack (Fernandes et al., 2012c). This has resulted to a serious decrease in the commercial, medicinal and nutritional value of edible mushrooms. Therefore, mushrooms are mainly used in the processed form (Jaworska and Bernás, 2009). Extending shelf-life is an imperative factor to increase the profitability and availability of any food product, since it offers the possibility of developing markets at a greater distance (Akram and Kwon, 2010) however, it is needful that the applied technology does not act itself as a source of chemical modifications.

Furthermore, as earlier stated, the fruit-bodies of mushrooms are highly perishable and most of the fruit-bodies produced are lost due to moisture loss, colour changes and of course poor preservation methods. Various mushroom preservation methods including blanching, freezing (Lyophilization and cryopreservation), steaming, oil or butter sautéing, drying, canning (sterilization), pickling and salting have been reported by different researchers (Chang & Miles, 2004; Uzunova-Doneva and Donev, 2005; Abatenh and Gizaw, 2018). However, each has shown at some point deficiency in performance and some, cost ineffective. For instance, drying often toughens or changes mushroom texture as well as resulting to the lost of colour, volatile flavours and aromas (Kaur et al., 2011; Rahart, 2017; Abatenh and Gizaw, 2018). Furthermore, it has been reported that mushrooms stored by blanching require further treatment like crisping and frying, otherwise quality is reduced over a short time, steaming can be somewhat more time-consuming and does not clean dirt, sand, and grit (Rahart, 2017). Canning was reported (Panagiota and Philippoussis, 2015) to be expensive and sometimes finicky equipment is necessary, filled with complicated processes, and requires very strict adherence to methods, procedures, and techniques. This is not a method to where one can cut corners. Under pickling, recipes must be proven and techniques adhered to rigidly. It is not a good medium for experimentation, as improper acid balance could lead to botulism or other serious food poisonings etc. The anti-bacterial and anti-browning properties of some chemical compounds such as: ascorbic acid, citric acid, hydrogen peroxide, sodium erythorbate, chlorine dioxide, sodium disoascorbate monohydrate, sodium D, L-isoascorbate and their derivatives along with hydrocolloid-based substances against fruit and vegetable spoilage have been reported (Cliffe-Byrnes and O'berrne, 2008; Simon and Gonzalez-Fandos, 2009). This could be an effective mushroom shelf life elongation method provided the right dosage and combination is maintained.

Therefore, determining the best and effective approach to store *Pleurotus plumonarius* sp. fresh, which is a highly relished mushroom species in Nigeria and sub-Saharan Africa, raises a concern to researchers. Improving their shelf life and quality characteristics will enhance marketability and value addition on food chain. Similarly, evolving preservation methods that are cheap and reliable especially to poor resource farmers is imperative in order to achieve food sustainability, which forms the basis for storability methods chosen in this trial. Hence, this study was carried out to investigate different preservation methods for fragile mushrooms so as to know the most effective method with retained nutritional values of the stored mushrooms when compared to the fresh ones.

Materials and Methods

Spawn mother culture was obtained from the Department of Biotechnology, Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State, Nigeria and multiplied (bulked up) in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.

Processes of Production of Mushroom Fruit-Bodies

The spawn multiplication was done following the method of Okwulehie and Okwujiako (2008). Sorghum grains were washed, parboiled and then allowed to drain completely. About 100g of the grains were thoroughly mixed with 5g of calcium carbonates and 5g of calcium sulphate for pH optimization and prevention of clumping respectively. The grains were autoclaved at 121°C for 45 minutes and then allowed to cool. The sterile sorghum were inoculated with the mother spawn and then incubated in the dark at room temperature for 2 weeks for mycelia running (ramification) (Baysal et al., 2003).

The substrate, which is straw of *Andropogon gayanus* was chopped into pieces of about 1-3cm average lengths and soaked 48hrs in tap water according to the method of Sharma et al. (2003). 1kg of completely drained prepared A. gayanus straw was packed into 2.5litres transparent plastic bucket perforated from bottom to the top. Buckets containing the substrates were pasteurized for one hour at 80°C in a gas-heated drum and allowed to cool overnight as suggested by Okwulehie and Nosike, (2015).

During inoculation, pasteurized substrates were thoroughly mixed with 10g of the spawn in transparent plastic buckets. The buckets containing inoculated substrates were placed on wooden racks and covered with black polythene sheets to

avoid contamination and to provide dark environment needed for vegetative growth of mycelia during incubation as recommended by Okwulehie et al. (2014).

During spawn run, cropping room was flooded with tap water to optimize relative humidity between 75-80% and temperature at 27±2^{O}C. Inoculated substrates were regularly checked against contamination until substrates were fully colonized by mycelia. When substrates were fully colonized by mycelia, covering sheaths were removed to allow for ample supply of oxygen and light required for primodia initiation. Air humidity was increased by spray-watering the substrates daily and was discontinued as soon as fruit bodies were formed according to Mwita et al. (2011).

Harvesting was done at maturity by hand-picking. The fruit-bodies which appeared mostly in bunches were picked by the stipe at the base and twisted gently to avoid injury. The harvested fruit-bodies were washed thoroughly under running tap water to reduce microbial load and adhering soil particles based on Melese and Workneh, (2015) recommendation.

Experimental design

The experiment was carried out in a completely randomized design (CRD). The set up was made up of ten (10) treatments with three (3) replicate. This gave a total of thirty (30) experimental units for one (1) week and a combined total of one hundred and twenty (120) experimental units for four (4) weeks.

Preservation methods

The prepared fruit-bodies were preserved using the following methods:

Chemical preservation

Citric acid was made into the concentration of 0.0%, 0.1%, 0.3% and 0.5% by diluting in the corresponding quantity of water. Two grams (2g) each of fresh fruit-bodies of P. pulmonarius (blanched and unblanched) were put in eight 473 ml bottles containing different concentration of citric acid according to the modified method of Ifeoluwa et al. (2015).

Drying

15g of the fruit-bodies were put in brown envelopes and oven dried at 50^{o}C for 4 hours, and then wrapped with aluminium foil to avoid moisture absorption. Another 15g of fruit-bodies were sun-dried in saucer pans for a period of 3 days.

Determination of mushroom shelf life

Shelf life was determined by running microbial test at 1week interval for all the mushroom samples during the experiment. This was done for 1 month as follows:

Nutrient agar (NA) and Sabourand Dextrose agar (SDA) were first prepared by measuring 39g of SDA and 28g of NA into a flask containing 1000ml of distilled water each, they were uniformly mixed by shaking and corked using cotton wool and aluminium foil. This was autoclaved at 121^{o}C for 45 minutes. After autoclaving the SDA and NA mixtures were poured into various well labelled petri dishes to cool and solidify.

Each of the preserved samples was diluted in ten-fold serial dilution technique as described by Joanne et al. (2010). Test tubes were set up in a rack and filled with 9ml of distilled water labelled as stock 10^{-1}, 10^{-2}, 10^{-3}, up to 10^{-12} respectively. An aliquot (1ml) was added using a pipette and transferred from the sample tube into the one labelled 10^{-1} and carefully homogenized. Similarly 1ml was drawn up from the 10^{-1} tube and transferred to the 10^{-2} tube. This dilution continued up to the last test tube labelled 10^{-4} and 10^{-5} from which 1ml was discarded. This procedure was carried out for all the samples except for the samples in solid forms where 1g was soaked in 10ml of sterile distilled water, properly homogenized and diluted in the above dilution technique.

Dilutions from 10^{-4} and 10^{-5} tube were inoculated into freshly prepared Nutrient agar (NA) and Sabourand Dextrose agar (SDA) plates for bacterial and fungal counts, respectively. The spread plate method of inoculation as described by Prescott et al. (2008) was used where 0.1ml of the dilutions (10^{-4} and 10^{-5}) were placed on the various agar plates and evenly spread over the entire plate using a flame sterilized glass rod. The inoculated plates were incubated at 35^{o}C for 24hrs and 72hrs for bacteria and fungi, respectively.

After incubation, the number of resulting colonies was counted using a colony counter and total microbial load (expressed as cfu/ml or cfu/g) was estimated using:

Microbial load = Reciprocal of dilution factor x Number of colonies counted

Dilution Factor (DF) = Initial dilution x Subsequent dilution x Volume of inoculums.

Where,

Cfu/ml = Colony forming unit per millilitre.

Cfu/g = Colony forming unit per gram.

Proximate analysis

Proximate analysis was carried out on each of the 10 samples. The protein, ash, fat, moisture and crude fibres were determined by the method of AOAC (2005). Carbohydrate content was calculated by difference as the nitrogen free extract (NFE), a method separately described by James (1995). Nitrogen free extract estimates non-fibrous carbohydrate such as sugars and starches. This was calculated by:

% NFE = 100 % - (a + b + c + d) %

Where,

a = protein, b = fat, c = fibre, d = ash.

Descriptive sensory evaluation was used in the screening of preserved mushroom based on their sensory quality characteristics. A questionnaire was administered to assess these attributes using a 9 point hedonic scale. A total of 10 panellists were involved in the sensory evaluation using the 9 point hedonic scales. The preserved mushroom samples were presented for panellist to evaluate the colour, texture, flavour and overall acceptability of the samples. All panellists were allowed to evaluate the samples for each quality feature using rating scale. All panellists were instructed to make their own individual assessments according to the evaluation criteria provided for each samples on the basis of colour, texture, flavour and overall acceptability. Finally, the scores of all judges were added and divided by the number of judges to find the final mean score.

Statistical analysis

The statistical package that was used in this experiment

is SPSS. Data obtained during the experiment were analyzed using ANOVA while the means were separated using Duncan New Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

It is important for a food product to conform with the microbiological criteria in order to certify that it is of good quality and will not pose any risk to the health of consumers. Mushrooms deteriorate easily due to high rate of respiration, and this shortens their shelf life (Valerie and David, 2008), hence, unsafe and unavailable for consumption. The role played by microbial populations in the postharvest quality of mushrooms has been reported (Soler-Rivas et al., 1999).

From the present result, the total heterophilic bacteria load count (Table 1) showed that bacteria load of the treated samples increased during storage irrespective of the treatments. The values obtained for the dried samples (SD and OV) were significantly different from blanched and unblanched samples preserved with citric acid. The total heterophilic bacteria count in week one ranged from 1.10 x 10^4 in SD to 3.00 x 10^5 in BC and UBC (control samples). The lower bacteria count on the dried samples could be attributed to the low moisture content which discouraged microbial activities (Okhuoya, 2011). Similar observation on Indian goose berry powder during storage was reported by Pareek and Kaushik (2012). The values obtained for the unblanched samples after one week showed significant difference ($P<0.05$) and ranged from 1.58 x 10^5 in UB0.5 to 3.00 x 10^5 in UBC. This steady decrease in bacteria load count observed in the samples preserved in higher concentration of citric acid solution confirms the antibacterial property of citric acid. Zhang et al. (2006) had reported a similar result in sweet potato. The same scenario was also observed for the blanched samples. The lower microbial load count value obtained in week one for blanched samples at 0.5 citric acid storage when compared to unblanched samples at the same concentration of citric acid showed significant difference ($P<0.05$), this ranged from 1.5 x 10^4 in B0.5 to 1.58 x 10^5 in UB0.5. This may be due to the fact that boiling temperature could have killed some of the microorganism during blanching. Similar result was reported by Fana et al. (2015) on orange fleshed sweet potato. Week 2 and week 3 followed the same trend.

Table 1. Total heterophilic bacterial load count of treated *Pleurotus pulmonarius* during storage

Treatment	Week 1	Week 2	Week 3	Week 4
SD	$1.1^{h}x10^{4}$	$1.5^{d}x10^{6}$	$7.8^{f}x10^{8}$	$>10^{9}$
OV	$6.15^{f}x10^{4}$	$4.65^{cd}x10^{6}$	$9.6^{e}x10^{8}$	$>10^{9}$
UB0.5	$1.58^{e}x10^{5}$	$4.5^{cd}x10^{6}$	$1.30^{d}x10^{9}$	$>10^{9}$
UB0.3	$2.09^{c}x10^{5}$	$1.10^{b}x10^{7}$	$1.83^{c}x10^{8}$	$>10^{9}$
UB0.1	$2.57^{b}x10^{5}$	$1.31^{b}x10^{7}$	$2.28^{b}x10^{9}$	$>10^{9}$
UBC	$3.00^{a}x10^{5}$	$2.11^{a}x10^{7}$	$2.90^{a}x10^{9}$	$>10^{9}$
B0.5	$1.5^{h}x10^{4}$	$2.7^{cd}x10^{6}$	$5.2^{g}x10^{8}$	$>10^{9}$
B0.3	$3.3^{g}x10^{4}$	$6.4^{c}x10^{6}$	$1.08^{e}x10^{9}$	$>10^{9}$
B0.1	$1.81^{d}x10^{5}$	$1.13^{b}x10^{7}$	$1.92^{c}x10^{9}$	$>10^{9}$
BC	$3.00^{a}x10^{5}$	$1.88^{a}x10^{7}$	$2.30^{b}x10^{9}$	$>10^{9}$

SD = sun dried, OV = oven dried, UB0.5 = unblanched at 0.5 conc., UB0.3 = unblanched at 0.3 conc., UB0.1 = unblanched at 0.1 conc., UBC = unblanched control, B0.5 = blanched at 0.5 conc., B0.3 = blanched at 0.3 conc., B0.1 = blanched at 0.1 conc., BC = blanched control. Values are mean of triplicate observation, values with different superscripts on the same column are significantly different ($P<0.05$)

Table 2. Total fungal load count on treated Pleurotus pulmonarius

Treatment	Week 1	Week 2	Week 3	Week 4
SD	$0.4^{i}x10^{4}$	$0.8^{h}x10^{6}$	$0.6^{g}x10^{8}$	$>10^{9}$
OV	$2.4^{h}x10^{4}$	$3.5^{g}x10^{6}$	$1.7^{f}x10^{8}$	$>10^{9}$
UB0.5	$8.1^{e}x10^{4}$	$6.4^{e}x10^{6}$	$4.4^{e}x10^{8}$	$>10^{9}$
UB0.3	$1.23^{e}x10^{5}$	$8.4^{d}x10^{6}$	$8.6^{d}x10^{8}$	$>10^{9}$
UB0.1	$1.82^{a}x10^{5}$	$1.15^{c}x10^{7}$	$1.10^{c}x10^{9}$	$>10^{9}$
UBC	$1.07^{d}x10^{5}$	$1.62^{b}x10^{7}$	$2.11^{a}x10^{9}$	$>10^{9}$
B0.5	$0.5^{i}x10^{4}$	$2.7^{g}x10^{6}$	$0.95^{fg}x10^{8}$	$>10^{9}$
B0.3	$3.9^{g}x10^{4}$	$5.1^{f}x10^{6}$	$3.8^{e}x10^{8}$	$>10^{9}$
B0.1	$7.0^{f}x10^{4}$	$8.0^{d}x10^{6}$	$7.9^{d}x10^{8}$	$>10^{9}$
BC	$1.52^{b}x10^{5}$	$2.50^{a}x10^{7}$	$1.92^{b}x10^{9}$	$>10^{9}$

SD = sun dried, OV = oven dried, UB0.5 = unblanched at 0.5 conc., UB0.3 = unblanched at 0.3 conc., UB0.1 = unblanched at 0.1 conc., UBC = unblanched control, B0.5 = blanched at 0.5 conc., B0.3 = blanched at 0.3 conc., B0.1 = blanched at 0.1 conc., BC = blanched control. Values are mean of triplicate observation, values with different superscript on the same column are significantly different ($P<0.05$)

The result for total fungal load count (Table 2) of the samples were significantly different (P<0.05). Similar observation to the bacteria load count was recorded for total fungi load count. Values obtained ranged from 0.40 x 10^4 in SD to 1.52 x 10^5 in BC for week one, 0.80 x 10^6 in SD to 2.50 x 10^7 in BC for week two, while in the third week it ranged from 0.60 x 10^8 in SD to 2.11 x 10^9 in UBC. The lower load count on the dried samples when compared to the other samples could be attributed to the low moisture content which discouraged microbial activities (Okhuoya, 2011). Pareek and kaushik (2012) reported similar observation on Indian goose berry powder. As with the result for bacteria load count, increase in the concentration of preservative used caused a sequential decrease in the load count of the samples. Blanched samples containing preservatives at different concentration also had lower load count compared to the unblanched samples.

The result obtained from the proximate composition of the fresh P. pulmonarius showed that the mushroom is a rich source of protein and fibre (Table 3). The lower value obtained for ash, fat and protein when compared to the results reported by some authors like Ayodele and Okhuoya (2009); Kuforiji et al. (2000) and Aletor (1995) could be attributed to the fact that the mushroom in this study was analyzed on fresh matter basis. However the protein and fibre content in this fresh mushroom was higher than that reported by Okwulehie et al. (2014) for mushroom, Hernandez et al. (2008) for tomato and Amandikwa (2012) for cocoyam. The value for the moisture content compared similarly to the result reported by Okwulehie et al. (2014) on mushroom. The high protein content of P. pulmonarius will help to enhance the protein level of food cooked with it thereby reducing the over dependence on meat and fish which are comparatively expensive for the low income earners where majority of our farmers in the tropics belong. The higher fibre contents is an added advantage as fibre have been reported to have the ability to lower the serum cholesterol level, heart disease, hypertension, constipation, diabetes and cancer (Ishida et al., 2000). The high moisture content is of disadvantage especially to storage, as high moisture content encourages microbial activities which bring about food degradation and spoilage (Okhuoya, 2011). The low carbohydrate content is of great advantage to diabetic patients that require low carbohydrate content food.

Table 3. Proximate composition of freshly harvested *Pleurotus pulmonarius*

Nutrient composition	Percentage (%)
Crude protein	10.18±0.46
Fat	2.23±0.04
Ash	2.50±0.00
Crude fibre	6.30±0.14
Moisture	72.63±0.11
Carbohydrate	6.18±0.67

Table 4 shows the proximate composition of the treated mushroom after one week of storage. The result showed significant difference in all the proximate parameters. However, the values obtained for the dried samples (OV and SD) were much higher when compared to blanched and unblanched samples irrespective of the concentration of the citric acid, for crude protein, fat, ash, crude fibre and carbohydrate. This might be as a result of moisture loss which in turn caused the nutrients to concentrate on the dried samples. Muyanja et al. (2012) reported similar observation on oyster mushroom. The values obtained for protein at 0.5 citric acid concentration ranged from 9.20 for unblanched to 9.65 for blanched samples which showed non significant (P>0.05) difference. Similar result was reported by Muyanja et al. (2012). The decrease in protein content of the blanched and unblanched samples at different concentration of the preserving solution suggests the

Table 4. Proximate composition of *Pleurotus pulmonarius* after one week of storage

Treatment	Crude protein	Fat	Ash	Crude fibre	Moisture	Carbohydrates
SD	29.40^{a}±0.00	6.80^{b}±0.06	11.25^{a}±0.07	14.43^{b}±0.11	9.70^{d}±0.07	28.43^{b}±0.17
OV	28.30^{b}±0.49	7.15^{a}±0.07	10.50^{b}±0.14	15.35^{a}±0.07	8.45^{e}±0.07	30.25^{a}±0.42
UB0.5	9.65^{c}±0.14	1.41^{c}±0.01	2.25^{c}±0.07	7.25^{d}±0.07	73.13^{c}±0.11	6.32^{f}±0.16
UB0.3	8.85^{d}±0.35	1.33^{d}±0.04	2.08^{cd}±0.11	7.00^{e}±0.00	73.28^{bc}±0.04	7.48^{def}±0.25
UB0.1	8.25^{e}±0.0	1.25^{def}±0.00	1.83^{ef}±0.04	6.73^{f}±0.04	73.62^{bc}±0.05	8.33^{cd}±0.04
UBC	7.40^{f}±0.14	1.20f^{g}±0.00	1.55^{g}±0.07	6.35^{h}±0.07	74.90^{a}±1.48	8.60^{cd}±1.62
BO.5	9.20^{cd}±0.00	1.30^{de}±0.00	1.90^{de}±0.00	7.45^{c}±0.07	73.82^{abc}±0.06	6.33^{f}±0.13
B0.3	8.75^{d}±0.00	1.28d^{ef}±0.04	1.68^{fg}±0.11	7.25^{d}±0.07	74.14^{abc}±0.08	6.92^{ef}±0.00
B0.1	8.25^{e}±0.07	1.23^{efg}±0.04	1.50^{g}±0.00	7.00^{e}±0.00	74.25^{abc}±0.00	7.78^{de}±0.11
BC	7.20^{f}±0.14	1.15^{g}±0.00	1.25^{h}±0.07	6.55^{g}±0.07	74.42^{ab}±0.11	9.43^{c}±0.25

SD = sun dried, OV = oven dried, UB0.5 = unblanched at 0.5 conc., UB0.3 = unblanched at 0.3 conc., UB0.1 = unblanched at 0.1 conc., UBC = unblanched control, B0.5 = blanched at 0.5 conc., B0.3 = blanched at 0.3 conc., B0.1 = blanched at 0.1 conc., BC = blanched control. Values are mean of triplicate observation ±SD, values on the same column with different superscripts are significantly different (P<0.05)

effectiveness of the preservative (citric acid). This may have been caused by the breakdown of protein into different amino acids by the acid (Zulqarnian et al., 2012). The fat and ash content were significantly different at various levels of preservation. This ranged from (1.15, 1.25) in BC to (7.15) in OV, and (11.25) in SD, respectively. The higher values recorded for the dried samples can be attributed to loss of moisture which concentrated the nutrient in the samples, while the lower values obtained in the blanched samples could be attributed to the leaching out of nutrient which happened during heating in water. Fana et al. (2015) reported similar result on orange fleshed sweet potato. Citric acid preserved the ash and fat content of the samples which was observed by the linear reduction in values with decrease in the concentration of the preservative solution used. Jebelli-Javan et al. (2015) also reported similar result on sliced button mushroom. The fibre content was also significant ($P<0.05$) for all the treatments. The values ranged from 6.55 in BC to 15.35 in OV. The higher value obtained for blanched treatment samples when compared to the unblanched could be attributed to the nutrient leaching out during heating in water which gave room for concentration of fibre in the samples. Similar observations was reported by Zhang et al. (2006) on fruits and vegetables, however, Agiriga et al. (2015) and Fana et al. (2015) had a contrary view. Moisture content was significantly different ($P<0.05$) for all treatment samples. It ranged from 8.45% in OV to 74.90% in BC. The low moisture value obtained for the dried samples could be due to loss of moisture during drying. The samples preserved in citric acid and blanched had higher moisture content compared to unblanched samples. This could be as a result of the fact that *Pleurotus pulmonarius* absorbed some of the water that was used in blanching before they were transferred into the preservation solution for storage. Fana et al. (2015), Zhang et al. (2006) and Agiriga et al. (2015) reported similar findings. The lower values obtained in samples stored in higher concentration of citric acid suggest that citric acid treatment prevented the absorption of more water by the sample but rather caused linear loss of water through osmosis. Mujanja et al. (2012) reported similar observation on oyster mushroom. Carbohydrate content ranged from 6.32% in UB0.5 to 30.25% in OV. The higher value of carbohydrate for the dried samples could also be as a result of low moisture content which concentrated the nutrient in the samples. Similar observation was reported by Mujanja et al. (2012).

The result (Table 5) obtained for the sensory evaluation one week after storage showed that colour scores obtained were significant ($P<0.05$). This ranged from 5.40 in OV to 7.40 in B0.5. The higher colour score of the blanched samples suggests that blanching treatment enhanced the colour of the samples during storage. Similar observation has been reported by Zhang et al. (2006) on orange fleshed sweet potato. The low score of OV suggests that the panellists had low preference for it. This could be as a result of the brown appearance which was as a result of milliard reaction caused by heating temperature (Surkiewicz et al., 1975). The texture score ranged from 5.60 in SD to 7.20 in UB0.5. Scores obtained for the other samples were significantly different ($P<0.05$) except for UB0.3 and B0.5 that had similar score (7.00). The texture score suggested that the panellist had higher preference with increase in the concentration of the preserving solution (citric acid). This supports an idea that citric acid can be used to preserve and enhance the texture quality of a food source. Similar result was recorded by Fana et al. (2015) on sweet potato. Values obtained for odour were significantly different which ranged from 7.20 in B0.5 to 5.20 in OV. Blanched samples had higher preference when compared to the unblanched samples. This could be as a result of low microbial load of the blanched samples. Microbial activities cause deterioration of food sources leading to release of foul odour as reported by Fana et al. (2015).

Table 5. Sensory evaluation of *Pleurotus pulmonarius* after one week storage

Treatment	Colour	Texture	Flavour
SD	$6.80^{abc}\pm0.79$	$5.60^{d}\pm1.07$	$5.40^{d}\pm0.97$
OV	$5.40^{d}\pm0.70$	$6.80^{abc}\pm1.23$	$5.20^{d}\pm1.14$
UB0.5	$7.00^{abc}\pm0.94$	$7.20^{a}\pm0.92$	$6.80^{ab}\pm0.92$
UB0.3	$6.60^{abc}\pm0.84$	$7.00^{bc}\pm0.94$	$6.40^{abc}\pm0.84$
UB0.1	$6.40^{bc}\pm0.97$	$6.60^{abc}\pm1.07$	$6.00^{bcd}\pm0.94$
UBC	$6.20^{c}\pm0.79$	$6.40^{abcd}\pm1.07$	$5.60^{cd}\pm0.97$
B0.5	$7.40^{a}\pm0.70$	$7.00^{ab}\pm0.82$	$7.20^{a}\pm0.79$
B0.3	$7.20^{ab}\pm0.79$	$6.60^{abc}\pm1.26$	$6.80^{ab}\pm0.92$
B0.1	$6.80^{abc}\pm0.79$	$6.20^{bcd}\pm0.42$	$6.40^{abc}\pm0.52$
BC	$6.40^{bc}\pm0.84$	$6.00^{cd}\pm0.47$	$6.00^{bcd}\pm0.47$

SD = sun dried, OV = oven dried, UB0.5 = unblanched at 0.5 conc., UB0.3 = unblanched at 0.3 conc., UB0.1 = unblanched at 0.1 conc., UBC = unblanched control, B0.5 = blanched at 0.5 conc., B0.3 = blanched at 0.3 conc., B0.1 = blanched at 0.1 conc., BC = blanched control. 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like or dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. Values are mean± SD, values on the same column with different superscripts are significantly different ($P<0.05$)

Two weeks after storage, results (Table 6) showed that the colour score ranged from 3.60 in BC to 6.40 in OV. The reduction in values when compared to week one may be as a result of higher microbial activity which deteriorated the samples. Similar trends was observed in the texture and odour/flavour values which ranged from 3.20 in UBC to 6.60 in B0.5 for flavour and 3.60 in BC to 6.40 in OV for texture. Similar reduction in preference was reported by Ifeoluwa et al. (2015).

Conclusions

Mushroom a rich source of protein, carbohydrate, fats and dietary fibre is very essential to health. Inference could be drawn from the present study that drying (oven drying and sun drying) of oyster mushrooms (*Pleurotus pulmonarius*) can lengthen their shelf life and retain their essential properties, control microbial populations during storage as shown by the low population of bacteria and fungi, and It could also be concluded that citric acid could be classified a good preservative as witnessed in the reduction of the microbial loads when its concentration increased.

The results from the research experience showed that mushrooms with longer shelf-life might be obtained if the concentration of citric acid is increased further than the 0.5% used in this study. This present research also showed that these preservation methods did not prolong the shelf life of *Pleurotus pulmonarius* beyond 7-14 days which demonstrates the need to combine preservation methods as a form of hurdle technology in order to extend the shelf life of mushrooms.

Table 6. Sensory evaluation of *Pleurotus pulmonarius* after two weeks of storage

Treatment	Colour	Texture	Flavour
SD	$6.20^{ab}\pm0.63$	$5.20^{bcd}\pm0.79$	$5.20^{bc}\pm0.79$
OV	$5.00^{cd}\pm0.94$	$6.40^{a}\pm0.52$	$4.80^{bc}\pm0.79$
UB0.5	$6.40^{ab}\pm0.97$	$6.00^{ab}\pm1.15$	$6.40^{ab}\pm0.84$
UB0.3	$5.80^{bc}\pm1.14$	$5.20^{bcd}\pm0.79$	$5.20^{bc}\pm0.79$
UB0.1	$5.20^{cd}\pm0.79$	$4.60^{de}\pm1.07$	$4.40^{cd}\pm0.70$
UBC	$4.60^{d}\pm0.70$	$4.20^{ef}\pm0.63$	$3.20^{e}\pm0.42$
B0.5	$6.80^{a}\pm0.79$	$5.60^{bc}\pm1.35$	$6.60^{a}\pm1.26$
B0.3	$6.20^{ab}\pm1.23$	$5.00^{cde}\pm0.82$	$5.60^{b}\pm0.97$
B0.1	$5.60^{bc}\pm1.07$	$4.20^{ef}\pm0.63$	$4.60^{d}\pm0.97$
BC	$5.00^{cd}\pm0.94$	$3.60^{f}\pm0.52$	$3.80^{de}\pm0.63$

SD = sun dried, OV = oven dried, UB0.5 = unblanched at 0.5 conc., UB0.3 = unblanched at 0.3 conc., UB0.1 = unblanched at 0.1 conc., UBC = unblanched control, B0.5 = blanched at 0.5 conc., B0.3 = blanched at 0.3 conc., B0.1 = blanched at 0.1 conc., BC = blanched control. 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like or dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. Values are mean± SD, values on the same column with different superscripts are significantly different (P<0.05)

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JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.3.9 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(3):171-181 (2019)

Astaxanthin biosynthesis: A two-step optimization approach and model construction with Response Surface Methodology and Artificial Neural Network

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Abstract

The first part of this research is investigating and comparing yield of a synthetic medium submerged three sugars (glucose, fructose and sucrose) at four different concentrations and solid fermentation systems with wheat bran and lentil waste for biosynthesis of astaxanthin (ASX) pigment by Xanthophyllomyces dendrorhous ATCC 24202 and Sporidiobolus salmonicolor ATCC 24259 microorganisms. The second part is modeling and optimizing the most efficient biosynthesis depending on waste, yeast and production variables consisted of moisture content, pH and temperature using a design matrix. The yields produced by X. dendrorhous were 51.88 µg of ASX/g glucose for the submerged medium with the least glucose, and 210.49 µg of ASX/g glucose for the wheat bran fermentation system. It was understood that the yield values of the submerged systems were lower and there was no requirement for the addition of any supplement to the waste systems. It was found that R^2=0.9869 was the highest value with the maximum predicted ASX amount of 109.23 µg of ASX/g wheat bran with X. dendrorhous using Artificial Neural Network modeling and the moisture content was the most significant production parameter.

Keywords: Astaxanthin biosynthesis, Neural network, Optimization, Response Surface Methodology, Submerged system

Introduction

The toxic and carcinogenic effects of the pigments produced synthetically reveal the importance of natural raw materials and biosynthesis for commercial production (Joshi et al., 2003; Duffosé et al., 2005; Gupta et al., 2011). Gupta et al. (2011) noted a food color market worth an approximate $1.2 billion globally, which has a 31% proportion of natural pigments. The boom in the global market and numerous research work points to the great economic potential for natural pigments, particularly those produced by microorganisms (Joshi et al., 2003, Gupta et al., 2011; Panesar et al., 2015). Among the natural carotenoid pigments, astaxanthin (ASX) has a very attractive commercial appeal due to its antioxidant power, economic value, wide usage, and health benefits for human (Naguib, 2000; Ramírez et al., 2001; Visser et al., 2003; Higuera-Ciapara et al., 2006; Amorim-Carillo et al., 2014; Dong et al., 2016; Niizawa et al., 2018). ASX is considered as the most powerful antioxidant and super-food nutritionally for human health. It is a valuable commercial product due to its marketing price varying from $2500-7000/kg (Panis and Rosales Carreon, 2016). The total value of ASX is predicted to reach to $1.1-1.5 billion in 2020 (Sujarit et al., 2017; Niizawa et al., 2018). ASX produced synthetically has safety and unfavorable (like biological functions) issues for human health. Besides, synthetic ASX has an unsustainable production and causes pollution (Panis and Rosales Carreon, 2016). Microbiologically produced ASX can be described as natural and replaced with the synthetic one. Safety of natural ASX consumption by a human is proven (Panis and Rosales Carreon, 2016; Schewe et al., 2017).

Physico-chemical conditions are very effective in the synthesis of microbial products. In the manufacturing of industri-

Cite this article as:
Dursun Saydam, D., Dalgic, A.C. (2019). Astaxanthin biosynthesis: A two-step optimization approach and model construction with Response Surface Methodology and Artificial Neural Network. Int. J. Agric. Environ. Food Sci., 3(3), 171-181.
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.9
Received: 29 June 2019 **Accepted:** 12 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 171-181
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

al products, there is a great deal of scientific research on the characteristics and conditions of the production medium. The raw material is a significant parameter for biosynthesis because it directly affects the production efficiency with its variety and nutritive content. It also plays an important role in the process and environmental issues. The variety and quantity of carbon and nitrogen sources of the production medium and water content are the fundamental parameters for the growth and target product synthesis of microorganisms. Additionally, temperature, pH, agitation, and inoculum size are the environmental parameters having an effect on microbial activities. The use of wastes as raw material sources for the production medium has been employed for a very long time in industrial processes worldwide. Compared to the synthetic media, the products synthesized by the utilization of the wastes are qualified and labeled as natural (Nigam and Pandey, 2009).

Sujarit et al. (2017) stated that the biosynthesis conditions have a great effect on ASX accumulation. Therefore, a way of natural characterized, safe and efficient ASX production should be submitted by considering optimization of the process, modeling the system, enhancement of the conditions and utilization of cheap sources (An et al., 2017; Schewe et al., 2017). In addition, optimization based on a design framework is a good way of determining which factor is the most important one. Statistical studies are necessary to be done to discuss and support the significancy of the considerations. Response surface and artificial neural network methodologies are commonly applied tools for modeling, optimization and statistical evaluations in biotechnological practices (Kalil et al., 2000). Production, modeling and optimization applications for enzymes, bio-surfactants, ethanol, gelling agents, pharmaceutical compounds, bio-fuels, pigments, and fermentation medium have been studied recently (Basri et al., 2007; Desai et al., 2008; Kashkouli et al., 2011; Nelofer et al., 2012; Zou et al., 2013; Pilkington et al., 2014; Dikshit and Tallapragada, 2015; Maran and Priya, 2015; Singh et al., 2015; del Rio-Chanona et al., 2016; Sehrawat et al., 2017; Wei et al., 2017; Shafi et al., 2018).

A growth-curve study is generally performed as a preliminary study in microbial processes, which is important for commercially high-grade ASX product in terms of monitoring growth phases of producer microorganisms and determining synthesis period and efficiency. Correlatively, investigation of these terms for ASX producers, *Xanthophyllomyces dendrorhous* ATCC 24202 and *Sporidiobolus salmonicolor* ATCC 24259 yeasts in synthetic media were aimed as the first stage of this study. The conditions of the synthetic media were optimized. In the second stage, wheat bran and lentil waste were used as solid raw materials for ASX biosynthesis. The effects of fermentation temperature, moisture content and pH variables on the biosynthesis were searched within the scope of an experimental design. Modeling and optimization of the biosynthesis were performed by Response Surface Methodology (RSM). The comparison of estimation capabilities of RSM and Artificial Neural Network (ANN) techniques for ASX response were performed. Statistical evaluation of the methodology results were carried out by root mean square error (*RMSE*), mean absolute error (*MAE*), and coefficient of determination (R^2).

Materials and Methods

Wastes and microorganism cultures

Wheat bran and lentil waste were sourced from Gaziantep, Turkey and kept in cold storage (+10 °C) in polyethylene packages. They were sieved to a size of 0.85 mm in order to obtain a uniform material. Freeze-dried forms of ATCC 24202 (*Xanthophyllomyces dendrorhous*) and ATCC 24259 (*Sporidiobolus salmonicolor*) were purchased from the American Type Culture Collection (Manassas, USA). The microorganisms were maintained in yeast malt extract broth (YMB) that has the following composition: 3 g/L of yeast extract (Merck, Germany), 3 of g/L malt extract (Merck, Germany), 5 g/L of peptone (Merck, Germany), 10 g/L of dextrose (Sigma-Aldrich, Germany). The growth of the yeasts with a 2% (v/v) inoculum size was performed in 20 °C-4.5 pH and 18 °C-6.0 pH conditions which are the optimum growth conditions of ATCC 24202 and ATCC 24259, respectively according to the ATCC protocol.

Growth curve determination

Growth parameters of the yeasts were determined to observe the phases of the growth and the fermentation period. *Optical density* (O.D.) was measured by a double-beam UV/VIS spectrophotometer (Lambda 25 UV/VIS spectrophotometer, USA) at 540 nm. Additionally, *viable cell* was determined by plate counting method and *biomass* was weighted as dry cell. Each measurement (Lopes et al., 2007; Aber et al., 2012) was carried out daily for 20 days. Experimental data of the growth parameters were plotted versus time. Natural logarithm of optical density value In [O.D.] versus fermentation period for each yeast was modeled by SigmaPlot Version 11.0 (Systat Software GmbH, Erkrath, Germany) program using Gompertz-4 Parameter equation:

$$y = y_0 + a^x \exp\left(-\exp\left(-\frac{x - x_0}{b}\right)\right)$$

Where y is response, x is time, a and b are coefficients.

Astaxanthin pigment analysis

Astaxanthin analysis was performed for the synthetic media content, and the forms of un-fermented and fermented contents of the wastes according to Babitha et al. (2007). A mixture of the sample and methanol (Sigma-Aldrich, Germany) at 1:4 ratio was centrifuged at 6000 rpm for 10 minutes, and the supernatant was analyzed at 474 nm using the UV/VIS spectrophotometer. The results were recorded as the mean of triplicate measurements.

Yeast growth and productivity in synthetic media

Synthetic media were prepared using no sugar (only containing nitrogenous compounds) and three different sugar types (glucose, fructose and sucrose) at 4 different concentrations (5 g/L, 10 g/L, 20 g/L and 40 g/L) measure the growth and product formation capabilities of the yeasts by keeping the amount of malt extract, yeast extract and peptone ingredients constant. Optical density values of the media during

the fermentation period and the ASX pigment produced in the synthetic media were measured at the last day of the fermentation period.

Experimental design and model construction of the fermentation systems

An experimental design was generated for each combination of yeast and waste based on the optimal growth conditions of the yeasts using Box-Behnken design (BBD) with the independent variables of temperature (x_1), moisture content (x_2) and pH (x_3). Levels coded as high (+), middle (0) and low (−) are given in Table 1. Seventeen experiments were conducted for each combination to produce astaxanthin (response). The experimental data were analyzed using RSM (Design-Expert Version 7.1.5, Minneapolis, USA) and ANN (MATLAB Version 7.10, USA) methodologies.

Table 1. Levels of the independent variables for Box-Behnken design

Microorganisms	Coded Levels								
	x_1			x_2			x_3		
	−1	0	+1	−1	0	+1	−1	0	+1
ATCC 24202	15	20	25	70	80	90	3.5	4.5	5.5
ATCC 24259	13	18	23	70	80	90	5.0	6.0	7.0

The ndividual and interaction effects of the process variables on the response are demonstrated in the equation below which is a second-order polynomial equation quadratic model:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x^2{}_j + \sum_{i=1}^{j-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j$$

Where y is the predicted response, β_0 is constant, β_j, β_{ij} and β_{jj} are the regression coefficients and x_j and x_i are the levels of the independent variables.

RSM requires a mathematical model such as quadratic for the data prediction. However, ANN uses vectors based on a function for this purpose. This specification provides the main advantage of ANN methodology (Baş and Boyacı, 2007; Singh et al., 2015). Non-linear mapping was performed by using the process parameters as the input variables and the response as the output variable. A Gausiian function with 0.75 spreadability was applied, which uses the equation below to estimate the data with a 3 input layer, one hidden layer with 17 nodes and 1 output layer (3-17-1) topology.

$$a_{hk} = \exp\left(- \frac{\| x_h - x_k \|^2}{\sigma_h{}^2} \right)$$

Where a_{hk} is basis function or activation of h-th unit in the hidden layer; x_h is unit center or n-dimensional position of the centre of h-th (n as input number); x_k mean or center of the function, σ_h is standard deviation or local scaling constant.

The prediction capability of the methodologies was investigated by *RMSE (root mean square error)*, *MAE (mean absolute error)*, and R^2 statistical measurements. R^2 values for RSM came out from the design program and for ANN were calculated by regression analysis tool in MS Excel program.

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (MR_{pre,i} - MR_{\exp,i})^2}$$

$$MAE = \frac{1}{N} \sum_{i=1}^{N} (MR_{pre,i} - MR_{\exp,i})$$

Where n is number of data, $R_{pre,i}$ is predicted model value, and $R_{exp,i}$ is experimental value.

Results

Growth curve study

The optical density, viable cell and biomass data obtained in the synthetic YMB medium are presented in Figure 1 for ATCC 24202 and ATCC 24259 yeasts. Based on the reasonable progress of the growth parameters, the fermentation period was determined to be ten days. Figure 2 shows the growth curves of the yeasts modeled with the data of In [O.D.] versus fermentation period. The results of the statistical evaluation of the modeling are given in Table 2. The R-squared values are quite high, 0.97 for ATCC 24202 and 0.96 for ATCC 24259, meaning a good fitness of the regression model.

Table 2. Modeling report of the growth curve determination

Sources	ATCC 24202	ATCC 24259
a	2.969	4282.1518
b	0.7998	2.4397
x_0	0.6075	–17.8474
y_0	14.4159	–4264.3628
R^2	0.9721	0.9618
Adj. R^2	0.9581	0.9427
Normality test*	0.3128	0.1285

*: passed at $p<0.05$

Effect of sugar types and concentrations

The media prepared with glucose (G), fructose (F) and sucrose (S) sugars at four different concentrations were investigated to see the effects of the sugar type and the concentration on the growth and product formation abilities of the selected yeasts. The optical density values (including viable cells, dead cells, and metabolites) of the yeasts are shown in Figure 3 and Figure 4. The optical density changes observed between 100 and 150 hours are striking for both yeasts. A decrease in cell concentration was seen between about 100 to 125 hours for ATCC 24202 yeast. There seems to be a few non-significant data points such as the increase in S5 after 150 hours in Figure 3, which is distinct because of the optical values including the viable cells, dead cells and metabolites. It can be stated that glucose at any concentration presents a high cell concentration for ATCC 24202 yeast. The O.D. values of ATCC 24259 yeast showed significant changes from 75 hours at any concentration of glucose and sucrose sugars (Figure 4). The cell concentrations at low fructose concentrations (5 and 10 g/L) were quite high for ATCC 24259 yeast. It is thought

that it took time for ATCC 24259 yeast to adapt to the F20 medium whereas there was no active growth on the F40 medium. ATCC 24202 yeast at no sugar medium showed a curve similar to the G40's and the growth trend of ATCC 24259 yeast with 'no sugar' medium resembles the trend obtained from the media of glucose and sucrose sugars in Figure 5.

It is understood that as the sugar concentration of the media increases, the amount of ASX decreases for both yeast as seen in Table 3. The ASX amount produced by ATCC 24202 in any medium is higher than that produced by ATCC 24259. Maximum amounts of 51.88 µg ASX/g glucose and 17.98 µg ASX/g fructose were measured for ATCC 24202 and ATCC 24259, respectively. It is seen that the amount of ASX is less in the absence of sugar than in other media for both yeasts. When the relationship between cell concentration and product formation is evaluated (Table 3), it could be concluded that there is no connection between them.

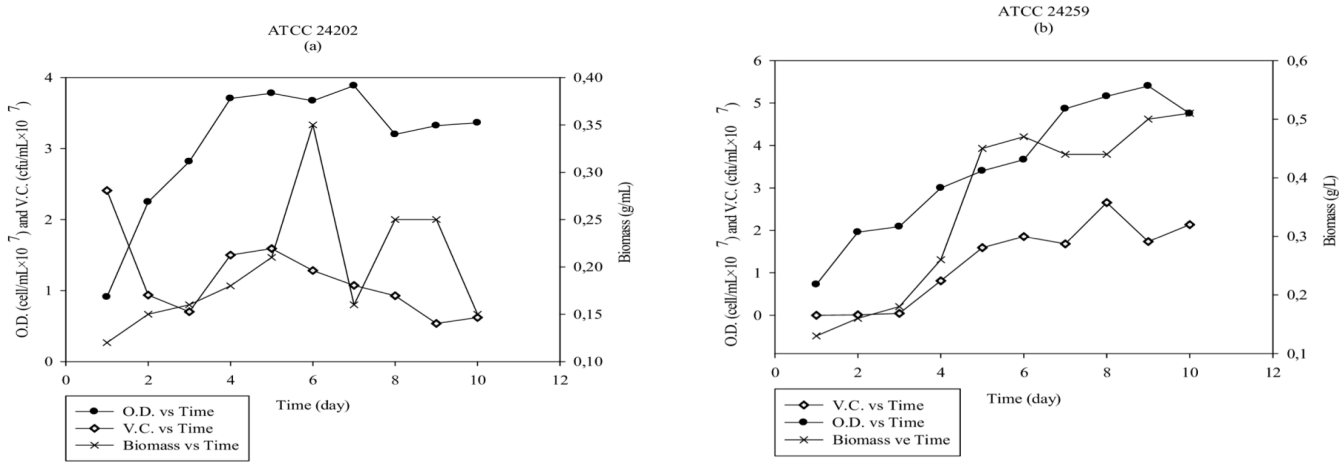

Figure 1. The growth parameters plotted for X. dendrorhous ATCC 24202 (a) and S. salmonicolor ATCC 24259 (b)

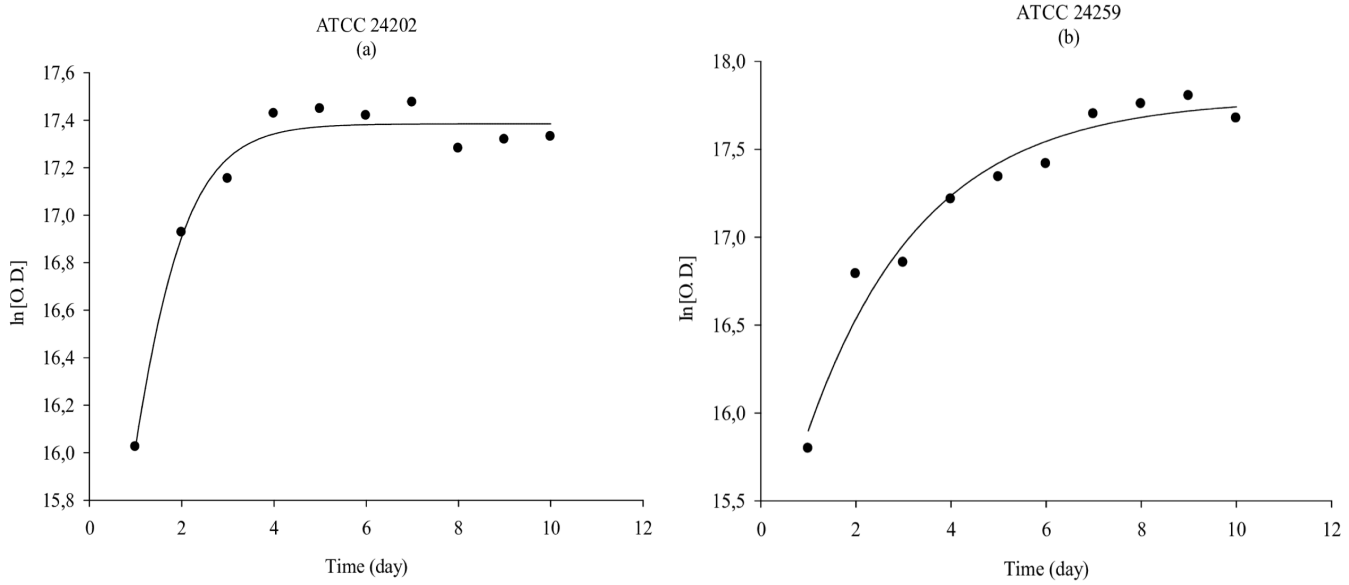

Figure 2. Modeled growth curves of for *X. dendrorhous* ATCC 24202 (a) and *S. salmonicolor* ATCC 24259 (b). The lines and dots indicate the model regression of estimated and experimental data, respectively.

Table 3. Astaxanthin yield and cell concentration in synthetic media

Sugar concentrations[a]	Astaxanthin concentration (mg ASX/L YMB)[b]		Astaxanthin amount (µg ASX/g sugar)[c]		Cell concentration (cell/mL×10^7)[d]	
	ATCC 24202	ATCC 24259	ATCC 24202	ATCC 24259	ATCC 24202	ATCC 24259
G5	2.08 ± 0.02	0.51 ± 0.02	51.88 ± 0.57	12.72 ± 0.44	1.42	2.57
G10	1.96 ± 0.02	0.6 ± 0.01	24.44 ± 0.22	7.53 ± 0.11	1.14	3.05
G20	1.87 ± 0.02	0.81 ± 0.0	11.71 ± 0.13	5.04 ± 0.02	3.72	2.69
G40	1.76 ± 0.02	0.75 ± 0.02	5.5 ± 0.05	2.34 ± 0.06	0.93	2.52
F5	1.88 ± 0.02	0.72 ± 0.01	46.93 ± 0.59	17.98 ± 0.23	1.24	3.32
F10	2.08 ± 0.03	0.68 ± 0.04	26.02 ± 0.34	8.49 ± 0.50	2.35	2.23
F20	2.42 ± 0.02	1.38 ± 0.02	15.13 ± 0.11	8.61 ± 0.16	1.69	2.94
F40	0.7 ± 0.02	1.19 ± 0.01	2.2 ± 0.07	3.73 ± 0.04	1.23	5.67
S 5	1.91 ± 0.02	0.34 ± 0.01	47.67 ± 0.49	8.39 ± 0.28	2.34	2.07
S10	1.9 ± 0.01	0.41 ± 0.02	23.71 ± 0.07	5.1 ± 0.29	1.46	4.26
S20	2.72 ± 0.01	0.37 ± 0.01	16.98 ± 0.05	2.33 ± 0.09	0.94	1.12
S40	1.82 ± 0.02	0.33 ± 0.02	5.7 ± 0.06	1.03 ± 0.05	1.8	2.03
No sugar	1.6 ± 0.01	0.27 ± 0.02	-	-	1.92	1.91

[a]: G: glucose, F: fructose, S: sucrose
[b]: Astaxanthin concentration: milligram astaxanthin/Liter yeast malt extract broth as mean value of duplicate results
[c]: Astaxanthin amount: microgram astaxanthin/gram sugar as mean value of duplicate results
[d]: Optical density values at 540 nm

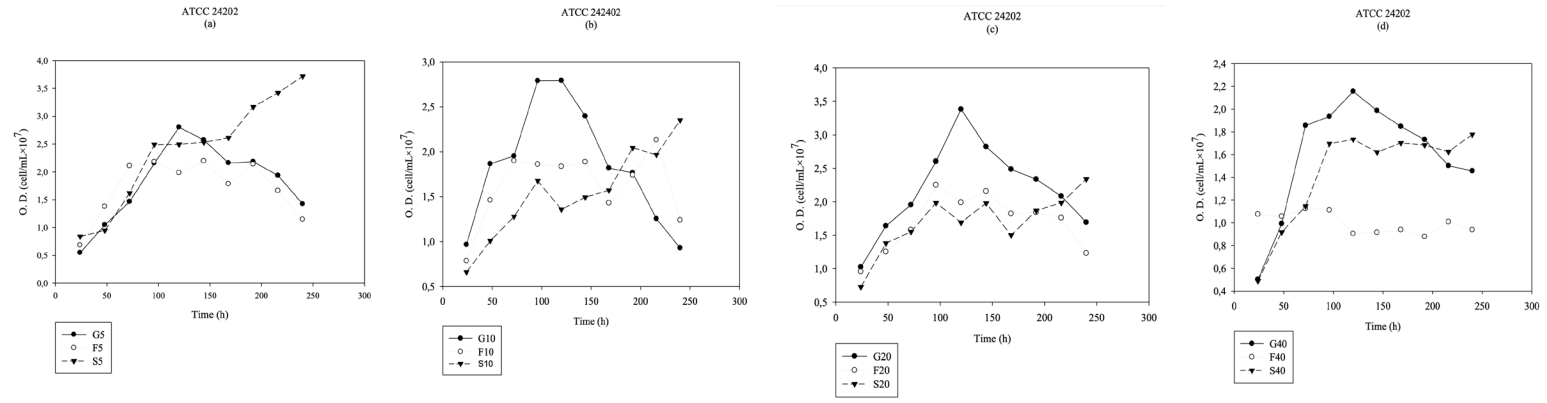

Figure 3. Time versus optical density values for *X. dendrorhous* ATCC 24202 at sugar concentrations of; a: 5 g/L, b: 10 g/L, c: 20 g/L, c: 40 g/L

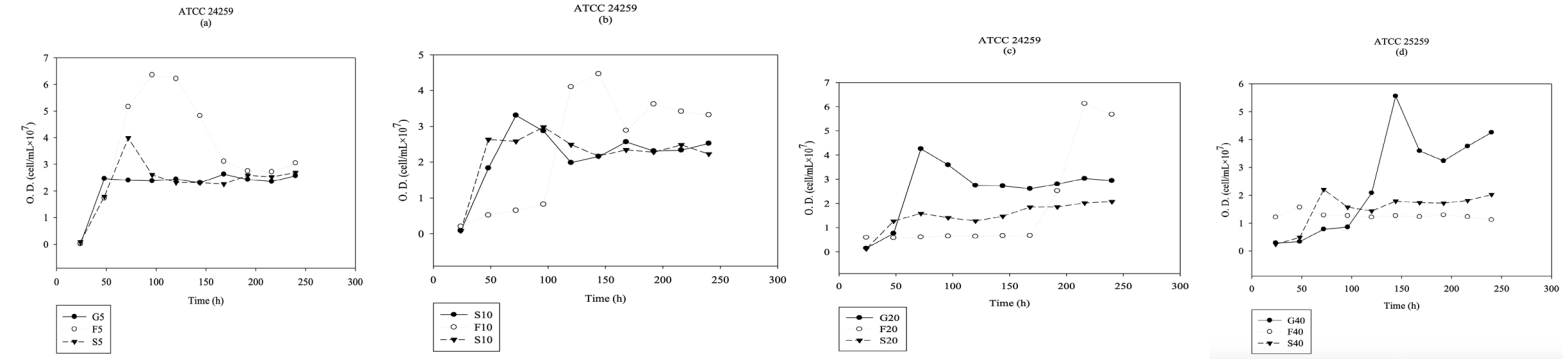

Figure 4. Time versus optical density values for *S. salmonicolor* ATCC 24259 at sugar concentrations of; a: 5 g/L, b: 10 g/L, c: 20 g/L, c: 40 g/L

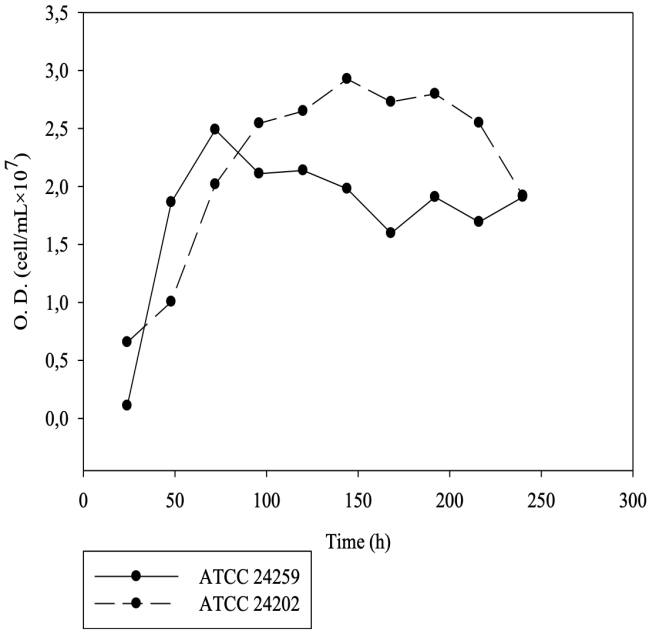

Figure 5. Time versus optical density values at no sugar medium for *X. dendrorhous* ATCC 24202 and *S. salmonicolor* ATCC 24259

Modeling and optimization study

The production of the ASX pigment from the wheat bran and lentil waste was achieved in the optimum growth conditions of the yeasts (Table 4). Based on the sugar content of the wastes, the wheat bran provided the highest ASX yield (201.49 µg ASX/g glucose) for both yeasts. After considering the ASX yield obtained from the wastes, an optimization study for both yeasts was conducted by generated BBD experimental design. The ASX yield of both yeasts can be seen in Table 5. The optimized conditions were determined depending on the actual maximum yield. For the fermentation system of the wheat bran and ATCC 24259 yeast, the maximum yield of 60.54 µg ASX/gm was obtained under the conditions of 23 °C, 90% and pH of 6.0. The maximum yield of 109.23 µg ASX/gm was synthesized from the wheat bran fermentation medium with ATCC 24202 yeast at the conditions of 20 °C, 90% and pH of 5.5, which was the data from previously published work elsewhere and used to compare with the other fermentation systems in this study. The wheat bran and ATCC 24202 yeast system again maximized the yield. Furthermore, 100.25 µg of ASX/gm was a quite high amount of ASX produced from the fermentation system of lentil waste and ATCC 24202.

Table 4. Astaxanthin yield produced in waste medium at optimum yeast growth conditions

Medium	Sugar content (g glu/gm)[a]	Astaxanthin amount (µg ASX/gm)[b]		Astaxanthin amount (µg ASX/g glu)[c]	
		ATCC 24202	ATCC 24259	ATCC 24202	ATCC 24259
Wheat bran	0.44 ± 0.01	87.83 ± 5.67	35.55 ± 1.19	201.49 ± 13.02	81.56 ± 2.72
Lentil waste	0.75 ± 0.0	59.33 ± 4.57	21.70 ± 0.51	79.02 ± 6.09	28.90 ± 0.67

[a]: Sugar content: gram glucose/gram waste as mean value of duplicate results
[b]: Astaxanthin amount: microgram astaxanthin/gram material as mean value of triplicate results
[c]: Astaxanthin amount: microgram astaxanthin/gram glucose as mean value of triplicate results
±: standard deviation

When the yields are compared in Table 4 and Table 5, it is seen that the product yield increased while the temperature and moisture content levels increased at the optimum pH value in the fermentation systems of ATCC 24202 yeast. The highest ASX yield was obtained at a lower temperature and moisture content value than the optimum ones at a constant pH value for the fermentation system of wheat bran and ATCC 24259. Increasing values of pH and moisture content parameters induced product formation for the fermentation system of lentil waste and ATCC 24259.

The ANOVA results and model coefficients for all the fermentation systems are shown in Table 6. The significant status (+) of 'model' is good, but not the same for 'lack of fit', which rates how the model chosen at the determined probability represents a fit between the experimental and predicted data. The quadratic model was fitted to the experimental data of all fermentation systems at $p<0.1$ with a minimum 0.82 correlation for the findings that shows the proportion of the total variation of the response that fitted the model. The effects of the independent variables depending on the model coefficients are also seen in Table 6. They are supportive outcomes of the model for the fitness.

Table 5. BBD design matrix and experimental astaxanthin data of two fermentation systems

Run	x_1	x_2	x_3	Astaxanthin amount (µg ASX/gm)			
				Wheat bran		Lentil waste	
				ATCC 24202	ATCC 24259	ATCC 24202	ATCC 24259
1	-1	0	-1	95.0 ± 2.5	30.5 ± 0.16	37.40 ± 1.0	14.75 ± 0.34
2	-1	0	1	55.9 ± 1.2	41.81 ± 2.47	31.50 ± 1.1	47.73 ± 6.29
3	-1	-1	0	34.98 ± 2.9	17.21 ± 0.48	11.53 ± 1.0	14.16 ± 0.74
4	-1	1	0	66.31 ± 1.8	51.56 ± 2.18	38.02 ± 3.1	36.85 ± 1.46
5	0	-1	-1	28.96 ± 2.9	11.6 ± 0.2	19.17 ± 0.0	9.44 ± 1.13
6	0	0	0	79.64 ± 5.2	37.26 ± 0.87	49.44 ± 0.0	20.06 ± 0.56
7	1	0	1	70.76 ± 4.5	35.37 ± 0.38	51.35 ± 5.60	16.22 ± 1.24
8	0	0	0	86.8 ± 8.7	36.62 ± 1.78	59.59 ± 3.1	14.05 ± 0.42
9	0	0	0	60.85 ± 1.6	32.78 ± 0.92	57.28 ± 8.6	25.3 ± 0.28
10	1	1	0	60.84 ± 2.2	60.54 ± 1.83	90.51 ± 1.0	36.11 ± 3.13
11	1	0	-1	72.84 ± 1.3	20.76 ± 0.71	64.22 ± 2.6	15.65 ± 0.45
12	0	-1	1	33.4 ± 5.3	11.49 ± 0.32	70.20 ± 52.4	16.04 ± 1.16
13	0	1	1	109.23 ± 12.1	51.83 ± 1.68	100.25 ± 0.0	35.82 ± 1.28
14	0	1	-1	84.46 ± 0.0	35.32 ± 1.19	75.15 ± 0.0	58.15 ± 1.41
15	0	0	0	88.99 ± 0.2	21.42 ± 0.56	61.12 ± 2.1	20.89 ± 0.56
16	1	-1	0	26.82 ± 2.8	16.76 ± 1.79	16.70 ± 0.8	13.05 ± 0.04
17	0	0	0	87.7 ± 8.2	19.9 ± 0.62	64.72 ± 5.4	20.54 ± 0.63

±: standard deviation

Table 6. ANOVA results and equation coefficients for each fermentation system

Tools	p<	Wheat bran		Lentil waste	
		ATCC 24202	ATCC 24259	ATCC 24202	ATCC 24259
Model	0.1	+	+	+	+
	0.05	–0.0507	+0.0136	+0.0206	–0.0523
	0.01	–	–	–	–
Lack of fit	0.1	–	–	+	+
	0.05	–0.1701	–0.6817	+0.0183	+0.026
	0.01	–	–	–	–
Std. deviation		15.67	7.48	13.61	8.8
Mean		67.26	31.34	52.83	24.4
C.V.%		23.3	23.86	25.76	35.86
PRESS		19561.22	2233.4	18867.08	7642.66
R^2		0.8246	0.8858	0.8696	0.82
Adj. R^2		0.5991	0.7389	0.7019	0.59
Coefficients (coded values)					
β_0		80.8	29.6	58.43	20.17
β_1		–2.62	–0.1	13.04	–4.06
β_2		24.59	17.77	23.29	14.28
β_3		–1.5	5.29	7.17	2.23
β_{12}		0.67	2.35	11.83	0.09
β_{13}		9.26	0.83	–1.74	–8.1
β_{23}		5.08	4.15	–6.48	–7.23
β_{11}		–11.97	5.74	–19.66	–0.7
β_{22}		–21.5848	1.1846	0.419	5.5748
β_{33}		4.8008	–3.2201	7.3457	4.1216

The results of the response data predictions of RSM and ANN for each experimental run are presented in Table 7. The lower RSME and MAE values and higher R^2 results revealed that the prediction capability of ANN methodology was better than RSM (Table 8). This result was depicted in Figure 6 and Figure 7 by visualizing the data distribution. The fitted lines obtained from the observations and the model calculations supported a good fit with ANN methodology. The fermentation systems of ATCC 24259 yeast indicated more successful predictions of the ASX yield depending on the correlation and error calculations.

Table 7. Predicted astaxanthin data of the methodologies for each fermentation system

Run	Wheat bran				Lentil waste			
	ATCC 24202		ATCC 24259		ATCC 24202		ATCC 24259	
	RSM	ANN	RSM	ANN	RSM	ANN	RSM	ANN
1	86.99	95.0	28.6	30.5	24.17	37.4	17.32	14.75
2	65.49	55.9	37.53	41.81	41.99	31.5	37.97	47.73
3	25.94	34.98	22.05	17.21	14.69	11.53	14.91	14.16
4	73.77	66.31	52.89	51.56	37.61	38.02	43.29	36.85
5	46.0	28.96	8.65	11.6	29.25	19.17	6.12	9.44
6	80.8	80.8	29.6	29.6	58.43	58.43	20.17	20.17
7	78.77	70.76	37.27	35.37	64.58	51.35	13.66	16.22
8	80.8	80.8	29.6	29.6	58.43	58.43	20.17	20.17
9	80.8	80.8	29.6	29.6	58.43	58.43	20.17	20.17
10	69.88	60.84	55.69	60.54	87.35	90.51	35.36	36.11
11	63.25	72.84	25.04	20.76	53.73	64.22	25.41	15.65
12	32.85	33.4	10.92	11.49	56.56	70.2	25.04	16.04
13	92.18	109.23	54.78	51.83	90.17	100.25	39.14	35.82
14	85.02	84.46	35.89	35.32	88.80	75.15	49.15	58.15
15	80.8	80.8	29.6	29.6	58.43	58.43	20.17	20.17
16	19.36	26.82	15.43	16.76	17.11	16.7	6.61	13.05
17	80.8	80.8	29.6	29.6	58.43	58.43	20.17	20.17

Table 8. Statistical evaluation results of RSM and ANN methodologies

Sources	Wheat bran				Lentil waste			
	ATCC 24202		ATCC 24259		ATCC 24202		ATCC 24259	
	RSM	ANN	RSM	ANN	RSM	ANN	RSM	ANN
RMSE	10.0551	5.6881	4.7979	4.0517	8.7337	2.7684	5.6143	1.9469
MAE	8.5645	2.4826	3.969	2.1026	7.1955	1.1929	4.4768	0.7325
R^2	0.8246	0.9439	0.8858	0.9185	0.8696	0.9869	0.8228	0.9787

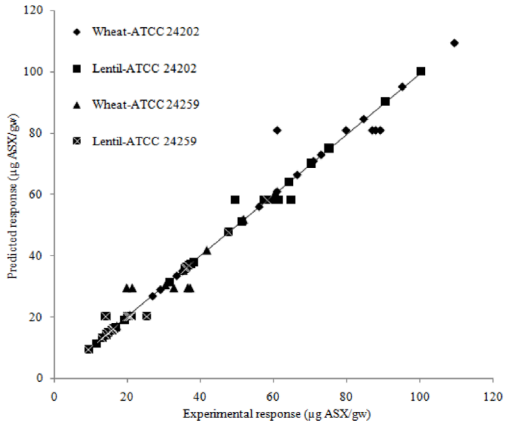

Figure 6. The fitness plot of the ANN model for all fermentation systems

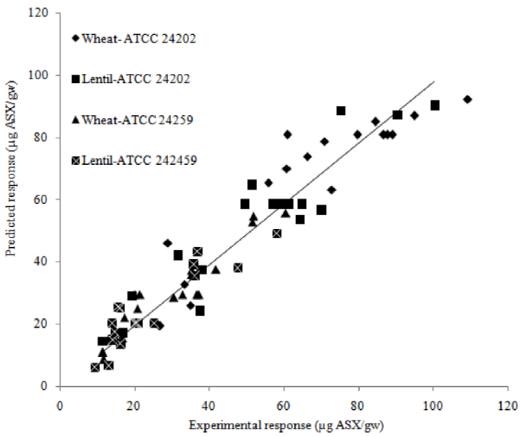

Figure 7. The fitness plot of the RSM model for all fermentation systems

Discussion

Cell growth is an exquisite and complex process which is realized by microorganisms utilizing elements such as carbon, nitrogen and oxygen to grow and produce metabolites. The microbiological growth depends on consuming substrates in the medium which is important for the targeted product formation. In the synthetic medium, the constituents are well known so that the growth may be followed easily. The period, interactions and product formation may also be easy to determine (Bailey and Ollis, 1986). The growths of the yeasts were exhibited in Figure 1 to observe the changes of viability and weight of the cells that are the fundamental items for cell kinetics (Bailey and Ollis, 1986).

It is a well-known phenomenon for the growth of microorganisms that a carbon source is essential, glucose is particularly fundamental and the concentration of the source determines the growth phases and period, metabolite production and limitations in growth. Besides, glucose and fructose are directly utilized by yeasts in their metabolic mechanisms. Sucrose may then be metabolized (Carlson, 1987). The concentration of fermentable sugars is a significant parameter for the growth rate of the microorganisms (Arroyo-López et al., 2009). The product formation of the yeasts, according to the sugar type and concentration -as given in Table 3- showed that it is not necessary to supply more substrate to the microorganism in order to reach a high production level and promote cell growth, which is an explanation supported by Bailey and Ollis (1986). The highest ASX produced by *X. dendrorhous* resulted from having glucose as the sugar type and at its lowest concentration. Meyer and du Preez (1994), Ramírez et al (2000) and Stoklasa et al. (2018) reported that high sugar concentration caused reduction of ASX synthesis. Johnson and Lewis (1979) studied different sugar types, including glucose and sucrose, to produce ASX with *X. dendrorhous* (formerly *P. rhodozyma*), and indicated that the concentration of ASX was higher in the sucrose medium than in the glucose medium. Guo et al. (2010) studied with different strains of *X. dendrorhous* and found that ASX yield is higher in a sucrose medium than glucose or fructose. The increasing sugar concentration revealed a substrate limitation on ASX production for both yeasts in the study, whereas there was no such effect for cell concentration. It has been reported that high glucose concentration inhibits the pigment production for *X. dendrorhous* by Hu et al. (2005). The 'no sugar' medium showed that other nutrients, particularly nitrogen, are required for product formation. The nitrogen source and its concentration are important parameters for ASX production and yield (Ramírez et al 2000; Ni et al., 2007; Guo et al., 2010).

It is very important that products with commercial importance -from cheap and highly nutritious substrates- can be manufactured on an industrial scale. Besides, the optimization of the process is required not only for synthetic media but also in media consisting of this kind of substrate in terms of raw material and environmental conditions due to the high product on goal (Stoklasa et al., 2018). Haard (1988) reported that he managed to produce much more ASX utilizing molasses with *X. dendrorhous* than the synthetic media prepared from glucose, sucrose and fructose sugars. However, a parallel result with the synthetic media was obtained where lower sugar content provided the maximum ASX yield (Table 3). Ni et al. (2007) stated that the optimization of the fermentation conditions for ASX manufacturing is very important. In previous studies, the maximum ASX amount from ATCC 24202 was reached at 19.7 °C and a pH of 6.0 using a synthetic medium within the scope of an experimental design generated by Ramírez et al. (2001). Ananda and Vadlani (2011) studied wheat bran to produce ASX using ATCC 24202. At the end of an 11 day fermentation period, they managed to produce 66.75 μg of ASX/g of substrate from wheat bran.

The yeast *S. salmonicolor* has been mainly studied for carotenoid production by Valduga et al. (2008; 2009): the maximum total carotenoids in the synthetic (913 μg/L) and agro-industrial (502 μg/L) media were produced by the yeast in approximately 100 hours; and the maximum concentration of 1.019 μg/L for the total carotenoids was obtained using the same yeast in a synthetic medium containing 40 g/L glucose at 25 °C, and an initial pH of 4.0 after the optimization with response surface methodology.

Pérez-Guerra et al. (2003) and Mitchell et al. (2004) emphasized that moisture content is the most critical parameter for solid-state fermentation systems. In parallel, the moisture content parameter is the most effective parameter for ASX production in all the fermentation systems studied in this work (Table 6). The interactions of the moisture content with the pH and temperature induced a higher ASX yield. Moreover, the interaction with high moisture content engendered an increase in the yield, whether or not the other parameter had a high or low value. The water content of the synthetic YMB media was higher than the waste media when prepared with 90% water. However, the amount of ASX produced in those waste media was higher (Table 4). It is thought that the nutrient content of the wastes has an enhancing effect on the yield whether or not the other parameters changed. Improving effects of medium ingredients such as vitamins and trace elements on ASX synthesis were reported by Shewe et al. (2017). The increasing temperature affected only the pigment production of *X. dendrorhous* in the lentil fermentation medium positively. In the wheat bran media fermented by the yeasts, the increasing temperature introduced favorable interactions with the pH and moisture content for the ASX production. The effect of pH on the ASX biosynthesis was positive for *X. dendrorhous* and wheat bran fermentation system. However, Shewe et al. (2017) reached the result of induced ASX production by lowering pH.

The ANN-based modeling approach was better in fitting the inputs/variables to the response in comparison to RSM. More accurate results in estimations using the ANN methodology can be understood by using the statistical (Table 8) and parity plots (Figure 6 and 7) for ASX production. It could be stated that ANN is a good and powerful tool for the modeling of the non-linearity of bioprocesses, whereas RSM is the most used method for the optimization of fermentation conditions. This statement is also supported by Shafi et al. (2018).

The media, including high levels and a 'no sugar' had an

adverse effect on ASX production in the submerged fermentation systems. The investigation of whether *S. salmonicolor* has potential as an ASX producer on an industrial scale resulted in the success of *X. dendrorhous*. The experimental design results showed that the solid fermentation system of the wheat bran and *X. dendrorhous* produced the highest ASX amount at the highest moisture content level. The lentil waste medium provided a quite high productivity and demonstrated that it could be a good resource for biosynthesis. The Artificial Neural Network was statistically determined to be the more effective and accurate modeling for astaxanthin biosynthesis from solid waste materials.

Acknowledgement

This study was funded by Scientific Research Foundation of Gaziantep University (BAP M.F.12.08).

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International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.3.10

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(3):182-190 (2019)

Effects of different hormone applications on phenological and pomological properties in some Raspberry (*Rubus idaeus* L.) species

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Abstract

The purpose of this study conducted in a raspberry garden in Karaagac central village of Usak province in 2017 and 2018 was to analyze the effects of phenological and pomological properties of different hormone applications on Heritage and Tulameen raspberry species in the ecology of Usak province. Our experiment was established as 3 replications with 10 plants in each replication. Hormones were applied as Giberallic acid (GA), Melatonin (Mel) and GA+Mel with 2 different doses (5 ppm and 10 ppm) and 2.5 ppm melatonin and 2.5 ppm GA mixture for GA+Mel 5 ppm and 5 ppm melatonin and 5 ppm GA mixture for GA+Mel 10 ppm twice before blooming and fruit set. When we analyze both species, we can see that blooming happens between 17th May and 18th June and the harvest is between 21st June and 12th September. In pomological measures, it was found that in both species fruit length was between 8.89 and 13.13 mm, fruit width was 9.76 and 13.68 mm and fruit weight was between 0.64 and 1.29 g. While pH is between 3.62 and 4.80 and SSC is between 9.27% and 13.82%, TEA is between 21.62% and 30.56%. While Total Phenolic content (ppm/GAE) is between 3.91 and 5.33, Total Flavonoid content (ppm/QE) is between 0.75 and 1.42 and Antioxidant activity (IC50) is between 43.66 and 175.66, vitamin C (ppm) is between 1009 and 2308.50 values. According to the results we obtained in our study, Mel 10 ppm application in Tulameen cultivar in terms of pomological measurements and Heritage varieties in terms of chemical results and hormone applications in 5 ppm dose can be suggested in general terms.

Keywords: Raspberry, Melatonin, Giberallic acid, Fruit quality

Introduction

Berry fruits include the genus and related species such as strawberries, raspberries, blackberries, gooseberries, ribes, blueberries, rosehips, blueberry-vaccinium, berberis vulgaris, blackthorns (Ağaoğlu, 2006) and they have very wide range in nature. Especially in Europe, Asia and North America in different climate areas they have sub-genus, species and inter-species hybrids (Onur, 2006). Although raspberry is a perennial plant in terms of its roots, its shoot is biennial and regeneration per year is its special characteristic that is not observed in many plants. In addition, another important characteristicis that it lays fruit in a short time. Raspberry is important for health. As its glucose structure is in the type of laevulose it is also suitable for the use of diabetic patients. Its juice has a positive effect in the flu and inflamatory diseases and also it is useful for rheumatic patients. In pharmacology the syrup obtained from its fruits is used for taste and odour for medicines. Its fruits are rich in vitamin C, organic acid and glucose. When consumed fresh, it is used as deuretic, appetiser, roborant and cathartic (Göktaş, 2011). It was revealed that ellagic acid, a phenolic acid with a strong anticancerogenic effect, had antiviral and antibacterial effects (Akiyama et al., 2001; Smerak et al., 2002). Ellagic acid is included most in red (Rubusideaus) and black (Rubusoccidentalis) raspberries among all fruits and vegetables and it has an anticancerogenic effect by inactivating cancer-causing chemicals in body (Stoner and Mukhtar, 1995). In addition, ellagic acid has an anti-aging effect. As a result of some studies, ellag-

Cite this article as:
Colak, A.M. and Saglam, B. (2019). Effects of different hormone applications on phenological and pomological properties in some Raspberry (Rubus idaeus L.) species.Int. J. Agric. Environ. Food Sci., 3(3), 182-190
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.10
Received: 04 August 2019 **Accepted:** 15 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 182-190
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

ic acid especially obtained from red raspberries was found to be blocking the development of some cancer cells (Glen and Halvorson, 2001; Kresty et al., 2001; McKenzie, 2000). Quercetin has anticancerogenic and antioxidant effects. The antioxidants such as quercetin and kaempferol may prevent the destructions by lipid peroxidation in cells of human body. Raspberry is an important source of quercetin and kaempferol flavonoids (Anonymous, 2002).

Giberallic acid has an important role in growth and development processes of a plant, germination, root elongation, leaf development and breeding. Giberallic acid is commonly considered as a compound which promotes the growth (Razem et al., 2006). Giberallic acid provides body elongation by stimulating cell proliferation and body elongation. It is effective in blooming and fruit coarsening. It grows the cells in elongation part and provides lateral elongation. It stimulates germination in seeds (Çelik, 1982; Hopkins, 1995). Giberallins are effective in the whole growth of a plant including leaves and roots as well as the body elongation. Direct application to the roots is not effective; however, the application that can reach to the tip of the shoots stimulates the development of young leaves and indirectly root development with the increase in photosynthesis (Salisbury and Ross, 1992).

In conducted studies so far it has been identified that melatonin functions as plant growth regulator and has been revealed that it has aging regulatory effects on rooting and shoot growth. It has an active role in the plant's response to the stress. It has been reported that melotonin directly acts like an antioxidant and provides an antioxidative response for the plant (Arnao and Hernandez-Ruiz, 2014; Tan et al., 2012; Zhang et al., 2013). An essential function of melatonin in plants is the defense against internal and environmental stress sources. Melatonin rate in plants is thought to be higher than the rate in animals. Melatonin has a role in the protection of chlorophyll, stimulation of photosynthesis and root development. Transgenic plants with improved melatonin content may lead to the attempts to increase plantive production in agriculture and improve overall health of people (Tan et al., 2012). Antioxidative effect of melatonin application has been presented in some plants (apples, rice and grapes) (Wang et al., 2012; Park et al., 2013; Vitalini et al., 2013). It has been reported that melatonin is a strong antioxidant and prevents the oxidative damage as a result of lipid peroxidation (Zhang et al.,1998; Longoni et al., 1988).

In the current study, by analyzing the effects of giberallic acid (GA) and melatonin (Mel) applicationson phenological and pomological structure in different species of raspberry plant with various use and consumption field and also serious benefits for human health in the ecology of Usak province, it is aimed to shed light for future studies that will investigate not only raspberry but also other berry fruits, make cotributions to the literature and provide new parameters to science.

Material and Method

Material

Plant Material

Our experiment was conducted in a 5-year-old Raspberry and blackberry garden with 5 decares of area in Mucevhir location of Karaağaç Village of Usak province with the coordinates of 38o38'51.54" north and 29o20'04.09" south. Heritage and Tulameen species which are 2 different latish raspberry (Rubus idaeus) species were used in our experiment. Our experiment was established as 3 replications with 10 plants in each replication. They are planted at 1.5 m. of interrow distance and 0.6 m. of intrarow distance.

Soil Properties of Experimental Area

Soil samples were taken from the experimental area and analyzed for physical and chemical properties in Uşak Provincial Directorate of Agriculture and Forestry Soil Laboratory (Table 1).

Table 1. Analysis Results of Experimental Area (Anonymous, 2018)

pH	Salt (micros\cm)	Lime (%)	Organic matter (%)	Saturation (ml)	Total N (%)	Useful P (ppm)	Useful K (ppm)
7.72	474	0.8	1.69	57	0.085	0.38	920

Although the raspberries do not have a special soil demand, they grow well in the organic matter-rich, light or medium-sized, deep, permeable, soil moisture-maintained drainage areas. pH of the soil should be between 6 and 7. Fertilization should be performed at the end of fall or at the end of winter in the form of N P K fertilization (Brand-Williams et al., 1995).

Method

There are seven application groups as Control, Giberallik acid (GA), Melatonin (Mel) and Giberallik acid + Melatonin (GA + Mel). Hormones were applied as GA 5 and 10 ppm, Mel 5 and 10 ppm and GA (2.5 ppm) + Mel (2.5 ppm) 5 ppm, GA3 (5 ppm) + Mel (5 ppm) 10 ppm mixtures before flowering and fruit attitudes before the two times.

Analyzed Properties in the Experiment

Phenological Properties

They are first blooming date, full blooming date, final blooming date, first harvest date, final harvest date, number of clusters, number of grapes in a cluster.

Pomological Properties

The yield per shoot and the fruit weight were calculated using the fruits taken randomly for each replication in harvest period in a scale with 0.1 g of sensitivity. Fruit length and width were calculated by a digital compass scaling the average length of 10 fruits taken accidently and average fruit length ofthe fruits belonging to the species was determined. pH was determined by Hanna brand of pH meter in the fruit juice obtained from randomly selected 10 fruits. Soluble solids content (SSC) was determined as % by a manual refractometer in the fruit juice obtained from randomly selected 10 fruits.

Titrable Acidity (TEA)

1 ml of fruit juice obtained from randomly selected 10 fruits was taken and completed to 50 ml with distilled water. For instance, it was titrated with sodium hydroxide (NaOH) until the pH value was 8.1. Calculations were determined as % in citric acid (Özdemir et al., 2001; Adak et al., 2003).

Determination of Vitamin C

The organic acid compositions of the samples were determined by Agilent brand 1260 model HPLC filtering the fruit juices with white tape filter paper first and then filtering by 25 micron of injector tip. For that purpose, ACE 5 C18 column (5µm, 250 mm x 4.6 mm) and UV detector were used. In the analysis carried out in the isocratic flow, 2% of KH2PO4solution was used as the mobile phase with orthophosphoric acid and the pH was adjusted to 2.3. In the analysis carried out at 30 °C in 0.9 mL/min flow rate and 10 µl of injection volume, organic acids were determined at 214 nm of wave length. Analysis period is 20 minutes. The amounts of organic acid components in the samples were calculated according to standard organic acid analysis results (Fadavi et al., 2005).

Identification of Total Phenolic Content (TPC)

By employing the Folin-Ciocalteu method, the TPC of blackberry juice extract was indicated. 4500 µL deionized water and 500 µL unsubtilised Folin-Ciocalteureagent were laced with 1000 µL extract. Following 60 seconds, 4000 µL of 7.5 % (w/v) aquatic Na2CO3 was mixed. And then, the solution was taken to 30 minutes of maturing period at 30 ºC, which was then followed by measuring the absorbance at 765 nm through employing an UV-Vis spectrophotometer. And the result was aligned with a gallic acid calibration curve. The all phenols were identified as gallic acid equivalents (mg gallicacid/g extract), the valves of which have been suggested as medium of triple assessment (Kähkönen et al.1999).

Identification of Total Flavonoid Content (TFC)The TFC of the plant extraction was identified by employing aluminum chloride colorimetricassay (Chang et al. 2002). To begin with, 0.5 ml aliquots of the extract and 0.01-1.0 mg/ml of quercetin were mixed with 2 ml of distilled water and then with 0.15 ml of sodium nitrite (5% NaNO2 w/v).Upon waiting for 6 minutes, 0.15 ml of it (10% AlCl3, w/v) was accompanied. The solutions were made to rest for 6 minutes more. The last volume was adjusted to 5 ml level by adding instantly the water under distillation, then it was mixed utterly and left to rest up to quarter of an hour. The absorbtion of each composition was identified at the level of 510 nm together with an empty tube as a controller. TFC was determined as mg quercetin equivalent to per gram of sample with the help of calibration curve of quercetin. Every test that indicates the level of extract was conducted for three times (n=3).

Idenetification of Antioxidant Activity (DPPH)

The DPPH (2.2-diphenyl-1-picrylhydrazyl) was conducted by employing Thaipong et al. technique (Thaipong et al. 2006). The existing solution was made up through solving 24 mg of DPPH into 100 ml of methanol which was then stocked at –20°C till necessity accurs(Brand-Williams et al. 1995). The working solution was derived by way of stirring 10 ml of existing solution with 45 ml of methanol so as to make the absorbtion of 1.1 ± 0.02 units at 515 nm emplaying the spectrophotometer Shimadzu UV Mini 1240. 150 µl plant extracts were put under reaction with 2,850 µl of the DPPH sol for an hour under the darkness. Afterwards, the absorbtion level was applied at 515 nm. The antioxidant level showed a demise in absorbtion value under the equation. The outcomes of the absorbtion were transformed into the table content via a standardized calibration curve. These outcomes were then noted in ascorbic acid equivalents (AAE). The extract which supplies 50% of radicalscavenging activity (IC50, the concentration of the sampletoscavenge 50% of the DPPH radicals) was counted up by the the graphic of scavengingpercentage against extract concentration. In order to achieve this goal, subtilization series (five different concentrations) were made up for every plant sample extract. The resulting valves were counted up and given in µg/ml.

Statistical Analysis

Analyses were made in SPSS 24.0 statistics software. Kruskal Wallis H test was used in the comparison of application groups. In case of significant differences among groups, Maan Witneey U test was used for dual comparisons instead of multiple comparison test. Maan Witneey U test was again used for the comparison of the plant in application.

Result and Discussion

Phenological Observations

We can see the phenological observation results of Heritage and Tulameen species in Table 2. While the first blooming date is 17th May in Heritage species, it is 22nd May in Tulameen species. Full blooming date in Heritage species is 31st May; however, it is 8th June in Tulameen species. When we look at the final blooming date, we can see that it is on 10thJune in Heritage species; however, it is on 18th June in Tulameen species. The first harvest in Heritage species in GA+Mel 5 ppm application is 21st June and it is 29th June in Control and GA+Mel 10 ppm applications, however, it is 23rd June in all the other applications. The first harvest started on 21st June in Tulameen species in Mel 5 ppm and 10 ppm applications and on 23rd June in all the other applications. We can see that final harvest date in Heritage and Tulameen species is 12th September.

Yıldız (2011) reported that in Heritage species first blooming was on 16th May, full blooming was on 19th May, final blooming was on 24th May, first harvest was on 20th June and final harvest was on 20th July; however, in Tulameen species first blooming was on 15th May, full blooming was on 24th May, final blooming was on 27th May, first harvest was on 20th June and final harvest was on 30th July. Aydemir; in a study in 2008, stated that in open-air cultivated Heritage I species first blooming was on 7th May, full blooming was on 11th May, final blooming was on 21st May, first harvest was on 11th June and final harvest was on 20th October; however, in Tulameen species first blooming was on 13th May, full blooming was on 17th May, final blooming was on 30th May, first harvest was on 19th June and final harvest was on 29th July. Ada (2014); in his/her study, stated that first blooming date was between 21st and 24th May, 2013, final blooming was between 3rd June and 23rd August, 2013, first harvest

was between 27th and 31st May, 2013 and final harvest was between 17th June and 1st October, 2013. Öz (2006) found the first blooming date between 7th and 13th May, full blooming date between 13th and 18th May, final blooming date between 20th and 24th May, first harvest between 8th and 17th June and final harvest between 9th and 23rd July. Pehluvan (2000) observed blooming in Heritage between 8th June,1999 and 15th June,2000, in Tulameen between 22nd June,1999 and 28th June, 2000. Cangi and İslam (2003) found that harvest period started in the first week of June and lasted until the end of September and as a conclusion Heritage species was found to be the most suitable species for the region. When these studies are analyzed, it can be seen that our study is more similar to Yıldız's study; however, the potential differences between the years among studies and the ecologies of the study zones should be considered.

Table 2. Phenological Observation Results of Heritage and Tulameen Species

Species	Applications	First Blooming	Full Blooming	Final Blooming	First Harvest	Final Harvest
Heritage	Control	17.05	31.05	10.06	29.06	12.09
	GA 5 ppm	17.05	31.05	10.06	23.06	12.09
	GA 10 ppm	17.05	31.05	10.06	23.06	12.09
	Mel 5 ppm	17.05	31.05	10.06	23.06	12.09
	Mel 10 ppm	17.05	31.05	10.06	23.06	12.09
	GA+Mel 5 ppm	17.05	31.05	10.06	21.06	12.09
	GA+Mel 10 ppm	17.05	31.05	10.06	29.06	12.09
Tulameen	Control	22.05	08.06	18.06	23.06	12.09
	GA 5ppm	22.05	08.06	18.06	29.06	12.09
	GA 10 ppm	22.05	08.06	18.06	23.06	12.09
	Mel 5 ppm	22.05	08.06	18.06	21.06	12.09
	Mel 10 ppm	22.05	08.06	18.06	21.06	12.09
	GA+Mel 5 ppm	22.05	08.06	18.06	23.06	12.09
	GA+Mel 10 ppm	22.05	08.06	18.06	23.06	12.09

When we look at Table 3 indicating the number of grapes in a cluster and the number of clusters in a plant in Heritage and Tulameen species, we can see that maximum number of clusters is in Mel 5 ppm with 5.67; however, minimum number of clusters is in GA+Mel 5 ppm group with 2.67 in Heritage species. In Tulameen species maximum number of clusters is observed in Mel 10 ppm with 17.33 and GA+Mel 10 ppm applications with 17.00. When we looked at the difference in the number of clusters in plants among the applications of two species, a statistically significant difference was observed in all applications except for GA 5 and 10 ppm doses (P<0,05). When we analyze the number of grapes in a cluster, approximately the same values (between 3.53 and 4.30) were statistically obtained in Heritage species and the highest values were obtained in Control, GA 5 ppm and Mel 5 and 10 ppm applications in Tulameen species. When we look at the difference in the number of clusters in plants among the applications of two species, we can see that there is a statistical difference in GA 10 ppm and GA+Mel 5 and 10 ppm applications (P<0,05).

Table3. The Number of Clusters in a Plant and Number of Grapes in a Cluster in Heritage and Tulameen Species

Application	Number of Clusters (number/shoot)			Number of Grapes in Cluster (number)		
	Heritage	Tulameen	P	Heritage	Tulameen	P
Control	4.67ab*	8.00b	0.010	3.90	4.03a	0.589
GA 5 ppm	4.33ab	12.67ab	0.162	3.77	3.60ab	0.422
GA 10 ppm	4.33ab	8.67b	0.249	3.73	2.97b	0.051
Mel 5 ppm	5.67a	13ab	0.018	3.53	3.27ab	0.502
Mel 10 ppm	5.00ab	17.33a	0.000	3.60	3.70ab	0.798
GA+Mel 5 ppm	2.67b	10.67b	0.026	4.07	2.93b	0.058
GA+Mel 10ppm	4.33ab	17.00a	0.000	4.30	2.87b	0.028
Overall Average	3.81	12.48	<0.0001	3.84	3.31	<0.0001

*There is a significant difference between applications at p<0,05 level

Aydın (2008); in his study,stated that the annual average number of clusters was 11.83in Heritage I and 10.77 in Heritage II species and 10.20 in Tulameen species; however, the number of grapes in a cluster was 6.32 in Heritage I, 6.05 in Heritage II and 5.54 in Tulameen species as the average of two years. While Yıldız (2011) found the average number of

clusters as 6.67 in Heritage and 5.35 in Tulameen species, he found the average fruit number in a cluster as 5.15 in Heritage species and 3.42 in Tulameen species. Öz (2006) found that the average cluster number per fruit was between 3.33 and 4.43 according to the data he obtained in 2004; however, the number of fruit clusters on annual shoots varied between 16 and 49.5. When we look at other research, we can see that in the current study the number of clusters is a bit more in Tulameen species in general; however, the number of grapes in clusters is less, but not much different.

Pomological Measurements

The fruit size, fruit width, fruit weight and yield per shoot are presented in Table 4 for the applications on the species used in the experiment. The maximum fruit length in Heritage species was obtained in Control and Mel 10 ppm applications. The highest value in the fruit length in Tulameen species was observed in Mel 10 ppm application. When the differences in fruit size of the plants among the applications of two species were analyzed, it was observed that there was a statistically significant difference in all applications except for Control (P<0,05). In the fruit width values of these two species, the highest values in Heritage species were in Control, GA 10 ppm and Mel 10 ppm applications. In Tulameen species approximately the same values were obtained in all the applications. When the differences in fruit width of the plants among the applications of two species were analyzed, it was observed that there was a statistically significant difference in all the applications except for Control and Mel 10 ppm applications (P<0,05). The highest values in fruit weight in Heritage species were in Control and Mal 10 ppm applications and they were in Mel 10 ppm and GA+Mel 10 ppm in Tulameen species. When the differences in fruit weight of the plants among the applications of two species were analyzed, it was observed that there was a statistically significant difference in all the applications except for Control application (P<0.05). While the highest values in yield amount per shoot in Heritage species were obtained fromControl, Mel 5 and 10 ppm applications, the highest values in Tulameen species were obtained from Mel 10 ppm application. When the differences in yield per shoot of the plants among the applications of two species were analyzed, it was observed that there was a statistically significant difference in all the applications except for GA 5 and 10 ppm applications (P<0,05).

Table 4. Length, Width, Weight and Yield per Shoot Measurement Results of Heritage and Tulameen Species

Application	Length (mm)			Width (mm)			Weight (g)			Yield per Shoot (g)		
	Heritage	Tulameen	P	Heritage	Tulameen	P	Heritage	Tulameen	P	Heritage	Tulameen	P
Control	10.5a*	10.74c	0.380	12.15a	13.2	0.238	0.91a	093c	0.724	84.17a	239.56d	0.000
GA 5 ppm	8.59b	11.56bc	0.005	9.76b	13.07	0.007	0.64b	1.10abc	0.006	45.87ab	775.60bc	0.166
GA 10 ppm	9.96ab	11.41bc	0.004	11.79a	13.21	0.038	0.79ab	1.0bc	0.011	58.72ab	254.67d	0.312
Mel 5 ppm	9.84ab	11.99abc	0.004	10.97ab	13.30	0.007	0.77ab	1.16ab	0.001	92.65a	648.44bcd	0.031
Mel 10 ppm	10.94a	13.13a	0.017	12.17a	13.68	0.121	0.94a	1.29a	0.005	87.42a	1427.60a	0.002
GA+Mel5 ppm	9.61ab	11.87abc	0.001	11.21ab	13.46	0.004	0.78ab	1.17ab	0.003	22.52b	381.42cd	0.028
GA+Mel 10ppm	9.81ab	12.45ab	0.001	11.24ab	13.54	0.004	0.75ab	1.27a	0.002	66.75ab	1051.98ab	0.002
Overall Average	9,93	12.03	<0.0001	11.38	13.37	<0.0001	0.80	1.15	<0.0001	65.46	489.33	<0.0001

*There are significant differences between applications at p<0,05 level

From Heritage species,Yıldız(2011) found the average fruit length as 15.63 mm, the average fruit width as 15.89 mm and the average fruit weight as 2.07 g. For Tulameen species, he/she found the average fruit length as 17.84 mm, the average fruit width as 18.69 and the average fruit weight as 2.88 g. According to Aydemir (2008), in Heritage I fruit length is 15.29 mm, fruit width is 15.47 mm and fruit weight is 2.14 g. In Tulameen species fruit length is 18.03 mm, fruit width is 15.55 mm and fruit weight is 2.05 g. In Heritage II species fruit length is 14.41 mm, fruit width is 16.38 and fruit weight is 2.03 g. Yılmaz (2007) found the fruit weight as 2.62 g, the fruit diameter as 16.14 and the fruit length as 16.75 mm. Ada (2014) in his study found the fruit length between 11.83 and 13.46 mm, the fruit width between 11.53 and 14.23 mm and the fruit weight between 1.31 and 1.70 g. Öz (2006) in his study in Tokat on Rubin raspberry found the average fruit length as 1.99 mm, fruit diameter as 13.49 mm and fruit weight as 1.28 g. While Pehluvan (2000) found the fruit weight in Heritage species as 2.23 g, he/she found it in Tulemeen species as 2.31 g. Cangi and İslam (2003) determined the fruit weight between 1.08 and 2.26 g and as a result found out that Heritage species was the most suitable species for the region. Küçükhüseyin (2017) determined the fruit weights of the species between 2.11 (Heritage) and 2.23 (Canby) g according to the average of two years. According to the study result, Heritage species stood out in terms of the analyzed properties. According to the data obtained by Eke (2017), it was determined that the fruit width in wild raspberries was 14.8 mm, in wild blackberries it was 12.8 mm, in wild blueberries it was 8.7 mm; the fruit length in wild raspberries was 13.0 mm, in wild blackberries it was 14.6 mm and in wild blueberries it was 9.4 mm; the fruit weight in wild raspberries was 12.7 g, in wild blackberries it was 11.8 g and in

wild blueberries it was 4.0 g. While Yıldız (2011); in his/her study, found the amount of yield per shoot in Heritage species in 2009 as 82.03 g, he/she found it as 60.17 g in 2010 and in Tulameen species he/she found it as 53.54 g in both 2009 and 2010. While Aydın (2008) obtained the yield per shoot as a result of his study in Heritage I in 2007 as 87.79 g, in 2008 as 96.86 g and in Heritage II in 2007 as 121.46 g and in 2008 as 127.54 g, he/she obtained it in Tulameen species in 2007 as 94.43 g and in 2008 as 104.72 g. Atila (2002); in his/her adaptation study, obtained 18.20 g of yield per shoot in Heritage I species, 22.40 g of yield per shoot in Heritage II and 50.30 g of yield per shoot in Tulameen species. Aydemir (2014); in his/her study,obtained the yield per shoot in outdoor cultivation in Heritage species in 2006 as 74.32 g, in 2007 as 24.90 g, in Tulameen species in 2006 as 51.01 g and in 2007 as 44.35 g and in Heritage II species in 2006 as 82.04 g and in 2007 as 46.47 g. When the current study carried out in Usak and the previous studies are compared, we can see that lower values in the fruit length, width and weight of Heritage and Tulameen species are obtianed in the current study; however, in the amount of yield per shoot, higher values are obtained in Tulameen species.

According to Table 5 which indicates some chemical properties of Heritage and Tulameen species, there is no significant difference in all the groups when we analyze TEA analysis results of Heritage species and near results are obtained in all applications in Tulameen species and there is no significant difference. When we look at the difference of TEA in plants among applications of two species, no significant difference is observed among the applications ($P<0.05$). No significant difference is obtained in pH analysis results of the applications in Heritage species and also there is no significant difference when pH analysis results are analyzed in Tulameen species. When we look at the pH difference in plants among applications of two species, we can see that there is a statistical difference in GA 5 ppm and GA+Mel 10 ppm applications ($P<0,05$). While the highest value is obtained in GA 5 ppm application in Heritage species in SSC values, the lowest value is obtained in Mel 5 ppm application. When the difference in SSC of the plants among the applications of two species is analyzed, we can seethat there is a statistical difference in GA 10 ppm, GA+Mel 5 ppm and GA+Mel 10 ppm applications ($P<0,05$).

Table 5.TEA, pH, SSC Measurement Results of Heritage and Tulameen Species

Application	TEA (%)			pH			SSC (%)		
	Heritage	Tulameen	P	Heritage	Tulameen	P	Heritage	Tulameen	P
Control	26.61	29.78a	0.5	4.15a	3.72a	0.552	11.93abc	10.92a	0.164
GA 5 ppm	27. 35	22.46a	0.060	4.80a	3.85a	0.008	13.82a	9.86bc	0.105
GA 10 ppm	26.75	21.61a	0.219	4.27a	4.03a	0.440	11.13abc	9.85bc	0.017
Mel 5 ppm	25.72	21.76a	0.219	4.37a	4.00a	0.258	10.04c	10.32ab	0.476
Mel 10 ppm	25.26	26.85a	0.5	4.28a	3.62a	0.133	10.58bc	11.18a	0.258
GA+Mel 5 ppm	30.56	26.73a	0.219	4.27a	3.68a	0.124	13.27ab	9.27c	0
GA+Mel 10 ppm	28.63	26.93a	0.5	4.54a	3.84a	0	12.11abc	10.53ab	0.023
Overall Average	27.27	25.16	<0.0001	4.39	3.83	<0.0001	11.66	10.23	<0.0001

*There are significant differences among the applications at p<0,05 level

Ada (2014) found SSC value between 14.4% and 16.3%, pH value between 2.2 and 2.6 and TEA value between 3.3% and 5.6%. While Yılmaz (2007) found 2.23 for TEA, 3.67 for pH and 13.87 for SSC, Aydemir (2008) in his/her study found pH value in Heritage I as 3.53, SSC value as 11.41 and TEA value as 2.97; however, he/she found pH value in Tulameen species as 3.72, SSC value as 12.57 and TEA value as 2.31. While Aydın (2008) in his/her study found pH value in Heritage as 2.81, SSC value as 9.45 and TEA value as 1.33, he/she found pH value in Tulameen as 3.26, SSC value as 7.16 and TEA value as 1.02. Öz (2006) found pH value as 3.60, SSC value as 11.26 and TEA value as 2.77 g/l. Küçükhüseyin (2017) determined soluble solid contentof the species between 9.70% (Aksu red) and 10.10% (Tulameen) and titrable acid rate between 2.43% (Tulameen) and 2.54% (Hollanda Boduru) according to the average of two years. According to the study results, Heritage species stood out in terms of the analyzed properties. Pehluvan (2000) found SSCin Heritage species as 10.23%, in Tulameen species as 9.48% and he/she found TEA in Heritage species as 4.09% and in Tulameen species as 2.97%. Cangi and İslam (2003) determined that soluble solid contentvaries between 10.30% and 13.80% and as a result it was decided that Heritage was the most suitable species for the region. Eke (2017) found SSC in wild raspberry as 13%, in wild blackberry as 11.1% and in wild blueberry as 10.2%; he/she found TEA in wild raspberry as 1.93%, in wild blackberry as 1.53% and in wild blueberry as 1.68 %. When the existing research is reviewed, it is seen that there are some similarities and differences with the current study. TEA values in the current study are close to the ones found in the studies of Yıldız, Aydemir andKüçükhüseyin. When pH values are analyzed, values close to the ones found the studies of Yılmaz, Aydemir and Öz are obtained in the current study; however, we can see that there are not too many differences with other studies. SSC values in the current study are lower than those reported by Ada, but we can see that they are close to the ones reported by

Aydemir and Yılmaz's studies and higher than those reported by Aydın and Küçükhüseyin.

According to the analysis results in Table 6, while the highest value in Heritage species in total phenolic content was found in GA 5 ppm application, the lowest value was found in GA+Mel 5 ppm application; the highest value in Tulameen species was found in GA+Mel 10 ppm and nearly the same values were found in all the other applications. When we look at the difference of total phenolic content in plants among applications of two species, a statistically significant difference was observed in all the applications except for GA+Mel 5 ppm (P<0,05). According to the results of the analysis of total flavonoid content in Heritage species, the highest value was obtained in all the applications except for Mel 10 ppm species and the highest value in Tulameen was obtained in GA and Mel 5 ppm applications and the lowest value was obtained in GA+Mel 5 ppm application. In the difference of total flavonoid content in plants among applications of two species, a statistically significant difference was observed in all the applications except for Control, GA 10 ppm, Mel 5 ppm and GA+Mel 10 ppm applications(P<0.05). According to antioxidant activity analysis results, the highest value in Heritage species was in Mel 5 ppm application; however, the lowest value was in Control group. While the highest value in Tulameen species was in Control group, the lowest value was in GA 10 ppm application. When the difference of antioxidant activity in plants among applications of two species was evaluated, a statistically significant difference was observed in all the applications except for Mel 10 ppm, GA+Mel 5 ppm and GA+Mel 10 ppm applications (P<0,05). In vitamin C values of Heritage and Tulameen species, while the highest value in Heritage species was observed in GA+Mel 10 ppm, the lowest value was observed in GA+Mel 5 ppm applications. While the highest value in Tulameen species was observed in GA 5 ppm and GA+Mel 5 ppm applications, the lowest value was observed in GA+Mel 10 ppm application. When the difference of vitamin C in plants among applications of two species was evaluated, no difference was observed among applications (P<0.05).

Table 6.Total Phenolic, Total Flavonoid, Antioxidant Activity and Vitamin C Measurement Results of Heritage and Tulameen Species

Application	Total Phenolic (ppm/GAE)			Total Flavonoid (ppm/QE)			Antioxidant Activity (IC50)			Vitamin C (ppm)		
	Heritage	Tulameen	P	Heritage	Tulameen	P	Heritage	Tulameen	P	Heritage	Tulameen	P
Control	4.46bc*	3.96b	0.02	1.42a	1.16ab	0.06	95c	153.66a	0.02	1365ab	1731.50ab	0.06
GA 5 ppm	5.33a	4.37b	0.04	1.15a	1.28a	0.04	137.50b	103.66b	0.04	1503ab	2221.50a	0.06
GA 10 ppm	4.54bc	4.09b	0.02	1. 29a	1.11ab	0.25	105bc	43.66c	0.02	1516.50ab	1560.50bc	0.5
Mel 5 ppm	4.65b	4.26b	0.02	1.13a	1.31a	0.08	175.66a	111.33b	0.02	1488.50ab	1200bc	0.06
Mel 10 ppm	4.25cd	4.49b	0.02	0.75b	1.186ab	0.02	128.33bc	115.66b	0.41	1455ab	1250.50bc	0.21
GA+Mel 5 ppm	3.91d	4.15b	0.13	1.34a	0.98b	0.02	109bc	107.66b	0.41	1314.50b	2308.50a	0.06
GA+Mel 10 ppm	4.19cd	5.25a	0.04	1.17a	1.12ab	0.5	130bc	119.33b	0.18	1866.50a	1009c	0.06

*There are significant differences among applications at p<0.05 level

Phenolic compounds (mg/100g in fresh fruit) were found as 113.73-177.6 mg (De Ancos et al., 2000), 192-359 mg (Anttonen and Karjalainen, 2005), 517 mg (Wada and Ou, 2002) and 330 mg (Proteggente et al., 2002) in another studies. The amount of anthocyanin (mg/100 in fresh fruit) was found as 65 mg (Wada andOu, 2002), 19-51 mg (Anttonen and Karjalainen, 2005) and 35.1-49.1 mg (Pantelidis et al., 2007). Antioxidant capacity was found by (µmol Trolox/g) Proteggente et al. (2002) as 18.49.When we look at the vitamin C values in Pahluvan'sstudy (200), it was determined that Heritage species had 28.92mg/100 g of vitamin C content, Tulameen species had 24.27mg/100 g of vitamin C content. In the current study, it was determined that Heritage and Summit species with two yields per year, Newburg species with only one yield per year could adapt to the region better than other species. Aydın (2008) found the vitamin C (mg/100g) amount as 21 mg in Heritage species; however, it did not find any values in Tulameen species. According to the chemical measurements, Eke (2017) obtained the phenolic matter (µg GAE/g ta) in wild raspberry as 1108, in wild blackberry as 1580 and in wild blueberry as 1308; he obtained the antioxidant(µmol TE/g ta) in wild raspberry as 14.95, in wild blackberry as 24.05 and in wild blueberry as 21.35; he obtained the anthocyanin (µg siy-3-glk/g ta) in wild raspberry as 203.36, in wild blackberry as 303.39 and in wild blueberry as 256.19. According to the results of Sezgin and Çelik (2015), tirable acidity value (TEA) was 0.71 g/100ml and antioxidant level was 2,100-2,240 µ mol TEAC. When some pomological properties of the current study conducted in Usak are compared with other studies, total phenolic contents found in the current study are higher. When vitamin C values are analyzed, it is seen that the values in Pehluvan's study are higher; however, there are not so many differences. In adaptation study by Aydın, the value found in Heritage species is very close to the value found in the current study.

Conclusion

According to the results in the current study, Mel 10 ppm application in Tulameen in terms of pomological measurements and Heritage species in general in terms of chemical results and the 5 ppm doses of hormone application in terms of hormone application can be recommended. While Tulameen species can be recommended for fresh consumption due to fruit size, Heritage species can be recommended for industry due to important chemical contents. In our study, while evaluating the

results obtained from the same or different results from other studies, the characteristics of the places where the studies are carried out, such as climate, soil, and time difference between these studies should be taken into consideration. While this kind of studies are carried out in different fruit species in the world, the studies on berry fruits have just become a current issue in our country and it is thought that our study will contribute to this topic. Both in our city Usak and in our country, the fact that raspberry and hormone studies go on gradually may include more new parameters to science.

Acknowledgement

This article is summarized from the master thesis of Büşra SAĞLAM. This study was supported by Uşak University Scientific Research Projects Coordination Unit (UBAP) 2017/TP033 project.

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International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.3.11

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(3):191-196 (2019)

Antioxidants, protein, oil content and fatty acids profiles of chia seeds (*Salvia hispanica* L.) genotype Tzotzol growing in three tropical ecosystems of Bolivia, Ecuador and Paraguay

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Abstract

Chia is a summer annual of the Lamiaceae. The objective of this study was to investigate the effect of growing location on the antioxidants content and composition in one genotype of chia, and the potential relationship with its major nutritional compounds, as protein, oil, and fatty acids. This study was carried out with black spotted chia seeds commercially grown in three different ecosystems, Tropical Rain Forest, Sub Humid Chaco, and Campo Cerrado, located in Ecuador, Bolivia and Paraguay, respectively. Flavonols quercetin, myrcetin, kaempherol, caffeic acid, chlorogenic acid, and SDG lignan compound presence was detected by chromatographic analysis. No significant ($P<0.05$) differences between seed origins= were found. Total oil content was significantly ($P<0.05$) higher in the seeds from Ecuador (34.2%) than all other locations, followed by the seeds from Bolivia (32.5%) which was significantly ($P<0.05$) higher compared to Paraguay (31.6%). The content of α linolenic fatty acid in seeds from Ecuador was significantly ($P<0.05$) higher compared to the seeds from all three locations. No significant correlation ($P<0.05$) between α -linolenic fatty acid and polyphenols content was detected. The results indicate that protein content, oil content and fatty acid profile characteristics of the chia are affected by the different ecological conditions of the tested ecosystems, which not affect the polyphenols content, and composition.

Keywords: *Salvia hispanica* L., Chia, Antioxidants, Fatty acids, Protein

Introduction

Chia (Salvia hispanica L.) is a summer annual of the Lamiaceae family. It was one of the basic foods of several Central American civilizations in pre-Columbian times. Tenochtitlan, the capital of the Aztec Empire, received 5B15,000 tons of chia annually, as a tribute from conquered nations (Codex Mendoza 1542). Chia seed was also part of holy ceremonies as an offering to the Aztec gods (Sahagun 1579). Apparently religious persecution, as well as the fact that it could not be grown in Europe, was essentially the reason of its disappearing for 500 years (Ayerza and Coates 2005a).

Chia oil contains one of the highest known concentrations of α linolenic fatty acid, up to 67.8% (Coates and Ayerza 1996). Recently, chia seed has become important for health and nutrition because its ω 3 fatty acid content promotes beneficial health effects in laboratory animals as well in humans (Ayerza and Coates 2005b; Vuksan et al. 2007). A number of studies have demonstrated good oxidative stability of chia seed when used as animal feed or as a food ingredient, with this being attributed to the high antioxidant activity of the phenolic compounds it contains (Taga et al. 1984; Reyes-Caudillo et al. 2007

Chia seed contains chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol flavonols. These compounds are both primary and synergistic antioxidants, and contribute in a major way to the strong antioxidant activity of chia (Taga et al. 1984; Castro Martinez et al. 1986). There is evidence that phenolic substances act as antioxidants by preventing the oxi-

Cite this article as:
Ayerza, R. (2019). Antioxidants, protein, oil content and fatty acids profiles of chia seeds (Salvia hispanica L.) genotype Tzotzol growing in three tropical ecosystems of Bolivia, Ecuador and Paraguay. Int. J. Agric. Environ. Food Sci., 3(3), 191-196
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.11
Received: 19 February 2019 **Accepted:** 16 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 191-196
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

dation of LDL lipoprotein, platelet aggregation, and damage of red blood cells (Cheynier 2005). Additionally, phenolics act as metal chelators, antimutagens and anticarcinogens, antimicrobial agents and clarifying agents (Proestos et al. 2005).

Recently Ayerza (2013) reported the detection of secoisolaricresorcinol diglucoside (SDG) compound in two genotypes of chia seeds. SDG is a lignan compound, which has antioxidant activity (Hosseinian 2006), and the great oxidative stability of chia oil could be attributed not just to the flavonols compounds content but to the SDG content as well.

Early papers demonstrated that location affected the oil, protein and fatty acid content of chia seed, presumably due to one or more environmental factors, such as temperature, light, soil type and available nutrients (Ayerza 2009, 2010, 2011). Thus, any possibility of variability in the polyphenols contents and composition needs to be explored. The objective of this study was to investigate the effect of growing location on its antioxidants content and composition in one genotype of chia, and its potential relationship with its major nutritional compounds, such as protein, oil, and fatty acids.

Materials and Methods

Samples

This study was carried out with black spotted chia seeds commercially grown in three different ecosystems, Tropical Rain Forest, Sub Humid Chaco, and Campo Cerrado, located in Ecuador, Bolivia and Paraguay, respectively (Table 1). The black spotted seeds belong to the Tzotzol variety as was reported by Ayerza and Coates (2005b).

Table 1. Locations where *Salvia hispanica* L. was grown.

Ecosystem	Country	Latitude	Elevation	Temp	Rainfall	Soil type
				Mean/year		
			m	EC	mm	
Sub-Humid Chaco	Bolivia	17°17' 00'' S.	265	24	1,157	Mollic planosols
Tropical Rain Forest	Ecuador	02E18' 00'' S.	300	25	>3,000	Regosol lateritico
Campo Cerrado	Paraguay	22E65' 59'' S.	168	23	1,600	Ultisol

Within the three ecosystems where the chia was grown, representative commercial fields were selected for sampling. The harvested seed was stored in 25 kg bags and random samples were taken. Samples were collected following the seed sample instructions of the Canadian Food Inspection Agency (2008). The samples were cleaned by hand and sent to the laboratory for analysis. The experimental design used was completely randomized, with six replications.

Chemical Analysis

Crude nitrogen of the chia seed samples was determined by standard micro Kjeldahl method and then converted to protein content using a 5.71 conversion factor (AOAC 1995).

Lipids were extracted from the samples according to the method described by Folch et al. (1957). Total lipids were then converted into fatty acid methyl esters using the IRAM 5 560II method (IRAM 1982), which is equivalent to ISO 5509 1978 item 6 (ISO 1978). Fatty acid methyl esters were separated and quantified by automated gas chromatography (Model 6890, GC; Hewlett Packard Co., Wilmington, DE, USA) equipped with flame ionization detectors and a 30 m 9 530 lm i.d. capillary column (Model HP FFAPFree fatty acid phase; Hewlett Packard Co., Wilmington, DE, USA). The temperatures of the oven, injector, and detector were set at 180, 290 and 330 EC, respectively. The fatty acid composition of each sample was determined by integrating the recorded peaks using Hewlett Packard Chem Station Software. Results were expressed as percentage of total fatty acids.

The peroxide values were determined by ISO 3960/1977 procedure; results were expressed as meq oxygen/kg (AOAC 2002).

Flavonol analysis performed using HPLC by methodology adapted from Chang et al. (1997); utilizing water acetonitrile (80:20) extract separated on a LiChrospher RP 18 column (Merck Chemicals, Basel, Switzerland), with mobile phase gradient elution of water acetonitrile (0 10 min 80:20, 14 25 min 63:37) employing a flow rate of 1.0 ml/min with detection at 270 nm. Caffeic acid analysis performed using HPLC by method adapted from Adzet et al. (1985); utilizing samples extracted into acetone and subjected to chromatography on a column (150x4.5mm) of Spherisorb C18 (5 µm) (Waters Corporation, Milford, MA, USA), eluted with a gradient mobile phase of 2.5% of acetic acid in aq. methanol with a linear gradient of 13 to 43% of methanol during 30 min, and detection by photo diode array (200 40nm) with UV detection at 325 nm. Isoresorcinol analysis performed using HPLC by method adapted from Charlet et al. (2002); utilizing acid hydrolysis, necessary for the release of lignan from their complex form to form free aglycone, subjected to separation on a Waters Symmetry C18 3ìm column (150x4.6mm) (Waters Corporation, Milford, MA, USA) eluted with a gradient mobile phase of water (95%), acetonitrile (5%) changing linearly in 20 min to water (50%), acetonitrile (50%), with diode array detection.

Statistical Analysis

A one way analysis of variance (ANOVA) was performed for oil, individual fatty acid content, protein content, peroxide value and polyphenols contents. When the F value was significant ($P<0.05$), means were separated using Student-Newman-Keuls Test (Cohort 2006). Additionally, correlation and regression analysis were undertaken to develop the relationship between measured parameters (Cohort 2006).

Results and Discussion

Total water, protein content, oil content, and peroxide value

Total water, protein content, oil content, and peroxide value, are summarized in Table 2.

Water content and peroxide value were not significantly (P

< 0.05) affected by location. All the

values were lower compared to those reported by Ayerza and Coates (2004) for chia seeds from Colombia, Peru and Argentina. However, all these values are within the range of two genotypes of chia grown in five different locations of Ecuador (Ayerza 2013).

Oil and protein contents, as a percentage of chia seed weight, showed significant

(P < 0.05) differences among locations. Total oil content was significantly (P<0.05) higher in the seeds from Ecuador than all other locations, followed by the oil content of seeds from Bolivia which was significantly (P<0.05) higher compared to that from Paraguay.

The seeds from Bolivian and Paraguayan ecosystems showed significantly (P < 0.05) higher protein content as a percentage of chia seed weight, compared to Ecuador. No significant (P < 0.05) difference in protein content was detected between the other two locations.

The results presented herein support the contention that ecosystem has a strong effect on the protein and oil content of chia seeds (Ayerza 1995, 2010, 2011), as it was reported for many other crops (Mohammed et al. 1987; Vollmann et al. 2007). A positive causeBeffect relationship between temperature and protein, and a negative one between temperature and oil content in oil seed crops such as soybean have also been reported (Thomas et al. 2003; Kumar et al. 2006).

Table 2. Water, protein, oil, and peroxide value of *Salvia hispanica* L.

Origin	Water	Protein	Oil	Peroxide Value
		%		meq of O_2/kg
Bolivia	5.9[a1]	23.1[a]	32.5[b]	0.425[a]
Ecuador	5.7[a]	19[b]	34.2[a]	0.605[a]
Paraguay	5.8[a]	23.25[a]	31.6[c]	0.415[a]
SD[2]	0.389	1.047	0.636	0.196

[1] Means in a column within a group with the same letter are not statistically different (P<0.05); [2] Least significant difference for P<0.05.

Results of the fatty acid compositional analyses

Results of the fatty acid compositional analysis by origin are presented in Table 3. Gas chromatography analysis of the oil composition of seeds from all locations detected the presence of α linolenic fatty acid, followed by linoleic, oleic, palmitic and stearic fatty acids. In addition, six more fatty acids were identified in all analyzed seed samples, myristic, arachidic, gadoleic, behenic, eracic, and lignoceric. However, as all of them were present just in traces, those fatty acids were omitted for this report.

Polyunsaturated ω 6 linoleic fatty acid, the second largest component of chia seed oil, was significantly (P < 0.05) lower in seeds from Ecuador than the other two locations; no significant (P < 0.05) differences were detected between seeds from Bolivia and Paraguay.

The main constituent in the oil was polyunsaturated ω 3-α linolenic fatty acid. The seed from Ecuador showed significant (P<0.05) higher content of α linolenic fatty acid comparing to all three locations. No significant (P<0.05) differences were detected between seeds from the other two locations. The present study confirmed that the fatty acid composition of chia oil is influenced by the effects of factors such as soil=s quality and climatic and weather conditions, as it was demonstrated in an early report (Ayerza 1995).

Table 3. Fatty acid composition of *Salvia hispanica* L.

Origin	Palmitic	Stearic	Oleic	Linoleic	α-Linolenic	ω-6:ω-3 rate	α-Linolenic
			% of total fatty acids				g/kg of seed
Bolivia	6.3[a1]	3.9[a]	8.95[a]	21.15[a]	58.5[b]	0.36[a]	19.01[b]
Ecuador	6.5[a]	3.6[b]	6.65[b]	17.5[b]	64.5[a]	0.27[b]	22.06[a]
Paraguay	7.3[a]	3.4[c]	8.85[a]	20.9[a]	59[b]	0.35[a]	18.64[b]
SD[2]	1.102	2.42	0.225	0.29	1.537	0.123	0.7

[1] Means in a column within a group with the same letter are not statistically different (P < 0.05); [2] Least significant difference for P < 0.05.

The seeds from Ecuador showed significantly (P<0.05) lower content of oleic and linoleic fatty acids, compared to seeds grown in the other two locations. Overall, the α-linolenic fatty acid was negatively correlated with linoleic and oleic fatty acids content; computed for these negative relationships, the regression coefficients (R2) and significance levels (P) were R2= 0.993 (P < 0.001), and R2= 0.994 (P < 0.001), respectively. The negative relationships of α linolenic fatty acid contents with the 18 C more saturated fatty acids, linoleic and oleic, were reported for a number of crops, such as almonds (Abdallah et al. 1998), chestnuts (Pires-Borges et al. 2007), soybeans (Thomas et al. 2003), flaxseed, a rich source of α linolenic fatty acid (Wakjira et al. 2004), and chia (Ayerza 2009, 2011). This strong inverse relationship is supported by the biosynthesis of α linolenic fatty acid through the process of desaturation of oleic fatty acid via linoleic fatty acid by the action

of desaturase enzymes (Thomas et al. 2003; Yaniv et al. 1995).

The ω 6:ω 3 ratio was significantly ($P < 0.05$) lower in oils from seeds grown in Ecuador compared to that of seeds grown in the other two locations. High dietary ω 6 and ω 3 fatty acid ratio has been identified as a risk factor of suffering a coronary heart disease, and a way of lowering the risk is to keep dietary ω 6:ω 3 fatty acid ratio as low as possible, the ratio of 1:1 being ideal (Simopoulos 2003). Western diets do not provide these ratios, mainly due to their high ω 6 fatty acid content. As source of ω 3, chia is consumed either as oil or as whole/ground seed. The significant ($P < 0.05$) lower ω 6:ω 3 rate (up to 25%), showed by seeds grown in the Ecuador location, compared with the other ones, could indicate an added health benefit for these seeds.

Polyphenols content and compositions

Polyphenols content and compositions are presented on Table 4. Chromatographic analysis found the polyphenols composition of seeds from the three locations. The presence of quercetin, myrcetin, kaempherol, caffeic acid, and chlorogenic acid flavonols, and the lignan compound SDG was detected. No significant ($P < 0.05$) differences between seeds origins were found. An exception was the caffeic acid content which showed differences between seed origins; these differences were significant ($P < 0.05$) among locations and showed a relationship of Paraguay > Ecuador > Bolivia. Weather this is just an anomaly or a result of environment is not know. The total flavonols amount found herein are not far of the 0.757-0.881 mg/g found for two chia sources reported by Reyes-Caudillo et al. (2007) or the 0.924-0.939 mg/g reported for the Totzol and Iztac genotypes, respectively (Ayerza 2013).

No significant difference ($P < 0.05$) in SDG content was found among seeds origins. The lignan SDG compound amount found herein is similar to the 0.405-0.424 mg/g determined by Ayerza (2013) for two different genotypes of chia grown in Ecuador. Since the discovery of their physiological value, lignans have been extracted from flax and other plants, in a variety of ways. Once extracted, lignans can be added to food or taken in a concentrated form, in an attempt to take advantage of their functionality and benefits (Comin et al. 2011). This content could indicate an added commercial benefit for chia seeds.

The lack of a positive correlation (data not shown) between α-linolenic fatty acid and poly phenol compounds are somewhat surprising because flavonols and lignans are effective antioxidants in oil, and it may be expected that the plant reacts to increased polyunsaturation by producing more polyphenols to protect the oil from oxidation. The absence of a direct relationship supports the proposition put forward by Dolde et al. (1996), that antioxidant=s concentration and the fatty acid profile are not causally related but influenced differently by independent external variables such as temperature or soil type as it was reported for other seed oil crops such as soybean and canola (Richards et al. 2008).

Table 4. Antioxidant content and composition in the seeds of Salvia hispanica L.

Origen	Flavonols						Lignans
	Myrcetin	Quercetin	Kaempherol	Chlorogenic acid	Caffeic acid	Total	SDG
	mg/g						
Bolivia	0.119[a1]	0.006[a]	0.024[a]	0.214[a]	0.141[c]	0.914[a]	0.409[a]
Ecuador	0.121[a]	0.006[a]	0.024[a]	0.218[a]	0.149[b]	0.924[a]	0.407[a]
Paraguay	0.121[a]	0.006[a]	0.025[a]	0.235[a]	0.156[a]	0.975[a]	0.432[a]
SD[2]	0.009	---	0.003	0.055	0.003	0.101	0.06

[1] Means in a column within a group with the same letter are not statistically different ($P < 0.05$); [2] Least significant difference for $P < 0.05$.

Conclusions

In summary, the results found herein indicate that protein content, oil content and fatty acid profile characteristics of the Tzotzol variety of chia are affected by the different ecological conditions of the ecosystems of this study, which not affected the flavonols and lignans content, and composition. Additional multi location and multiyear trials are required to confirm this polyphenols compound=s stability to the ecosystem=s differences, and to understand the biochemical bases for these phenomena. The results also indicate that caution needs to be exercised before chia is introduced as a crop in a new area, since location can have a significant impact on seed's protein content, oil content and composition.

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International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.3.12

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(3):197-203 (2019)

Determination of morphological and pomological characteristics of pomegranate (*Punica granatum* L.) genotypes grown in Diyarbakır

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Abstract

The aim of this study was to determine the pomological and morphological characteristics of pomegranate genotypes grown in Çermik and Dicle districts of Diyarbakır province. As a result of the study carried out on 10 genotypes; fruit weights were found between 198.8 and 366.0 g, seed hardness of the fruit was medium-hard in eight genotypes, hard in two genotypes, aril yield ranged from 58.1 to 70.0% and fruit juice volume was between 63.9 and 135.7 ml. The upper peel color was purple in four genotypes, pink-red in two genotypes, orange-red in one genotype and orange in three genotypes. The bottom peel color is orange-red in seven genotypes and orange in three genotypes. Aril color was dark purple in three genotypes, purple in two genotypes, medium red in two genotypes and pink-red in three genotypes. The acid content was found to be between 0.65 and 1.21% and the amount of total soluable solid (TSS) varied between 15.0 and 21.0%. It was concluded that all 10 genotypes were promising and these genotypes showed superior characteristics.

Keywords: Pomegranate, Pomological properties, Morphology, Dicle, Çermik

Introduction

Pomegranate (*Punica granatum* L.) is the most important species of *Myrtiflorae* order and *Punicaceae* family. Pomegranate, thought to have been brought to Southern Europe by the Carthaginians, is known as *Malum punicum* (Carthagia apple). The name pomegranate, which is derived from the words grained apple, is named "Pomegranate" in English and "Granadapfel" in German (Onur, 1988). In the Middle Ages, the name *Punica granatum* was derived from the term *Pomuni granatum* (apple with seeds) (La Rue, 1980). Many holy books mention pomegranate fruit. In Egypt, Greek and Roman legends, "pomegranate" fruit is mentioned.

Pomegranate has been produced in South Asia and Southwest Asia for thousands of years. Pomegranate is widely growing Afghanistan, China, Morocco, Palestine, India, Iraq, Iran, Israel, Italy, North and South Cyprus, Egypt, Syria, Saudi Arabia, Thailand, America and Tunisia. Compared to other fruit species, production and consumption rate is less than others (Özbek, 1977, Dokuzoğuz and Mendilcioğlu, 1978; Onur, 1983, Özgüven and Yılmaz., 2000).

Many ingredients like starch, mannitol, punicic acid, anthocyanin, polyphenolic, isopelletierin alkaloids, triterpenes, resinous substances, acids, tannins and alkaloids can be found in various structures of pomegranate tree which are root, stem, branch bark, seeds and fruits. Pomegranate is generally used to strengthen the body and heart. It is also used for ceasing diarrhea, cough, constipation, stomach burns and vomiting. It has also been used in folk medicine for centuries due to its antipyretic, diuretic, antipyretic in febrile diseases and prevention of vascular obstruction (Saleh et al., 1964; Onur, 1983; Anesini and Perez, 1993; Ponce-Macotela et al., 1994; Zhang; et al., 1995; Yılmaz et al., 1995; Mavlyanov et al., 1997).

Cite this article as:
Cicek, M., Pakyurek, M., Celik, F. (2019). Determination of morphological and pomological characteristics of pomegranate (*Punica granatum* L.) genotypes grown in Diyarbakır. Int. J. Agric. Environ. Food Sci., 3(3), 197-203
DOI: https://dx.doi.org/10.31015/jaefs.2019.3.12
Received: 02 June 2019 **Accepted:** 12 September 2019 **Published:** 27 September 2019
Year: 2019 **Volume:** 3 **Issue:** 3 (September) **Pages:** 197-203
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

In recent years, understanding of the benefits to human health of pomegranate and increasing economic value have become the leading causes of significant developments occurring in pomegranate cultivation in Turkey. Propagation of pomegranates can be done by seed, layering, cuttings, bottom shoots and budding. Pomegranate is one of the most suitable fruit species can be grown in arid and semi-arid climatic conditions owing to its drought-resistant characteristics.

Turkey is one of the most important pomegranate producer countries in the world and has a rich variety of varieties and genotypes. The suitability of ecological conditions, the abundance of land, domestic and foreign demands increase pomegranate production rapidly. Turkey has a total of 502.000 tons of pomegranate production as of 2017. Besides, 13.662.000 trees are in the yield period in the existing pomegranate farms, and 3.123.000 trees have not yet invested in yield (Anonymous, 2017). Southeast Anatolia Region, on the other hand, ranks third after the Mediterranean and Aegean Region in terms of pomegranate production due to its climatic characteristics (Özgüven and Yılmaz, 2000). Total pomegranate production area in Southeastern Anatolia is 71.980 da. Production amount is 53.352 tons, the total number of trees is 3.564.392 and average yield amount per tree is 21 kg (Anonymous, 2016).

Diyarbakır, the most developed city in terms of agriculture and industry in Southeastern Anatolia, has a continental climate. Genetic resources of the region include pomegranate types that have superior properties in terms of yield and quality. No study on the identification and selection of these pomegranate genotypes have been conducted. With this first study, it was aimed to identify these genotypes grown in Çermik and Dicle districts of Diyarbakır province, to make them a standard genetic variety, to protect them, to preserve them for breeding studies and to expand their cultivation.

Material and Methods

Samples are taken from different pomegranate genotypes grown naturally in Çermik and Dicle districts of Diyarbakır province. The selection criteria to study the pomegranate trees are considered to be abundant and regular in yield, to display good vegetative development, to show adequate flowering, to yield high fruit set, to offer short flowering period, to be adapted to local climate for ripening period, to produce large fruity, reddish, thin-peeled, aromatic, juicy, soft seeded, to be resistant against diseases and pests. The trees presenting superior properties in these criteria are selected.

The villages where the pomegranate cultivation was widespread were determined under the guidance of Çermik and Dicle district Directorate of Agriculture in Diyarbakır. The villages were visited; producers were interviewed and existing types were determined consequently. The identified types are given numbers according to the district code. May-June is the flowering time depending on the local climate and September-November is the harvest time of the pomegranate. Crown height (cm), crown width (cm), trunk number, trunk circumference (cm), branching frequency, cold damage, first foliation date, flowering date and harvesting date of the trees were noted. Depending on these parameters; phenological, morphological and pomological characteristics of genotypes were established. At the harvest, five fruit samples were taken from each predetermined trees and transported in cloth bags. Then, these samples were analyzed in the laboratory of Gap International Agricultural Research and Training Center.

In genotypes, fruit weight (g), fruit length (mm), fruit width (mm), fruit juice yield (fruit juice quantity/fruit weight x100), fruit density (g/cm3), fruit volume (ml), calyx radius (mm)), calyx length (mm), peel thickness (mm), aril color (red and pink), bottom peel color (green, greenish-yellow, yellow), peel thickness (mm), upper peel color (pink, red), weight of the 100 arils, number of chamber, appearance of the calyx (apparent, less pronounced, moderate pronounced), the easiness of aril separating (easy, moderate, difficult), the taste of the fruit (sweet, sour), fruit pulp weight (g), fruit shape index (fruit length/fruit width), aril yield (total aril weight/fruit weight x 100) and total seed weight were measured or calculated. Besides, total soluable solid (%), pH and titratable acidity (%) contents of fruits were determined (Onur 1983; Yılmaz et al. 1995).

The promising genotypes were defined depending on pomological and morphological characteristics through weighed grading method. This method attributes greater percentage values to the properties which are considered to be important. The sum of these values is 100. Genotypes were evaluated according to this scoring system. Utilizing this method, seed hardness was scored as 15%, aril yield 15%, fruit weight 15%, fruit juice volume 15%, upper peel color 10%, aril color 10%, titratable acid ratio 10%, and TSS (total soluable solid) were 10%.

Results and Discussion

Genotype-specific phenological, morphological and pomological features of each are shown in the tables Table 1, Table 2, Table 3.

Results of Fieldwork

This study was carried out in two villages of Çermik and Dicle districts of Diyarbakır province in vegetation period of 2015-2016. Field studies revealed the physical properties of trees. Pomegranate cultivation in the before mentioned districts is widespread in regions where irrigation opportunities are available. Cultivation operations (pruning, fertilizing, irrigation, agricultural spraying, soil tillage, etc.) are not performed by the definition. Failure to perform cultivation related procedures by the definition directly affects the quality and the yield of fruits. Irrigation has a very important place in pomegranate orchards. Irrigation of the pomegranates orchards is provided by various natural water sources. However, irrigation arcs and channels are not consolidated, so water cannot be transported as desired for long distances. This leads to cracks on pomegranate due to irregular irrigation. Cracks on pomegranate is a big problem in terms of marketing. Pomegranates grown in Çermik and Dicle are generally composed of local genotypes.

Physical Properties of Fruits

Physical properties of fruit samples belonging to pome-

granate genotypes were evaluated and the mean values of these values are given in Table 1. Accordingly, the average weight of fruit samples, the lowest 198.8 g (21 ÇR 30) and the highest 366.0 g (21 ÇR 42) were determined. Four genotypes were between 150 and 225 g (40%) and six genotypes were between 225 and 375 g (60%). On the other hand, in a study conducted in the Mediterranean Region, it was found that fruit weights ranged from 213 to 806 g in genotypes (Onur, 1983). Fruit weights in the pomegranates of the Aegean Region ranged between 186 and 499 g (Dokuzoğuz and Mendilcioğlu, 1978). It was reported that the average fruit weight of Mardakyanlı cultivar in Azerbaijan was 237.5 grams and that this value was between 160 and 232 g in other varieties (Onur, 1983). In a study on the adaptation of pomegranates in the Mediterranean Region, it was found that the fruit weight ranged from 411 to 566 g (Yılmaz et al., 1992). In a study on adaptation of pomegranates in the Aegean Region, it was reported that the fruit weight varied between 260 and 308 g (Yılmaz et al., 1995). In our study, it is known that the weight and development of fruit vary depending on many factors, and it is seen that the promising pomegranate types we have selected are superior in terms of fruit weight compared to some studies conducted in other regions and remain lower than others. The average fruit length value was 58.7 mm (21 ÇR 30) and the highest was 79.7 mm (21 ÇR 03). Five genotypes were found to be between 60.0 and 67.0 mm (50%), four genotypes were between 67.0 and 75.0 mm (40%), and one genotype was between 75.0 and 90.0 mm (10%). The lowest fruit width was 68.1 mm (21 DC 27) and the highest was 86.9 mm (21 ÇR 42). Additionally, it was found between 67.0 and 75.0 mm (50%) in five genotypes and between 75.0 and 90.0 mm (50%) in five genotypes. In the study conducted in the Mediterranean region in 1998, the widest fruit width was determined as 96.83 mm in the 01 N 06 Evci variety (Özgüven et al., 2000). In the study carried out in Kırıkhan district of Hatay; fruit width was reported to be 80-94 mm (Polat et al., 1999). The fruit width values obtained in our study were similar between the values obtained in other studies conducted in Turkey.

The mean minimum fruit volume value was 108.3 ml (21 DC 18) and the highest value was 378.9 ml (21 ÇR 42). It also was found between 200 and 250 ml (60%) in six genotypes, 250 and 300 ml (30%) in three genotypes and 300–400 ml (10%) in one genotype. The lowest fruit density values were found to be 0.85 g/ml (21 ÇR 30) and 1.05 g/ml (21 DC 32). It was between 0.78 and 0.85 g/ml (10%) in one genotype and 0.85–1.20 g/ml (90%) in nine genotypes. The calyx radius was 9.5 mm (21 DC 07) and the highest value was 13.8 mm (21 DC 32). It was detected between 9.0 and 10.5 mm (10%) in one genotype, 10.5 and 12.5 mm (40%) in four genotypes, and 12.5–15.0 mm (40%) in five genotypes. The calyx length was found to be 17.5 mm (21 DC 07) and the highest value was 25.0 mm (21 ÇR 30). Three genotypes were found to be between 15.0 and 20.0 mm (30%) and seven genotypes were between 20.0 and 25.0 mm (70%). Mars and Marrakchi (1999) in a study in Tunisia, they found that the calyx length varies between 12.00 and 21.00 mm. For our genotypes, the lowest juice volume was found 63.9 ml (21 ÇR 30) and the highest value was 135.7 ml in genotype 21 ÇR 51, while the two genotypes were between 60.0 and 85.0 ml (20%) and the eight genotypes were between 85 and 140 ml (80%). Al-Maiman and Ahmad (2002) found that the fruit juice volume is 156 ml in their study for variety named as Taifi. Gündoğdu (2006) showed up that the juice volume was between 76.0 and 170.0 ml. Our results are similar to the results of other studies. Fruit taste was determined to be sour (20%) in two genotypes and sweet (80%) in eight genotypes. Fruit aril color was determined as dark purple (30%) in three genotypes, purple (20%) in two genotypes, medium red (20%) in two genotypes and pink-red (30%) in three genotypes. In a study conducted in Çukurca district of Hakkâri in 2008, 20 genotypes were examined. According to this study, aril colors were found to be white in three genotypes, light pink in 10 genotypes, pink in five genotypes and red in two genotypes (Özatak, 2010). Eight genotypes were easy (80%) and two genotypes were medium (20%) in easiness of aril separating.

The lowest weight of the 100 arils was found to be 40.3 g (21 DC 27) and 47.4 g (21 ÇR 45). Eight of ten genotypes were between 40.0 and 45.0 g (80%) and two of ten genotypes were between 45.0 and 50.0 g (20%). In a previous study conducted in Kırıkhan district of Hatay the weight of the 100 arils was measured between 29.0 and 50.0 g (Polat et al., 1999). In another study conducted in the Pervari district of Siirt, weight of the 100 arils was found as 26.50-45.90 g (Gündoğdu, 2006). The values obtained in our study shows similarity in terms of pomological features. In our study, the lowest aril yield was discovered as 58.1% (21 ÇR 48) and the highest value was 70.0% (21 ÇR 42). Previous studies revealed that aril yield was found to be 54-73% in Kırıkhan district of Hatay (Polat et al., 1999). In our study, two genotypes out of ten showed 2.2-3.0 mm (20%) peel thickness eight genotypes displayed 3.0-4.5 mm (80%) peel thickness. In another study carried out in Hizan district, it was found that the peel thickness ranged between 1.3 and 2.8 mm (Yıldız et al., 2003). In our examination, the upper peel color was purple (40%) in four genotypes, pink-red (20%) in two genotypes, orange-red (10%) in one genotype and orange (10%) in three genotypes. The bottom peel color was determined as orange-red (70%) in seven genotypes and orange (30%) in three genotypes. Seed hardness was determined as medium-hard (80%) in eight genotypes and hard (20%) in two genotypes. In the study conducted in the Pervari district of Siirt in 2002, the hardness of the fruits was found to be hard in 12 genotypes, medium-hard in 11 genotypes and soft in two genotypes (Gündoğdu, 2006).

The number of calyx was 7 (90%) in nine genotypes and 8 (10%) in one genotype. The external appearance of the calyx was found to be significant in six genotypes (60%), moderate prominence in two genotypes (20%), and not prominent in both genotypes (20%). According to the another study, the number of calyx was 6 in eight genotypes, 7 in eight genotypes, and 8 in six genotypes. The appearance of the calyx was found to be significant in 17 genotypes and less prominent in eight genotypes. The appearance of the calyx was found to be significant in 25 genotypes (Gündoğdu, 2006). The fruit shape index was found to be the lowest at 0.85% (21 ÇR 30 and 21 ÇR 45) and

the highest 0.93% (21 ÇR 42). In addition, between 0.85 and 0.90% (70%) values were obtained in seven genotypes and between 0.90 and 0.95% (30%) in three genotypes.

Chemical Properties of Fruits

The chemical properties of the fruit samples taken from 10 selected pomegranate genotypes were evaluated and the mean values are given in Table 1. The amount of total soluable solid (TSS) in fruit samples was determined between 10 and 15% (10%) in one genotype and between 15-23% (90%) in nine genotypes. In previous studies, the TSS was found to be between 13.3 and 16.9% in Tunisia, 13.0–16.0% in the Mediterranean Region, 12.8–15.9% in the Aegean and Southeastern Anatolia regions, 14.3–15.8% in the Kırıkhan district (Hatay), 10.0–17.0% in Hizan (Bitlis), 13.0–25.0% in the Pervari district of Siirt, 12.2-17.6% in Çukurca, respectively (Mars and Marrakchi, 1999; Yılmaz et al., 1992; Polat et al., 1999; Yıldız et al., 2003; Gündoğdu et al., 2010; Muradoğlu et al., 2006). Compared to other findings, the TSS values of the pomegranates of the Diyarbakır region are higher than those of the pomegranates grown in other regions. Similarly, Pervari pomegranates present analog values for TSS. In our study, pH values were between 3.3 and 3.6 (70%) in seven genotypes and 3.6–4.0 (30%) in three genotypes. Gündoğdu (2006) revealed that pH values of fruit juice was between 3.30 and 3.56 in 14 genotypes and 3.56–3.90 in 11 genotypes of the samples. The measures were repeated in the same study for next year. The pH of the fruit juice was found 3.6-4.0 in 8 genotypes, 4.00-4.40 in 17 genotypes (Gündoğdu, 2006). The pH values obtained in our study were in parallel with the values of different studies. As another important feature, we checked vitamin C levels in our study. Vitamin C levels in fruit samples were between 70 and 90 mg/100 g in eight genotypes (80%) and 90–120 mg/100 g in two genotypes (20%). Former examination conducted in Siirt were exhibited 18–78 mg/100 g vitamin C level (Kazankaya et al., 2007). It is plausible to conclude that vitamin C values of our study are higher than those obtained in the study in Siirt. Titratable acid content was between 0.5 and 0.9% in seven genotypes (70%) and 0.9–1.3 in three genotypes (30%). In a study conducted in the Mediterranean region, the amount of titratable acid content of fruit juice varied between 0.13 and 1.63 (Yılmaz et al., 1992). In another study conducted in the Mediterranean region, the minimum acid content was determined as 0.20% in genotype 07 N 15; the highest amount of acid was determined to be 2.00% in 33 N 12 genotype (Özgüven et al., 2000). The titratable acid ratio content of the genotypes we studied was much lower than the values obtained in other studies.

Conclusion

According to the results, the average fruit weight of the genotypes was ranged between 198.8 and 366.0 g. Fruit length, width, volume, and density were detected between 58.7-79.7 mm, 68.1–86.9 mm,108.3–378.9 ml and 0.85–1.05 g/ml, respectively. The calyx radius and the length of the calyx were ranged from 9.5 to 13.8 mm and 17.5 to 25.0 mm, respectively. The volume of the fruit juice was determined between 63.9 and 135.7 ml. Fruit taste was as certained as sour in two genotypes and as sweet in eight genotypes. Aril color was determined as dark purple in three genotypes, purple in two genotypes, medium red in two genotypes and pink-red in 3 genotypes. The easiness of aril separating was determined as easy in eight genotypes and medium in two genotypes. Aril weight and aril yield were found between 40.3–47.3 g and 58.1–70.0%, respectively. The thickness of the peel was found between 2.36-3.87 mm. The upper peel color was purple in four genotypes, pink-red in two genotypes, orange-red in one genotype and orange in three genotypes. The bottom peel color was orange-red in seven genotypes and orange in three genotypes. Seed hardness was medium hard in eight genotypes and hard in two genotypes. The external appearance of the calyx in the selected genotypes was found to be significant in six genotypes, moderate in two genotypes and not significant in two genotypes. The number of chamber of the fruits was 7 in nine genotypes and 8 in one genotype. The fruit shape index was found to be between 0.85–0.93%. Vitamin C values were determined between 62 and 110 mg/100 g. The total soluble solid (TSS) ranged from 15.0 to 21.0%. The pH value of the fruits was determined between 3.42 and 3.83%. The titratable acidity of fruit juice varied between 0.65 and 1.21%.

Pomegranate cultivation has been carried out in the Dicle and Çermik districts of the Diyarbakır province where the study was carried out for many years. Up to now, there is no genetic selection study conducted on pomegranate genotypes. In this study, it was uncovered that pomegranate genotypes grown in the region have superior fruit properties. Pronounced quality criteria of pomegranate are big fruit, big arils, juiciness, soft seeds, tastiness, and aroma. The genotypes we have studied have been of higher quality than previously studied ones so far. The fruit size, the volume of fruit juice, taste, ease of graining affects its production and consumption. These parameters are the reasons to choose for table consumption and pomegranate syrup production. Considering these, pomegranates in the region are of importance.

The Dicle and Çermik districts of the Diyarbakır province has optimal climatic conditions for pomegranate cultivation. This is a major opportunity for pomegranate producers. However, the producers do not have the necessary technical knowledge about pomegranate cultivation. This prevents producers to benefit from the climatic advantage. To resolve this problem; universities, research institutes, provincial and district directorates of agriculture and other agricultural organizations should be in cooperation and organize training activities for producers. The economic value of pomegranate has promising potential for them. If cultivation and production become widespread, it might propose an alternative source of livelihood for the people of the region. Currently, we have not encountered any registered pomegranate genotype in our study. In this region, selective breeding activities should be accelerated as soon as possible and genotypes which are well adapted, highly efficient and have superior properties should be registered. These genotypes and varieties should be recommended to producers who will establish new pomegranate orchards.

Table 1. Physical and Chemical Properties of 10 Pomegranate Genotypes (Average values).

Properties	21 ÇR 03	21 ÇR 30	21 ÇR 42	21 ÇR 45	21 ÇR 48	21 ÇR 51	21 DC 07	21 DC 18	21 DC 27	21 DC 32
Fruit Weight (g)	201.6	198.8	366.0	250.4	232.8	249.4	239.7	218.7	203.2	234.3
Fruit Length (mm)	63.8	58.7	79.7	68.1	64.7	66.8	68.5	67.1	60.8	69.6
Fruit Width (mm)	72.7	69.0	86.9	79.8	70.5	76.5	78.9	74.6	68.1	77.8
Fruit Volume (ml)	218.1	232.4	378.9	265.3	244.6	254.1	276.4	237.9	207.7	222.5
Fruit Density (g/ml)	0.92	0.85	0.97	0.94	0.95	0.98	0.87	0.92	0.97	1.05
Calyx Radius (mm)	11	11.8	13.7	13.6	11.6	12.8	9.5	10.9	13.2	13.8
Calyx Length (mm)	23.4	25.0	24.9	21.0	19.3	22.3	17.5	19.3	21.6	23.1
Fruit Juice Volume (ml)	64.7	63.9	103.4	110.6	102.3	135.7	127.5	108.3	117.8	108.7
Fruit Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sour	Sour
Aril Color	Dark Purple	Dark Purple	Pink - Red	Pink - Red	Pink - Red	Dark Purple	Purple	Purple	Medium Red	Medium Red
Aril Weight (g)	44.1	42.9	46.2	47.4	44.2	40.5	41.6	42.6	40.3	42.1
Aril Yield (%)	61.9	56.5	70.0	63.2	58.1	59.3	62.7	66.8	61.5	60.9
Upper Peel Color	Purple	Purple	Pink - Red	Pink - Red	Purple	Purple	Orange	Orange-Red	Orange	Orange
Bottom Peel Color	Orange-Red	Orange-Red	Orange-Red	Orange-Red	Orange-Red	Orange-Red	Orange	Orange-Red	Orange	Orange
Peel Thickness (mm)	2.44	2.36	3.71	3.54	3.87	3.03	3.34	3.41	3.21	3.30
Seed Hardness	Medium Hard	Medium Hard	Medium Hard	Medium Hard	Medium Hard	Medium Hard	Medium Hard	Medium Hard	Hard	Hard
Dry Matter Ratio (%)	29.7	26.2	29.5	22.7	23.4	26.0	28.9	27.3	38.8	29.7
Fruit Pulp (g)	104.3	88.2	115.4	123.3	112.8	120.8	117.3	101.9	129.0	135.4
Number of Caliyx	7	7	7	8	7	7	7	7	7	7
Clarity of Caliyx Appearance	Clear	Clear	Medium Clear	Clear	Not Clear	Clear	Clear	Clear	Medium Clear	Not Clear
Easiness of Aril Separating	Easy	Easy	Easy	Easy	Easy	Easy	Easy	Easy	Medium	Medium
Fruit Shape Index	0.88	0.85	0.93	0.85	0.92	0.87	0.87	0.90	0.89	0.89
Vitamin C (mg/100 g)	77	62	90	75	87	93	71	89	110	86
TSS (%)	16	20	19	18	21	17	16	19	17	15
pH (%)	3.76	3.42	3.55	3.44	3.51	3.40	3.83	3.52	3.81	3.55
Titratable Acid Ratio (%)	0.9	0.85	0.76	0.83	0.88	0.65	0.92	0.81	1.07	1.21

Table 2. Phenological and Morphological Properties of 10 Pomegranate Genotypes.

Properties	21 ÇR 03	21 ÇR 30	21 ÇR 42	21 ÇR 45	21 ÇR 48	21 ÇR 51	21 DC 07	21 DC 18	21 DC 27	21 DC 32
Crown Width (cm)	318.2	305.8	221.3	274.2	298.6	274.1	388.0	330.8	237.9	276.8
Trunk Number (No)	4	5	4	3	3	4	2	5	4	6
Tree Girth (cm)	16-16-19-20	12-13-15-15-16	13-14-16-17	11-12-12	13-14-16	14-15-17	21-24	12-13-13-14-15	13-13-14-16	15-15-16-17
Density of the Branches	Frequent	Frequent	Frequent	Semi Frequent	Semi Frequent	Frequent	Medium Frequent	Frequent	Frequent	Frequent
Cold Damage	No	No	No	No	No	No	No	No	No	No
First Foliation Date	Apr-18	Apr-15	Apr-24	Apr-25	Apr-15	Apr-11	Apr-14	Apr-8	Apr-11	Apr-17
Flowering Date	May-24	May-17	May-26	May-29	May-18	May-20	May-23	May-17	May-19	May-21
Harvest Date	Oct-21	Oct-19	Oct-25	Oct-21	Oct-14	Oct-23	Oct-26	Oct-25	Oct-23	Oct-24

Table 3. Weighted Grading Method Scores of 10 Pomegranate Genotypes.

Genotype Name	21 ÇR 03	21 ÇR 30	21 ÇR 42	21 ÇR 45	21 ÇR 48	21 ÇR 51	21 DC 07	21 DC 18	21 DC 27	21 DC 32
Score (%)	61.6	53.5	85.1	67.2	63.7	76.8	70.7	67.9	65.8	66.3

Acknowledgement

This article was adapted from Master Thesis belongs to Mehmet Çiçek.

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