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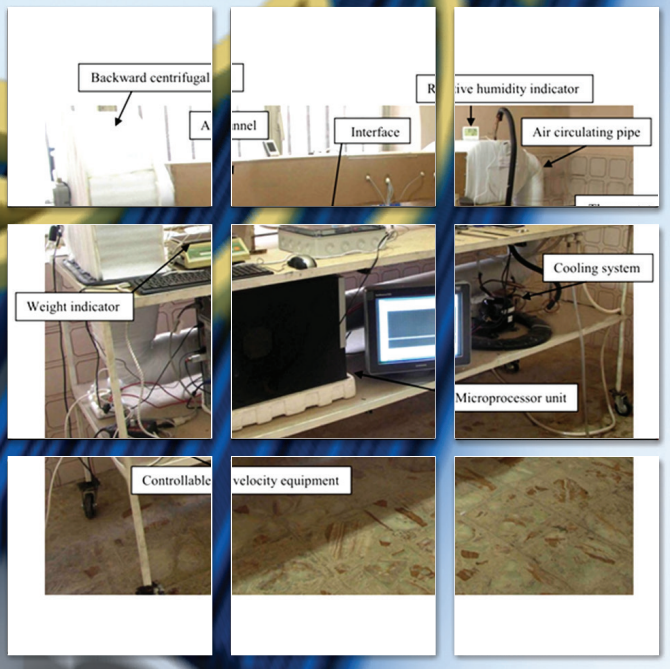

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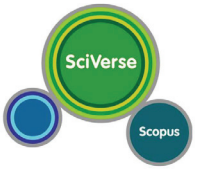

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Agricultural Performances of Some Safflower (*Carthamus tinctorius* L.) Lines Developed by Single Plant Selection Method

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ABSTRACT

This study was carried out to determine seed and oil yield with their components of some safflower lines and varieties under Eskisehir ecological conditions. This research was conducted at the experimental fields of Transitional Zone Agricultural Research Institute (TZARI), during 2010, 2011 and 2012. Both twenty-one safflower lines and four varieties (Yenice, Dincer, Remzibey and Balci) originating from TZARI-Eskisehir were evaluated in this research. The lines used in this study are developed by using single plant selection method. Three years data were collected and analyzed according to randomized block design with three replications. Means of seed yield, number of head per plant, head diameter, 1000 seed weight, oil content, oil yield were found 1330.3-1990.9 and 1210.1 kg ha^{-1}, 11.2-12.3 and 9.6 number $plant^{-1}$, 2.28-2.42 and 2.54 cm, 41.6-45.7 and 44.1 g, 36.1-36.6 and 35.6%, 470.9-730.0 and 430.0 kg ha^{-1} in 2010, 2011 and 2012, respectively. According to all years and combined analysis results of this study, lines GE-ES-YA-36-36, GE-ES-YA-36-7 in terms of seed yield, lines GE-ES-YA-36-30, GE-ES-YA-36-25, GE-ES-YA-36-26, GE-ES-YA-36-27 in terms of oil content and lines GE-ES-YA-36-36, GE-ES-YA-36-7, GE-ES-YA-36-4 terms of oil yield were listed at the highest statistical group. As a result of this study, it was decided that these lines could be candidate varieties with regard to these different characteristics.

Keywords: Safflower; Selection; Lines; Seed yield; Oil yield

Tek Bitki Seleksiyonu Islahı ile Geliştirilmiş Bazı Aspir (*Carthamus tinctorius* L.) Hatlarının Tarımsal Performansları

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ÖZET

Bu çalışma, Eskişehir ekolojik koşulları altında, bazı aspir hat ve çeşitlere ait tane ve yağ verimleri ile komponenetlerinin belirlenmesi amacı ile yürütülmüştür. Araştırma, 2010, 2011 ve 2012 yıllarında, Geçit Kuşağı Tarımsal Araştırma

Enstitüsü (GKTAE) deneme tarlalarında gerçekleştirilmiştir. Çalışmada, GKTAE-Eskişehir tarafından geliştirilmiş 21 adet hat ve 4 standart çeşit (Yenice, Dincer, Remzibey ve Balcı) kullanılmıştır. Çalışmada kullanılan hatlar tek bitki seleksiyon ıslahı yöntemi ile elde edilmiştir. 3 yıla ait veriler, tesadüf blokları deneme desenine uygun olarak analiz edilmiştir. Araştırmada, ortalama tane verimi, bitkide tabla sayısı, tabla çapı, 1000 tane ağırlığı, yağ oranı, yağ verimi değerleri 2010, 2011 ve 2012 yıllarında sırasıyla, 1330.3-1990.9 ve 1210.1 kg ha^{-1}, 11.2-12.3 ve 9.6 adet bitki^{-1}, 2.28-2.42 ve 2.54 cm, 41.6-45.7 ve 44.1 g, 36.1-36.6 ve 35.6%, 470.9-730.0 ve 430.0 kg ha^{-1} olarak bulunmuştur. Yıllara ve birleştirilmiş analiz sonuçlarına göre; tane verimi bakımından GE-ES-YA-36-36, GE-ES-YA-36-7, yağ oranı bakımından GE-ES-YA-36-30, GE- ES-YA-36-25, GE- ES-YA-36-26, GE-ES-YA-36-27, yağ verimi bakımından ise; GE-ES-YA-36-36, GE-ES-YA-36-7, GE-ES-YA-36-4 hatları istatistiki olarak ilk grupta yer almıştır. Çalışma sonuçlara göre, bu hatların belirlenen farklı özellikler bakımından aday çeşit olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Aspir; Seleksiyon; Hat; Tane verimi; Yağ verimi

1. Introduction

Turkey is undergoing an increasing loss of foreign exchange for many years due to the ongoing oil deficit in every year. To solve this problem, it is necessary to add other oilseed crop in production, beside of the increase yield of existing oilseed crops. Safflower has capable of wide adaptation, high drought resistance and can grow in arid areas (Omidi et al 2012; El-Lattief 2012). Safflower oil is of great importance as well as important for human nutrition as a raw material for biodiesel (Bergman & Charles 2008; Mündel 2008; Sujatha 2008; Uher 2008). Taken into consideration cultivation requests of safflower, it can be cultivating easily. It is a suitable plant applied to fallow and wheat cultivating systems in Turkey (Kose et al 2011).

In our country, although safflower production area was about 2,000 ha in 1970's, it gradually decreases about 30-40 ha at the beginning of the 2000's (TSI 2014). This situation has negative effect of the safflower breeding programs in Turkey. In last year, recognizing the importance of the safflower cause to increase production and breeding researches. According to data, in Turkey, safflower harvested area was 29,259 ha, production quantity is 45,000 tons and yield 1,530 kg ha^{-1}, which was above the world yield average (FAO 2014).

Breeding program, in accordance with the different objectives, is designed for developing new varieties and is used different breeding method. Selection is one of the oldest breeding procedures and is the basis of all crop improvement. Essentially, selection is a process, either natural or artificial, by which individual plants or groups of plants are sorted out from mixed population (Poehlman 1978). Nowadays, single plant selection method is mostly used in safflower breeding program (Fernandez Martinez et al 1986). In this method single plants are selected according to the morphological and quality parameters from population until obtaining breeding objectives.

This study aimed to estimate the performances of 21 safflower lines, which are used in this study are developed by using single plant selection method as compared to varieties for releasing new safflower varieties.

2. Material and Methods

Field trials were performed in 2010, 2011 and 2012 by using 21 safflower lines and 4 varieties (Yenice, Dincer, Remzibey and Balci) developed by Transitional Zone Agricultural Research Institute (39° 45'' 57' N, 30° 24'' 5' E) in Turkey.

These lines derived from 4 different population P-010, P-029, P-101, P-201 originated from Turkey, Balikesir; China, Qinghai; Turkey, Eskisehir; Turkey Isparta, respectively. The efficiency of a selection program mainly depends on the degree of genetic variation and heritability of a trait (Falconer & Mackay 1981; Shinwari et al 2014). A significant level of phenotypic variation was noticed among the population for most of the characters. Lines were

developed by using single plant selection method their characteristic and origin are given in Table 1. This method is regarded as the most effective for varietal development in safflower (Singh & Nimbkar 2007). According to the method, individual plants of each genotype were selected in terms of some morphological and agronomic parameters (flower colour, spines, earliness, plant height, head number per plant and number of seed per head) correlated to seed yield and oil content (Khidir 1974; Patil et al 1994; Malleshappa et al 2003; Alizadeh 2005; Omidi et al 2012; Rudolphi et al 2012). Each year selected single plants replanted next year as a new generation. When the selection reached S_7, the plots were harvested in bulk and genotype evaluated screening nursery especially seed yield, oil content and oil yield. Material selection was ended in S_8.

Experimental location has typically a steppe climate with temperature differences between day and night and dries in summer, relatively rainy winter. The weather conditions during the experimental period are presented in Table 2. Soil is clayey and neutral in reaction. It is poor in organic matter and reach in available potassium and phosphorus content. Trials were set up in randomized complete block design with three replications; 80 kg ha^{-1} nitrogen and 60 kg ha^{-1} phosphorus were applied at seeding. Plots were 5 m long, with 45 cm between rows and 5-10 cm between plants within rows after thinning. A length of 45 cm on both sides of the

Table 1- Some morphological characteristics of the genotypes

Genotype	*Line/Variety*	*Flower colour*	*Spines*	*Genotype*	*Line/Variety*	*Flower colour*	*Spines*
GE-36-2	Line	Yellow	Spiny	GE-36-25	Line	Yellow-Orange	Spiny
GE-36-3	Line	Yellow-Orange	Spiny	GE-36-26	Line	Yellow-Orange	Spiny
GE-36-4	Line	Yellow-Orange	Spiny	GE-36-27	Line	Yellow	Spiny
GE-36-6	Line	Yellow	Spiny	GE-36-28	Line	Yellow-Orange	Spiny
GE-36-7	Line	Yellow	Spiny	GE-36-29	Line	Yellow	Spiny
GE-36-8	Line	Yellow	Spiny	GE-36-30	Line	Yellow-Orange	Spiny
GE-36-9	Line	Yellow-Orange	Spiny	GE-36-34	Line	Yellow-Orange	Spiny
GE-36-10	Line	Yellow	Spiny	GE-36-36	Line	Yellow-Orange	Spiny
GE 36-11	Line	Yellow-Orange	Spiny	BALCI	Variety	Yellow	Spiny
GE-36-12	Line	Yellow	Spiny	REMZİBEY	Variety	Yellow-Orange	Spiny
GE-36-13	Line	Yellow-Orange	Spiny	DİNÇER	Variety	Orange-Red	Spineless
GE-36-14	Line	Yellow-Orange	Spiny	YENİCE	Variety	Red	Spineless
GE-36-17	Line	Yellow	Spiny				

Table 2- Monthly and growing season mean temperature, rainfall and relative humidity in 2010, 2011 and 2012

Month	*Mean temperature (°C)*				*Rainfall (mm)*				*Relative humidity (%)*			
	Long term	*2010*	*2011*	*2012*	*Long term*	*2010*	*2011*	*2012*	*Long term*	*2010*	*2011*	*2012*
March	4.9	5.9	3.7	1.5	33.4	32.6	20.0	56.4	82.3	85.5	88.0	87.7
April	10.4	9.2	7.2	12.0	35.2	23.9	56.9	22.1	85.6	84.3	91.0	72.6
May	14.9	15.2	0.5	14.4	43.3	20.7	145.8	80.9	75.9	70.4	87.7	83.3
June	18.9	18.1	16.6	20.0	28.6	79	9.4	0	80.4	82.8	84.6	71.6
July	21.5	22	21.6	22.8	13.5	7.4	8.5	5.5	69.2	75.4	70.8	68.1
August	21.1	24.4	20.0	20.8	6.4	0.9	0	3.5	71.2	66.2	73.5	65.1
Mean	15.3	15.8	11.6	15.3	-	-	-	-	77.4	77.4	82.6	74.7
Total	-	-	-	-	160.4	164.5	240.6	168.4	-	-	-	-

rows in each plot was left as border effects. Date of planting is made in the month of March every three years. The trial was performed under natural conditions without irrigation. During growing season weeds were controlled by hand. Plants were harvested in August.

Samples of each plot were obtained to determine seed yield (kg ha^{-1}), number of heads per plant (number), head diameter (cm), 1000 seed weight (g), oil content (%) and oil yield per hectare (kg ha^{-1}). Oil content of genotype was determined by using Soxhlet apparatus. Oil yield was calculated by multiplying oil content and the seed yield of each plot. Analysis of variance (ANOVA) was performed with the statistical package JMP 5.0.1 (SAS 1989-2002). Statistically significant differences among the mean values were determined with the least significant difference (LSD) test at the 0.05 level.

3. Result and Discussion

In this study, significant differences were determined between the genotypes for all the traits investigated in three years of the study and according to the three-year combined analysis, which implied genetic variation existed for these traits. The analysis demonstrated that significant difference existed between the years expect for head diameter. Year x genotype interaction was found important for all the traits investigated (Table 3, 4 and 5). It is because that there was some environmental factor in

Table 3- Mean values and statistics group of seed yield and number of head per plant studied some safflower lines and varieties in 2010, 2011, 2012 and 2010-2012

Genotype[§]	*Seed yield (kg ha^{-1})*				*Number of head per plant*			
	2010	*2011*	*2012*	*2010-12*	*2010*	*2011*	*2012*	*2010-12*
GE-ES-YA-2	1390.0 a-f	1841.7 c-g	1180.5 c-f	1470.2 f-ı	11.2 b-f	10.8 f-j	9.6 b-g	10.5 d-ı
GE-ES-YA-3	1330.9 a-f	1682.6 d-g	1260.5 cd	1420.9 g-j	10.7 c-f	11.4 d-ı	10.6 a-d	10.9 c-g
GE-ES-YA-4	1490.8 a-c	1920.8 b-g	1560.7 ab	1660.2 b-f	11.0 b-f	12.9 a-f	11.2 ab	11.7 b-d
GE-ES-YA-6	1450.2 a-d	1723.1 d-g	1600.0 a	1590.2 c-g	12.0 a-c	11.9 b-h	11.7 a	11.9 a-c
GE-ES-YA-7	1530.9 ab	2327.6 a-c	1630.0 a	1830.2 ab	11.4 b-e	13.4 a-f	10.0 b-f	11.6 b-d
GE-ES-YA-8	1280.1 c-g	2117.7 b-d	1130.7 c-f	1510.2 d-h	10.5 c-f	14.2 a-c	9.4 d-h	11.4 b-e
GE-ES-YA-9	1300.2 c-g	1728.0 d-g	1020.0 d-g	1350.0 h-k	11.0 b-f	11.7 c-h	7.9 h	10.2 e-ı
GE-ES-YA-10	1410.1 a-e	1893.1 b-g	1240.3 c-e	1510.6 d-h	12.1 a-c	10.8 e-j	8.2 g-h	10.4 e-ı
GE-ES-YA-11	1360.0 a-f	2042.8 b-e	1000.0 e-g	1460.8 f-ı	10.6 c-f	12.8 a-g	8.9 e-h	10.8 c-h
GE-ES-YA-12	1440.0 a-d	2658.6 a	1280.0 cd	1790.3 a-c	12.6 ab	14.8 a	9.3 d-h	12.2 ab
GE-ES-YA-13	1370.3 a-f	2403.0 ab	1020.5 d-g	1600.1 c-g	13.2 a	14.4 ab	9.2 d-h	12.3 ab
GE-ES-YA-14	1400.4 a-e	2388.3 ab	1270.3 cd	1680.9 b-e	13.2 a	14.9 a	11.0 a-c	13.0 a
GE-ES-YA-17	1180.1 fg	2034.8 b-e	960.7 fg	1390.4 g-j	9.7 fg	12.6 a-g	8.5 f-h	10.3 e-ı
GE-ES-YA-25	730.2 h	2081.5 b-d	850.7 g	1220.3 jk	8.7 g	12.6 a-g	8.9 e-h	10.1 f-ı
GE-ES-YA-26	1080.7 g	1405.1 g	980.0 fg	1150.8 k	9.9 e-g	10.2 g-j	8.2 g-h	9.4 ı
GE-ES-YA-27	1260.1 d-g	1536.8 e-g	1070.3 c-g	1290.0 ı-k	10.2 d-g	9.7 h-j	9.6 b-g	9.8 g-ı
GE-ES-YA-28	1500.0 a-c	2333.6 a-c	1320.0 bc	1710.8 b-d	10.9 c-f	14.3 a-c	9.9 b-f	11.7 b-d
GE-ES-YA-29	1300.3 c-g	1817.0 c-g	1300.7 bc	1470.6 f-ı	11.8 a-d	13.7 a-d	9.4 d-h	11.6 b-d
GE-ES-YA-30	1320.1 b-f	1935.4 b-f	1240.7 c-e	1500.1 e-h	11.0 b-f	13.5 a-e	8.7 e-h	11.1 b-f
GE-ES-YA-34	1290.0 c-g	2029.6 b-e	1140.4 c-f	1480.8 e-ı	10.3 d-g	8.3 j	10.2 a-e	9.6 h-ı
GE-ES-YA-36	1560.0 a	2683.9 a	1740.0 a	1990.5 a	12.0 a-c	13.1 a-f	10.3 a-e	11.8 bc
YENİCE	1210.7 e-g	1446.7 f-g	1110.7 c-g	1260.0 jk	10.9 b-f	8.9 ıj	9.9 b-f	9.9 f-ı
DİNÇER	1400.4 a-f	2031.9 b-e	1090.7 c-g	1510.1 e-h	11.9 a-d	13.8 a-d	10.3 a-e	12.0 a-c
REMZİBEY	1340.2 a-f	1793.3 d-g	1000.0 e-g	1370.8 h-j	11.6 a-d	11.8 b-h	9.4 c- h	11.0 c-g
BALCI	1340.0 a-f	2113.3 b-d	1200.7 c-f	1550.3 d-h	11.1 b-f	11.7 c-h	9.8 b-g	10.9 cg
Mean	1330.3	1990.9	1210.1	1510.4	11.2	12.3	9.6	11.0
Genotype	**	**	**	**	*	**	*	**
Year				**				*
Genotype x Year				**				**

[§], means in the same column followed by the same letters were not significantly different at 0.05 level using LSD test; * and **, significant at the 5 and 1% level, respectively

Table 4- Mean values and statistics group of head diameter and 1000 seed weight studied some safflower lines and varieties in 2010, 2011, 2012 and 2010-2012

Genotype§	*Head diameter (cm)*				*1000 seed weight (g)*			
	2010	*2011*	*2012*	*2010-12*	*2010*	*2011*	*2012*	*2010-12*
GE-ES-YA-2	2.33 b-g	2.17 gh	2.63 a-d	2.38 c-g	42.8 d-ı	47.5 a-d	44.5 c-f	45.0 c-ı
GE-ES-YA-3	2.60 ab	2.17 gh	2.27 e-g	2.34 e-g	41.7 f-j	45.0 b-f	43.4 d-f	43.4 g-j
GE-ES-YA-4	2.33 b-g	2.45 b-f	2.87 ab	2.55 bc	39.8 h-k	43.8 e-h	39.5 ıj	41.0 kl
GE-ES-YA-6	2.13 f-ı	2.38 d-g	2.20 g	2.24 g	43.2 c-h	46.5 a-e	47.9 ab	45.9 c-e
GE-ES-YA-7	2.10 g-ı	2.40 c-g	2.23 fg	2.24 g	47.8 ab	47.6 a-d	48.4 a	47.9 ab
GE-ES-YA-8	2.30 c-h	2.40 c-g	2.63 a-d	2.44 c-f	42.2 e-j	45.7 a-f	44.5 c-f	44.1 e-j
GE-ES-YA-9	2.20 e-ı	2.40 c-g	2.70 a-c	2.43 c-f	39.3 ı-k	44.6 c-f	44.2 d-f	42.7 jk
GE-ES-YA-10	2.62 a	2.80 a	2.90 a	2.77 a	39.0 jk	48.6 ab	47.3 a-c	45.0 c-h
GE-ES-YA-11	2.10 g-ı	2.57 a-d	2.33 d-g	2.33 e-g	45.3 a-e	49.1 a	45.8 a-e	46.7 a-c
GE-ES-YA-12	2.40 a-f	2.27 e-h	2.70 a-c	2.46 c-e	43.5 c-g	47.6 a-d	47.4 a-c	46.2 b-d
GE-ES-YA-13	2.03 hı	2.40 c-g	2.27 e-g	2.23 g	46.5 a-c	45.2 b-f	45.6 a-e	45.8 c-f
GE-ES-YA-14	2.13 f-ı	2.10 h	2.47 c-g	2.23 g	46.4 a-d	47.9 a-c	45.9 a-d	46.7 a-c
GE-ES-YA-17	2.17 e-ı	2.50 b-e	2.57 b-e	2.41 c-g	41.1 f-k	45.9 a-e	44.6 c-f	43.9 f-j
GE-ES-YA-25	2.10 g-ı	2.20 f-h	2. 53 c-f	2.27 fg	34.4 lm	44.1 d-g	42.7 e-h	40.4 l
GE-ES-YA-26	2.20 e-ı	2.53 b-d	2.49 c-g	2.41 c-g	41.3 f-k	42.2 f-h	39.7 h-j	41.1 kl
GE-ES-YA-27	2.19 e-ı	2.27 e-h	2.53 c-g	2.33 e-g	42.3 e-j	46.2 a-e	45.3 b-f	44.6 d-j
GE-ES-YA-28	2.23 d-ı	2.50 b-e	2.43 c-g	2.39 c-g	40.0 g-k	45.8 a-f	45.9 a-d	43.9 e-j
GE-ES-YA-29	2.21 e-ı	2.27 e-h	2.60 a-d	2.36 d-g	39.3 ı-k	40.8 gh	40.2 g-j	40.1 l
GE-ES-YA-30	2.40 a-f	2.40 c-g	2.50 c-g	2.43 c-f	32.5 m	40.2 h	35.7 k	36.2 m
GE-ES-YA-34	2.53 a-c	2.53 b-d	2.90 a	2.66 ab	39.1 jk	43.6 e-h	38.8 jk	40.5 l
GE-ES-YA-36	2.00 ı	2.70 ab	2.33 d-g	2.34 e-g	48.0 a	48.9 a	48.3 ab	48.4 a
YENİCE	2.30 c-h	2.63 a-c	2.67 a-c	2.53 b-d	38.0 kl	48.2 a-c	42.9 d-g	43.0 ı-k
DİNÇER	2.42 a-e	2.50 b-e	2.50 c-g	2.47 b-e	44.2 b-f	45.90 a-e	45.3 a-f	45.1 c-g
REMZİBEY	2.50 a-d	2.43 c-f	2.50 c-g	2.48 b-e	41.2 f-k	45.67 a-f	42.3 f-ı	43.1 h-j
BALCI	2.40 a-f	2.53 b-d	2.70 a-c	2.54 bc	40.6 g-k	44.80 c-f	45.7 a-e	43.7 g-j
Mean	2.28	2.42	2.54	2.41	41.6	45.7	44.1	43.8
Genotype	**	**	**	**	**	**	**	**
Year				ns				*
Genotype x Year				*				*

§, means in the same column followed by the same letters were not significantly different at 0.05 level using LSD test; * and **, significant at the 5 and 1% level, respectively; ns, not significant

year effect which was responsible for differences in cultivar responses to this trait. Means of seed yield, number of head per plant, head diameter, 1000 seed weight, oil content, oil yield were found 1330.3-1990.9 and 1210.1 kg ha^{-1}, 11.2-12.3 and 9.6 number plant^{-1}, 2.28-2.42 and 2.54 cm, 41.6- 45.7 and 44.1 g, 36.1-36.6 and 35.6%, 470.9-730.0 and 430.0 kg ha^{-1} in 2010, 2011 and 2012, respectively (Table 3, 4 and 5).

3.1. Seed yield

To increase seed yield is one of the main aim in breeding research of field crops and it is also important to determine high yielding genotype in safflower. Environmental condition is as important as genotype on grain yield, therefore breeders want to tested performance lines and varieties developed in different years and location.

When the results examined, mean of seed yield were found 1330.3 kg ha^{-1} in 2010, 1990.9 kg ha^{-1} in 2011, 1210.1 kg ha^{-1} in 2012 (Table 3). It was determined that the second year had higher seed yield than the others. The reason is that experimental location took high rainfall, especially, during the early stages of development in 2011 (Table 2)**.** It was emphasized that seed yield was significantly affected water supply and rain, particularly during early stage of safflower (Agasimani et al 1997; Uslu et al 2002).

Considering grain yield mean values, the highest seed yield was obtained from line GE-36-36 all years of the study and three-year averages and this line gave 1560.0 kg ha^{-1}, 2683.9 kg ha^{-1}and 1740.0 kg ha^{-1} seed yield in 2010, 2011 and 2012, respectively. Based on the average of the combined values of three years, GE-ES-YA-36-36 (1990.5 kg ha^{-1}), GE-ES-YA-36-7 (1830.2 kg ha^{-1}), GE-ES-YA-36-12 (1790.3 kg ha^{-1}) belonged to the same group, with respect to seed yield (Table 3). To consider of this trial value of the varieties; Balci reached 1550.3 kg ha^{-1} and Dincer (1510.1 kg ha^{-1}), Remzibey (1370.8 kg ha^{-1}) and Yenice (126.0 kg ha^{-1}) followed. When the result examined, large number of lines passed the varieties in terms of seed yield. Genotype and ecological factors are two major factors, which are influential on seed yield. Beside of this seed yield was the most affected trait from the environment (Camas & Esendal 2006). The evaluation of seed yield values demonstrated that variations existed between the lines and varieties. These variations were considered may have been from the reactions of the different cultivars and lines to different ecological conditions. It was reported that, seed yield varied 1164.0-2810.0 kg ha^{-1} Bergman et al (1989); 920.0-1050.0 kg ha^{-1} Muralidharudu & Nagaraj (1990); 1030.0-1250.0 kg ha^{-1} Bayraktar (1995); 1300.0-2700.0 kg ha^{-1} Reinbrecht et al (2005); 1330.0-2394.0 kg ha^{-1} Koutroubas & Papadoska (2005); 1107.5-1823.8 kg ha^{-1} Camas et al (2007); 1706.0-3111.0 kg ha^{-1} Kizil et al (2008); 971.0-1585.0 kg ha^{-1} Beyyavas et al (2011) and 1602.0-2167.0 kg ha^{-1} Zarei et al (2011). In this research differences and similarities between the results of the previous researches referred above and also in this trial might be concerned with genetic diversity of the genotypes, different ecological condition and agronomic application.

3.2. Yield components

In breeding programs, new varieties developed are selected by using various yield components are used to determine seed yield (Omidi et al 2012). Although the number of head per plant affected by environmental conditions, it is important trait influence the yield (Consentino et al 1997; Omidi Tabrizi 2000; Beyyavas et al 2011). Weiss (2000) is reported to obtain high seed yield, well-developed 12 to 14 heads per plant are sufficient. In this research, number of heads per plant ranged between 8.7 and 13.2 in first year, 8.3 and 14.9 in second year, 7.9 and 11.7 in third year of the study (Table 3). The three-year averages, number of heads per plant ranged between 9.4 number plant^{-1} (GE-ES-YA-36-26) and 13.0 number plant^{-1} (GE-ES-YA-36-14). Dajue & Mündel (1996) emphasize that number of heads per plant is very strongly linked to yield in safflower. Chaudhary (1990) showed that number of heads per plant could be used determined to high seed yielding varieties with 50 safflower lines. The highest number of heads per plant had been obtained by the researchers were Cazzato et al (2001), 30.0 number plant^{-1}; Beyyavas et al (2011), 19.5 number plant^{-1} and Ada (2013) 23.7 number plant^{-1} in their research. The investigated result in this trial showed differences between previous data. This is the result of different environmental condition and agronomic application. Zarei et al (2011) reported that number of head per plant greatly affected from environmental conditions particularly plant density.

The result of variances analysis examined there is no significant difference existed between the years for head diameter. These result showed that head diameter is not effect different year condition whereas significantly varied with genotypes. According to the Table 4 average of the combined values of three years, head diameter of the lines and the varieties ranged between 2.23 cm (GE-ES-

YA-36-13) and 2.77 cm (GE-ES-YA-36-10). Ashri et al (1976) were reported that head diameter can vary considerably without affecting seed yield; studied on safflower germplasm collection consisted of 900 lines.

When the results examined, 1000 seed weight of genotype ranged between 32.5-48.0 g in 2010, 40.2 and 49.1 g in 2011, 35.7 and 48.4 g in 2012 (Table 4). The genotypes investigated in this study, displayed significant variation. This diversity was considered to reaction of genotypes to different ecological conditions in this study. Beyyavas et al (2011) reported that genetic structure and ecological factors are two major factors, which are influential on 1000 seed weight. Acharya et al (1994) reported that 1000-seed weight had positively effect on seed yield and also indicated the influence of this character was greater than the other characters in

Table 5- Mean values and statistics group of oil content and oil yield studied some safflower lines and varieties in 2010, 2011, 2012 and 2010-2012

	Oil content (%)				*Oil yield (kg ha^{-1})*			
Genotype[§]	*2010*	*2011*	*2012*	*2010-12*	*2010*	*2011*	*2012*	*2010-12*
GE-ES-YA-2	37.0 ef	36.6 d-h	37.3 d-f	37.0 cd	510.4 a-e	670.4 b-h	440.4 c-g	540.4 c-ı
GE-ES-YA-3	36.4 e-h	37. 3 b-g	35.4 hı	36.4 d-f	480.6 b-f	620.6 e-ı	440.8 b-g	520.0 e-j
GE-ES-YA-4	38.6 a-d	39.9 a-b	37.3 d-f	38.6 a	570.8 a	760.7 a-g	580.4 a	640.3 ab
GE-ES-YA-6	35.1 hı	36.4 d-ı	33.8 k-m	35.1 gh	500.9 a-e	620.6 e-ı	540.0 ab	550.9 c-g
GE-ES-YA-7	35.9 f-h	36.5 d-h	35.2 ı	35.9 e-g	550.2 ab	840.8 a-c	570.4 a	650.8 a
GE-ES-YA-8	37.4 b-e	38.3 a-d	36.6 fg	37.4 b-c	470.9 b-f	810.2 a-e	410.6 c-k	560.9 b-f
GE-ES-YA-9	35.4 g-ı	36.1 d-j	34.8 ıj	35.4 fg	460.1 c-g	620.4 e-ı	350.5 g-k	480.0 h-j
GE-ES-YA-10	35.8 f-h	35.0 f-j	36.6 fg	35.8 e-g	500.6 a-e	660.3 c-h	450.6 b-e	540.2 c-ı
GE-ES-YA-11	37.4 c-e	38.6 a-d	36.1 gh	37.4 b-d	500.8 a-e	790.0 a-f	360.1 f-k	550.3 c-h
GE-ES-YA-12	34.1 ı-k	34. 0 h-k	34.2 jk	34.1 hı	490.2 b-f	900.5 a	430.8 c-h	610.1 a-d
GE-ES-YA-13	34.2 ıj	34.7 g-j	33.7k-m	34.2 hı	470.0 c-f	830.4 a-d	340.6 h-k	550.0 c-ı
GE-ES-YA-14	32.8 kl	34.2 h-k	31.4 o	32.8 j	460.0 c-g	810.9 a-e	400.0 c-k	560.0 c-g
GE-ES-YA-17	38.8 ab	40.3 a	37.3 d-f	38.8 a	450.8 c-g	820.2 a-e	360.0 f-k	540.7 c-ı
GE-ES-YA-25	39.2 a	40.4 a	38.0 a-c	39.2 a	280.8 h	830. 9 a-d	320.5 jk	480.4 g-j
GE-ES-YA-26	39.2 a	39.9 a-c	38.5 a	39.2 a	420.5 fg	550.9 hı	370.7 d-k	450.4 jk
GE-ES-YA-27	38.4 a-d	37.8 a-e	39.0 a	38.4 ab	480.4 b-f	580.0 g-ı	410.8 c-j	490.4 f-j
GE-ES-YA-28	35.7 f-h	37.3 c-g	34.1 j-l	35.7 e-g	530.5 a-c	870.2 ab	450.1 b-f	610.9 a-c
GE-ES-YA-29	36.5 e-g	35.4 e-j	37.6 c-e	36.5 c-e	470.4 b-f	640.0 d-h	490.1 a-c	530.5 c-ı
GE-ES-YA-30	39.3 a	40.3 a	38.3 ab	39.3 a	520.0 a-d	770.9 a-g	470.8 bc	590.2 a-e
GE-ES-YA-34	37.3 de	37.6 b-f	37.0 ef	37.3 cd	480.2 b-f	760.3 a-g	420.4 c-ı	550.6 c-h
GE-ES-YA-36	33.6 jk	33.8 ı-k	33.3 l-m	33.6 ıj	520.4 a-c	900.3 a	570.9 a	660.9 a
YENİCE	31.5 lm	30.0 l	33.0 mn	31.5 k	380.3 g	430.4 ı	360.8 e-k	390.5 k
DİNÇER	31.2 m	32.1 kl	30.3 p	31.2 k	430.8 e-g	650.2 c-h	330.2 ı-k	470.4 ıj
REMZİBEY	33.0 jk	33.7 jk	32.2 no	33.0 j	440.2 d-g	600.6 f-ı	320. 2 k	450.7 jk
BALCI	38.7 a-c	38.3 a-d	39.0 a	38.7 a	510.6 a-e	810.1 a-e	470.1 b-d	590.9 a-d
Mean	36.1	36.6	35.6	36.1	470.9	730.0	430.0	540.7
Genotype	**	**	**	**	**	**	**	**
Year				**				**
Genotype x Year				*				**

[§], means in the same column followed by the same letters were not significantly different at 0.05 level using LSD test; * and **, significant at the 5 and 1% level, respectively

relation to seed yield. According to average of three years, GE-ES-YA-36-36 (48.4 g), GE-ES-YA-36-7 (47.9 g), GE-ES-YA-36-14 and GE-ES-YA-36-11 (46.7 g) belonged to the same statistic group in terms of 1000 seed weight. The first two lines were also included in the first group with the highest seed yield. These results showed that 1000 weight seed was reliable components influencing seed yield.

3.3. Oil content

The value of safflower oil is dramatically increased nowadays, economically, oil content of seed is important for safflower production and considered as the major factors, affecting the success of safflower introduction in new production areas (Vorpsi et al 2010). Therefore, improving seed yield as well as the oil content of safflower has become importance in breeding program. According to this research, oil content of genotype ranged between 31.2% and 39.3% in the first year, 30.0% and 40.4% in the second year, 30.3% and 39.0% in the third year of the study (Table 5). Compared the lines and the varieties, it was observed that oil content of lines increased and most of them exceed the varieties. This situation is the result of selection made in terms of oil content along breeding program and the difference between the lines and varieties is strongly due to the genotype, beside of environmental condition. Consentino et al (1997), Johnson et al (1999), Uysal et al (2006), Zhang & Chen (2005), Koutroubas & Papadoska (2005), Gawand et al (2005), Arslan & Kucuk (2005) and Kose (2013) were reported that oil content varied between 33.4-43.4%; 13-46%; 23.7-26.9%; 23.8-40.3%; 26.7-35.7%; 26.3-28.5%; 31.3-36.3% and 30.6-38.7%, respectively. These results showed some similarity and differences in our results depend on genotype, trial and environmental condition. Hang & Evans (1985) reported that oil content mostly depends on the genotype, beside of this climatic factor and agronomic practices also affected it (Esendal & Tosun 1972; Pascal Villalobos & Alburquerque 1996; Rahamatalla et al 2001). When the combined values examined GE-ES-YA-36-30 (39.3%), GE-ES-YA-36-25 and GE-ES-YA-36-26 (39.2%), GE-ES-YA-36-17 (38.8%), GE-ES-YA-36-4 (38.6%), GE-ES-YA-36-27 (38.4%) had the first statistic group and remarkable in terms of oil content.

3.4. Oil yield

The oil yield calculated on the seed yield and oil content of genotypes, affected by these two factors. When the examined the result, oil yield of genotype ranged between 280.8 and 570.8 kg ha^{-1} in 2010, 430.4 and 900.5 kg ha^{-1} in 2011, 320.2 and 580.4 kg ha^{-1} in 2012 (Table 5). It is clear that highest oil yield obtained in the second year, similar in seed yield. This result revealed that the increase of oil yield was primarily associated with the increase of seed yield. According to average of three years, lines GE-ES-YA-36-36 with 660.9 kg ha^{-1}, GE-ES-YA-36-7 (650.8 kg ha^{-1}), GE-ES-YA-36-4 (640.3 kg ha^{-1}), GE-ES-YA-36-28 (610.9 kg ha^{-1}), GE-ES-YA-36-12 (610.1 kg ha^{-1}) and variety Balci (590.9 kg ha^{-1}) were in the same statistic group, with respect to higher oil yield per hectare. The result from the present study indicated that oil yield of safflower has been affected of genotype oil content, seed yield and ecologic conditions under which the experiments were carried out. For oil yield, the previous studies were recorded such data as 390.6-514.4 kg ha^{-1}, Rajput et al (2007); 322 to 460 kg ha^{-1} Gawand et al (2005) and 416-701 kg ha^{-1} Koutroubas & Papadoska (2005). The finding of the present study is in parallel with the results of other researchers.

4. Conclusions

This study indicated that large number of line had higher performance than varieties in terms of yield, yield components, oil content and oil yield. In this case, the result of selection of lines showed superior performance compared to the varieties since S_5 generation.

Based on all three years and combined analysis results of this study, lines GE-ES-YA-36-36, GE-ES-YA-36-7 in terms of seed yield, lines GE-ES-YA-36-30, GE-ES-YA-36-25, GE-ES-YA-36-26, GE-ES-YA-36-27 in terms of oil content and

lines GE-ES-YA-36-36, GE-ES-YA-36-7, GE-ES-YA-36-4 in terms of oil yield were listed at the highest statistical group. Consequently, it was decided that these lines could be candidate varieties with regard to these different characteristics.

References

Acharya S, Dhaduk L K & Maliwal G L (1994). Path analysis in safflower (*Carthamus tinctorius* L.) under conserved moisture conditions. *Gujarat Agricultural University Research Journal* **20**: 154-157

Ada R (2013). Cluster analysis and adaptation study for safflower genotypes. *Bulgarian Journal of Agricultural Science* **19**: 103-109

Agasimani C A, Patil R & Hand Radder G E (1997). Recent advances in agronomy of safflower (*C. tinctorius* L.) in India. In: *Proceedings of IVth International Safflower Conference,* 2-7 June, Bari, Italy, pp. 77-82

Alizadeh K (2005). Evaluation of safflower germplasm by some agronomic characteristics and their relationships on grain yield production in the cold dry land of Iran. *International Journal of Agriculture Biology* **3**: 389-391

Arslan B & Kucuk M (2005). Oil content and fatty acid composition of some safflower cultivars in Van, Turkey. In: *Proceedings of VIth International Safflower Conference,* 6-10 June, Istanbul, Turkey, pp. 167-175

Ashri A, Urie A L & Zimmer D E (1976). Evaluation of the world collection of safflower, *Carthamus tinctorius L*. variability of capitulum width and outerin volucral bract dimension. *Euphytica* **25**: 225-229

Bayraktar N (1995). The seed yield and yield component in the third progeny of safflower hybrids. *Journal of Field Crops Central Research Institute* **1**: 23-29

Bergman J W & Charles R F (2008). Evaluation of safflower and other oil seed crops grown in the United States Northern Plains region for biofuels/biobased products. In: *Proceedings of VIIth International Safflower Conference*, 3-6 November, Wagga Wagga, Australia

Bergman J, Carlson G, Kushnak G, Riveland N R, Stallknecht G, Welty L E & Wichman D (1989). Registration of "Finch" safflower. *Crop Science* **29**: 829

Beyyavas V, Haliloglu H, Copur O & Yilmaz A (2011). Determination of seed yield and yield components of some safflower (*Carthamus tinctorius* L.) cultivars, lines and populations under the semi-arid conditions. *African Journal of Biotechnology* **10**: 527-534

Camas N & Esendal E (2006). Estimates of broad sense heritability for seed and yield components of safflower. *Hereditas* **143**: 55-57

Camas N, Cirak C & Esendal E (2007). Seed yield, oil content and fatty acids composition of safflower (*Carthamus tinctorius* L.) grown in Northern Turkey Conditions. *Journal of Faculty of Agriculture* **22**(1): 98-104

Cazzato E, Borazio L & Corleto A (2001). Grain yield, oil content and earliness of flowering of hybrids and open-pollinated safflower in southern Italy. In: *Proceedings of Vth International Safflower Conference*, 23-27 July, Wiliston, U.S.A, pp. 185-189

Chaudhary S K (1990). Path analysis for seed yield in safflower (*Carthamus tinctorius* L.) in acid soil under mid altitude conditions. *International Journal Tropical Agriculture* **8**: 129-132

Consentino S L, Copani V, Cammarat M & Riggi E (1997). Relations between metereological parameters, yield and seed oil content in safflower (*Carthamus tinctorius* L.) in Mediterranean environment. In: *Proceedings of IVth International Safflower Conference*, 2-7 June, Bari, Italy, pp. 149-152

Dajue L & Mündel H (1996). Safflower (*Carthamus tinctorius* L.) promoting the conservation and use of underutilized and neglected crops. International Plant Genetic Resources Institute, Rome, Italy, pp. 1-83

El-Lattief E A (2012). Evaluation of 25 safflower genotypes for seed and oil yields under arid environment in upper Egypt. *Asian Journal of Crop Scienc*e **4**: 72-79

Esendal E & Tosun F (1972). Research on the yield, phonological, morphological and seed characteristics of some native and foreign varieties of safflower (*Carthamus tinctorius* L.) under Erzurum Ecological Conditions. *Atatürk University Journal of Faculty of Agriculture* **3**: 93-115

Falconer D S & Mackay T F C (1981). Introduction to Quantitative Genetics, Longman. Harlow, UK

FAO (2014). The Food and Agriculture Organization of the United Nations. http://faostat.fao.org/site/567/default.aspx#ancor (Access date: 05.05.2015)

Fernandez Martinez J, Dominguez Gimenez J, Jimenez A & Hernandez L (1986). Use of the single seed descent method in breeding safflower (*Carthamus tinctorius* L.). *Plant Breeding* **97**: 289-378

Gawand P B, Reddyand B N & Tambe S I (2005). Evaluation of productivity of safflower cultivars under moisture and nutrient management in rainfeed vertisols. In: *Proceedings of VIth International Safflower Conference*, 6-10 June, Istanbul, Turkey, pp. 205-209

Hang A N & Evans D W (1985). Deficit sprinkler irrigation of sunflower and safflower. *Agronomy Journal* **77**: 588-592

Johnson R C, Bergman J W & Flynn C R (1999). Oil and meal characteristics of core and non-core safflower accessions from the USDA collection. *Genetic Resources and Crop Evolution* **46**: 611-618

Khidir M O (1974). Genetic variability and interrelationship of some quantitative characters in safflower. *The Journal of Agricultural Science* **83**: 177-202

Kizil S, Cakmak O, Kirici S & Inan M (2008). A comprehensive study on safflower (*Carthamus tinctorius* L.) in semi-arid conditions. *Biotechnology & Biotechnological Equipment* **22**(4): 947-953

Koutroubas S D & Papadoska D K (2005). Adaptation, grain yield and oil content of safflower in Greece. In: *Proceedings of VIth International Safflower Conference*, 6-10 June, Istanbul, Turkey, pp. 161-167

Kose A (2013). Research on yield and quality characteristics of some safflower (*Carthamus tinctorius* L.) lines and varieties under dry and irrigated conditions in Turkey. *Journal of Food Agriculture & Environment* **11**(1): 553-556

Kose A, Hatipoglu H, Kose T F, Arslan H, Kosar F & Karakus M (2011). Determining the performances in Eskisehir and Sanliurfa conditions. In: *GAP VIth Agriculture Congress,* 9-12 May, Sanliurfa, Turkey, pp. 286-291

Malleshappa S M, Hiremath I & Ravikumar R I (2003). Negative associations between important quantitative traits in safflower (*Carthamus tinctorius* L.). *Sesame and Safflower Newsletter* **18**: 80-84

Muralidharudu Y & Nagaraj H (1990). Effect of location and genotype on safflower oil and its quality. *Field Crops Abstracts* **43**(5): 3516

Mündel H H (2008). Major achievements in safflower breeding and future challenges. In: *Proceedings of Products, VIIth International Safflower Conference*, 3-6 November, Wagga Wagga, Australia

Omidi A H, Hassan A, Khazaei H, Monneveux P & Stoddard F (2012). Effect of cultivar and water regime on yield and yield components in safflower (*Carthamus tinctorius* L.). *Turkish Journal of Field Crops* **17**: 10-15

Omidi Tabrizi A H (2000). Correlation between characters and path analysis for grain and oil yield in spring safflower. *Sesame and Safflower Newsletter* **15**: 78-80

Pascal Villalobos M J & Alburquerque N (1996). Genetic variation of a safflower germplasm collection grown as a winter crop in Southern Spain. *Euphytica* **3**: 327-332

Patil V D, Reddy M V S & Nerkar Y S (1994). Efficiency of early generation selections for yield and related characters in safflower (*Carthamus tinctorius* L.). *Theorotical and Applied Genetic*s **89**: 293-296

Poehlman, M J (1978). Breeding Field Crops. The Avi Publishing Company, U.S.A

Rahamatalla A B, Babiker E E, Krishna A G & El-Tinay A H (2001). Changes in fatty acids composition during seed growth and physicochemical characteristics of oil extracted from four safflower cultivars. *Plant Foods Human Nutrient* **56**: 385-395

Rajput G R, Khawale V S, Mankar P S, Sharma S K & Kumbhare M D (2007). Effect of sowing dates and nutrient management in safflower. *Journal of Soils and Crops* **17**(1): 110-113

Reinbrecht C, Barth S, VonWithzke-Ehbrecht S, Frick C, Elfadl E, Kahnt G, Becker H C & Claupein W (2005). Screening of a worldwide safflower collection adaptation to humid temperate climates and cultivation in organic farming. In: *Proceedings of VIth International Safflower Conference,* 6-10 June, Istanbul, Turkey, pp. 236-242

Rudolphi S, Becker H C, Schierholt A & Witzke-Ehbrecht S (2012). Improved estimation of oil, linoleic and oleic acid and seed hull fractions in safflower by NIRS. *Journal of American Oil Chemists Society* **89**: 363-369

Shinwari Z K, Rehman H & Rabbani M A (2014). Morphological traits based genetic diversity in safflower. *Pakistan Journal of Botany* **46**(4): 1389-1395

Singh V & Nimbkar N (2007). Safflower (*Carthamus tinctorius* L.). In: R J Singh (Ed), *Genetic Recources Chomosome Engineering and Crop Improvement*, CRC Press, Baco Raton, pp. 167-194

Sujatha M (2008). Biotechnological interventions for genetic improvement of safflower. In: *Proceedings*

*of VII*th *International Safflower Conference*, 3-6 November, Wagga Wagga, Australia

TSI (2014). Turkish Statistical Institute. http://www.tuik.gov.tr/bitkiselap/bitkisel.zul. (Access date: 05.05.2015)

Uher J (2008). Safflower in European Floriculture. In: *Proceeding of VII*th *International Safflower Conference*, 3-6 November, Wagga Wagga, Australia

Uslu N, Tutluer I, Taner Y, Kunter B, Sagel Z & Peskircioglu H (2002). Effects of temperature and moisture stress during elongation and branching on development and yield of safflower. *Sesame and Safflower Newsletter* **17**: 117-122

Uysal N, Baydar H & Erbas S (2006). Determination of agricultural and technological properties of safflower (*Carthamus tinctorus* L.) lines developed from Isparta population. *Suleyman Demirel University Journal of Faculty of Agriculture* **1**(1): 52-63

Vorpsi V, Harizaj F, Bardhi N, Vladi V & Dodona E (2010). *Carthamus tinctorius* L, the quality of safflower seeds cultivated in Albania. *Research Journal of Agricultural Science* **42**(1): 326-331

Zarei G, Shamsi H & Fazeli F (2011). Effect of planting density on yield and yield components of safflower cultivars in spring. *International Scholarly and Scientific Research & Innovation* **5**(12): 118-120

Zhang Z & Chen Y (2005). Studies on adaptability of safflower germplasms in Xinjiang China. In: *Proceedings of IV*th *International Safflower Conference,* 6-10 June, Istanbul, Turkey, pp. 132-139

Weiss E A (2000). Safflower. In: *Oilseed Crops*, Blackwell Science, Victoria, Australia, pp. 93-129

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An Evaluation of the Performance of Forced Air Cooling on Cooling Parameters in Transient Heat Transfer at Different Layers of Pomegranate

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ABSTRACT

The quality of horticultural products can be promoted using high techniques. One of these methods is precooling applied before storage and leads to increased shelf and storage life of the fruit. For this reason, the effect of forced air cooling was conducted to investigate the cooling rate at the center (aril), spongy tissue (peel) and leathery skin (rind) of pomegranate (*Punica granatum* L.). Airflow velocity as an effective factor in cooling products at three levels of 0.5, 1, and 1.3 m s^{-1} and temperature of 7.2 °C was considered. Cooling parameters including lag factor and cooling coefficient were calculated from experimental data. Then, half-cooling time and seven-eighths cooling time were obtained at different layers of pomegranate. Cooling heterogeneity was analyzed at different air velocity and at different layers of pomegranate. The results showed that increase in air velocity from 0.5 to 1.3 m s^{-1}, reduced the half-cooling time and seven-eighths cooling time. After 5000 seconds, the change of air velocity had a slight influence on decreasing temperature of different layers of pomegranate. Cooling heterogeneity at the air velocity of 0.5 m s^{-1} was low and then increased at the air velocity of 1 m s^{-1}. Finally, at the air velocity of 1.3 m s^{-1}, it was declined. The overall results illustrate that the applied methodology in this research, which explains unsteady heat transfer in the cooling process, can be performed in pomegranate or similarly shaped fruits.

Keywords: Precooling; Forced air cooling; Heat transfer; Pomegranate

Nar'ın Farklı Katmanlarında Geçici Isı Transferindeki Soğutma Parametreleri Üzerinde Zorlanmış Hava ile Soğutma Performansının Değerlendirilmesi

ESER BİLGİ

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ÖZET

Bahçe tarımında yetiştirilen ürünlerin kalitesi, ileri teknikler kullanılarak muhafaza edilebilir. Bu yöntemlerden birisi de depolamadan önce uygulanan ön soğutma ile meyvenin raf ve depolama ömrünün uzatılmasıdır. Bu nedenle, nar (*Punica granatum* L.)'ın merkezinde (tanede), süngerimsi dokusunda (iç zarda) ve derisinde (kabukda) zorlanmış hava ile soğutmada, soğutma hızının etkilerini araştırmak amacıyla bu çalışma ortaya konulmuştur. Ürünlerin soğutulmasında etkili faktör olarak 0.5, 1 ve 1.3 m s^{-1} değerlerindeki üç farklı hava hızı ve 7.2 °C sıcaklık değeri dikkate alınmıştır. Deneysel verilerden, ısı transferi direnç faktörü ve soğutma katsayısını içeren soğutma parametreleri hesaplanmıştır. Ayrıca, narın farklı katmanlarındaki yarı soğuma süresi ve sekizde yedi soğuma süreleri elde edilmiştir. Soğutma heterojenliği, farklı hava hızlarında ve narın farklı katmanlarında analiz edilmiştir. Sonuçlar, hava hızının 0.5 m s^{-1}'den 1.3 m s^{-1} arttığında, yarı soğuma süresinin ve sekizde yedi soğutma süresinin azaldığını göstermiştir. 5000. saniyeden sonra hava hızındaki değişimin, narın farklı katmanlarındaki sıcaklığın azalması üzerinde etkisi oldukça hafif olmuştur. Soğutma heterojenliği, 0.5 m s^{-1} hava hızında düşük olup sonraki 1 m s^{-1} hava hızında artmıştır. Sonuç olarak, 1.3 m s^{-1} hava hızı uygun bulunmamıştır. Genel sonuçlar, soğutma sürecindeki kararsız ısı iletimini açıklayan bu araştırmada uygulanan metodolojinin, nar veya benzer şekildeki meyvelerde de uygulanabileceğini göstermektedir.

Anahtar Kelimeler: Ön soğutma; Zorlanmış hava ile soğutma; Isı transferi; Nar

1. Introduction

Pomegranate (*Punica granatum* L.) is a plant from the family of Punicaceae (Fadavi et al 2006) and Iran is the main origin of planting this fruit. In order to supply domestic and export markets, the control of effective factors in reducing fruit quality needs to be considered. Temperature is one of the factors contributing to the rise of postharvest life in the horticultural products. Spoilage of harvested crops is proportional to their respiration rate which depends on temperature (Kader 2002). Studies have shown that, for every 10 °C reduction in temperature of the product, respiration rate diminishes by 2-4 times (Golob et al 2002). So, temperature management is crucial to maintain the quality of fruits during the postharvest period. This parameter should be managed using different techniques. Precooling is one of the most effective methods for enhancing the quality and freshness of the product, in which biochemical reactions and microbiological growth are reduced (Baird & Gaffney 1976; Ginsburg et al 1978; Dincer & Akaryildiz 1993; Thompson et al 1998; Brosnan & Sun 2001). In precooling, heat is reduced in fruit and vegetable after harvest to prepare it quickly for transport and storage. In order to evaluate a precooling system, cooling rate and cooling uniformity are required. Cooling rate is measured by calculating half-cooling time; but, the temperature of cooling medium should be homogeneous (Goyette et al 1996). Precooling methods differ based on the factors such as cooling time, water contact with product, performance of energy consumption, and rate of water loss. Given the above considerations, cooling methods can be divided into the following methods: forced air cooling, room cooling, vacuum cooling, hydrocooling, evaporative cooling, and ice cooling (Brosnan & Sun 2001).

Dennis (1984) and Hass et al (1976) stated that cooling rate with forced air cooling primarily depends on air velocity encountering the product and this is the only controllable parameter among other variables, because factors such as size, shape and physical characteristics of the product are unchangeable. Cold air temperature is a limiting factor that can not be reduced below a certain point because of frost.

Lambrinos et al (1997) found that increasing air velocity from 2 to 3.65 m s^{-1} decreased the cooling time up to 3 to 6 times based on the product packaging. This result was also confirmed by Emond et al (1996), who reported that seven-eighths cooling time, with increasing cooling airflow from 0.002 to 0.004 m^3 s^{-1} kg^{-1} of product, was reduced by 30-40%. Kumar et al (2008) reported that cold air could increase the cooling rate significantly in

tomatoe and orange. Rapid cooling is one of the advantages of forced air cooling (Thompson et al 1998), which could change the velocity of cold air and then increase cooling rate. Investigation of heat transfer on an individual pomegranate provides more precise results on the fruit behavior in terms of cooling time. Few studies have been conducted on unsteady heat transfer on pomegranate. The objective of the present study was to evaluate the velocity of cold air as an effective factor in the rate of cooling, estimate cooling parameters, and precise determination of cooling time at the different layers of a pomegranate, namely Rabab. The results could be used as a guideline in the design of processing and cooling systems in pomegranate in order to enhance efficiency and prevent fruit loss.

2. Material and Methods

The pomegranate fruits (*Punica granatum* L. cv. Rabab) were prepared from Arsenjan city, Fars province, Iran. This kind of pomegranate is used for export. Tests were performed on three parts of the pomegranate: arils, spongy tissue (peel) and leathery skin (rind). The samples were maintained for 24 h in the oven at 105 °C. Moisture content (w.b.) of the three parts of pomegranates on average was 81.87, 79.56, and 66.48% in arils, peel, and rind, respectively. Thickness measurements of the pomegranate layers were performed using a digital caliper. Air velocity was measured using an anemometer (Lutron-YK, 80AM, Taiwan) and then, the air velocities of 0.5, 1 and 1.3 m s^{-1} were selected for experiments. To perform the precooling operations, an instrument including a backward centrifugal fan (single inlet, 0.03×0.01 m^2, 220V, 1400 RPM, 160W), air tunnel and cooling system was designed and built at University of Tabriz, Iran, presented in Figure 1.

In order to optimize the use of energy, a polyethylene tube with the diameter of 0.15 m was

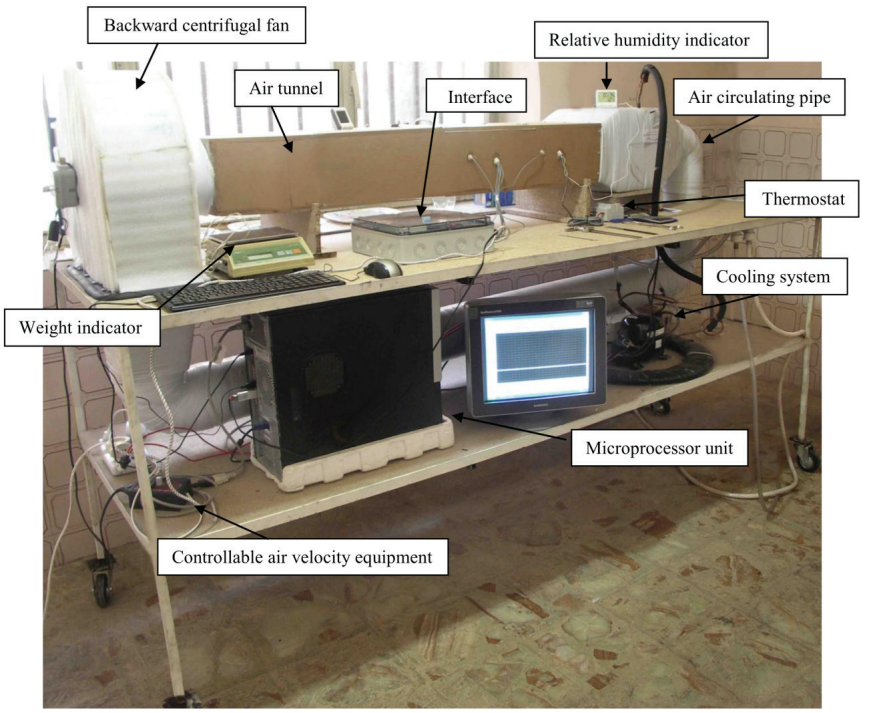

Figure 1- Experimental set up of the forced air cooling

used for air circulation. In each experiment, 6 pt100 sensors (0.003 m length, 0.002 m width, and 0.0011 m height) were placed in different parts of a single pomegranate. Sensors were placed as follows: (a) one sensor in the center of each fruit, (b) one at 50% of radius, (c) one in the peel, and (d) one in fruit rind. Also, two sensors recorded air temperature before and after the sample test. Placement of the entrance of the sensors was insulated. The temperatures were read and displayed per second. Accuracy of each sensor was ±0.1 °C. Temperatures were recorded for each sensor separately in LabVIEW software, version 2010. Before testing, the samples were placed at homogeneous temperature (25 °C) until the temperature of all parts of the sample became almost the same. Then, the sample (single pomegranate) was placed on a pedestal in the tunnel and cooling operations was started. Cooling operations were continued until the central temperature of pomegranate reached to 10 °C and then the apparatus was turned off. The temperature was adjusted at 7.2 °C inside the tunnel by means of a digital thermostat.

The main assumptions for conducting this experiment were as follows:

1. The test samples were homogeneous and isotropic.
2. Test conditions was conducted under unsteady state heat transfer.
3. Product temperature was the same in different parts of the fruit.
4. Thermophysical properties of the pomegranate and air temperature were constant in the tunnel (ρ_p= 970 kg m^{-3}, k_p= 0.52 W m^{-1} °C^{-1}, and $C_{p,p}$= 3606.07 J kg^{-1} °C^{-1}) .
5. Heat of respiration was ignored due to rapid cooling of the product.

In order to analyze the cooling process, calculation of the cooling parameters is essential. The dimensionless temperature inside the fruit is calculated using the product temperature at any time (T), initial product temperature (T_i), and cooling medium temperature (T_a), (Dincer 1995).

$$\theta = \frac{T - T_a}{T_i - T_a} \quad (1)$$

Dimensionless temperature is expressed in the form of cooling parameters including cooling coefficient (C) and lag factor (J) as (Thompson et al 1998).

$$\theta = J\exp(-Ct) \quad (2)$$

Cooling coefficient (C) explains the ability of cooling a product; it represents the change in product temperature per unit time at any moment (R) for the difference between the product temperature and cooling air (dT) and its value is negative. Also, C is the slope of the cooling curve (Kumar et al 2008).

$$C = R / dT \quad (3)$$

Lag factor (J) implies resistance to heat transfer from the product to the surroundings (Dincer, 1995). The value of this parameter is between 1 and 2 in the center of the fruit sample. These two parameters were determined by fitting the data to the dimensionless temperature and cooling the time curve.

The cooling rates are denoted by half-cooling time (H) and seven-eights cooling time (S). By substituting θ= 0.5 for H and θ= 0.125 for S in Equation (2), H and S were calculated by following equation (Ngcobo et al 2013).

$$H = [\ln(2J)/C] \quad (4)$$

$$S = [\ln(8J)/C] \quad (5)$$

The half-cooling time is used in practical applications (Dincer 1995) and seven-eighths cooling time is used for commercial cooling operations, because this time is close to the temperature of storage and transport (Guillou 1960). Parameters J and C are independent from the initial product temperature and, during the cooling process, will remain constant.

Effect of different velocities on cooling uniformity and temperature distribution were

calculated by cooling heterogeneity parameter according to the following equation (Dehghannya et al 2011).

$$Cooling\ heterogeneity = S_d / \overline{T} \quad (6)$$

Where; S_d is standard deviation and $\overline{T}$ is mean instantaneous temperature. This index can be calculated during cooling time and at different layers of pomegranate at certain times at each point of the product.

To evaluate the effect of cooling operations on fruit weight loss, this parameter was measured. Before and after testing, each fruit was weighed by a digital scale at the accuracy of 0.01 g. Weight loss was calculated based on the difference between initial (W_i) and final (W_f) weight.

$$Percent\ of\ weight\ loss = \frac{W_i - W_f}{W_i} \times 100 \quad (7)$$

Finally, least significant difference (LSD) test was used to test differences between means (p= 0.05).

3. Results and Discussion

3.1. Cooling rate

Cooling rate and cooling parameters of the pomegranate layers are shown at the air velocities of 0.5, 1 and 1.3 m s^{-1} in Table 1 (a, b, c).

At any air velocity, with increasing the radius of pomegranate from the center to the rind (outer shell), the lag factor decreased and cooling coefficient enhanced. Finally, half-cooling times and seven-eights cooling times decreased. Change of the lag factor depends on shape, size, and thermal characteristics of the product. Also, a lag factor greater than 1 causes an internal resistance to heat

Table 1- Cooling parameters in different layers of pomegranate (a, b, c)

V= 0.5 m s^{-1} (a)						
Placement of sensors	*J*	*C* (s^{-1})	*H (s)*	*S (s)*	R^2	**E (%)*
Center	1.1749	0.00028	3051.18	8002.23	0.9936	3.27
50% of radius	1.0069	0.00031	2258.14	6730.06	0.9996	0.88
Peel	0.9959	0.00033	2087.10	6288.89	0.9994	1.40
Rind	0.8685	0.00032	1725.50	6057.67	0.9964	2.99
V= 1 m s^{-1} (b)						
Placement of sensors	*J*	*C* (s^{-1})	*H (s)*	*S (s)*	R^2	**E (%)*
Center	1.2033	0.00031	2832.95	7304.87	0.9955	3.35
50% of radius	0.9761	0.00033	2027.14	6228.03	0.9994	2.06
Peel	0.9461	0.00036	1771.50	5622.32	0.9991	2.09
Rind	0.9450	0.00043	1480.41	4704.35	0.9975	0.95
V= 1.3 m s^{-1} (c)						
Placement of sensors	*J*	*C* (s^{-1})	*H (s)*	*S (s)*	R^2	**E (%)*
Center	1.1618	0.00031	2719.73	7191.65	0.9936	3.34
50% of radius	1.0182	0.00041	1734.59	5115.80	0.9989	1.95
Peel	0.9135	0.00041	1469.94	4851.15	0.9971	3.02
Rind	1.0619	0.00053	1421.15	4036.79	0.9913	6.47

*, maximum difference between the experimental and regression data

transfer from the product to airflow (Dincer 1995). As a result, in the center of product and the radius close to the center, the heat transfer was conducted more slowly than the other layers (Table 1). Cooling coefficient (*C*) and cooling rate were enhanced with increasing the air velocity. Increasing in air velocity from 0.5 to 1.3 m s^{-1}, reduced the half-cooling time and seven-eights cooling time up to 10.86% and 10.13% in the center (aril) and by 17.64% and 33.36% in the outer layer (rind), respectively. These were consistent with the findings by Dincer (1995) and Castro et al (2005). It should be also noted that shape and size were the factors that could affect rate of cooling. The experiments conducted by Castro et al (2005) showed that increasing the air velocity led to increasing of cooling rate. These experiments were carried out by the polymer spheres which were similar in shape, size, and thermal properties. Considering the fact that the pomegranates which were tested, were not exactly the same in terms of shape, cooling parameters could be partially affected (*J* & *C*). The results corresponded to those by Dincer (1995) who stated that shape, size, and thermal properties affected the lag factor of product. Cooling curves (cooling models) at different layers of pomegranate are shown in Figure 2.

The mean air and product temperatures were 7.2 and 22.2 °C, respectively. With approaching the end of the cooling process, the slope of the curves decreased in all the curves. In other words, at the end of the cooling time, the reduction of temperature was slower due to the lower temperature difference between the layers of pomegranate and cold air temperature. Dimensionless temperature, θ, of less than 0.2 had a slight effect on the cooling rate at different velocities in the center and 50% of radius of the pomegranate. However, the cooling rate had a small effect on the spongy tissue (peel) and the outer shell (rind) at the dimensionless temperature of less than 0.1. After 5000 seconds, the changes of air velocity had a small effect on reducing the temperature of the different layers of the center to the rind of the fruit. These results were compatible with the reports by other researchers (Kumar et al 2008). The value of the lag factor of greater than 1 occurred in the center (1.1749, 1.2033, and 1.1618), which represented an internal resistance to heat transfer against the airflow. At the velocity of 1.3 m s^{-1} and in the outer layer (rind), the lag factor increased (1.0619), which was probably due to partial differences in the thermophysical properties of the product layers. However, with increasing the cooling coefficient up to 0.00053, the ability of heat transfer at the rind layer improved; therefore, the half-cooling time and seven-eights cooling time reduced. Cooling of the fruit started with time delay of the initial cooling time (0-500 seconds) and in the center of the product, which led the beginning of the cooling curve to become flat. The reason for this subject may be the distance from the center of the pomegranate to the cold air in the cooling time of the product. Lindsay et al (1983) findings showed that the center of potatoe, which was at the top layers relative to the cold air, was cooled with a time lag.

Seven-eighths cooling time (*S*) is a part of the half-cooling time (*H*) (Henry & Bennett 1973). The range of *S* was 2.5-3.5 *H* in this study (Table 2).

In the systems where cooling rate is rapid, temperature changes in the product (center) are slower than the surface temperature changes in rind (outer shell). In such cases, the limiting factor is heat conduction from the center to surface of the product. This state alters the relative difference between *S* and *H*.

Table 2- The ratio of *S*/*H* in different parts of pomegranate

Airflow velocity (m s^{-1})	*Center*	*50% of radius*	*Peel*	*Rind*
0.5	2.62^{a}±0.02*	2.98^{a}±0.13	3.01^{b}±0.04	3.51^{a}±0.15
1	2.58^{a}±0.03	3.07^{a}±0.11	3.17^{ab}±0.24	3.18^{b}±0.18
1.3	2.64^{a}±0.06	2.95^{a}±0.09	3.30^{a}±0.13	2.84^{c}±0.14

*, mean (± SD). Values in the same column with the same letter(s) are not significantly different, using LSD test at an alpha level of 5%

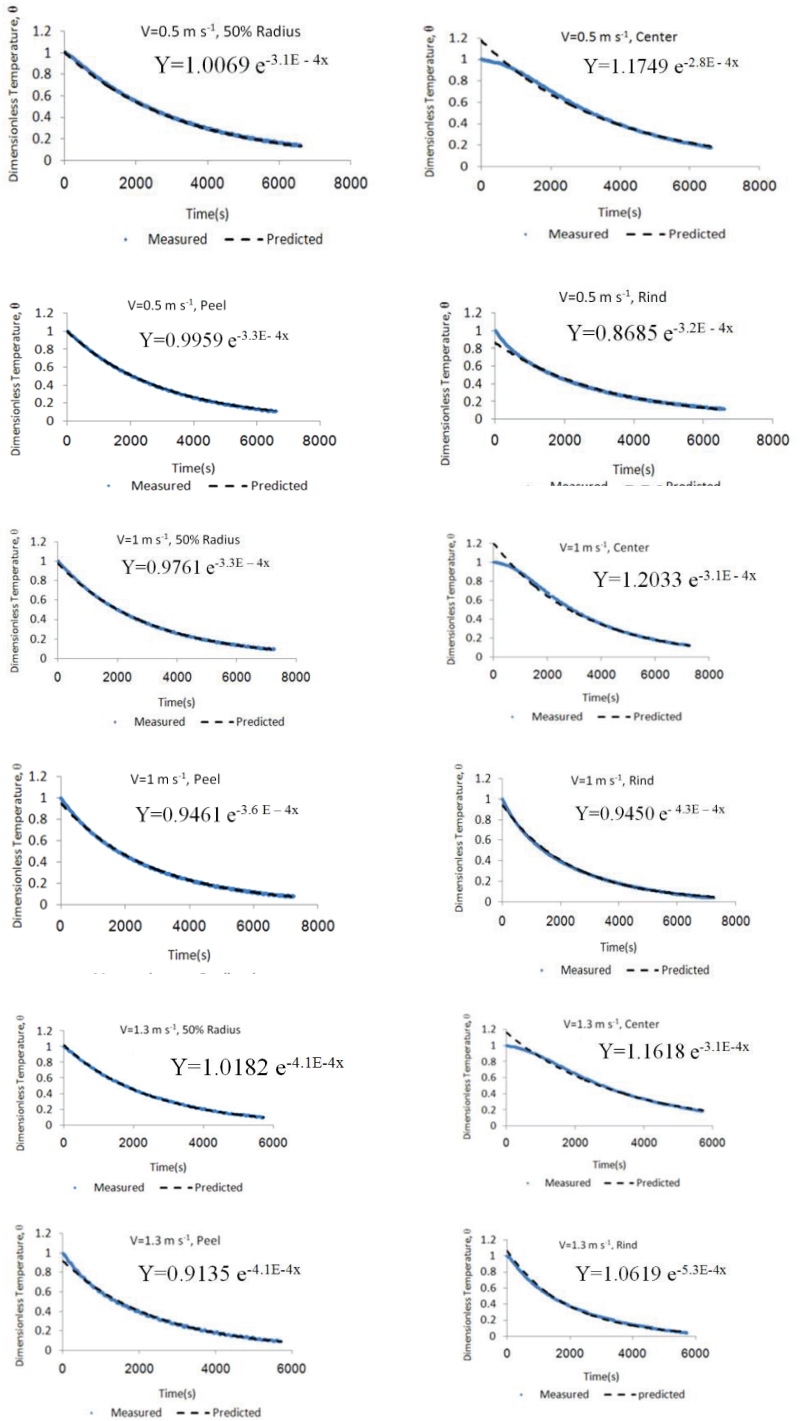

Figure 2- Cooling curves (experimental and regression) in different parts of pomegranate

3.2. Cooling heterogeneity

According to Figure 3, cooling heterogeneity was low at different layers at the air velocity of 0.5 m s^{-1}. Then, with increasing air velocity to 1 m s^{-1}, it was increased and again decreased at 1.3 m s^{-1}.

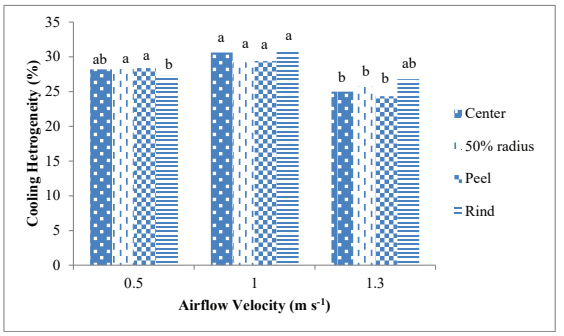

Figure 3- Cooling heterogeneity at three levels of airflow velocity in different parts of pomegranate (values with the same letter(s) are not significantly different, using LSD test at an alpha level of 5%)

Castro et al (2005) conducted an experiment on the spheres of polymer and reported that heterogeneity at the air velocity of 0.03 m s^{-1} was 0.505. This value was less than the air velocity of 0.128 m s^{-1} with the cooling heterogeneity of 0.534 and then, by increasing the air velocity to 1.043, m s^{-1} the cooling heterogeneity was reduced (0.409) due to the influence of gravity at lower air velocity. However, the half-cooling time at the airflow velocity of 0.03 m s^{-1} was 2.6 times higher than the time for the airflow velocity of 0.128 m s^{-1}. Castro et al (2004) also reported that the airflow velocity of 0.024 m s^{-1} in contact with plastic sphere created more uniform distribution of air than the air velocity of 0.111 m s^{-1}. They mentioned that the probable reason can be the natural convection effect. Results of the present research showed an inversion in the air velocity of 0.5 to 1 m s^{-1}. It was expected that heterogeneity would reduce with increasing air velocity, which was observed from the air velocity 1 to 1.3 m s^{-1}. The lowest heterogeneity obtained at the air velocity of 1.3 m s^{-1} that showed the temperature distribution at the layers of pomegranate was more uniform than the other velocities. Based on Figure 2, after the cooling period of time (5000 seconds), approximately all layers of the pomegranate got the same temperature; so, the heterogeneity of the various layers reduced. Results of the experiments by Dehghanniya et al (2011) also indicated that the column of the layers of plastic spheres became almost isothermal after a certain period of time and the cooling heterogeneity of layers decreased.

3.3. Weight loss of pomegranate

Based on the experimental data, the influence of airflow velocity on the weight loss of pomegranate fruit was low (Table 3). Therefore, this parameter paid no attention to the cooling calculations.

4. Conclusions

Cooling efficiency is generally evaluated based on two parameters: (1) rapid cooling (by reduction in half and seven-eights cooling time) and (2) cooling uniformity (by reduction in cooling heterogeneity). Based on these two parameters:

1- The trend of cooling curves against time was exponential for all the layers of pomegranate.

2- The half and seven-eights cooling time were reduced considerably at all layers of pomegranate

Table 3- Weight loss at different airflow velocity

Airflow velocity (m s^{-1})	*Initial weight (g)*	*Final weight (g)*	*Percent of weight loss*
0.5	$334.13^{b}\pm3.52^{*}$	$333.39^{b}\pm3.19$	0.22^{a}
1	$347.46^{ab}\pm2.93$	$346.74^{ab}\pm3.15$	0.21^{a}
1.3	$355.47^{a}\pm4.07$	$354.90^{a}\pm3.99$	0.16^{a}

*, mean (± SD). Values in the same column with the same letter(s) are not significantly different, using LSD test at an alpha level of 5%

with increasing airflow velocity in the range of 0.5 to 1.3 m s^{-1}, which proved the direct efficacy of airflow velocity on cooling rate. This effect may be due to the change in the heat transfer coefficient.

3- The lowest value of cooling heterogeneity was at the highest air velocity (1.3 m s^{-1}) that made temperature distribution more uniform.

4- After a specific time (5000 seconds), the influence of airflow velocity was low on cooling rate. Thus, the consumption of energy could be reduced by decreasing the airflow velocity in commercial applications.

5- The results showed that the applied method in this experiment could be used for pomegranate or similarly shaped fruits, which clearly and without complex calculations could explain the unsteady heat transfer in the cooling process.

6- The air velocity of 1.3 m s^{-1} is recommended for forced air precooling operations at different layers of pomegranate.

Abbreviations and Symbols	
C	Cooling coefficient, s^{-1}
H	Half-cooling time, s
J	Lag factor, dimensionless
S	Seven-eights cooling time, s
ρ_p	Product density (kg m^{-3})
k_p	Thermal conductivity of product (W m^{-1} ºC^{-1})
$C_{p,p}$	Specific heat capacity of product (J kg^{-1} ºC^{-1})
T	Product temperature at any time, ºC
T_a	Cooling medium temperature, ºC
T_i	Initial product temperature, ºC
$\overline{T}$	Mean instantaneous temperature, ºC

References

Baird C D & Gaffney J J (1976). A numerical procedure for calculating heat transfer in bulk loads of fruits or vegetables. *ASHRAE Transactions* **82**(2): 525-540

Brosnan T & Sun D (2001). Precooling techniques and applications for horticultural products-a review. *International Journal of Refrigeration* **32**: 154-170

Castro L R, Vigneault C & Cortez L A B (2004). Effect of container opening area on air distribution during precooling of horticultural produce. *Transactions of the ASAE* **47**(6): 2033-2038

Castro L R, Vigneault C & Cortez L A B (2005). Cooling performance of horticultural produce in containers with peripheral openings. *Postharvest Biology and Technology* **38**: 254-261

Dehghannya J, Ngadi M &Vigneault C (2011). Mathematical modeling of airflow and heat transfer during forced convection cooling of produce considering various package vent areas. *Food Control* **22**: 1393-1399

Dennis C (1984). Effect of storage and distribution conditions on the quality of vegetables. *Acta Horticulturae* **163**: 85-104

Dincer I (1995). Airflow precooling of individual grapes. *Journal of Food Engineering* **26**: 243-249

Dincer I & Akaryildiz E (1993). Transient temperature distributions within spherical products with internal heat generation and transpiration. *International Journal of Heat and Mass Transfer* **36**: 1998-2003

Emond J P, Mercier F, Sadfa S O, Bourre M & Gakwaya A (1996). Study of parameters affecting cooling rate and temperature distribution in forced air precooling of strawberry. *Transactions of the ASAE* **39**(6): 2185-2191

Fadavi A, Barzegar M & Azizi M H (2006). Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varities grown in Iran. *Journal of Food Composition Analysis* **19**: 676-680

Ginsburg L, Combrink J C & Truter A B (1978). Long and short term storage of table grapes. *International Journal of Refrigeration* **1**(3): 137-142

Golob P, Farrell G & Orchard G E (2002). *Crop Postharvest Science and Technology, Principles and Practices*. Volume 1. Blackwell Publishing Company, Oxford, UK, pp. 554

Goyette B, Vigneault C, Panneton B & Raghavan G S V (1996). Method to evaluate the average temperature at the surface of horticultural crop. *Canadian Agricultural Enigineering* **38**(4): 291-295

Guillou R (1960). Forced air fruit cooling *Transactions of the ASAE* **3**(2): 16-18

Hass E, Felsenstein G, Shitzer A & Manor G (1976). Factors affecting resistance to airflow through packed fresh fruit. *ASHRAE Transactions* **82**(2): 548-554

Henry F E & Bennett A H (1973). Hydraircooling vegetables products in unit loads. *Transactions of the ASAE* **16**(40): 731-739

Kader A A (2002). *Posthaevest technology of horticultural crops*. Cooperative Extension of University of California, Division of Agricultural and Natural Resources, University of California, Davis, CA, Publication No. 3311

Kumar R, Kumar A & Murthy U N (2008). Heat transfer during forced air precooling of perishable food products. *Biosystems Engineering* **99**: 228-233

Lambrinos G, Assimaki H, Manolopoulou H, Sfakiotakis E & Porlimgis J (1997). Air precooling and hydrocooling of Hayward Kiwifruit. *Acta Horticulturae* **444**: 561-566

Lindsay R T, Neale M A & Messer H J M (1983). Ventilation rates for positive ventilation of vegetables in bulk bins. *Journal of Agricultural Engineering Research* **28**(1): 33-44

Ngcobo M E K, Delele M A, Opera U L & Meyer C J (2013). Performance of multi-packaging for table grapes based on airflow, cooling rates and fruit quality. *Journal of Food Engineering* **116**: 613-621

Thompson J F, Mitchel F G, Rumsey T R, Kasmire R F & Crisosto C H (1998). *Commercial cooling of fruits, vegetables, and flowers.* University of California, Agriculture and Natural Resources, Davis, DANR Publication 21567

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Effects of Irrigation Programs Formed by Different Approaches on the Yield and Water Consumption of Black Cumin (*Nigella sativa* L.) under Transition Zone in the West Anatolia Conditions

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ABSTRACT

This study was carried out to determine the effects of different irrigation programs obtained by pan evaporation and water balance methods on some yield and vegetative parameters and water consumption of black cumin in the experiment field located in the center of Cobanlar District in Afyonkarahisar, Turkey during 2013 and 2014 growing seasons. In the experiment, the irrigation treatments were formed with 3 different irrigation intervals (SA3: 3 days, SA5: 5 days, and SA10: 10 days) and 4 different irrigation water levels as 0% (I_0: non-irrigated) 50%, 75% (I_{50}, I_{75}: deficit irrigation) and 100% (I_{100}: full irrigation) of the cumulative evaporation amount measured from class A pan in the first year and, of the required water amount to replenish the available soil moisture to the field capacity in the 0.60 m soil depth in the second year. The highest and the lowest evapotranspiration (ET) values were determined as 387.6 mm in SA3-I_{100} and as 166.9 mm in I_0, respectively. The highest seed yield was obtained in SA5-I_{100} with an amount of 1700.6 kg ha^{-1} while the lowest seed yield was obtained in I_0 with an amount of 722.2 kg ha^{-1}. The highest total water use efficiency (WUE) and irrigation water use efficiency (IWUE) values were calculated from SA5-I_{50} as 5.11 kg ha^{-1} mm^{-1} and 4.80 kg ha^{-1} mm^{-1}, respectively. While the yield response factor values (ky) were obtained as 0.75 (SA3), 0.80 (SA5) and 0.50 (SA10) for different irrigation interval, the mean ky value was determined as 0.68 according to all treatments of both years. Since the values of the yield and vegetative parameters and ET in same irrigation programs formed by two different methods were close to each other, the both methods can be used for irrigation of black cumin.

Keywords: Evapotranspiration; Drip irrigation; Water use efficiency; Yield response factor

Batı Anadolu Koşullarındaki Geçiş Bölgesi Altında Farklı Yaklaşımlar ile Oluşturulan Sulama Programlarının Çörekotu (*Nigella sativa* L.) Bitkisinin Verim ve Su Tüketimine Etkileri

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ÖZET

Bu çalışma, buharlaşma kabı ve toprak nem dengesine göre elde edilen farklı sulama programlarının çörekotu bitkisinin verim ve vejetatif özellikleri ile su tüketimine etkisini belirlemek amacıyla 2013-2014 yetişme sezonlarında, Afyonkarahisar İli Çobanlar İlçesi merkezinde yer alan deneme alanında yürütülmüştür. Çalışmada, sulama konuları 3 farklı sulama aralığı (SA3: 3 gün, SA5: 5 gün ve SA10: 10 gün) ve ilk yıl A sınıfı buharlaşma kabında ölçülen yığışımlı buharlaşma miktarının, ikinci yıl ise 0.60 m toprak derinliğindeki mevcut nemi tarla kapasitesine çıkarmak için gerekli olan sulama suyu miktarının % 0 (I_0: sulama yapılmayan), % 50, % 75 (I_{50}, I_{75}: kısıntılı sulama) ve % 100 (I_{100}: tam sulama)'ünün uygulandığı 4 farklı sulama suyu düzeyi konularından oluşturulmuştur. En yüksek ve düşük bitki su tüketimi (ET) değerleri sırasıyla, SA3-I_{100} konusundan 387.6 mm ve I_0 konusundan 166.9 mm olarak saptanmıştır. Çalışmada, en yüksek tohum verimi 1700.6 kg ha^{-1} ile SA5-I_{100} konusundan elde edilirken, en düşük 722.2 kg ha^{-1} ile I_0 konusundan elde edilmiştir. Denemede en yüksek toplam su kulanım randımanı (WUE) ve sulama suyu kullanım randımanı (IWUE) değerleri SA5-I_{50} konusundan sırasıyla, 5.11 kg ha^{-1} mm^{-1} ve 4.80 kg ha^{-1} mm^{-1} olarak hesaplanmıştır. Verim tepki etmeni (ky) değerleri farklı sulama aralığı konularından 0.75 (SA3), 0.80 (SA5) ve 0.50 (SA10) olarak elde edilirken, her iki yılın değerleri göz önüne alındığında tüm konular için ortalama ky değeri 0.68 olarak belirlenmiştir. İki farklı yöntemle oluşturulan aynı sulama programlarından elde edilen verim, vejetatif özellikler ve ET değerlerinin birbirine yakın olması, çörekotu bitkisinin sulanmasında her iki yönteminde kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Bitki su tüketimi; Damla sulama; Su kullanım randımanı; Verim tepki etmeni

1. Introduction

Black cumin (*Nigella sativa* L.) which has an important place among medicinal and aromatic plants is an annual herbaceous plant from *Ranunculaceae* family. Because of the fixed oil, volatile oil and other nutrients in its seed, black cumin is highly valued in health and food industries. Nowadays, due to the consumption needs of the rapidly increasing world population, black cumin, the consumption in the world based on very old ages, has a quite large market which is about $ 60 billion (Kumar 2009). Within the total production of 3520 tons in 326.1 ha area in Turkey, Afyonkarahisar ranks third with 180 tons of production in 12.3 ha area after Bursa and Sivas provinces. In recent years in Turkey, despite the decrease in the production of black cumin, consumption is rapidly increasing. Black cumin export declined from 160 tons ($ 243 thousand) to 57 tons ($ 224 thousand) whereas import increased from 50 tons ($ 25 thousand) to 2933 tons ($ 2.8 million) between 2001 and 2014 years (TUIK 2015). The vast majority of medicinal and aromatic plants are collected as wild from nature in Turkey as in other areas of the world, and their cultivation is not performed (Baydar 2013). When the mentioned import and export amounts are taken into consideration, the increasing of production amount by the culture of the black cumin plant and irrigated agriculture is expected to contribute to the farmers by meeting the demands of both domestic and foreign markets. Water is one of the most important sources for the agricultural production has become quite limited in both quantity and quality aspects due to the rapidly increasing population and industrialization. Especially, as demand for irrigation water increases in arid and semi-arid regions, the need for water-yield functions revealing the relationship between the yield and irrigation water and evapotranspiration, used to determine the optimum irrigation management also increases. Evapotranspiration is crucial in the economic use of water, the preparation of irrigation programs and the management of agricultural water (Yazar et al 2002; Panda et al 2004). The pan evaporation method is practicable for farmers and commonly used method for the irrigation scheduling of plants because of the simple application and close correlation between evapotranspiration and pan evaporation (Kanber 1984; Wang et al 2009). For inadequate water conditions, with the using of irrigation techniques called as deficit irrigation, it is aimed to save the inputs such as irrigation water,

labor and energy, as well as to increase the water use efficiency (WUE) which means the yield obtained from a unit of water (English & Raja 1996). The yield response factor (ky), which is an important parameter showing the linear relationship between the relative evapotranspiration deficit and the relative yield decrease, is a very important guide for the determination whether a plant is tolerate or sensitive by taking into consideration the response to water shortage (Doorenbos & Kassam 1986). Despite many studies performed on the yield and vegetative parameters of the black cumin plant (Kalcin 2003; Ozel et al 2009; Akgoren 2011; Taqi 2013; Safaei et al 2014), in a limited number of the studies especially performed on the irrigation practices and water-yield functions in deficit irrigation conditions. While Ghamarnia et al (2010) obtained the seasonal plant water consumption within 414-1461 mm and water use efficiency (WUE) values within 0.49-1.39 kg ha^{-1} mm^{-1}, Ghamarnia & Jalili (2013) determined the same values as 193-645 mm and 0.11-1.87 kg ha^{-1} mm^{-1}, respectively.

Since the black cumin plant is generally cultivated in unirrigated conditions, the reaction of the plant to scheduled irrigation is very important in terms of yield and the optimal use of water. In this study, it was aimed to determine the effects of irrigation programs obtained by pan evaporation and water balance methods on some yield and vegetative parameters, seasonal evapotranspiration and water-yield functions of black cumin.

2. Material and Methods

2.1. Experimental area, climate, soil and irrigation water features

The experiment was conducted in a field located in Cobanlar District of Afyonkarahisar Province (38º 41' 59.07" N, 30° 47' 34.03'' E and 1013 m altitude). Since the experimental area is away from the sea and surrounded by mountains, it has a typical continental climate. Some climate data for the long-term and vegetation periods of experimental years were given in Table 1.

Table 1- Monthly some climate data in the growing season for the long-term and the experiment years

Climate data	*Year*	*Month*					*Average*
		March	*April*	*May*	*June*	*July*	
Temperature (ºC)	2013	7.8	11.2	17.7	20.6	21.9	15.8
	2014	7.0	12.0	15.1	18.6	23.5	15.2
	Long-term*	5.4	10.3	15.0	19.1	22.3	14.4
Sunshine duration (hour)	2013	5.6	7.0	9.4	11.2	13.2	9.3
	2014	5.3	6.3	8.3	9.8	10.7	8.1
	Long-term	5.1	6.2	8.2	10.0	11.2	8.1
Precipitation (mm)	2013	29.2	34.6	22.2	11.6	57.2	154.8
	2014	25.4	17.6	66.2	52.4	0.4	162.0
	Long-term	43.5	46.9	47.6	35.0	19.0	192.0
Relative humidity (%)	2013	58.5	62.2	51.9	48.0	47.7	53.7
	2014	74.3	63.9	68.3	67.3	52.4	65.2
	Long-term	73.0	69.1	54.1	62.8	66.4	65.1
Wind speed ($m\ s^{-1}$)	2013	2.1	1.8	1.8	2.0	2.1	2.0
	2014	3.1	2.8	2.6	2.4	2.5	2.7
	Long-term	2.0	2.2	1.8	1.7	2.2	2.0
Evaporation (mm)	2013	-	101.0	191.8	237.3	249.3	779.4
	2014	-	86.2	137.7	180.2	266.6	670.7
	Long-term	2.0	73.2	145.4	188.1	241.3	650.0

*, between the years 1970 and 2013

Some physical properties of the experimental area soil were presented in Table 2. Available soil water holding capacity was 102.09 mm for 0-60 cm. The irrigation water used in the experiment was obtained from the well located next to the field with 1.8 L s^{-1} flow rate and C_3S_1 qualified.

Table 2- Soil physical characteristics of the experimental area

Soil depth (cm)	*Bulk density (g cm^{-3})*	*Texture*	*Field capacity (%)*	*Wilting point (%)*	*Water holding capacity (%)*	*(mm)*
0-30	1.32	CL	28.63	17.78	10.85	42.96
30-60	1.46	SCL	24.71	11.21	13.50	59.13
60-90	1.35	SCL	29.15	11.68	17.47	70.75

2.2. Experiment treatments, planting, cultural practices and design of drip irrigation system

The study was carried out according to the factorial arrangement in completely randomized plots design with three replications. In the experiment, while class A pan was used in the first year (2013), the available moisture at 60 cm soil depth was monitored with the gravimetric method in the second year (2014) in order to determine the amount of the irrigation water. The two approaches widely used for forming the irrigation program, were compared with use of actual data obtained from each experimental years. The irrigation treatments were formed with 3 different irrigation intervals (SA3: 3 days, SA5: 5 days, and SA10: 10 days) and 4 different irrigation water levels for each irrigation interval as 0% (Non-irrigated: I_0) 50%, 75% (Deficit irrigation: I_{50}, I_{75}) and 100% (Full irrigation: I_{100}) of the amount of the measured cumulative evaporation in the first year and, of the amount of required water to replenish the available soil water to the field capacity in the second year.

The experimental area consisted of a total of 30 plots. The area of each plot was 1.62 m^2 (0.90x1.80 m), and the total area was 208.0 m^2 (10.4x20.0 m). In order to prevent the interaction between irrigation treatments, 1 m space was left between the plots. Besides, two rows in each plot were left out of the assessment due to the edge effect, and the remaining area formed the harvest plots.

Black cumin (*Nigella sativa L.*) seed sowing was performed in the plots in the last week of March in both years (25 March 2013-26 March 2014) to be 0.2 kg per hectare. Following the germination, plants were diluted to ensure 15x10 cm plant spacing. The nutrient requirement of plants was determined according to the soil analysis (no K_2O was needed; P_2O_5 and N were applied at a rate of 55 kg ha^{-1} and 40 kg ha^{-1}, respectively). A half of the total nutrient amount was given during the preparation of soil, and remaining amount was given with the fertigation technique as equal amounts during the irrigation period by dividing into the number of irrigation. Engineering and operating principles of the drip irrigation system was designed according to the fundamentals given by Kanber (2010). Thus, emitter spacing was 20 cm, dripper discharge was 4 L h^{-1}, PE lateral diameter was 16 mm, main pipe diameter was 50 mm, and a single lateral was placed for each two rows.

2.3. Irrigation water, evapotranspiration and water-yield functions

While the amount of the applied irrigation water to the treatments was calculated using Equation 1 in the first year (2013), Equation 2 was used in the second year (2014). The amount of irrigation water determined as depth terms, was calculated in volume terms by multiplying with the parcel unit area and the percentage of the wetted area during the applications (Equation 3).

$$I = Epan \times Kp \times Sd \quad (1)$$

$$I = (FC - AW) \times Sd \quad (2)$$

$$V = I \times A \times WA \quad (3)$$

Where; I, irrigation water amount (mm); Epan, cumulative evaporation amount from the class A pan during the irrigation interval according to the treatments (mm); Kp, pan coefficient (taken as 0.77 based on the principles given by Doorenbos & Pruitt (1977)); Sd, irrigation water level; FC, field capacity (mm); AW, available water in the soil within 60 cm depth before irrigation applications (mm); V, irrigation water as volume (L); *A*, plot area (m^2); WA, percentage of the wetted area (67%).

The evapotranspiration for the treatments was calculated using Equation 4 based on the water balance (James 1988).

$$ET = I + P + Cp \pm rSW - Dp - Rf \quad (4)$$

Where; ET, evapotranspiration (mm); P, precipitation (mm); Dp, deep percolation (mm); rSW, change in the soil water storage in the 60 cm depth (mm); Rf, runoff (mm); Cp, amount of water entering the root zone with capilaric rise (mm).

Regression analyzes were performed by establishing graphical relations between total irrigation water and seed yield values, and evapotranspiration and seed yield values. Besides, Equation 5, 6 and 7 were used to calculate the water use efficiency (WUE), irrigation water use efficiency (IWUE), and the coverage ratio of the applied irrigation water to the evapotranspiration (*IRc*) relating to the treatments (Howell et al 1990).

$$WUE = Y / ET \quad (5)$$

$$IWUE = (Y - Y_0) / I \quad (6)$$

$$IRc = (I / ET) \times 100 \quad (7)$$

Where; Y, seed yield obtained from the treatments (kg ha^{-1}); Y_0, seed yield obtained from I_0 (kg ha^{-1}).

The yield response factor (ky) representing the decrease in the black cumin seed yield which occurred as a result of the decrease in the unit evapotranspiration was determined using Equation 8 (Doorenbos & Kassam 1986).

$$(1 - Ya / Ym) = ky\,(1 - ETa / ETm) \quad (8)$$

Where; Ya, actual seed yield regarding the treatments (kg ha^{-1}); Ym, maximum seed yield (kg ha^{-1}); Eta, actual seasonal evapotranspiration regarding the treatments (mm); Etm, maximum seasonal evapotranspiration (mm).

2.4. The effective root depth, some yield, and vegetative parameters

In order to determine the effective root depth of black cumin, the full irrigation treatment (I_{100}) was monitored, and the depth consumed 85% of the amount of water required for normal development of the plants was taken into account (Kanber 1997). Plant height (cm), the number of branches (number $plant^{-1}$), and plant capsule number (number $plant^{-1}$) values were determined by measuring and counting before harvest. Following the harvest, seed number in capsules (number $capsule^{-1}$) was determined by counting and seed yield (kg ha^{-1}) was determined by dividing the unit area of the total weight. In addition, thousand seed weight (g) was determined by multiplying the weight of 100 seeds which was measured with sensitive scales with 10, and the harvest index (%) was determined by the proportion of the weight of seeds harvested from 10 plants with the weight of the unharvested plant.

2.5. Statistical analysis

In order to determine the effects of the irrigation treatments on the some yield and vegetative parameters of black cumin for each year, data were performed to the variance analysis using Minitab® 16.2.4 computer software package, and Tukey's comparison test was used to determine the differences between the mean values ($P<0.05$).

3. Results and Discussion

3.1. Irrigation water, evapotranspiration and yield

The irrigation period was between 23rd May and 12th July 2013 in the first year, and between 25th May and 17th July 2014 in the second year. The highest irrigation water amount was applied in SA5-I_{100} as 255.6 mm, and the lowest irrigation water amount was applied in SA3-I_{50} as 97.0 mm

(Table 3). It can be said that the reason for the fact that the total irrigation water amount in the second year was lower according to the first year, that pan evaporation methods were used to determine the irrigation water amount in the first year, and that the amount of precipitation was higher than both the long-term value and the value in the first year of the vegetation period (from May to June).

ET values varied at both different irrigation water amounts and irrigation intervals. The highest ET values were calculated in SA3-I_{100} in both years as 334.8 and 387.6 mm, respectively, whereas the lowest ET values were measured in I_0 in which no irrigation was applied in the both years as 212.8 and 166.9 mm, respectively (Table 3). In general, ET values obtained from same irrigation programs formed by different methods among the years were similar. ET values increased with the amount of applied irrigation water increased. Additionally, higher ET values were also obtained from more frequent irrigation (3 days) compared to less frequent irrigation (5 and 10 days) in the treatments where applied similar water amount. While the applied irrigation water amount in the study was less than those obtained by some other researchers, ET values were similar in general (Ghamarnia & Jalili 2013; Ghamarnia et al 2010).

Soil water content was close to the field capacity (FC) at 143rd day of the year (2013) and 145th day of the year (2014) in all treatments. Then, soil water contents before irrigation showed a tendency to decrease in all treatments (Figure 1). A dramatic decrease was observed in the soil water content values in the I_0 and I_{50} treatments and the values were close to or lower than permanent wilting point (PWP). Soil water content decreased with

Table 3- Irrigation water, evapotranspiration, water use efficiencies

Year	*Treatments*	*I* (mm)	*ET* (mm)	*Yield* (kg ha^{-1})	*WUE* (kg ha^{-1} mm^{-1})	*IWUE* (kg ha^{-1} mm^{-1})	*IRc* (%)
2013	I_0	-	212.8	812.5 f	3.82	-	-
	SA3-I_{50}	122.5	265.0	983.8 e	3.71	1.40	46.2
	SA3-I_{75}	183.8	324.4	1137.0 d	3.50	1.77	56.7
	SA3-I_{100}	245.0	384.1	1222.2 c	3.18	1.67	63.8
	SA5-I_{50}	127.8	278.9	1425.7 b	5.11	4.80	45.8
	SA5-I_{75}	191.7	334.8	1487.4 ab	4.44	3.52	57.3
	SA5-I_{100}	255.6	372.5	1562.6 a	4.19	2.93	68.6
	SA10-I_{50}	127.8	242.7	1146.4 d	4.72	2.61	52.6
	SA10-I_{75}	191.7	275.8	1186.5 d	4.30	1.95	69.5
	SA10-I_{100}	255.6	323.1	1243.2 c	3.85	1.69	79.1
2014	I_0	-	166.9	722.2 f	4.33	-	-
	SA3-I_{50}	97.0	287.4	1073.2 e	3.73	3.62	33.8
	SA3-I_{75}	145.5	352.8	1361.7 c	3.86	4.40	41.2
	SA3-I_{100}	194.0	387.6	1461.0 bc	3.77	3.81	50.1
	SA5-I_{50}	104.6	276.4	1183.2 de	4.28	4.41	37.8
	SA5-I_{75}	166.9	322.8	1491.5 b	4.62	4.61	51.7
	SA5-I_{100}	209.2	359.7	1700.6 a	4.73	4.68	58.2
	SA10-I_{50}	104.6	255.4	1085.4 e	4.25	3.47	41.0
	SA10-I_{75}	166.9	290.3	1271.3 d	4.38	3.29	57.5
	SA10-I_{100}	209.2	350.5	1362.2 c	3.89	3.06	59.7

increasing irrigation interval in all treatments. Average depletions of the available soil water holding capacity in 60 cm deep root zone during the vegetative season for full irrigation treatments were 29% (SA3), 40% (SA5) and 60% (SA10) in 2013 and 22% (SA3), 29% (SA5) and 50% (SA10) in 2014.

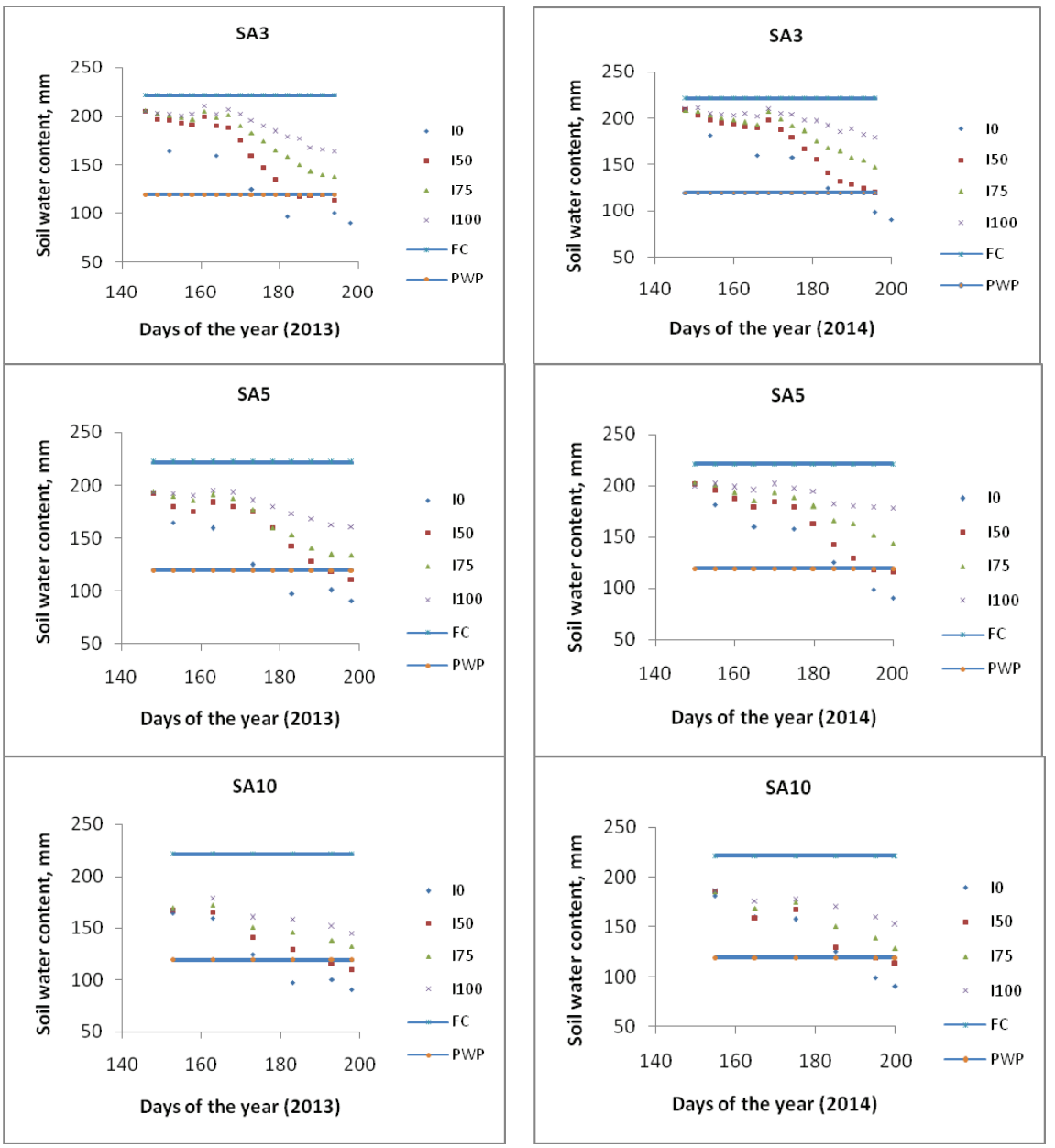

Figure 1- Soil water contents in 60 cm deep root zone before each irrigation

Seed yield varied between 722.2 (I_0) and 1700.6 kg ha^{-1} (SA5-I_{100}) as seen in Table 3. The difference among the treatments was found to be statistically significant in each experimental year ($P<0.05$). While statistically the highest yield was obtained from SA5-I_{100} in both years, SA5-I_{75} was in the highest yield group with 1487.4 kg ha^{-1} in the first year and followed SA5-I_{100} with 1491.5 kg ha^{-1} in the second year. There was no significant difference between the yields obtained from same irrigation programs formed by different methods in both years. Black cumin seed yields reacted positively to an increase in the amount of irrigation water, and the effect of irrigation on the yield was observed to be highly significant compared to the conditions in which no irrigation was applied. While the results of this study were similar to those of Ahmed & Haque (1986), Ozel et al (2009), Ghamarnia et al (2010), Al-Kayssi et al (2011) and Ghamarnia & Jalili (2013) in terms of seed yield, the values obtained in this study were higher than those of Kalcin (2003) and Safaei et al (2014). Furthermore, the values obtained from the treatments with 5 days irrigation interval were generally found to be higher than others in which similar or equal amount of irrigation water was applied. The reason for this, it may be associated that the black cumin commonly cultivated in unirrigated conditions and resistant to drought could show negative response to more intensive and the frequency irrigation applied in SA3 compared to SA5. El-Mekawy (2012) found that the yield of black cumin in treatments that irrigation were applied 4 or 6 days intervals after flowering stage till harvesting time were higher than treatments with 2 days irrigation interval after flowering stage. In this study, irrigation covered generally the period after flowering time due to the realization of flowering on the 163rd day of the year. Therefore, these results were in line with those of El-Mekawy (2012). Furthermore, the lower yield obtained in SA10 compared to SA5 could be due to water stress caused by low irrigation frequency which increased depletion of the available soil water holding capacity. The similar results were also reported by Al-Kayssi et al (2011).

3.2. Water-yield functions

3.2.1. Water use efficiencies

The highest and the lowest WUE values were calculated from SA5-I_{50} and SA3-I_{100} as 5.11 and 3.18 kg ha^{-1} mm^{-1}, respectively in the first year, and from SA5-I_{100} and SA3-I_{50} treatments as 4.73 and 3.73 kg ha^{-1} mm^{-1}, respectively in the second year (Table 3). In addition, the highest IWUE was calculated in SA5-I_{50}, while the lowest was calculated in SA3-I_{50}. In general, while WUE values were similar in both years, IWUE values in the first year were lower than those of the second year. The reasons of the differences can be explained with the higher total irrigation water amount applied in the first year because of pan evaporation methods used to form irrigation programs and less precipitation amount in the first year. The average WUE and IWUE values obtained in 5 days irrigation interval (4.56 and 3.85 kg ha^{-1} mm^{-1}) were higher than the other irrigation intervals. The WUE values calculated in the study were higher than WUE values found by Ghamarnia et al (2010) and Ghamarnia & Jalili (2013). Although ET values were similar to those of the mentioned previous studies, this difference in WUE was may have been caused by the higher yields as a result of the variety of the seed, climatic differences and cultivation practices. While the coverage ratio of the applied irrigation water to the evapotranspiration (IRc) was determined between 79.1% (SA10-I_{100}) and 33.8% (SA3-I_{50}). The highest IRc values were obtained in the highest irrigation interval (SA10) and full irrigation treatments (I_{100}) according to both high irrigation frequency and deficit irrigation treatments. IRc values of the first year were higher than those of the second year was due to the plant water requirement was met more by precipitation in the second year because of higher precipitation.

3.2.2. Graphical relationships

In the experiment, linear relationships were obtained between the irrigation water amount and yield (R^2= 0.520), and between the evapotranspiration and yield (R^2= 0.660) at 0.1% significance level (Figure 2). Although the black cumin yield could be

achieved even in the non-irrigated conditions, the relationships were observed that when irrigation water and ET increased, yield also increased.

3.3. Yield response factor

While SA5-I_{100} treatment was taken into consideration for ETm and Ym values in calculating mean yield response factor because of the highest yield obtained and no water stress observed, full irrigation (I_{100}) treatments were considered in calculating yield response factor for different irrigation intervals. The yield response factor (ky) values were calculated in SA3, SA5 and SA10 as 0.75, 0.80 and 0.50, respectively and mean ky of all treatments was also calculated as 0.68 (Figure 3). Although the ky value obtained in the high irrigation interval (SA10) was lower than those obtained in the low irrigation interval (SA3, SA5), all ky values were lower than 1. According to this result, the yield would decrease by only 0.68 units as a result of one unit deficit in the ET, it shows that black cumin plant is resistant to water deficit. It can be recommended that the deficit irrigation programs should be applied in the regions with limited water resources in order to increase the yield for black cumin plant which is widely cultivated under non-irrigated conditions especially.

3.3.1. The effective root depth, yield and vegetative parameters

The effective root depth of black cumin plant was determined as 27.25 cm. The effective root depth determined in the study was consistent with the findings reported by Taqi (2013) that the total root length of black cumin varied between 52.87 and 60.80 cm. For both years of the experiment, plant height, the number of branches, capsules, seeds in each capsule and a thousand seed weight values were obtained between 23.3 and 68.1 cm, between 3.7 and 7.6 number plant^{-1}, between 5.9 and 17.9 number plant^{-1}, between 42.3 and 94.3 number capsules^{-1}, and between 1.82 and 2.13 g, respectively (Table 4). According to some yield and vegetative parameters, significant differences were found among the treatments, and the yield

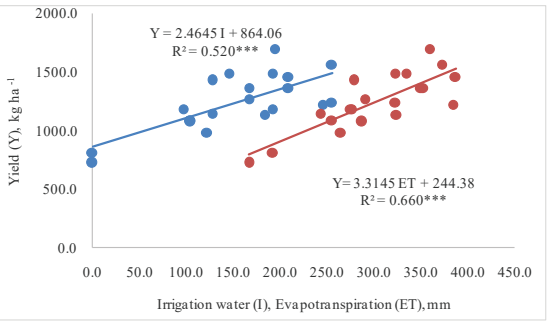

Figure 2- The relationships between irrigation water and yield, and evapotranspiration and yield

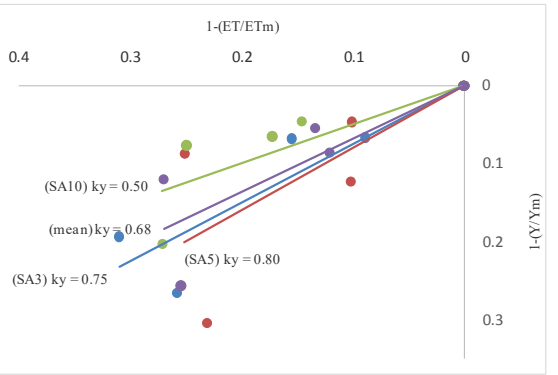

Figure 3- Yield response factor (ky) for all treatments and different irrigation intervals

and vegetative parameters of black cumin increased with the irrigation water amount ($P<0.05$). Except the harvest index value, while the values recorded in I_0 (non-irrigated) were generally constituted the lowest group, the highest values were recorded in I_{100} and followed by I_{75}. In addition, the values obtained from SA5 were generally higher than those obtained from SA3 and SA10. It was found out that the harvest index declined inversely to the increase in the irrigation water level due to the higher growth of the stem through irrigation, and it increased in the plots where less and no irrigation was applied due to the less growth of the stem. Although the findings obtained from the this study were generally higher than the findings of many researchers due to the controlled irrigation, it can be said that the

Table 4- Some yield and vegetative parameters of black cumin

Year	*Treatments*	*Plant height (cm)*	*Number of branches (number plant^{-1})*	*Number of capsules (number plant^{-1})*	*Seed number (number capsule^{-1})*	*Thousand seed weight (g)*	*Harvest index (%)*
2013	I_0	23.3 e	3.7 g	5.9 d	42.3 h	1.82 f	23.0 a
	SA3-I_{50}	41.0 d	4.0 fg	7.7 d	57.8 g	1.93 e	18.0 e
	SA3-I_{75}	48.9 c	4.4 ef	12.7 c	71.4 f	1.94 e	17.8 ef
	SA3-I_{100}	51.6 b	4.7 e	13.1 c	77.7 e	2.00 c	17.2 f
	SA5-I_{50}	47.5 c	5.3 cd	16.6 a	85.7 bc	1.82 f	16.9 g
	SA5-I_{75}	51.3 b	5.6 c	15.6 b	86.5 b	2.03 bc	19.0 d
	SA5-I_{100}	53.5 a	7.6 a	17.9 a	94.3 a	2.12 a	18.0 e
	SA10-I_{50}	48.0 c	4.8 de	11.2 c	80.5 d	2.05 b	17.0 fg
	SA10-I_{75}	50.2 bc	5.6 c	11.8 c	83.6 c	2.10 ab	21.4 b
	SA10-I_{100}	51.2 b	6.6 b	12.3 c	77.7 e	1.97 d	20.7 c
2014	I_0	49.1 h	4.5 d	7.5 f	74.1 e	1.95 de	28.0 a
	SA3-I_{50}	55.4 g	6.3 c	10.7 e	76.4 d	1.97 d	26.8 ab
	SA3-I_{75}	57.1 f	7.3 ab	13.0 bc	78.2 c	2.01 c	20.3 f
	SA3-I_{100}	59.0 e	7.3 ab	13.7 ab	81.2 b	2.03 bc	22.5 d
	SA5-I_{50}	64.9 d	6.5 c	11.7 cd	75.5 de	2.01 c	27.5 a
	SA5-I_{75}	65.7 c	6.5 c	13.4 b	80.3 b	2.08 b	20.9 e
	SA5-I_{100}	65.9 c	7.0 b	14.4 a	83.2 a	2.13 a	18.0 g
	SA10-I_{50}	58.6 e	7.0 b	11.3 d	74.3 e	1.94 e	25.6 bc
	SA10-I_{75}	67.2 b	7.2 ab	12.4 c	77.7 c	1.98 d	24.0 cd
	SA10-I_{100}	68.1 a	7.5 a	12.8 bc	77.9 c	2.05 b	22.3 d

difference with the findings of other researchers was based on the irrigation, soil and climatic features (Ghamarnia et al 2010; Akgoren 2011; Taqi 2013; Ghamarnia & Jalili 2013; Safaei et al 2014).

4. Conclusions

Consequently, it was revealed that irrigation was inevitable to obtain higher yield in black cumin plant which generally grows under non-irrigated conditions. Considering the water-yield functions, it can be recommended that the irrigation interval should be 5 days, irrigation water amount should be applied to meet full evapotranspiration requirement under conditions where the water is sufficient, or the maximum deficit should not exceed 25% of full irrigation under limited water conditions in the irrigation schedules for black cumin. In addition, both of the pan evaporation and water balance methods can be used for irrigation of black cumin, since the values of the yield and vegetative parameters and ET in the same irrigation programs formed by two different methods were close to each other. Especially, pan evaporation which is simple method to obtain irrigation programs, can be preferred because of low cost, labor and easy operation.

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References

Ahmed N U & Haque K R (1986). Effect of row spacing and time of showing on the yield of black cumin (*Nigella sativa* L.). *Bangladesh Journal of Agricural Research* **11**(1): 21-24

Akgoren G (2011). Agricultural features of some black cumin (*Nigella sativa* L.) populations. MSc Thesis, Osman Gazi University (Unpublished), Eskisehir

Al-Kayssi A, Shibab R M & Mustafa S H (2011). Impact of soil water stress on Nigellone oil content of black cumin seeds grown in calcareous-gypsiferous soils. *Agricultural Water Management* **100**: 46-57

Baydar H (2013). The Science and Technology of Medicinal and Aromatic Plants. 1st Ed. Süleyman Demirel University, Isparta

Doorenbos J & Pruitt W O (1977). Crop water requirements. *Irrigation and Drainage Paper,* No. 24 FAO, Rome, pp. 179

Doorenbos J & Kassam A H (1986). Yield response to water. *Irrigation and Drainage Paper,* No. 33. FAO, Rome, pp. 193

El-Mekawy M A M (2012). Growth and yield of *Nigella sativa* L. plant influenced by sowing date and irrigation treatments. *American-Eurasian Journal of Agriculture & Environmental Science* **12**(4): 499-505

English M & Raja S N (1996). Perspectives on deficit irrigation. *Agricultural Water Management* **32**: 1-14

Ghamarnia H & Jalili Z (2013). Water stress effects on different Black cumin (*Nigella sativa* L.) components in a semi-arid region. *International Journal of Agronomy and Plant Production* **4**(4): 753-762

Ghamarnia H, Khosravy H & Sepehri S (2010). Yield and water use efficiency of *(Nigella sativa L.)* under different irrigation treatments in a semi arid region in the West of Iran. *Journal of Medicinal Plants Research* **4**(16): 1612-1616

Howell T A, Cuenca R H & Solomon K H (1990). Crop yield response. In: *Management of Farm Irrigation Systems*. ASAE, Madison, Wisconsin, pp. 312

James L G (1988). Principles of farm irrigation system design. John Wiley and Sons. Inc., New York, pp. 543

Kalcin F T (2003). Effect of plant density on yield and yield components on two black cumin species (*Nigella sativa* L. *Nigella damascena* L.). MSc Thesis, Ankara University (Unpublished), Ankara

Kanber R (1984). Çukurova koşullarında açık su yüzeyi buharlaşmasından yararlanarak birinci ve ikinci ürün yerfıstığının sulanması [in Turkish]. Bölge Topraksu Araştırma Enstitüsü Yayınları **114**(64), Tarsus, Türkiye

Kanber R (1997). Irrigation. 1st Ed. Cukurova University, Academic Press, Adana, pp. 140-142

Kanber R (2010). Field Irrigation Systems. 1st Ed. Cukurova University, Academic Press, Adana, pp. 402-490

Kumar S A (2009). Plants-based Medicines in India. Retrieved in February, 15, 2013 from http://pib.nic.in/feature/feyr2000/fmay2000/f240520006.html

Ozel A, Demirel U, Guler I & Erden K (2009). Effect of different row spacing and seeding rates on black cumin (*Nigella sativa* L.) yields and some agricultural characters. *University of Harran Journal of the Faculty of Agriculture* **13**(1): 17-25

Panda R K, Behera S K & Kashyap P S (2004). Effective management of irrigation water for maize under stressed conditions. *Agricultral Water Management* **66**: 181-203

Safaei Z, Azizi M, Maryam Y, Aroiee H & Davarynejad G (2014). The effect of different irrigation intervals and anti-transpiration compounds on yield and yield components of black cumin (*Nigella sativa* L.). *International Journal of Advanced Biological and Biomedical Research* **4**(2): 326-335

Taqi H (2013). Determination of important agricultural and quality characters of some black cumin (*Nigella sativa* L.) populations under Samsun ecological conditions. MSc Thesis, Ondokuz Mayıs University (Unpublished), Samsun

TUIK (2015). Turkish Statistical Institute, Retrieved in February, 20, 2015 from http://www.tuik.gov.tr/

Wang Z, Liu Z, Zhang Z & Liu X (2009). Subsurface drip irrigation scheduling for cucumber (*Cucumis sativus* L.) grown in solar greenhouse based on 20 cm standard pan evaporation in Northeast China. *Scientia Horticulturae* **123**: 51-57

Yazar A, Sezen S M & Gencel B (2002). Drip irrigation of corn in the Southeast Anatolia project (GAP) area in Turkey. *Irrigation and Drainage* **51**: 293-300

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Development of a Small-sized and Low-cost Attitude Measurement Unit for Agricultural Robot Application

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ABSTRACT

The objective of this study was to develop a small-sized and low-cost unit to provide attitude measurements for light-loaded, small-sized and cost effective agricultural robot application. The attitude measurement unit comprised an electronic control unit (ECU) and a gyroscope and an accelerometer within a small-sized and low-cost IMU. In order to avoid the measurement limitations of a single sensor, a self-adaptive complementary filter and a Kalman filter were discussed and compared for sensor fusion. By comparison, in respect of preventing angle drift and maintaining dynamic characteristics, the Kalman filter has the significant advantage, especially in dynamic motion. In the comparison with a highly precise aviation-level fiber optic gyroscope (FOG), the results showed that the static angle drift was restrained by Kalman filter which reached the performance of the FOG. And in the series of farm experiments, the dynamic characteristic of the developed attitude measurement unit is close to the FOG performance in the sub-degree level. This is an acceptable accuracy for light-loaded, small-sized and cost effective agricultural robot application such as agriculture drone, greenhouse robots, harvesting robot arm and so on.

Keywords: IMU sensor; Complementary filter; Kalman filter; Sensor fusion; Attitude estimation

Tarımsal Robot Uygulamaları için Küçük Boyutlu ve Düşük Maliyetli Konum Ölçüm Ünitesinin Geliştirilmesi

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ÖZET

Bu çalışmanın amacı; hafif, küçük boyutlu ve uygun maliyetli tarımsal robot uygulamaları için konum ölçümlerini sağlayan küçük boyutlu ve düşük maliyetli bir ünitenin geliştirilmesidir. Konum ölçüm ünitesi; bir elektronik kontrol ünitesi (ECU), bir jiroskop ile küçük boyutlu ve düşük maliyetli atalet ölçüm ünitesi (IMU) içeren bir ivmeölçerden

oluşmaktadır. Tek bir sensörün ölçüm sınırlamalarından kaçınmak amacıyla, sensör birleştirmeleri için otomatik ayarlı bir tamamlayıcı filtre ve Kalman filtresi ele alınmış ve karşılaştırılmıştır. Karşılaştırmada, açı kaymasının önlenmesi ve dinamik özelliklerin muhafazası bakımından Kalman filtresinin, özellikle dinamik hareket nedeniyle önemli avantaja sahip olduğu ortaya çıkmıştır. Sonuçlar havacılıkta kullanılan yüksek hassasiyetli bir fiber optik jiroskop (FOG) ile karşılaştırıldığında, Kalman filtresi tarafından belirlenen statik açı kayması sonuçlarının FOG'un performansına yaklaşıldığını göstermiştir. Tarla denemelerinde, geliştirilen konum ölçüm ünitesinin dinamik karakteristiği FOG'un performansına yakın bulunmuştur. Bulunan bu sonuçlar; tarımsal amaçlı kullanılan insansız hava araçları (dronlar), sera robotları, hasat robot kolları gibi hafif, küçük boyutlu ve uygun maliyetli tarımsal robot uygulamaları için kabul edilebilir bir hassasiyettir.

Anahtar Kelimeler: IMU sensör; Tamamlayıcı filtre; Kalman filtre; Sensör birleştirme; Konum tahminleme

1. Introduction

The role of robotics in precision agriculture (PA) is becoming more and more important with the development of electronic technology. By combining with various advanced sensors, it has become possible for agricultural machines to do farming tasks autonomously. A global positioning system (GPS) has recently been used extensively in autonomous navigation for providing position information. For higher navigation accuracy, attitude sensors such as a geomagnetic direction sensor (GDS), FOG and an inertial measurement unit (IMU) can be utilized to correct the GPS position information. There have been a considerable number of studies on application of GPS with attitude sensors (Kise et al 2001; Noguchi et al 2001; Inoue et al 2009). Much progress has also been made in large-scale mechanization, which has advantages of high strength workload and labor cost reduction. However, now more attention is being given to small-sized smart agricultural machines (Robinson 2012). In order to keep the soil loose, there is a need to develop light-weight, small-sized, low-power and low-cost systems for agricultural robotic applications. Therefore, the development of a small-sized and low-cost attitude measurement system is necessary.

Two sensors, a gyroscope and an accelerometer, were used to measure the attitude of an agricultural robot. A gyroscope is an inertial sensor for measuring orientation based on the principles of angular momentum. However, because of noise jamming, temperature variation and unstable force moment, system drift error will occur and increase with time. Therefore, a gyroscope cannot be reliably used for a long time. Otherwise, an accelerometer is a device that measures proper acceleration. When the accelerometer is motionless, the attitude angles can be calculated on the basis of the acceleration of gravity component in every axis via trigonometric functions. However, an accelerometer cannot distinguish between the acceleration of gravity and external acceleration. Therefore, in the case of frequent variable accelerated motion and a bumpy outdoor field, the use of only an accelerometer is not appropriate for calculating attitude angle. Thus, the use of only a gyroscope or an accelerometer is not appropriate for a farming operation over a long period and in a complex environment.

The objective of this study was to develop a small-sized and low-cost unit including an ECU and an IMU to provide attitude measurement with acceptable accuracy for light-loaded, small-sized and low-priced agricultural robot application. Data processing used the sensor fusion principle including a self-adaptive complementary filter and a Kalman filter to integrate data from the gyroscope and accelerometer in the IMU.

2. Material and Methods

2.1. Hardware platform

For a small agricultural machine development, size and price are the two main parameters that must be

considered in the design idea. In this study, a small-sized and low-cost IMU (S4E5A0A0A1, Seiko Epson) was used as the inertial sensor. This IMU with six degrees of freedom is compact (24×24×10 mm^3) and only weighs 7 grams. It is composed of a triaxial micro-electro-mechanical system (MEMS) accelerometer, a triaxial quartz-MEMS (QMEMS) gyroscope and a temperature sensor. Outputs of the IMU include chip temperature, triaxial angular rates and linear accelerations in real time. The main performance and specifications of the IMU are shown in Table 1.

Table 1- Main performance and specifications of the IMU

Parameters	*Type*	*Unit*
Gyroscope		
Dynamic range	±300	deg s^{-1}
Initial error	0.5	deg s^{-1}
In-run bias stability	6	deg h^{-1}
Angular random walk	0.2	Deg $\sqrt{hr^{-1}}$
Noise density	0.004	deg s^{-1} $\sqrt{Hz^{-1}}$
Accelerometer		
Dynamic range	±3	G
Initial error	8	mG
In-run bias stability	0.1	mG
Velocity random walk	0.04	mG sec^{-1} $\sqrt{hr^{-1}}$
Noise density	0.06	mG $\sqrt{Hz^{-1}}$, rms

An ECU (Due, Arduino) was used for collecting and processing IMU data for attitude estimation. This ECU is based on the Atmel SAM3X8E ARM Cortex-M3 CPU. The 32-bit ARM core is fast enough for communicating with the IMU, to realize the sensor fusion algorithm and outputting results of the attitude measurement data to peripheral equipment by serial port. Figure 1 shows an application of the attitude measurement unit communicating with a computer.

In the IMU, an accelerometer was used for measuring triaxial linear acceleration. Figure 2 shows the coordinates and tilt angle of the accelerometer. Coordinate *O-XYZ* is the geodetic

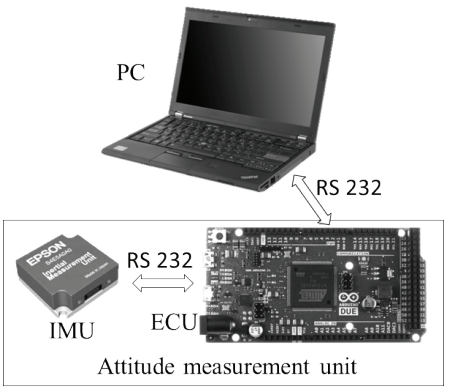


Figure 1- Schematic diagram of the attitude measurement unit

coordinate system, and coordinate *o-xyz* is the IMU body-fixed coordinate system. The tilt angle between the *OX* axis and *ox* axis is called pitch, and the tilt angle between the *OY* axis and *oy* axis is called roll. As is well known, in nature, the acceleration of gravity vector is directed to the center of the earth. The value measured by an accelerometer is the projection addition of acceleration of gravity and absolute acceleration (Chen et al 1994). Thus, when the IMU remains steady and Corioli's acceleration and noise can be ignored, the relationship among the components of acceleration of gravity can be described as shown in Equation (1).

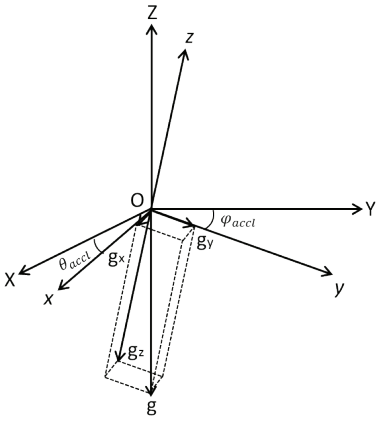


Figure 2- Attitude angle of the accelerometer in coordinate

$$g = \sqrt{g_x^2 + g_y^2 + g_z^2} \quad (1)$$

Where; g, acceleration of gravity; g_x, g_y and g_z, acceleration components of gravity in the *ox*, *oy* and *oz* axes, respectively.

Based on trigonometric functions, the formulas of attitude angle can be obtained by Equation (2) and Equation (3).

$$\theta_{accl} = -\arcsin(\frac{g_x}{g}) \quad (2)$$

$$\varphi_{accl} = \arctan(\frac{g_y}{g_z}) \quad (3)$$

Where; θ_{accl} and φ_{accl}, denote the pitch and roll angles, respectively.

However, the angle between the *OZ* axis and *oz* axis, denoted as yaw, is in the horizontal plane. The angle is orthogonal to the acceleration of gravity. Thus, projection on the horizontal plane cannot be obtained, and therefore the yaw cannot be calculated from the accelerometer. In addition, the environment in which the agricultural robot works is complex. There will be a random noise in measurements by the accelerometer when there is strenuous accelerated motion.

The gyroscope can measure the angular rate of the IMU. The triaxial angles Φ_{gyro} can therefore be obtained by angular rate via an integral as shown in Equation (4).

$$\Phi_{\text{gyro}} = \int \omega_{\text{gyro}} \text{dt} \quad (4)$$

Where; ω_{gyro} is the measured angular rate; *dt* is the gyroscope measurement sampling period.

Because of the temperature variation, unstable moment of force and noise jamming denoted by σ, the gyroscope will accumulate drift error that will become larger with time, as can be seen in Equation (5).

$$\Phi_{gyro} = \int (\omega_{gyro} + \sigma)\ dt \quad (5)$$

Thus, attitude can be measured by using only an accelerometer or a gyroscope, but each sensor has the measurement limitation. The accelerometer has a motion bandwidth problem, and the gyroscope has angle drift error when operation time is long.

2.2. Sensor fusion method

To avoid the measurement limitations by using only an accelerometer or a gyroscope, the sensor fusion method was used to integrate data from the accelerometer and the gyroscope. Figure 3 shows a block diagram of the sensor fusion methods. In this study, two methods were discussed and compared. One is a self-adaptive complementary filter and the other one is a Kalman filter.

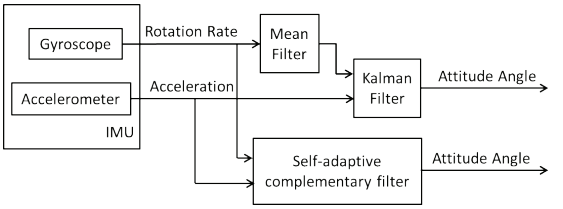


Figure 3- Block diagram of the sensor fusion methods

Because of the high-pass characteristics of gyroscope and low-pass characteristics of accelerometer, the complementary filter can combine the advantages from the both sensors. A student group which was sponsored by Edgerton center at MIT (Colton 2007) described the complementary filter as Equation (6).

$$angle_{i+1} = (1-k)\cdot(angle_i + \omega_{gryo}\cdot dt) + k\cdot angle_{acc} \quad (6)$$

Where; k, filter weight coefficient; $angle_{acc}$, angle calculated by accelerometer.

In order to make the complementary filter work better, the choice of is important. When is small, the estimation result depends on the angular rate, the influence from accumulate drift error is big. When is big, the estimation result depends on acceleration, the influence from accelerated motion is big. Therefore, based on the state of motion, a self-adaptive complementary filter was designed as Equation (7). The determination of k_i was based on Equation (8).

$$angle_{i+1} = (1-k_i)\cdot(angle_i + \omega_{gryo}\cdot dt) + k_i\cdot angle_{acc} \quad (7)$$

$$k_i = \begin{cases} 0, & when\,|a_i - g| > \lambda \\ k_{\max} & when\,|a_i - g| < \lambda \\ p\cdot|a_i - g| + q & when\,\lambda < |a_i - g| < \lambda \end{cases} \quad (8)$$

Where; g, acceleration of gravity; a_i, resultant acceleration; parameters λ and λ are based on environment noise and motion state; p and q, determined by the parameters λ, λ and the extremum of weight coefficient k_{max}.

The Kalman filter (Kalman 1960) is another popular filter which is used on data fusion processing. In order to prevent the high frequency measured noise on the gyroscope, the mean filter is used to eliminate the outlier signal as a pre-process of the angular rate obtained by the gyroscope in this low-cost IMU. The mean filter is expressed in Equation (9).

$$\omega_{\text{gyro}} = \frac{\sum_{t=1}^{n} \omega_t}{n} \quad (9)$$

Where; n, window size of the mean filter. In consideration of the ECU computing scale and time delay, n= 3 was used in this study.

The Kalman filter is optimal when process noise and measurement noise can be modelled by white Gaussian noise (St-Pierre & Gingras 2004). As mentioned above, the relationship between attitude angle and angular rate is derivative relation. The system can therefore be described as a discrete-time state equation expressed in Equation (10).

$$X(k \mid k-1) = A \cdot X(k-1 \mid k-1) + B \cdot U(k) \quad (10)$$

Where; A, system transition matrix $A = \begin{bmatrix} 1 & -Ts \\ 0 & 1 \end{bmatrix}$; B, system control matrix $B = \begin{bmatrix} Ts \\ 0 \end{bmatrix}$; T_s, sampling period; $X(k|k-1)$, system state in moment k estimated by state k-1; $U(k)$, exogenous state control input in moment k ($U(k)$= 0 in this study). The covariance of $X(k|k-1)$ is shown in Equation (11).

$$P(k \mid k-1) = A \cdot P(k-1 \mid k-1) \cdot A^T + Q \quad (11)$$

Where; Q, covariance matrix of the system process noise $Q = \begin{bmatrix} q\_accl & 0 \\ 0 & q\_gyro \end{bmatrix}$, in which q_accl is the noise covariance of the accelerometer; q_gyro, noise covariance of the gyroscope; matrix A^T, transpose of matrix A.

The optimal estimation $X(k \mid k)$ in state k is calculated by Equation (12).

$$X(k \mid k) = X(k \mid k-1) + k_g(k) \cdot (Z(k) - H \cdot X(k \mid k-1)) \quad (12)$$

Where; H, observation matrix, H=[1 0]; $k_g(k)$, Kalman gain which is updated by Equation (13).

$$k_g(k) = \frac{P(k \mid k-1) \cdot H^T}{H \cdot P(k \mid k-1) \cdot H^T + R} \quad (13)$$

Where; R is a covariance matrix of error in measurement by the accelerometer. In order to update the Kalman filter, the covariance equation is updated by Equation (14).

$$P(k \mid k) = (I - k_g(k) \cdot H) \cdot P(k \mid k-1) \quad (14)$$

Where; I, unit matrix, $I = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$.

Based on recursive functions from Equation (7) to Equation (11), it is possible to calculate iteratively to find the optimal estimate attitude angle.

3. Results and Discussion

In order to verify the validity of the sensor fusion methods that combines the accelerometer and gyroscope, which are better than using each sensor alone, two indexes of the sensors were chosen to evaluate performance. One was the drift error and the other was the dynamic attitude angle. According to the electrical specifications of the IMU, the initial parameters of the Kalman filter and complementary filter are shown in Table 2.

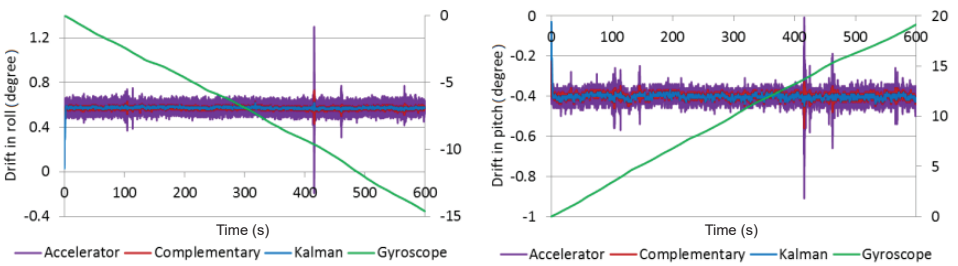
Table 2- Initial parameters of the Kalman filter and complementary filter

Kalman filter				*Complementary filter*		
Parameter	*Ts*	*A*	*Q*	λ	λ	k_{max}
Value	0.008 s	$\begin{bmatrix} 1 & -0.008 \\ 0 & 1 \end{bmatrix}$	$\begin{bmatrix} 0.001 & 0 \\ 0 & 0.003 \end{bmatrix}$	0.2G	0.004G	0.2
Parameter	*R*	*X0*	*P0*	*p*	*q*	
Value	[0.03]	$\begin{bmatrix} 0 \\ 0 \end{bmatrix}$	$\begin{bmatrix} 0.04 & 0.04 \\ 0.04 & 0.04 \end{bmatrix}$	-0.918	0.204	

3.1. Filters comparison

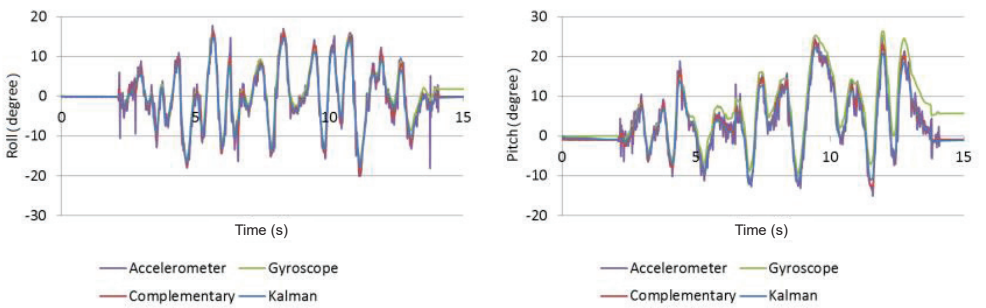
Drift error is directly related to measurement accuracy and stability of the measurement system. In this study, we kept the IMU static in a horizontal plane and then compared the performances using the gyroscope alone, the accelerometer alone and two sensor fusion methods. Figure 4 shows a comparison of results for drift during a period of 10 minutes. Figure 4(a) shows drift in the roll direction, and Figure 4(b) shows drift in the pitch direction. In this coordinate system, the abscissa is the sampling time in seconds. The ordinate is drift angle in degrees. Because of error accumulation, drift measured by gyroscope alone continuously increases to -14.6 degrees in the roll direction and to 19.1 degrees in the pitch direction. Drift measured by the accelerometer alone does not increase with time, but data oscillate in a wide range area in both roll and pitch directions. When the self-adaptive complementary filter and Kalman filter were used, drift was almost zero and was smoother than the data from the accelerometer.

The dynamic characteristic is therefore a very important index to evaluate the attitude measurement unit. Similar to the above evaluation steps of drift error, the dynamic attitude angle data was logged when rotating the attitude measurement unit in different rotation directions. Figure 5 shows a comparison of results for dynamic attitude angles when using the gyroscope alone, the accelerometer alone and two sensor fusion methods. In this coordinate system, the abscissa is the sampling time in seconds. The ordinate is the dynamic attitude estimated angle in degrees. Because the IMU worked in a random variable motion,



(a) Drift in roll direction (b) Drift in pitch direction

Figure 4- Comparison for drift when using the gyroscope alone, accelerometer alone and two sensor fusion methods



(a) Dynamic attitude in roll direction (b) Dynamic attitude in pitch direction

Figure 5- Comparison for dynamic attitude angles when using the gyroscope alone, the accelerometer alone and two sensor fusion methods

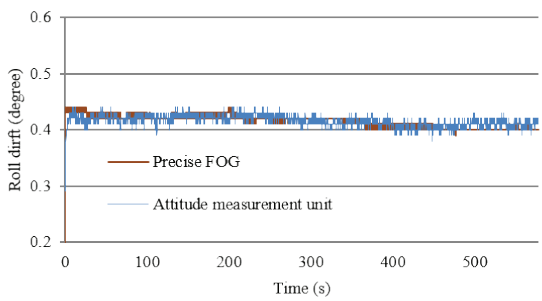
we can see that the dynamic curve measured by the accelerometer is not stable, a lot of spurious signal errors superpose on the dynamic curve, especially at the position where motion direction is changed. In a short time, the data drift obtained by the gyroscope alone is not very obvious. However, at the end of the sampling time, the attitude angle cannot return to the original value. The drift errors are 1.8 degrees in the roll direction and 5.7 degrees in the pitch direction. Otherwise, the dynamic curve which was estimated by the self-adaptive complementary filter was better than the performance of each sensor alone. A large numbers of spurious signals were removed, but some parts of wide oscillations still exist, especially on the data wave crest. On the other hand, the dynamic curve estimated by Kalman filter is much more robust and smooth.

3.2. System performance comparison

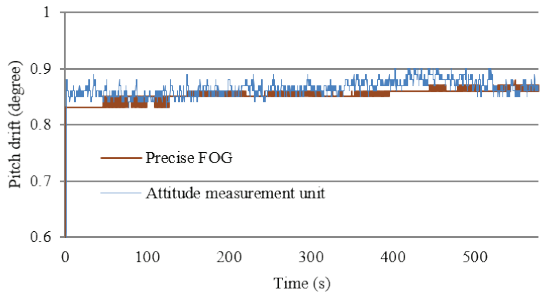
Through the above comparative analysis, it was found that the Kalman filter processing was superior to the single sensor measurement and the complementary filter. For in-depth discussion, we compared the developed small-sized and low-cost attitude measurement unit based on Kalman filter processing with a highly precise aviation-level FOG attitude measurement equipment (JCS7401A, Japan Aviation Electronics Industry), which can output attitude angles directly with accuracy of ±0.2 degrees and digital measured resolution of 0.1 degrees.

At first, the attitude measurement unit and the FOG were fixed together on the same plane and drift data were logged. Figure 6 shows a comparison of results for drift in the roll and pitch direction during a period of 10 minutes. The drift data show that the performance of the attitude measurement unit is very close to that of the precise FOG. The drift data of the two devices are both stable in the range of 0.1 degrees. That is to say the developed attitude measurement unit can inhibit the angle drift for providing high static stability.

As shown by results presented in Figure 6, data obtained by the sensor fusion method not only inherits the little drift characteristics from the accelerometer but also inherits the transient



(a) Drift in roll direction



(b) Drift in pitch direction

Figure 6- Comparison for drift when using the developed attitude measurement unit and precise FOG

stability from the gyroscope. Now, we compared the dynamic attitude angle obtained from both the attitude measurement unit using the Kalman filter and the highly precise FOG. In order to ensure high accuracy of comparison as far as possible, the attitude measurement unit and FOG were fixed together and in the same coordinate. The set-up situation is shown in Figure 7.

As it was done when comparing the dynamic angles measured by the gyroscope alone, the accelerometer alone and the two sensors fusion methods, we randomly rotated the attitude measurement unit and the FOG synchronously in different rotation directions and logged the data. Figure 8 shows that the measurement of dynamic attitude angle by the attitude measurement unit coincides well with that by the highly precise FOG. Based on statistics, the RMS errors are 0.3 degrees in the roll direction and 0.4 degrees in the pitch

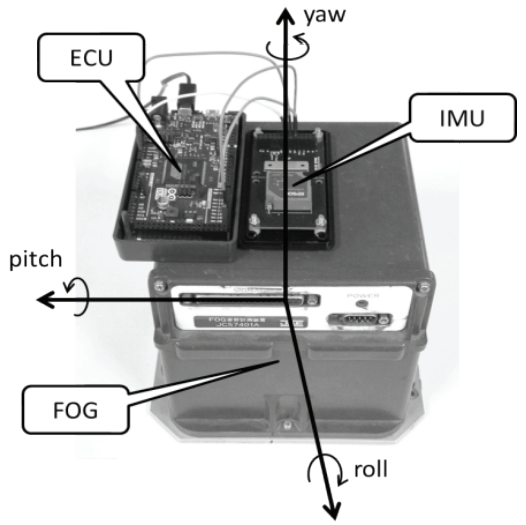
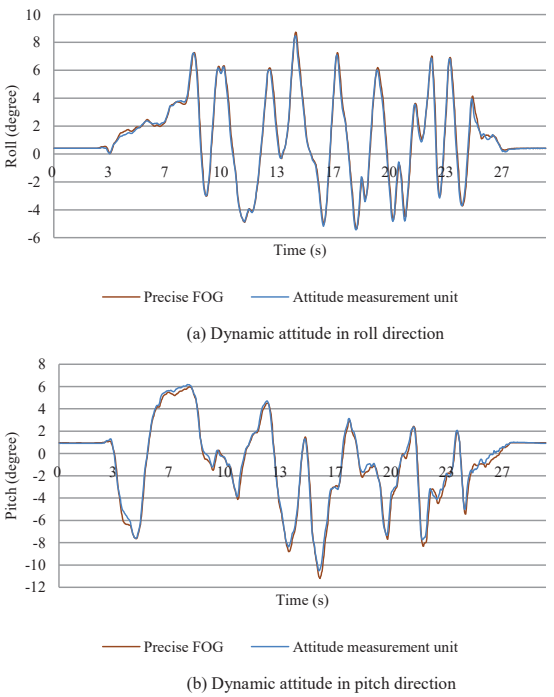


Figure 7- Fixation situation of the attitude measurement unit and FOG



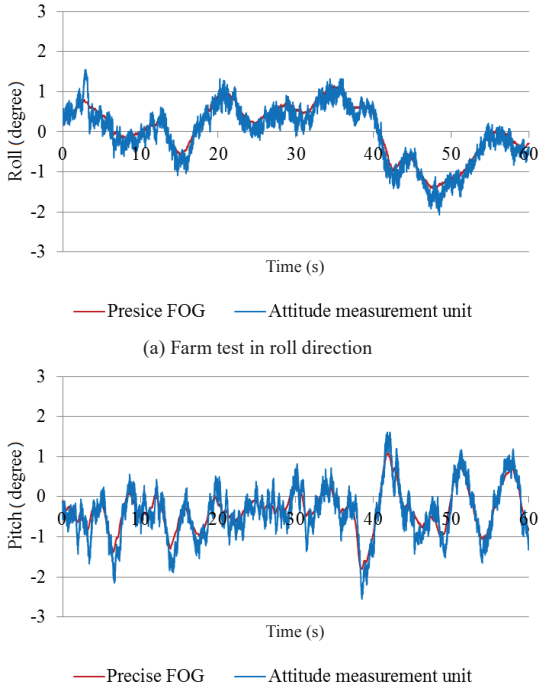
(a) Dynamic attitude in roll direction

(b) Dynamic attitude in pitch direction

Figure 8- Comparison for dynamic attitude angle when using the attitude measurement unit and the precise FOG

direction. Several errors which are bigger than 1 degree appeared once in a while. The maximum error in the roll direction is -1.3 degrees and the maximum error in the pitch direction is 1.4 degrees.

As we know, the farming conditions are complex for agricultural machinery such as the level and smooth of field surface, temperature, humidity and so on. Experiment is the sole criterion for testing the developed attitude measurement unit. A developed robot combine harvester (Zhang et al 2013) was utilized to attach the attitude measurement unit and precise FOG. The experiment was executed in the farm of Hokkaido University. This robot combine harvester could run autonomously under a predetermined map. The developed attitude measurement unit and the high precise FOG recorded the attitude of the robot respectively. The tests were conducted in different running speeds of robot of 0.5, 0.7 and 1.0 ms^{-1}. Figure 9 shows the



(a) Farm test in roll direction

(b) Farm test in pitch direction

Figure 9- Comparison for farm test when using the attitude measurement unit and the precise FOG

record result of robot attitude when robot run in speed of 0.7 ms^{-1}. The dynamic behaviour of attitude measurement unit is close to the precise FOG. The RMS errors are 0.2 degrees in the roll direction and 0.3 degrees in the pitch direction. However, the measurement noise of the attitude measurement unit is obvious in the sub-degree level which is not good as the FOG. The noise of ripple wave on the attitude angle was brought from the engine vibration. The similar phenomenon also appeared in the tests of speed of 0.5 and 1.0 m s^{-1}. Table 3 summarizes the errors of the attitude measurement unit compared to the precise FOG in different robot running speed. That means the developed attitude measurement unit need to be improved much more on the inhibition of high-frequency noise.

Table 3- Errors of dynamic attitude angle in different robot running speeds

Speed ($m\ s^{-1}$)	*Error (degree)*					
	Roll			*Pitch*		
	Max	*RMS*	*Mean*	*Max*	*RMS*	*Mean*
0.5	0.4	0.1	-0.1	0.6	0.2	-0.1
0.7	0.8	0.2	0.1	1.0	0.3	0.1
1.0	0.6	0.2	-0.1	1.3	0.3	-0.1

4. Conclusions

In this study, a small-sized and low-cost electronic unit was developed to provide attitude estimation with acceptable accuracy for light-loaded, small-sized and low-priced agricultural robot applications. This attitude measurement unit was composed of a small, low-cost IMU and an ECU by using sensor fusion methods. Based on the characteristic of gyroscope and accelerator, a self-adaptive complementary filter and a Kalman filter were discussed and compared. According to the comprehensive evaluation of drift error and dynamic motion, the Kalman filter is better to compensate the IMU drift, improved noise immunity and reduced measurement error. In the comparison with a highly precise FOG, the drift of attitude measurement unit approximates to the performance of the FOG. Finally, the results of farm test in dynamic characteristic shows that the performance of attitude measurement unit is close to the precise FOG, but the noise of ripple wave on the attitude angle exist. This issue needs to be solved in the future research. In a word, this developed attitude measurement unit can be applied to the low-cost small-sized harvesting robot arm, the greenhouse robots and agriculture drones.

References

Chen J, Lee S & Debra D B (1994). Gyroscope free strapdown inertial measurement unit by six linear accelerometers. *Journal of Guidance, Control and Dynamics* **17**(2): 286-290

Colton S (2007). The balance filter. Retrieved in May, 15, 2016 from http://web.mit.edu/first/segway/#how

Inoue K, Nii K & Zhang Y (2009). Tractor guidance system for farm work using DGPS and gyroscope. *Journal of Robotics and Mechatronics* **21**(3): 394-402

Kalman R E (1960). A new approach to linear filtering and prediction problems. *Transactions of the ASME-Journal of Basic Engineering* **82**(Series D): 35-45

Kise M, Noguchi N, Ishii K & Terao H (2001). Development of agricultural autonomous tractor with an RTK-GPS and a FOG. *Proceedings of the 4th IFAC Symposium on Intelligent Autonomous Vehicles*, 5-7 September, Sapporo, pp. 103-108

Noguchi N, Reid J F, Zhang Q, Will J D & Ishii K (2001). Development of robot tractor based on RTK-GPS and gyroscope. *ASAE Annual Meeting*, 29 July-1 August, Sacramento, Paper No: 011195

Robinson L (2012). Think smart and small for the future of crop protection. Retrieved in May, 10, 2016 from http://www.fwi.co.uk/articles/26/06/2012/133520/think-smart-and-small-for-the-future-of-crop-protection.htm

St-Pierre M & Gingras D (2004). Comparison between the unscented Kalman filter and the extended Kalman filter for the position estimation module of an integrated navigation information system. *2004 IEEE Intelligent Vehicles Symposium*, 14-17 June, Parma, pp. 831-835

Zhang Z, Noguchi N, Ishii K, Yang L & Zhang C (2013). Development of a robot combine harvester for wheat and paddy harvesting. *5th IFAC Conference on Bio-Robotics*, 27-29 March, Sakai, Japan, pp. 45-48

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The Hydraulic and Economic Performance Analysis of On-Demand Pressurized Irrigation Systems: A Case Study in Turkey

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ABSTRACT

In this study, COPAM (Combined Optimization and Performance Analysis Model) software revealing optimum design possibilities and performance analysis of pressurized irrigation systems, was applied to on-demand pressurized irrigation system in Uludag University Agricultural Application and Research Centre, Bursa, Turkey. The system reliability, hydrant pressure heads, upstream elevation, discharges and pipe diameters related to this irrigation system were analyzed with COPAM software which have a variety of analysis tools. Analysis results showed that there were no deficiencies of performance in the hydrant level of the examined system. Furthermore, pipe diameters of the existing irrigation network were recalculated with COPAM as an alternative scenario and the system performance was reanalyzed based on the new pipe diameters obtained. As a result of this analysis, it wasn't seen any difference in the system performance, although total pipe cost was reduced by 16%.

Keywords: Irrigation system performance; Hydrants characteristics; System reliability; Cost analysis

İstek Yöntemiyle İşletilen Basınçlı Sulama Sistemlerinin Hidrolik ve Ekonomik Performans Analizi: Türkiye'den Bir Durum Çalışması

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ÖZET

Bu çalışmada basınçlı sulama sistemlerinin optimum tasarım olanaklarını belirleyen ve performans analizini yapan COPAM (sınıflandırılmış karakteristik eğriler modeli) yazılımı, Uludağ Üniversitesi Tarımsal Uygulama ve Araştırma Merkezi'nde istek yöntemi ile işletilen basınçlı sulama şebekesine (Bursa, Türkiye) uygulanmıştır. Ele alınan sulama sistemine ilişkin sistem güvenirliliği, hidrant basınç yükleri, kaynak yüksekliği, debi ve boru çapları, geniş analiz araçlarına sahip olan COPAM yazılımı ile analiz edilmiştir. Analiz sonuçları, incelenen sistemde hidrant düzeyinde performans eksikliği olmadığını göstermiştir. Ayrıca, mevcut sulama şebekesinin boru çapları, alternatif bir senaryo olarak COPAM yazılımı ile yeniden hesaplanmış ve elde edilen yeni boru çaplarına göre sistem performansı tekrar analiz edilmiştir. Bu analiz sonucunda toplam boru maliyetinin % 16 oranında azaltılmasına rağmen, sistemin performansında herhangi bir farklılık görülmemiştir.

Anahtar Kelimeler: Sulama sistem performansı; Hidrant karakteristikleri; Sistem güvenilirliği; Maliyet analizi

1. Introduction

It is known that pressurized irrigation systems have more advantages compared with open channels commonly used in irrigated agriculture. Pressurized irrigation systems provide more productive use of water at farm level and keep losses at minimum level (Barutçu & Özcan 2011). Therefore, more area can be irrigated with the same amount of water and the measurement of the amount of water conveyed with these systems can be performed more easily and correctly.

On-demand is mostly preferred as operating method for pressurized irrigation systems, because it is based on conveying water to the irrigated area continuously. The system is based on the principle to provide the required amount of water to irrigation networks within appropriate time. These systems can keep water continuously in the valves of piped systems. The advanced technology is generally used in irrigation networks which water is distributed based on on-demand method. Human interference is at minimum level when the system is operated with automation principles in particular. Farmers can control the frequency and duration of irrigation better (Akyol 2012).

One of the most important issues in the design of on-demand irrigation systems is the calculation of the system discharge. These kinds of discharge can change rapidly depending on the crop pattern, climatic conditions, and on-farm irrigation efficiency and the farmers' demands. The development of the system and on-demand operation performance of irrigation systems require to consider flow regimes changing during the project (Lamaddalena 1997). When considered that initial investment costs of pressurized irrigation systems are high, low level savings can even reach to a remarkable level for these systems. Thus, the prevalence of computer programs and ease of use of the presented programs obligate to deal with pressurized irrigation systems within a system approach (Beyribey & Balaban 1992).

There is a tendency to pressurized irrigation systems in Turkey nowadays. Especially, the design and performance analysis of on-demand pressurized irrigation systems have a great importance in this tendency. Recently, many computer simulation models have been developed to reach this purpose around the world. There are many computer models such as COPAM (Lamaddalena 1997), EPANET (Rossman 2000), GESTAR (Estrada et al 2009), ICARE (CTGREF 1979) and AKLA (Lamaddalena 1997; Lamaddalena & Sagardoy 2000) developed by different researchers and based on their own modelling principles to fulfill performance analyses of on-demand pressurized irrigation systems. Several studies have been published on this subject. Calejo et al (2008) evaluated the hydraulic performance of the Lucefecit irrigation network using both ICARE and AKLA simulation models. Lamaddalena & Khila (2012) showed that the characteristic curve of the irrigation network can be obtained using the COPAM software. However, the hydraulic and economic performance analyses of on-demand pressurized irrigation systems have not been sufficiently researched in Turkey. Therefore, the purpose of this study is to reveal optimum design possibilities of the pressurized irrigation systems by applying COPAM model to an on-demand pressurized irrigation system in Turkey for determining the hydraulic system performance and, so, to demonstrate deficiencies by analyzing the performance in terms of system discharge, hydrant pressure heads, pipe diameters of the network and pipe costs.

2. Material and Methods

2.1. The case study network

The COPAM was applied to the pressurized irrigation network of Göbelye, the Faculty of Agriculture at Uludag University, Bursa (Figure 1). Irrigation area has altitudes of the highest 104.0 m and the lowest 63.9 m, its average altitude is 85.0 m, and the average slope of the field is 5%. The irrigation water used is supplied from Göbelye Dam which is situated within Uludag University Görükle Campus and has 125 ha irrigation area and the network discharge 217 L s^{-1}. Irrigation water is conveyed to hydrants

with piped irrigation system by pumping from the sluice gate to reservoir located on the highest point of irrigation area by two electro pumps. And, it is then distributed to irrigated area from reservoir with the help of gravity. The pressurized irrigation system is designed to be operated by on-demand method and it has 40 hydrants. The elevation of the existing reservoir above sea level is 140 m. The minimum necessary pressure head of each hydrant is H_{min} 25 m. In the system, 9 of total 40 hydrants have discharges of 5 L s^{-1} and 31 hydrants have discharges of 10 l s^{-1}. There are also 14 nodes in the project. High density polyethylene (HDPE) pipes were used in the system.

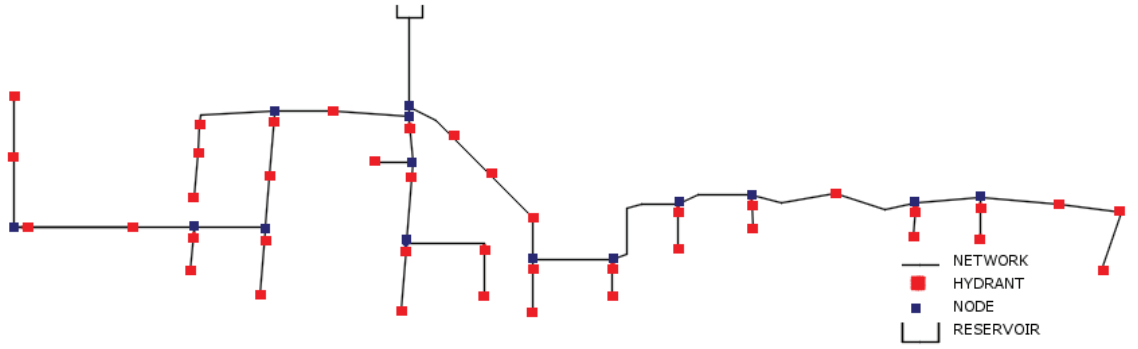

Figure 1- Layout of irrigation network

2.2. Hydraulic performance analysis

The COPAM software was used to carry out the hydraulic performance analysis of the system in the study. Also, the pipe diameters of the system were recalculated and the system performance analysis was repeated with COPAM as an alternative scenario.

There are three different configurations of COPAM; calculation of discharge, calculation of pipe diameter, and analysis. There are two modules (Clément and random) in the structure of discharge calculation, a module (optimization) under the structure of pipe diameter calculation and also two modules (configurations and hydrants) under the analysis structure. COPAM performs an optimization with Labye's iterative discontinuous method (ELIDM) extended for several flow regimes models (SFR) for the selection of pipe diameter (Labye 1981; Ait Kadi et al 1990). Further details about COPAM software can be found in Lamaddalena (1997) and Lamaddalena & Sagardoy (2000).

In this study, it was identified that there are 1000 random hydrant configurations that operate simultaneously depending on the system discharge varying between the range of 50 and 300 L s^{-1}. The fact that the number of the examined configurations is high has increased the accuracy of the calculated demand curves. It was identified that the upstream elevation above sea level is 140 m and the required minimum pressure in hydrants is 25 m in the system. To analyze by the model; each hydrant on irrigation system and their elevations above sea level, their distances from the initial point and between them were determined in the first. The technical data such as the diameter and length of pipes related to irrigation system were obtained from the irrigation project. The head losses which occurred at the pipes in regard to the positions of the hydrants on the field were calculated for each of the potential discharge values in order to determine the curves of irrigation system. The head losses occurring at the irrigation system were calculated in Equation 1 by using Darcy-Weisbach equation (Lamaddalena & Sagardoy 2000).

$$Y = 0.000857(1+2\gamma D^{-0.5})^2 Q^2 D^{-5} \quad L = uQ^2 L \qquad (1)$$

Where; γ, roughness parameter of Bazin (expressed by $m^{0.5}$); Q, pipe discharge (m^3 s^{-1}); u, dimensional coefficient of resistance (m^{-1} s^2); L, the length of pipe (m). The roughness coefficient of Bazin was taken as 0.05 for HDPE pipes used (Lamaddalena & Sagardoy 2000). AKLA model was used for the reliability analysis of each hydrant with respect to a minimum pressure head H_{min} of 25 m.

2.3. Cost analysis

In this study, an economic analysis was carried out in order to determine the change in the existing and recalculated total pipe cost according to alternative scenario. For this purpose, calculations were made using both existing and recalculated (by COPAM) pipe diameters and lengths of the irrigation system. Unit cost for every different HDPE pipe diameter was obtained from market research, and the total pipe cost was estimated by multiplying the pipe diameter and the total pipe length.

3. Results and Discussion

3.1. Hydraulic analysis of irrigation system

3.1.1. Upstream elevation-discharge analysis

The indexed characteristic curves for existing system and alternative scenario are given in Figure 2. The system was tested to determine what percentage of the configurations created in different discharges (50, 100, 150, 200, 217, 250 and 300 L s^{-1}) and elevations (60, 70, 80, 90, 100, 110, 120, 130 and 140 m) would be met by this system. This test has clearly demonstrated whether the system provides the optimum efficiency with less discharge and upstream elevation under the condition that it meets all of the configurations and the network will be sufficient how to increase the system discharge and upstream elevation under the condition that it meets an insufficent part of the configurations. Figure 2a indicates the configuration analysis curves of the irrigation system. It was drawn for the upstream elevation (140 m) corresponding to the available curved system discharge (217 L s^{-1}). It is seen that set point is located on the characteristic curve with P_0 100% according to the available data. It was found that all of the evaluated configurations were met. That is, it is seen that all system discharge meet 100% of the discharge required for all of the evaluated configurations and there is no deficiency of discharge in terms of the configuration. For alternative scenario, it is seen that the characteristic curve of set point P_0 drops to 95% approximately as understood in the figure when the system discharge and elevation are compared for 1000 random hydrant configurations in Figure 2b. This means that as a high value, 95% of the examined configurations are fully satisfied.

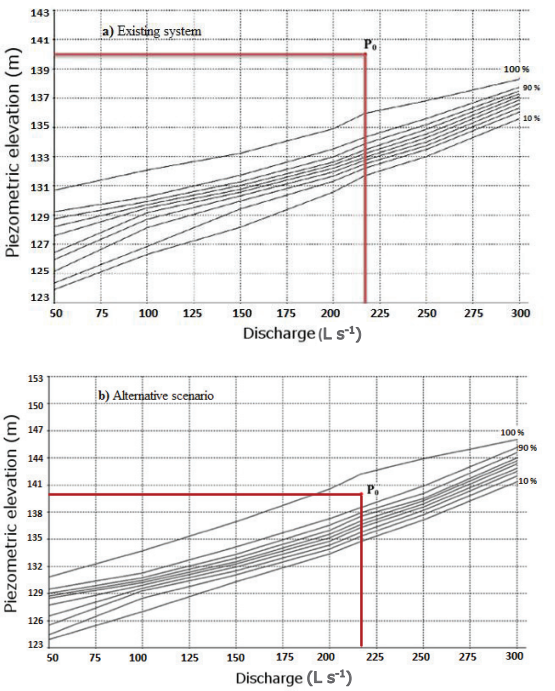

Figure 2- Characteristic curves (for 1000 random hydrant configurations)

3.1.2. Hydrant analysis (percentage unsatisfied hydrant-discharge)

The relative pressure values corresponding to hydrant numbers are given in Figure 3. Figure 3 helps to identify the hydrants subject to insufficient pressure and assess the insufficiency range. Figure

3a demonstrates that this hydrant is satisfactory, in terms of the pressure if its relative pressure value is above zero or this hydrant is unsatisfied if its relative pressure value is below zero. The insufficiency increases when points become closer to -1.0. All hydrants are satisfied in terms of pressure in our study as shown in Figure 3a. The curve obtained by excluding 10% probability of the results is used to decide whether there is deficit of the relative pressure in a hydrant (Figure 3a). This graphic was also obtained depending on the design discharge and upstream elevation. It was concluded that there was no any problem arising from deficit of the relative pressure in hydrants as shown in Figure 3a which it is also supported by reliability test given Figure 4a.

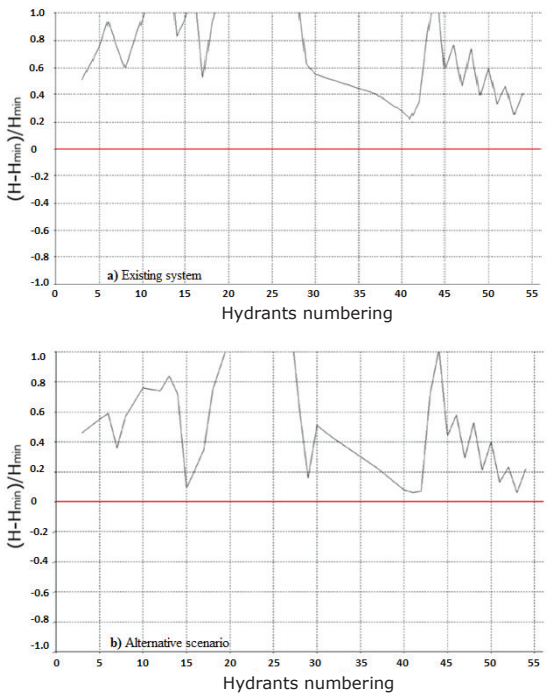

Figure 3- The relative pressure corresponding to the hydrant numbers

The different results of hydrant analysis were obtained for the new pipe diameters calculated according to alternative scenario (Figure 3b). The relative pressure values corresponding to hydrant numbers are given in Figure 3b. Figure 3b was obtained by excluding 10% probability of the results of the relative pressure values corresponding to hydrant numbers. The figure was obtained depending on the design discharge and upstream elevation. It is seen that there is scarcely any hydrant which its relative pressure value is below zero in Figure 3b and their relative pressure are above zero to all hydrants formed by excluding 10% probability in Figure 3b. This situation shows that there is not unsatisfied hydrant in the system in terms of pressure.

3.1.3. Reliability test (reliability-hydrant numbers curve)

Any deficiency on the hydrants is defined as the failure of the system. The failure in a pressurized irrigation system is expressed by the decrease in the minimum pressure head of the hydrant required for suitable field irrigation. The reliability value of the analyzed irrigation network is between 0 and 1, and it is a desirable condition that this value is to be 1 or closer to 1. The analyzed irrigation system becomes reliable, because all hydrants have the value of 1 as shown in Figure 4a. The results of the reliability analysis in 217 L s^{-1} discharge and 1000 random configurations associated with the alternative scenario are seen in Figure 4b. When the distributions of the hydrant reliabilities were investigated on the graphic, all hydrants except two-hydrant were determined to be reliable. According to the results of the reliability analysis, it can be easily stated that there is not the risky hydrant on the system.

3.1.4. Hydrant analysis in different upstream elevations (percentage unsatisfied hydrant-discharge)

The location of hydrants on on-demand pressurized irrigation systems, create variable pressure heads and this is essential for pumping station to meet the requirement of minimum pressure head on each hydrant. This situation shows that there are more than one irrigation-demand curve on on-demand pressurized irrigation systems (Planells et al 2001;

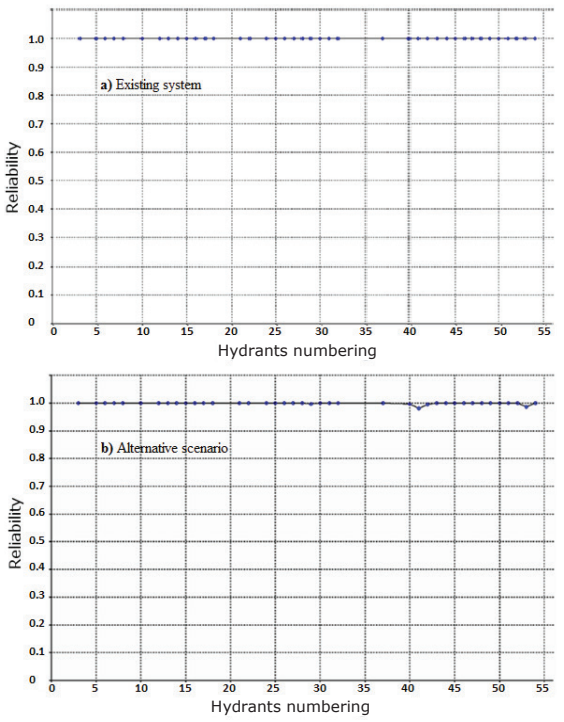

Figure 4- Reliability test (in 217 L s^{-1} discharge and 1000 random configuration)

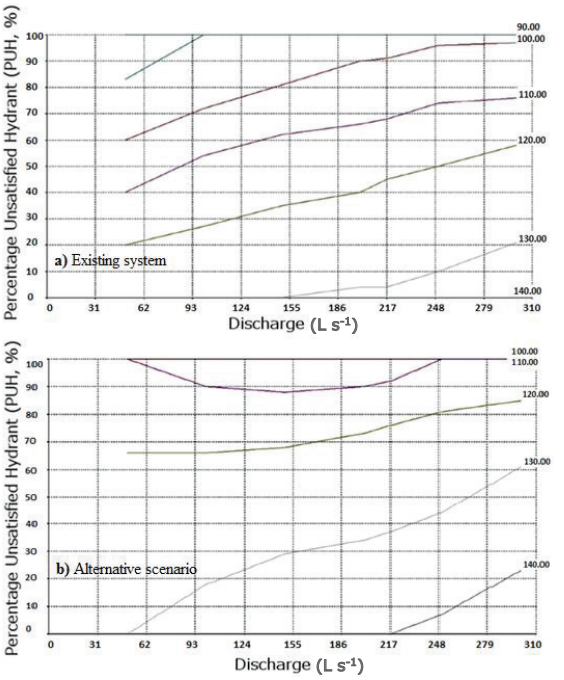

Figure 5- Percentage unsatisfied hydrant (PUH, %) (for conditions that upstream elevation above sea level ranges from 60 to 140 m)

Pérez et al 2002). Figure 5 was obtained with the selection of 10% probability curve for points in different elevations ranging from 60 m to 140 m. This figure has demonstrated the discharge corresponding to percentage change of the unsatisfied hydrants for different upstream elevation values above sea level and 10% probability of occurrence of PUH (percentage unsatisfied hydrants). This figure provides information whether it is necessary to reduce or increase upstream elevation above sea level to obtain a less PUH value. All range of PUH (100%-0%) varies between 90 m and 130 m respectively for upstream discharge 217 L s^{-1}. Thus, it is seen that all hydrants in the irrigation network are unsatisfied in terms of pressure when upstream elevation above sea level is 90 m or none of the hydrants are unsatisfied in terms of pressure when upstream elevation is about 130 m as shown in Figure 5a. As a result, it is realized that adequate pressure can be provided to all hydrants with the available network discharge even if upstream elevation of the irrigation system is designed to be about 130 m.

The model was operated for the different elevations above sea level ranging from 60 m to 140 m and the percentage unsatisfied hydrant curves (Figure 5b) were obtained by selecting 10% probability curve for alternative scenario. All range of PUH for upstream discharge 217 L s^{-1} (100%-0%) are between 110 m and 140 m respectively. Therefore, it is seen that all hydrants in the irrigation network become unsatisfied in terms of pressure in case that upstream elevation above sea level is 110 m as seen in Figure 5b and none of the hydrants become unsatisfied in terms of pressure in the case that upstream elevation is 140 m.

As a result, it was presented that there was not unsatisfied hydrant with 140 m upstream elevation in the hydrant analysis performed in the same system

discharge and upstream elevation with smaller pipes in diameter calculated by COPAM without using the available pipe diameters of the irrigation network in the alternative scenario. According to these results, it was observed that pipe diameters reduced by 15%. When the new pipe diameters were calculated in the program instead of pipe diameters of the existing irrigation system, it was seen that there were smaller diameters than the available pipe diameters and almost the same analysis results were obtained with the smaller pipes in diameter. This result is an important finding for evaluating the cost of the system.

3.2. Economic assessment

The irrigation system cost, as well as the optimum system design, should be considered for cost-effective use of financial resources. The results of an economic evaluation based on pipe diameters and lengths in irrigation system are shown in Table 1. The total pipe cost for existing conditions and alternative scenario was US$ 570257 and US$ 477396, respectively. The total pipe cost decreased by recalculation of pipe diameters according to alternative scenario. Based on the results of the present study, a decrease of 16% in irrigation system cost without being a significant decrease in hydraulic performance, compared with existing condition, could be obtained with alternative scenario. Considering pipe diameters which were computed using optimization techniques of COPAM, it is understood that pipe diameters of the existing system was not selected properly.

4. Conclusions

COPAM was useful in evaluating the performance of the pressurized irrigation system Göbelye. It was concluded that the system could operate with 100% efficiency in the discharge value (217 L s^{-1}), its set point. None of the hydrant configurations tested in the study had unsatisfied hydrant, and there was any problem arising from pressure insufficiency for the sufficiency of the system in terms of pressure in the hydrant level. It was found that hydrants located on the irrigation system are reliable. When pipe diameters were calculated with COPAM according to the available other data about the system as if the inspected system had been redesigned, it was reached to a striking conclusion that the system performance could be provided without any unsatisfied hydrant by using smaller pipes in diameter. Thus, it is possible to state that the irrigation network could

Table 1- Cost analysis for pipe diameters

Pipe diameter (mm)	*Pipe cost ($ m^{-1})*	*Total pipe length (m)*		*The change in total pipe length (m)*	*Total pipe cost ($)*		*The change in the total pipe cost ($)*
		Existing system	*Calculated with COPAM*		*Existing system*	*Calculated with COPAM*	
450	175	1072	538	-534	187600	94150	-93450
400	140	1009	160	-849	141287	22400	-118887
355	110	278	1140	-862	30580	125391	94811
315	87	723	1044	321	62924	90857	27932
250	55	738	780	43	40563	42926	2364
225	44	1333	1035	-298	58645	45556	-13090
160	22	1105	1348	242	24316	29645	5329
125	14	418	350	-68	5852	4903	-949
110	11	1681	1961	280	18491	21568	3077
Total		8357	8357	0	570257	477396	-92681

be established with lower investment cost (16%) in terms of pipe diameter without any loss in its hydraulic performance. It can be stated that COPAM developed to apply the performance analysis of on-demand pressurized irrigation networks is very useful for determining the hydraulic performance of the system and the system design. COPAM allowing the simulation of the possibilities arising from many variables can be used especially in the project designs of the large-scale on-demand pressurized irrigation systems leading to the high investment costs and so the appropriate system designs can be performed. The COPAM can be recommended for the institutions which are involved in designing and operating the pressurized irrigation systems.

References

Ait Kadi M, Abdellaoui R, Oulhaj A & Essafi B (1990). Design of large-scale collective sprinkler irrigation projects for on demand operation: A holistic approach. In: *Proceedings of the 14th International Congress on Irrigation and Drainage*, 30 April-04 May, Rio de Janeiro, Brazil, pp. 59-78

Akyol A (2012). Basınçlı sulama şebekesinde proje debisi hesaplamasına ilişkin yöntem karşılaştırması. Yüksek lisans tezi, Ege Ünivesitesi Fen Bilimleri Enstitüsü (Basılmamış), İzmir

Barutçu F & Özcan T M (2011). Basınçlı sulama sistemlerinde değişken hızlı pompalarla enerji kazanım olanaklarının araştırılması. *Çukurova Üniversitesi Fen ve Mühendislik Bilimleri Dergisi* **26**(1): 196-208

Beyribey M & Balaban A (1992). Basınçlı sulama dağıtım sistemlerinin optimizasyonu. Ankara Üniversitesi Ziraat Fakültesi Yayınları, No: 1248, Ankara

Calejo M J, Lamaddalena N, Teixeria J L & Pereira L S (2008). Performance analysis of pressurized irrigation systems operating on-demand using flow-driwen simulation models. *Agricultural Water Management* **95**: 154-162

CTGREF (1979). Programme ICARE-Calcul des Caractéristiques Indicées. Note Technique 6, CTGREF Division Irrigation, Aix-en-Provence

Estrada C, González C, Aliod R & Paño J (2009). Improved pressurized pipe network hydraulic solver for applications in irrigation systems. *Journal of Irrigation and Drainage Engineering* **135**(4): 421-430

Labye Y (1981). Iterative discontinuous method for networks with one or more flow regimes. In: *Proceedings of the International Workshop on Systems Analysis of Problems in Irrigation, Drainage and Flood Control*. 30 November-14 December, New Delhi, pp. 31-40

Lamaddalena N (1997). Integrated simulation modeling for design and performance analysis of on-demand pressurized irrigation systems. PhD Thesis, Technical University of Lisbon, Portugal

Lamaddalena N & Sagardoy J A (2000). Performance analysis of on-demand pressurized irrigation systems. FAO Irrigation and Drainage Paper No: 59, Roma

Lamaddalena N & Khila S (2012). Energy saving with variable speed pumps in on-demand irrigation systems. *Irrigation Science* **30**: 157-166

Pérez P C, Alandi P P, Alvarez F O & Martin Benitez M T (2002). Management and over-exploitation risk of water resources in semi-arid zones. *Annual International ASAE Meeting Presentation*, Paper No: 022192

Planells P, Tarjuelo J M, Ortega F & Casanova I (2001). Design of water distribution networks for on-demand irrigation. *Irrigation Science* **20**: 189-201

Rossman L (2000). Epanet 2 user's manual. Environmental Protection Agency, Cincinnati

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Hook Selectivity for Bluefish (*Pomatomus saltatrix* Linneaus, 1766) in Gallipoli Peninsula and Çanakkale Strait (Northern Aegean Sea, Turkey)

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ABSTRACT

This study was conducted to determine the selectivity of the hooks used for bluefish (*Pomatomus saltatrix* Linneaus, 1766) in the Gallipoli Peninsula and the Dardanelles between 2006 and 2009 fishing seasons (November to September). Bluefish were fished with hooks sized 1, 1/0, 2/0, 3/0, 4/0 and in sum; 1210 bluefish were caught. The hook no 2/0 caught the highest number of fish (344 fish, 20.43%) and the hook no 1 caught the least (35 fish, 2.89%). Length frequency distribution of bluefish, which were caught with different hook sizes, was used in SELECT method and according to the results; the normal scale model gave the best fit for selectivity. The normal scale model was used to calculate model length (ML) and spread value (SV) of each hook size. Model length and spread value were found as follows; 19.18 cm ML and 4.44 SV for hook no. 1; 21.88 cm ML, 5.07 SV for hook no 1/0; 24.14 cm ML, 5.59 SV for hook no. 2/0; 27.02 cm ML, 6.26 SV for hook no. 3/0; 28.19 cm ML, 6.53 SV for hook no. 4/0, respectively. Because the minimum landing size (MLS) for bluefish has been stipulated as 20.0 cm (TL) in the Turkish Fishery Regulations, the use of hook no. 2/0 or bigger hook sizes can be recommended for fishing of bluefish.

Keywords: Bluefish; Çanakkale Strait; Hook selectivity; SELECT method

Gelibolu Yarımadası ve Çanakkale Boğazı'nda (Kuzey Ege Denizi, Türkiye) Lüfer Balığı için (*Pomatomus saltatrix* Linneaus, 1766) İğne Seçiciliği

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ÖZET

Bu çalışma, Gelibolu Yarımadası ve Çanakkale Boğazı'ndaki lüfer balığı (*Pomatomus saltatrix* Linnaeus, 1766) avcılığında kullanılan olta iğneleri seçiciliklerini belirlemek için, 2006-2009 balıkçılık sezonunda, lüfer göç zamanı

olan Eylül, Ekim ve Kasım ayları arasında gerçekleştirilmiştir. Lüfer balıklarının avcılığı 1, 1/0, 2/0, 3/0, 4/0 numara iğneler ile gerçekleştirilmiş ve 1210 adet lüfer balığı yakalanmıştır. En fazla birey 2/0 numara iğne ile (344 birey, % 20,43) en az birey ise 1 numaralı iğne ile (35 birey, % 2,89) elde edilmiştir. Farklı iğne numaraları ile avlanan lüfer balığının boy frekans dağılımları kullanılarak uygulanan SELECT metoduna göre en uygun model, normal scale model olarak saptanmıştır. Normal scale modele göre kullanılan iğne büyüklükleri için hesaplanan optimum yakalama boyları (OYB) ve eğrinin genişlikleri (EG), sırasıyla, 1 no'lu iğne için 19,18 cm OYB ve 4,44 EG; 1/0 no'lu iğne için 21,88 cm OYB ve 5,07 EG; 2/0 no'lu iğne için 24,14 cm OYB ve 5,59 EG; 3/0 no'lu iğne için 27,02 cm OYB ve 6,26 EG ve 4/0 no'lu iğne için 28,19 cm OYB ve 6,53 EG'dir. Lüfer balıklarının Türkiye'deki minimum avlanma boyu 20 cm olduğu için lüfer avcılığında 2/0 numara ve daha büyük iğnelerin kullanımı önerilebilir.

Anahtar Kelimeler: Lüfer balığı; Çanakkale Boğazı; İğne seçiciliği; SELECT yöntem

1. Introduction

In fisheries management, knowledge of size selectivity can be used for the estimation of incidental mortality (i.e. mortality of discards and escapees); yield-per-recruit analysis; age and length-based population models; estimation of population length frequencies; length at age (Millar & Fryer 1999). Therefore, the estimations of size selectivity of fishing gears offer remarkable information for the conservation and optimum exploitation of fisheries resources (Beverton & Holt 1957; Hilborn & Walters 1992; Quinn & Deriso 1999). Although the size selectivity of fishing gears such as trawls and gill nets is well known, there is still a gap between the size selection curves of hand lines. Not only logistic type models, typically used to describe the selectivity of trawls, but also unimodal models used in gillnet selectivity studies have been used in hook selectivity studies (Clark 1960; Erzini et al 1996; 1998; 2006), (Millar & Holst 1997; Sousa et al 1999; Czerwinski et al 2010; Campbell et al 2014; Öztekin et al 2014; Ateşşahin et al 2015).

Bluefish (*Pomatomus saltatrix*) is a migrant species, which has wide geographical distribution, except for the northern and central-Pacific Ocean (Briggs 1960; Wilk 1977). It is also one of the most important commercial fish species in Turkey, and particularly fished during alimental and spawning migration between the Black Sea and Aegean Sea. Fishing activity is intensive, especially due to purse seine, trawling net, hand lines, encircling gill and trammel net (Ceyhan & Akyol 2005; Acarlı et al 2013). The production made in Turkey is observed to have risen to one-third of the world's production during some years (Ceyhan & Akyol 2006). Unfortunately, recently, bluefish population has shown substantial declines (Robillard et al 2009) and some researchers (Akyol & Ceyhan 2007; Özdemir et al 2009) reported over-fishing pressure on the species. For these reasons, in some countries like Brazil, Australia and Tunisia, the fishing of bluefish was subjected to some regulations in order to allow the proper management of this resource (Dhieb et al 2006). In Turkey, although current stock levels are uncertain, there are indications (i.e. smaller average sizes of individuals, lower catch per unit effort according to the years) of the fact that stocks have declined due to fishing pressure. Recently, there has been much discussion concerning the state of bluefish stocks, because of declining catches (Acarlı et al 2013).

Although, a great deal of research has been published on the biology of this species (Lassiter 1962; Conand 1975; Van der Elst 1976; Champagnat 1983; Krug & Haimovici 1989; Barger 1990; Graves et al 1992; Haimovici & Krug 1992; Terceiro & Ross 1993; Lucena & O'Brien 2001; Salerno et al 2001; Sipe & Chittenden 2002; Ceyhan et al 2007; Cengiz et al 2012), there is no information about the selectivity of the hooks used for bluefish all over the world. This study aims to determine the selectivity of hooks sized 1, 1/0, 2/0, 3/0 and 4/0 used for bluefish in the Gallipoli Peninsula and the Dardanelles.

2. Material and Methods

2.1. Study area

The Çanakkale Strait is a strategic natural transition point where pelagic fish populations migrate from the Black Sea to the Aegean and the Mediterranean Sea and in the opposite direction for the purposes of feeding and reproduction. In this bi-directional pass along the Dardanelles during certain periods of the year, migratory fish schools are very important fishing potentials for small coastal fishermen and big coastal fishing boats. Atlantic mackerel *Scomber scombrus* (Linnaeus 1758), chub mackerel *Scomber japonicus* (Houttuyn 1782), bluefish and horse mackerel *Trachurus trachurus* (Linnaeus 1758) which are also known as Migratory Fish species are intensively fished during the period from early September to late February known as winter fishery and the whole summertime (Ünsal 2010). Therefore, the Çanakkale Strait has a special importance for the coastal fishery of Turkey (Zengin 2013).

Bluefish samples were collected between 2006 and 2009 fishing seasons (November to September) from the Çanakkale Strait, at depths ranging from 1 m to 40 m (Figure 1).

Fishing lines and hook sizes used in the present study were designated in accordance with those of fishermen. Fishing lines for bluefish were used in the study and all the lines had the same features except for hook sizes. Leaders with 150 cm length and 0.4 mm diameter which were equipped with hooks between no. 2 and 4 were knotted to 0.6 mm diameter main line with swivels. Hooks were equipped to with 10 cm long leaders between two swivels for flexibility in currents. Hooks were tied to 120 cm long lines and each hook was interspaced in about 3 cm. Hooks sized 13 were used with every fishing tackle. Straight shank hooks (Mustad 3282) sized 1, 1/0, 2/0, 3/0 and 4/0 were determined for main hooks and then equipped together with hooks sized 13 (Figure 2).

By virtue of bluefish's predatory behaviors, hooks were used along with live bait. The round sardinella (*Sardinella aurita* Valenciennes 1847), big-scale sand smelt and blotched picarel **(***Spicara maena* Linnaeus 1758**)** were used as baits. Different baits were used since bluefish were attracted to different kinds of

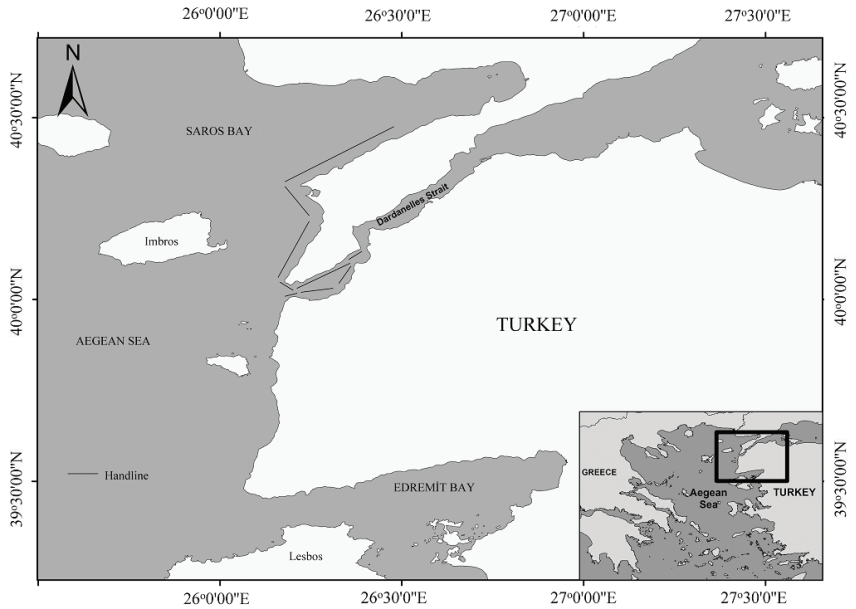

Figure 1- Study area (Northern Aegean Sea, Turkey)

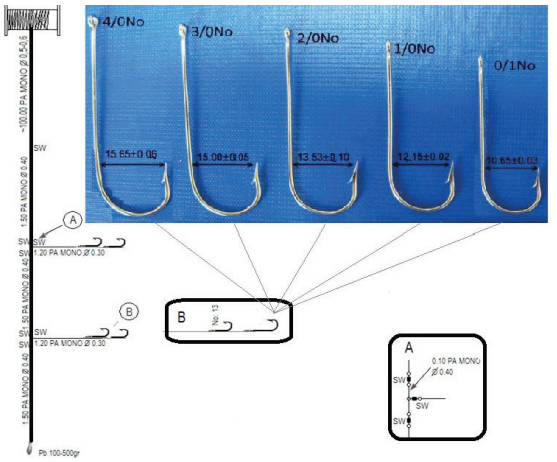

Figure 2- Hooks used for fishing bluefish and gap measures (cm) (hook gaps were determined by calculating the mean value of the gap between 60 hooks in each box)

baits in a day time. During fishing, only one type of bait was used not to affect catching efficiency. Due to their higher efficiency during daytime, live baits were preferred for sampling. However, sardine was also used as bait in case of an absence of live baits. Five fishing tackles were set with the same specifications; yet with different hook sizes differring between size 1, 1/0, 2/0, 3/0 and 4/0, respectively. The period was required for fishing bluefish with line differs between 5 to 10 minutes. The position of bait on hook does not affect the period. Fishing tackles were used alternatively to prevent the lack of fishing efficiency caused by fishermen, every 60 minutes. Samples were measured to the nearest cm (total length) and fish mouth gaps were measured during selectivity studies with 0.01 mm precised digital caliper to determine whether there is a relation between mouth gaps and hook sizes.

SELECT (select each length class' catch total) method was used to evaluate the data related to fish hooks (Millar 1992). This method assumes that the number of fish having length l and caught with a hook sized j has a n_{lj} Poisson distribution, and is defined by Equation 1.

$$n_{lj} \approx n_{lj} \approx \text{Pois}\,(p_j \lambda_l r_j(l)) \qquad (1)$$

Where; λ_l, abundance of fish sized l and caught by hook; p_j (l), relative fishing intensity (the relative abundance of fish sized l that hook sized j can catch). The Poisson distribution of the number of fish sized l and caught by fishing gear having hook sized j is defined as $p_{j(l)}\lambda_l$. $r_{j(l)}$ is the selectivity curve for the hook sized j.

Log-likelihood of n l_j is expressed as Equation 2.

$$\sum_l \sum_j \{n_l \log[p_j\, \lambda_l\, r_j\,(l)] - p_j\, \lambda_l\, r_j\,(l)\} \qquad (2)$$

Gillnet (generalized including log-linear N estimation technique) program (Constant 1998) was used for the analysis of the obtained data. The program calculates the selectivity parameters of five different models based on the SELECT method and by comparing the model deviances; the lowest one is chosen for the best model (Millar & Holst 1997; Millar & Fryer 1999). The equations used in the SELECT models are as follows.

Normal location (Equation 3).

$$exp\left(-\frac{\left(L-k.m_j\right)^2}{2\sigma^2}\right) \qquad (3)$$

Normal scale (Equation 4).

$$exp\left(-\frac{\left(L-k_1.m_j\right)^2}{2k_2^2.m_j^2}\right) \qquad (4)$$

Log-normal (Equation 5).

$$\frac{1}{L}exp\left(\mu+log\left(\frac{m_j}{m_1}\right)-\frac{\sigma^2}{2}-\frac{\left(log(L)-\mu-log\left(\frac{m_j}{m_1}\right)\right)^2}{2\sigma^2}\right) \qquad (5)$$

Gamma (Equation 6).

$$\left(\frac{L}{(\alpha-1).k.m_j}\right)^{\alpha-1} exp\left(\alpha-1-\frac{L}{k.m_j}\right) \qquad (6)$$

Bi-normal (Equation 7).

$$exp\left(-\frac{\left(L-k_1.m_j\right)^2}{2k_2^2.m_j^2}\right)+c.exp\left(-\frac{\left(L-k_3.m_j\right)^2}{2k_4^2.m_j^2}\right) \qquad (7)$$

The Kolmogorov-Smirnov (K-S) test was used to determine differences between size frequency distributions of fish caught by hooks in various sizes (Sigeal & Castellan 1989; Karakulak & Erk 2008).

3. Results and Discussion

The field studies were carried out with hooks sized 1, 1/0, 2/0, 3/0, 4/0 and a total of 1210 samples were caught during these studies. In terms of hook sizes, the highest number of bluefish were caught with hook no. 2/0 (344 fish, 20.43%) and the least bluefish were caught with hook no. 1 (35 fish, 2.89%). The number of bluefish caught by each differently sized hook and minimum, maximum, mean lengths and standart error are displayed in Table 1.

Table 1- The numbers and length values of bluefish according to hook sizes

Hook			*Total length (cm)*		
numbers	*n*	*%*	*Minimum*	*Maximum*	*Mean ± S.E.*
1	35	2.89	19.5	24.1	21.74±0.16
1/0	313	25.87	14.9	36.5	23.05±0.22
2/0	344	28.43	13.8	45.2	22.18±0.28
3/0	301	24.88	14.8	41.9	27.78±0.28
4/0	217	17.93	20.5	49.0	30.33±0.33

S.E, standart error

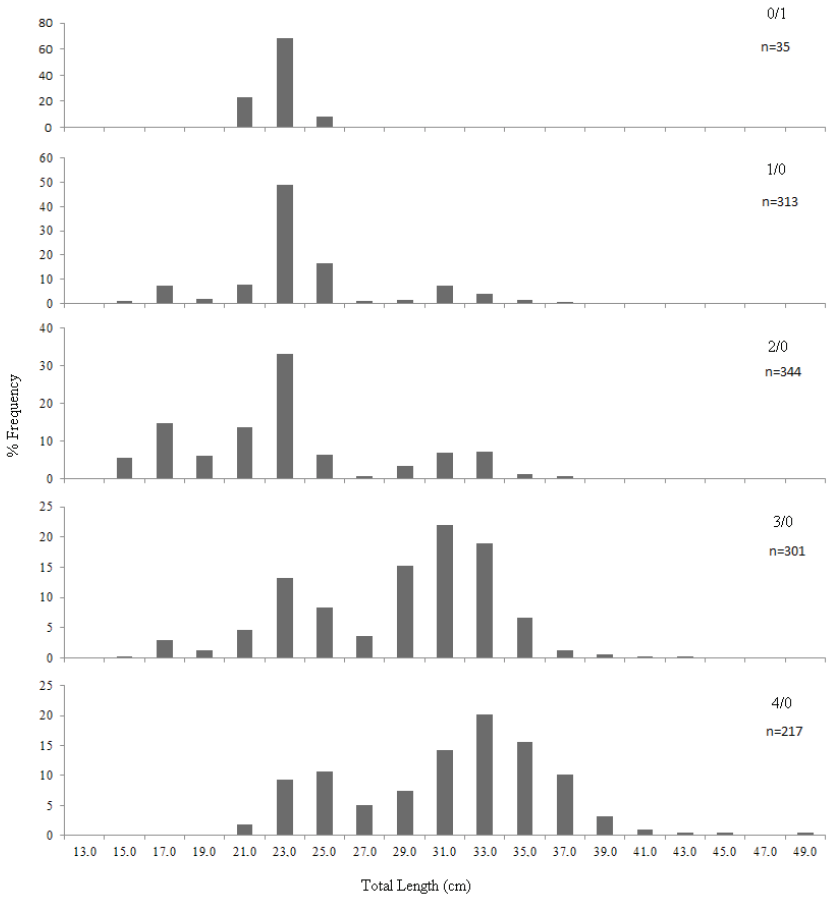

Figure 3 - Length frequency distribution of Bluefish (*Pomatomus saltatrix* Linnaeus, 1766) for each hook size

The catch size-frequency distributions are given in Figure 3 for each hook size used for fishing bluefish. Larger hook sizes have the greater mean length of the captured fish.

The length frequency distributions of the bluefish caught by the 5 different hook sizes (all hook sizes combined) were significantly different, except for the combination of hooks sized 1-1/0 (Table 2).

The normal scale model with the lowest deviance was given the best fit in comparison to the deviances of five models of SELECT (Table 3).

According to the normal scale model, the modal lengths and spread values of bluefish for each hook size are presented in Table 4. Model length accurately increases as the hook number increases.

Table 4- Modal lengths and spread value of bluefish for each hook size according to the normal scale model

Hook numbers	*Model length (cm)*	*Spread value (cm)*
1	19.18	4.44
1/0	21.88	5.07
2/0	24.14	5.59
3/0	27.02	6.26
4/0	28.19	6.53

Table 2- Results of the Kolmogorov-Smirnov test

		Kolmogorov-Smirnov test		
Hook numbers	*Hook numbers*	*D max*	*Critical values*	*Decision*
1	1/0	0.102	0.242	H_0 Not Reject
1	2/0	0.265	0.241	H_0 Reject
1	3/0	0.691	0.243	H_0 Reject
1	4/0	0.804	0.248	H_0 Reject
1/0	2/0	0.219	0.106	H_0 Reject
1/0	3/0	0.528	0.110	H_0 Reject
1/0	4/0	0.620	0.120	H_0 Reject
2/0	3/0	0.507	0.107	H_0 Reject
2/0	4/0	0.622	0.118	H_0 Reject
3/0	4/0	0.234	0.121	H_0 Reject

H_0, there are no significant differences between length frequency distribution (α= 0.05, K= 1.36)

Table 3- The SELECT model parameters estimates for hook selectivity

Model	*Equal fishing powers*			*Fishing power α hook size*			
	Parameters	*Modal deviance*	*p*	*Parameters*	*Modal deviance*	*p*	*Degree of freedom*
Normal location	k= 1.765±0.021 σ= 5.112±0.158	565.99	0.0000	k= 1.849±0.021 σ= 5.222±0.163	561.62	0.0000	70
Normal scale	k1= 1.801±0.025 k2= 0.417±0.015	561.88	0.0000	k1= 1.896±0.022 k2= 0.407±0.014	561.83	0.0000	70
Gamma	α= 20.902±1.442 k= 0.087±0.005	573.69	0.0000	α= 21.902±1.423 k= 0.087±0.005	573.69	0.0000	70
Log normal	μ1= 2.949±0.012 σ= 0.220±0.007	590.37	0.0000	μ1= 2.998±0.011 σ= 0.220±0.007	590.37	0.0000	70
Bi-normal	not calculated	-	-	not calculated	-	-	-

The selectivity curves of bluefish for each hook size according to the normal scale model are presented in Figure 4.

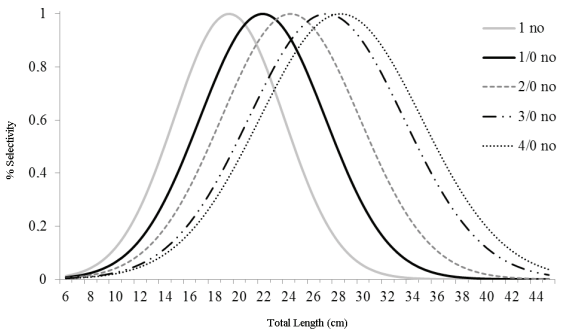

Figure 4- The selectivity curves of bluefish for each hook size according to the normal scale model

Due to the Turkish Fishery Regulation (TFR 2012); the minimum landing size of the bluefish is 20 cm. The hooks used in this study was highly selective and had no pressure on bluefish stocks except for the hook sized 1 according to the model length (cm) given in Table 4.

To ensure sustainable improvements in fisheries management, regulations on specific fishing gears must be put in order correspondingly with selectivity studies. The normal scale model gave the best fit for the data obtained in this study. It has been observed that estimated model lengths were increasing in parallel with the increase in hook sizes.

Hook and bait are the main factors that affect catching efficiency when fishing is conducted with line (Kaykaç et al 2003). Some particular studies reported that hook size does not have a significant effect on selectivity (Bertrand 1988; Clarke et al 2001). This conclusion is derived from the fact that bigger fish have bigger mouth gaps. On the other hand, some other studies reported that hook size has an effect on selectivity. Considering both of these facts, it is determined that smaller hook size catches smaller fish and bigger hook size catches bigger fish (Cortez-Zaragoza et al 1989; Otway & Craig 1993; Grixti et al 2007). In the present study; hooks sized 4/0 caught the largest fish size group with the length of 49 cm while hooks sized 2/0 caught the second largest fish size group with the length of 45.2 cm. This partially indicates resemblance with previous studies. Hook size and type that will be used on lines have significant importance on the resemblance of fishing efforts.

Erzini et al (1998) stated that increasing the size of hook used on lines causes lower fish catching numbers. On the contrary, our results showed that the hook size 4/0, which is the biggest size, had the largest number of fish caught with 217 individuals while the smallest hook sized 1 caught 35 individuals. This difference occurred due to the consideration of different species. Bjordal (1981) reported that small sized hooks have higher catching efficiency compared to bigger sized hooks. The results obtained in this study, except for the hook sized 1, are mostly in accordance with alternate studies.

4. Conclusions

An efficient management strategy could not apply for bluefish population spreading over Turkey until nowadays. Fishing gears must be improved for the preservation of fish stocks. Improving the selective features of fishing gears make a significant contribution to the preservation of natural fish stocks and to avoid catching fish with undesired length (Kalaycı 2001). More species based studies on hook sizes should be carried out to prevent catching fish under length of the first spawning due to the use of small hook size. The use of small sized hooks that catch fish under length of the first spawning must be inhibited and legislated with government laws and regulations. There are many selectivity studies on gill net or trawling but no study focused on fish hooks (Give some selectivity studies references about selectivity of other fishing methods). Woll et al (2001) stated that the methods of gill net selectivity can be applied to fish hook selectivity and that fish can be caught in nets from different parts of their bodies; yet with fishhook they can only be angled from their mouths.

While fishing bluefish is allowed for those being longer than 30 cm in USA (Muller 2001). A research conducted in the Marmara Sea and the Straits in Turkey was displayed the first reproduction fork length as 25.4 cm for bluefish (Ceyhan et al 2007). Unfortunately, the minimum landing size (MLS) for bluefish is 20.0 cm (TL) in the Turkish Fishery Regulation (TFR 2012). The 20.0 cm length limit applied is not effective in preserving bluefish stocks. For this reason, using of hook sized 2/0 or higher can be recommended. Considering the importance of the bluefish catching in Turkey's fishery, the effective precautions such as size selectivity, catching quote, fishing effort control should be implemented. Additionally, in parallel with the developments in fisheries management and studies based on selectivity; the detection of the first reproduction length of commercial fish species and the use of specific fishing gears for certain species are of great importance. Therefore, the landing of individuals under the length at first maturity can be prevented and fish stocks can be preserved.

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References

Acarlı D, Ayaz A, Özekinci U & Öztekin A (2013). Gillnet selectivity for bluefish (*Pomatamus saltatrix*, L. 1766) in Çanakkale Strait, Turkey. *Turkish Journal of Fisheries and Aquatic Sciences* **13**: 349-353

Akyol O & Ceyhan T (2007). Exploitation and mortalities of bluefish (*Pomatomus saltatrix* L.) in the Sea of Marmara, Turkey. *Journal of Applied Biological Sciences* **1**: 25-27

Ateşşahin T, Duman E & Cilbiz M (2015). Selectivity and catch efficiency of three spinner hook sizes in angling for rainbow trout *(Oncorhynchus mykiss)* (Walbaum 1792) in Karakaya Dam Lake (Eastern Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* **15**: 851-859

Barger L E (1990). Age and growth of bluefish *Pomatomus saltatrix* from the Northern Gulf of Mexico and US South Atlantic coast. *Fishery Bulletin* **88**: 805-809

Bertrand J (1988). Selectivity of hooks in the handline fishery of the Saya de Malha Banks (Indian Ocean). *Fisheries Research* **6**: 249-255

Beverton J R H & Holt S J (1957). On the Dynamics of Exploited Fish Populations. *Ministry of Agriculture, Fisheries and Food.* Fishery Investigation Series 2, **19**, pp. 533

Bjordal A (1981). Engineering and fish reaction aspects of longlining. A review. *ICES CM Documents*; 1981/B: 35

Briggs J C (1960). Fishes of world-wide (circumtropical) distribution. *Copeia* No. **3**: 171-180

Campbell M D, Pollack A G, Driggers W B & Hoffmayer E R (2014). Estimation of hook selectivity of red snapper and vermilion snapper from fishery-independent surveys of natural reefs in the Northern Gulf of Mexico. *Marine and Coastal Fisheries* **6**(1): 260-273

Cengiz Ö, Özekinci U & Öztekin A (2012). The relationships between total lenght-otolith length of bluefish, *Pomatomus saltatrix*, (Linnaeus, 1766) from Gallipoli Peninsula and Dardanelles (North-eastern Mediterranean, Turkey). *Iğdır University Journal of the Institute of Science and Technology* **2**: 31-34

Ceyhan T & Akyol O (2005). The hand lines used in bluefish (*Pomatomus saltatrix* L., 1766) fishery in Marmara Region. *Ege University Journal of Fisheries and Aquatic Sciences* **22**: 351-355

Ceyhan T & Akyol O (2006). Age distribution and relationship between fork length and otolith length of bluefish (*Pomatomus saltatrix* L., 1766) in the Sea of Marmara. *Ege University Journal of Fisheries and Aquatic Sciences* **23**: 369-372

Ceyhan T, Akyol O, Ayaz A & Juanes F (2007). Age, growth and reproductive season of bluefish (*Pomatomus saltatrix*) in the Marmara Region, Turkey. *ICES Journal of Marine Science* **64**: 531-536

Champagnat C (1983). Peche, biologie et dynamique du tassergal (*Pomatomus saltatrix* Linnaeus, 1766) sur les Cotes Senegalo-Mauritaniennes. *Travaux et Documents du l'ORSTOM* **168**: 1-279

Clark J R (1960). Report on selectivity of fishing gear, In Fishing effort, and effect of fishing on resources and the selectivity of fishing gear. *ICNAF Special Publication* **2**: 27-36

Clarke M, Connolly P & Bracken J (2001). Aspects of reproduction of the deep water *sharks Centroscymnus coelolepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *Journal of the Marine Biological Association of the United Kingdom* **81**(6): 1019-1029. doi: 10.1017/S0025315401005008

Conand C (1975). Maturite sexuelle et fecondité du Tassergal, *Pomatomus saltator* (L., 1776), Poissons-Pomatomidae. *Bulletin De l'Institut Fondamental d'Afrique Noire (A)*. **37**: 395-466

Constant (1998). Gillnet software, Denmark www. Constat.dk.

Cortez-Zaragoza E, Dalzell P & Pauly D (1989). Hook selectivity of yellowfin tuna (*Thunnus albacares*) caught off Darigayos Cove, La Union, Philippines. *Journal of Applied Ichthyology* **1**: 12-17

Czerwinski I A, Gutiérrez-Estrada J C, Escofet M C S & Hernando J A (2010). Hook selectivity models assesment for *black spot seabream*. Classic and heuristic approaches. *Fisheries Research* **102**: 41-49

Dhieb K, Ghorbel M, Jarboui O & Bouain A (2006). Interactions between reproduction and fisheries in bluefish (*Pomatomus saltatrix)*, from Gulf of Gabes (Tunisia). *Cybium* **30**: 355-364

Erzini K, Gonçalves J M S, Bentes L, Lino P G & Cruz J (1996). Species and size selectivity in a Portuguese multispecies artisanal long-line fishery. *Ices Journal of Marine Science* **53**: 811-819

Erzini K, Gonçalves J M S, Bentes L, Lino P & Ribeiro J (1998). Species and size selectivity in a *''red" sea bream* longline "metier" in the Algarve (Southern Portugal). *Aquatic Living Resources* **11**: 1-11

Erzini K, Goncalves J M S, Bentes L, Moutopoulos D K, Casal J A H, Soriguer M C, Puente E, Errazkın L A & Stergıou K I (2006). Size selectivity of trammel nets in southern European small-scale fisheries. *Fisheries Research* **79**: 183-201

Graves J E, McDowell J R, Beardsley A M & Scoles D R (1992). Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. *Fishery Bulletin* **90**: 703-710

Grixti D, Conron S D & Jones P L (2007). The effect of hook/bait size and angling technique on the hooking location and the catch of recreationally caught black bream *Acanthopagrus butcheri*. *Fisheries Research* **84**: 338-344

Haimovici M & Krug L C (1992). Alimentação e reprodução da enchova *Pomatomus saltatrix* no litoral sul do Brasil. *Revista Brasileira de Biologia* **52**: 503-513

Hilborn R & Walters C J (1992). Quantitative Fisheries Stock Assessment: Choice, Dynamics, and Uncertainty. Chapman Hall. New York. pp. 570

Kalaycı F (2001). Dip paraketasında kanca büyüklüğünün seçicilik üzerine etkisi. Yüksek lisans tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Samsun

Karakulak F S & Erk H (2008). Gill net and trammel net selectivity in the northern Aegean Sea, Turkey. *Scientia Marina* **72**(3): 527-540

Kaykaç M H, Ulaş A, Metin C & Tosunoğlu Z (2003). Olta balıkçılığında düz ve çapraz iğnelerin av etkinliği üzerine bir araştırma. *Ege Üniversitesi Su Ürünleri Dergisi* **20**: 227-231

Krug L C & Haimovici M (1989). Age and growth of enchova *Pomatomus saltatrix* in Southern Brazil. *Atlantica* **11**: 47-61

Lassiter R R (1962). Life history aspects of the bluefish, *Pomatomus saltatrix*, from the Coast of North Carolina. MSc Thesis, North Carolina State College, North Carolina

Lucena F M & O'Brien C M (2001). Effects of the gear selectivity and different calculation methods on estimating growth parameters of bluefish, *Pomatomus saltatrix* (Pisces: Pomatomidae), from Southern Brazil. *Fishery Bulletin* **99**: 432-442

Millar R B (1992). Estimating the size-selectivity of fishing gear by conditioning on the total catch. *Journal of the American Statistical Association* **87**(420): 962-968

Millar R B & Holst R (1997). Estimation of gillnet and hook selectivity using loglinear models. *ICES Journal of Marine Science* **54**: 471-477

Millar R B & Fryer R J (1999). Estimating the size-selection curves of towed gears, traps, nets and hooks. *Reviews in Fish Biology and Fisheries* **9**: 89-116

Muller R G (2001). The 2000 Update of the Quota and Stock Assesment of Bluefish, *Pomatomus saltatrix,* on Florida's Atlanci Coast. Florida fish and Wildlife Conservation Commission. Florida Marine Research Institute pp. 24

Otway N M & Craig J R (1993). Effects of hook size on the catches of undersized snapper *Pagrus auratus*. *Marine Ecology Progress Series* **93**: 9-15

Özdemir S, Erdem E, Özdemir Z B & Şahin D (2009). Estimation of population parameters from lenght composition of pelagic species caught in the Black Sea: Horse mackerel (*Trachurus trachurus*), Bluefish (*Pomatomus saltatrix*) and Allis shad (*Alosa alosa*). *Fırat University Journal of Science* **21**: 1-8

Öztekin A, Özekinci U, Ayaz A, Cengiz Ö, Altınağaç U & Aslan A (2014). The mouth opening-length relationship and the selectivity of bottom longline used for greater Forkbeard (*Phycis blennoides* B. 1768) fishing in Saros Bay (Northern Aegean Sea). *Ege Journal Fisheries Aquatic Sciences* **31**(1): 41-45

Quinn T J & Deriso R B (1999). Quantitative Fish Dynamics. Oxford University Press, New York, New York. pp. 542

Robillard E, Reis C S & Jones C M (2009). Age-validation and growth of bluefish (*Pomatomus saltatrix*) along the East Coast of the United States. *Fisheries Research* **95**: 65-75

Salerno D J, Burnett J & Ibara R M (2001). Age, growth, maturity and spatial distribution of bluefish, *Pomatomus saltatrix* (Linnaeus), off the Northeast Coast of the United States, 1985-96. *Journal of Northwest Atlantic Fishery Science* **29**: 31-39

Sigeal J & Castellan N S (1989). Non parametric statistics for the behavioural sciences. Statistics Series, 2nd Edition. Mc Graw Hill, New York

Sipe A M & Chittenden M E Jr (2002). A comparison of calcified structures for ageing bluefish in the Chesapeake Bay region. *Transactions of the American Fisheries Society* **131**: 783-790

Sousa F, Isidro E & Erzini K (1999). Semi-pelagic longline selectivity for two demersal species from the azors: The black spot sea bream (*Pegallus bogaraveo*) and the bluemouth rockfish (*Helicolenus dactylopterus dactylopterus*). *Fisheries Research* **41**: 25-35

Terceiro M & Ross J L (1993). A comparison of alternative methods for the estimation of age from length data for Atlantic coast bluefish. *Fishery Bulletin* **91**: 534-549

TFR (2012). Turkish Fisheries Regulations, Number **3/1**(2012-65): 1-112

Ünsal A (2010). Boğaz'ın beş efendisi: Lüfer, palamut, levrek, tekir ve istavrite dair. Yapı Kredi Yayınları, No: 3230, 1. Baskı, İstanbul, Aralık, s. 191

Van der Elst R (1976). Gamefish of the east coast of southern Africa. 1. The biology of the elf, *Pomatomus saltatrix* (Linnaeus), in the coastal waters of Natal. Investigational Report Oceanographic Research Institute, South African Association of Marine Biological Research **44**: 1-59

Wilk S J (1977). Biological and fisheries data on bluefish, *Pomatomus saltatrix* (Linnaeus), 56 pp, US National Marine Fisheries Service, Northeast Fisheries Science Center, Sandy Hook Laboratory Technical Series Report, 11

Woll A K, Boje J, Holst R & Gundersen A C (2001). Catch rates and hook and bait selectivity in longline fishery for Greenland halibut at East Greenland. *Fisheries Research* **51**: 237-246

Zengin (2013). An overview of the status of recreational fisheries in Turkey: Samples of Galata Bridge, the Dardanelles, and Lake Abant. *Yunus Arastırma Bülteni* **2013**(2): 51-65

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Bazı Organik Besin Kaynaklarının Cin Mısırın (*Zea mays* L. *everta*) Tane Verimine Etkisi

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ÖZET

Bu çalışma 16 farklı organik besin kaynağının cin mısırının (*Zea mays* L. *everta*) tane verimine etkisini belirlemek amacıyla Diyarbakır'ın Çermik ilçesinde 2010 ve 2011 yıllarında yürütülmüştür. Araştırma tesadüf blokları deneme desenine göre 3 tekerrürlü olarak kurulmuş ve bitkisel materyal olarak Ant-Cin 98 cin mısırı çeşidi kullanılmıştır. Araştırmada geleneksel yöntem dışında organik besin maddeleri olarak torf, kompost, sığır gübresi, tavuk gübresi, at gübresi, koyun gübresi, güvercin gübresi, solucan gübresi, deniz yosunu gübresi + sığır gübresi, kompost + humik asit, sığır gübresi + humik asit, tavuk gübresi + humik asit, at gübresi + humik asit, koyun gübresi + humik asit, torf + humik asit kullanılmıştır. Her iki yılda da uygulamaların cin mısır verimini istatistiksel olarak önemli ölçüde etkilediği belirlenmiştir ($P \leq 0.01$). İki yılın ortalamasında en yüksek cin mısır tane verimi sırasıyla; deniz yosunu + sığır gübresi, at gübresi + humik asit, koyun gübresi + humik asit uygulamalarından elde edilmiştir. Bu uygulamaların tane verimleri sırasıyla; 526.54 kg da^{-1}, 516.85 kg da^{-1} ve 497.07 kg da^{-1} olmuştur. Deniz yosunu + sığır gübresi, at gübresi + humik asit, koyun gübresi + humik asit ve güvercin gübresi uygulamalarına ait tane veriminde, geleneksele göre sırasıyla % 9.47, % 7.45, % 3.34 ile % 0.52'e varan verim artışı oluşmuştur. Çalışmada ayrıca ekonomik kârlılık durumu da belirlenmiştir. Dekardan en fazla net kâr sağlayan uygulama 2010 yılında at gübresi + humik asit (2280.64 TL da^{-1}), 2011 yılında ise at gübresi (2545.82 TL da^{-1}) uygulamaları olmuştur. Verim, kalite ve net ekonomik kârlılık göz önüne alındığında; at gübresi, tavuk gübresi, kompost, sığır gübresi, koyun gübresi ile humik asit uygulamaları organik cin mısır tarımında kullanılabilir.

Anahtar Kelimeler: Cin mısır; Organik gübre; Humik asit; Tane verimi

Effects of Some Organic Nutrient Sources on Grain Yield of Popcorn (*Zea mays* L. *everta*)

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ABSTRACT

This study was carried out to determine effect of sixteen different organic nutrition sources to grain yield of popcorn (*Zea mays* L. *everta*) in Çermik district of Diyarbakır province of Turkey between 2010 and 2011 years. The research was established according to Randomized Complete Block Designs (RCBD) with 3 replicates and Ant-Cin 98 popcorn variety was used as crop material in the study. Organic nutrition sources were torf, compost, cattle manure, chicken manure, horse manure, sheep manure, pigeon manure, vermicompost, seaweed + cattle manure, compost + humic acid, cattle manure + humic acid, chicken manure + humic acid, horse manure + humic acid, sheep manure + humic acid and torf + humic acid except conventional. In both years, applications affected significantly popcorn yield ($P \leq 0.01$). According to average of two years, the highest popcorn grain yields were found at seaweed + cattle manure, horse manure + humic acid and sheep manure + humic acid applications. Grain yields of these applications were 526.54 kg da^{-1}, 516.85 kg da^{-1} and 497.07 kg da^{-1}, respectively. Seaweed + cattle manure, horse manure + humic acid, sheep manure + humic acid and pigeon manure of applications were caused a 9.47%, 7.45%, 3.34%, 0.52% increase in yield compared with the conventional application, respectively. Also economic analysis was performed in this study. The greatest net profits were obtained from horse manure + humic acid application (2280.64 TL da^{-1}) in 2010 while horse manure (2545.82 TL da^{-1}) application in 2011. According to yield, quality and economic net profit; horse manure, chicken manure, compost, cattle manure, sheep manure and humic acid applications can be used in organic popcorn farming.

Keywords: Popcorn; Organic manure; Humic acid; Grain yield

1. Giriş

Cin mısırı patlatılarak doğrudan insan beslenmesinde kullanılmaktadır. Ülkemizde cin mısırın ekiliş alanı, üretim ve tüketim miktarı ile ilgili herhangi bir istatistiğe rastlanmamıştır. Adana, Çanakkale, Adapazarı, Antalya, Isparta ve Burdur illeri çevresinde, Ege ve Akdeniz Bölgelerinde ekiminin yapıldığı bildirilmektedir (Kün 1997). Mısır tarımının yapıldığı bölgelerde cin mısırının yetiştirilebileceği belirtilmektedir (Öktem 1997; Ülger 1998). Cin mısırının tüketimi son yıllarda artış göstermiş olup üretime olan talepte her geçen gün artmaktadır. Tüketicilerin organik olarak yetiştirilmiş ürünlere rağbet etme eğilimleri sürekli artış göstermektedir. Cin mısırının doğrudan insanlar tarafından tüketilmesi nedeniyle organik cin mısırı ürününe olan talebin daha fazla olduğu görülmektedir. Önümüzdeki yıllarda organik yetiştirilmiş cin mısırına olan talebin artarak devam edeceği tahmin edilmektedir. GAP bölgesinde sentetik kimyasalların az kullanılması, doğal şartlarda çeşitli ürünlerin yetiştiriliyor olması, tarım alanlarının kirlenmemiş olması nedeniyle büyük bir organik tarım potansiyeli bulunmaktadır.

Organik tarımda kullanılabilecek en önemli bitki besin maddesi kaynakları büyükbaş, küçükbaş ve kanatlı hayvan gübreleri ile bitkisel atıkların olduğu bilinmektedir. Son yıllarda organik tarımda kullanılan gübrelerin yelpazesi genişlemiş ve kompost, humik ve fulvik asit, leonardit gibi organik materyallere ilave olarak içerisinde çeşitli mikroorganizma türleri, enzimleri ve yosun ekstraktları içeren gübreler ticari boyutta üretilmeye başlanmıştır.

Çalışma konusu ile ilgili olarak dünyanın farklı bölgelerinde yapılan araştırmalarda farklı sonuçlar elde edilmiştir. Anaç & Okur (1996), organik gübre olarak Biofarm (sertifikalı organik gübre) ve çiftlik (sertifikasız) gübresinin deneme topraklarına uygulanması ile mısır bitkisinin kuru ağırlık, mineral içeriği ve veriminin kontrole göre belirgin artış gösterdiğini ve istatistiksel anlamda önemli olduğunu ($P<0.001$) bildirmişlerdir. Delate & Combardella (2000), geleneksel ve organik gübrenin mısır bitkisinin verimi üzerine etkisini belirlemek amacıyla bir çalışma yapmışlardır. Araştırmacılar yürüttükleri çalışma sonucunda, organik olarak yetiştirilen mısır bitkisinden elde ettikleri verimin (903.1 kg da^{-1}), geleneksel olarak

yetiştirilen mısır bitkisine nazaran (884.3 kg da^{-1}) daha fazla olduğunu bildirmişlerdir.

Neill & Robinson (2001), geleneksel, sığır gübresi, tavuk gübresi ve herhangi bir gübrenin uygulanmadığı bir çalışma sonucunda, sığır gübresinin uygulandığı parsellerden elde edilen tane veriminin diğer uygulamalara göre daha iyi sonuç verdiğini ifade etmişlerdir. Wang et al (2003), organik gübrenin % 26, sentetik gübrenin de mısır verimine % 74 katkı sağladığını bildirmişlerdir. Sharif et al (2004), en yüksek tane verimini organik gübre ve inorganik gübreye humik asit ilave edilmesi sonucunda elde etmişlerdir. Prasanna et al (2007), en yüksek tane verimini solucan gübre uygulamasından; Shafiq-ur-Rehman et al (2008) ise kimyasal gübrenin uygulandığı parsellerde en yüksek tane verimini elde ettiklerini bildirmişlerdir.

Bu çalışmada, organik tarıma yönelik kullanılan bazı organik besin kaynaklarının cin mısırın tane verimi üzerine etkisini ve ekonomik anlamda en uygun organik besin kaynağını belirlemek amaçlanmıştır.

2. Materyal ve Yöntem

Araştırma Diyarbakır ili Çermik İlçesi Aşağışeyhler köyünde 2010 ve 2011 yıllarında II. ürün koşullarında yürütülmüştür. Araştırmanın yürütüldüğü 2010 ve 2011 yılları ile uzun yıllara ait iklim değerleri Çizelge 1'de verilmiştir. Ekimden önce deneme alanından 0-30 cm derinlikten toprak örneği alınarak toprağın fiziksel ve kimyasal özellikleri belirlenmiştir. Deneme alanının bazı fiziksel ve kimyasal özellikleri Çizelge 2'de verilmiştir. Çizelge 2'de görüleceği üzere deneme alanının toprağı kırmızı-kahverengi

Çizelge 1- Diyarbakır ilinde 2010, 2011 ve uzun yıllar ortalamasına ait yağış sıcaklık ve nispi nem değerleri

Aylar	*Yıllar*	*Minimum sıcaklık (ºC)*	*Maksimum sıcaklık (ºC)*	*Ortalama sıcaklık (ºC)*	*Yağış (mm)*	*Nispi nem (%)*
	2010	14.9	40.8	27.2	8.0	47.6
Haziran	2011	13.2	37.9	26.3	14.6	33.9
	Uzun yıllar	16.9	33.7	26.3	7.2	36.0
	2010	18.0	44.0	32.7	0.0	34.3
Temmuz	2011	18.4	45.0	31.5	0.2	22.6
	Uzun yıllar	21.7	38.5	31.2	0.7	27.0
	2010	18.0	43.6	32.4	0.0	32.2
Ağustos	2011	16.0	43.5	31.2	0.0	22.3
	Uzun yıllar	21.0	38.1	30.3	0.3	27.0
	2010	13.6	41.2	26.8	3.0	44.7
Eylül	2011	12.8	38.1	25.6	1.9	28.5
	Uzun yıllar	16.0	33.1	24.8	2.6	31.0
	2010	7.3	30.0	17.6	49.2	61.8
Ekim	2011	3.0	32.8	17.4	57.4	52.5
	Uzun yıllar	10.1	25.3	17.2	30.8	48.0
	2010	1.0	26.1	12.0	0.0	57.4
Kasım	2011	-4.7	19.9	6.6	104.0	61.1
	Uzun yıllar	3.6	15.9	9.3	54.6	68.0

Kaynak: Diyarbakır Meteoroloji Bölge Müdürlüğü (Anonim 2011).

Çizelge 2- Denemenin yapıldığı alanın 0-30 cm'deki toprak özellikleri

Derinlik (cm)	*Bünye*	*Toprak rengi*	*pH*	*Su ile doygunluk (%)*	*Toplam tuz (%)*	*Organik madde (%)*	*$CaCO_3$ (%)*	*Yarayışlı P_2O_5 (kg da^{-1})*	*Yarayışlı K_2O (kg da^{-1})*
0-30	Killi-tınlı	Kırmızı-kahve	7.4	64.9	0.03	1.19	9.08	2.75	82.05

olup, toprak tekstürü ise killi-tınlıdır. Deneme alanı toprağının tuz oranı düşük, organik madde ve fosfor miktarı yetersiz, potasyum ve kireç oranı fazla ve hafif alkali karakterde olduğu belirlenmiştir.

Çalışmada bitkisel materyal olarak Ant-Cin-98 cin mısır çeşidi kullanılmıştır. Denemede organik yöntemlerle üretilmiş ve organik tarımda kullanılabilirlik sertifikası olan organik materyallerden yararlanılmıştır. Denemede kullanılan organik gübre materyalleri organik tarımın esasları ve uygulanmasına ilişkin yönetmeliğinin 20. maddesi gereğince arazi üzerine verilecek azami saf azot miktarına göre (17 kg da^{-1}) hesaplanarak uygulanmıştır (Resmi gazete 2010). Geleneksel gübre uygulamasında dekara saf olarak 17 kg da^{-1} azot, 8 kg da^{-1} fosfor ve potasyum verilmiştir. Organik besin maddeleri olarak torf, kompost, sığır gübresi, tavuk gübresi, at gübresi, koyun gübresi, güvercin gübresi, solucan gübresi, deniz yosunu gübresi + sığır gübresi, kompost + humik asit, sığır gübresi + humik asit, tavuk gübresi + humik asit, at gübresi + humik asit, koyun gübresi + humik asit, torf + humik asit kullanılmıştır. Araştırmada kullanılan besin kaynakları azot içeriği ile dekara atılan gübre miktarları Çizelge 3'te verilmiştir.

Deneme kurulan alanın organik tarıma uygun hale getirilmesi için, deneme alanına 2008 ve 2009 yıllarında buğday ekilmiş, hiçbir kimyasal gübre ve ilaç uygulanmadan buğday yetiştirilerek hasat edilmiştir. Ekimden önce toprak goble disk ve ardından diskaro ile işlenerek ekime hazır hale getirilmiştir. Deneme Yurtsever (1984)'e göre tesadüf blokları deneme desenine göre üç tekrarlamalı olarak kurulmuştur. Her parsel (5 m x 2.80 m) 4 sıradan meydana gelmiştir. Ekimde sıra arası mesafeler 70 cm ve sıra üzeri mesafeler 20 cm olmuştur. Besin kaynaklarının çoğu ekim öncesi ve ekimle birlikte, deniz yosunu gübresi ise bir kısmı ekim öncesi toprağa, geri kalanı ise bitkiler 20 cm boya ulaştıktan sonra birer hafta ara ile üç kez olmak üzere yapraktan uygulanmıştır. 15-30 Haziran tarihleri arasında cin mısır tohumları 5-6 cm derinliğe elle ekilmiştir. Çıkış için yeterli düzeyde nem bulunmadığı için ekimden sonra yağmurlama sulama, yetişme süresi boyunca da karık usulü sulama ile parsellere eşit miktarda su verilmiştir. Parseller arası su geçişini engellemek

Çizelge 3- Organik besin kaynaklarının azot içeriği ile dekara uygulanan miktarları

	Besin kaynakları	*N içeriği (%)*	*Uygulanan miktar*
1	Geleneksel gübre (üre)	46	36.96 kg da^{-1}
2	Torf	1.2	1416 kg da^{-1}
3	Kompost	2.5	680 kg da^{-1}
4	Sığır gübresi	3.5	486 kg da^{-1}
5	Tavuk gübresi	3.0	567 kg da^{-1}
6	At gübresi	2.0	850 kg da^{-1}
7	Koyun gübresi	2.0	850 kg da^{-1}
8	Güvercin gübresi	6.0	283 kg da^{-1}
9	Deniz yosunu + sığır gübresi	2.0+3.5	51.5 kg da^{-1} + 457 kg da^{-1}
10	Solucan gübresi	1.5	1133 kg da^{-1}
11	Kompost + humik asit	2.5	680 kg da^{-1} + 140 g da^{-1}
12	Sığır gübresi + humik asit	3.5	486 kg da^{-1} + 140 g da^{-1}
13	Tavuk gübresi + humik asit	3.0	567 kg da^{-1} + 140 g da^{-1}
14	Koyun gübresi + humik asit	2.0	850 kg da^{-1} + 140 g da^{-1}
15	Torf + humik asit	1.2	1416 kg da^{-1} + 140 g da^{-1}
16	At gübresi + humik asit	2.0	850 kg da^{-1} +140 g da^{-1}

için parseller arasında 2 metre boşluk bırakılmış ve parsellerin etrafı sedde ile çevrilmiştir. Bitki koruma ve bakım yöntemi olarak doğal kültürel önlemler (çimlenmeden sonra tekleme ardından traktör ve el çapası) yapılmıştır. Ayrıca mısır koçan kurdu zararlısına karşı *Trichogramma sp*. faydalı böceği kullanılmıştır. Hasat sırasında parselin her iki başından 0.5 m ve her iki kenarında bulunan birer sıra kenar tesiri olarak atılarak hasat edilmiş ve ortadaki iki sıradan değerler alınmıştır. Elde edilen değerler Totemstat-C paket programı kullanılarak varyans analizine ve Duncan çoklu karşılaştırmasına tabii tutulmuştur (Açıkgöz et al 2004). Ayrıca kullanılan besin maddelerinin hangisinin daha ekonomik olduğunu belirlemek için Vuruş et al (2000)'ın belirttiği şekilde ekonomik analiz de yapılmıştır.

3. Bulgular ve Tartışma

3.1. Tane verimi

Organik olarak yetiştirilen cin mısırda tane verimi ile ilgili varyans analiz değerleri Çizelge 4'de görülmektedir. Araştırmada yıllar ayrı ayrı ve birleştirilerek varyans analizine tabi tutulmuştur. Tane verimi bakımından besin kaynakları arasındaki farklılık 2010 yılı ve 2011 yılında % 1 düzeyinde önemli bulunmuştur. İki yılın birleştirilmiş varyans analizinde ise tane verimi bakımından, yıllar, besin kaynakları ve yıl x besin kaynakları interaksiyonu % 1 düzeyinde istatistiki olarak önemli bulunmuştur (Çizelge 4). Tane verimine ait ortalama değerler ve Duncan çoklu karşılaştırma testine göre oluşan gruplar Çizelge 5'te verilmiştir. 2010-2011 yılı

Çizelge 4- Tane verimine ait 2010, 2011 ve 2010-2011 birleştirilmiş varyans analizi tablosu

2010						
Varyasyon kaynakları	*Serbestlik derecesi*	*Kareler toplamı*	*Kareler ortalaması*	*Hesaplanan F değeri*	*Tablo değeri % 5*	*Tablo değeri % 1*
Tekerrür	2	586.351	293.176	0.604 öd	3.320	5.390
Besin kaynakları	15	76752.585	5116.839	10.548 **	2.010	2.700
Hata1	30	14552.362	485.079			
Genel	47	91891.298				
Değişim katsayısı	CV= % 5.33					
2011						
Varyasyon kaynakları	*Serbestlik derecesi*	*Kareler Toplamı*	*Kareler ortalaması*	*Hesaplanan F değeri*	*Tablo değeri % 5*	*Tablo değeri % 1*
Tekerrür	2	1544.856	772.428	1.602 öd	3.320	5.390
Besin kaynakları	15	70876.194	4725.080	9.801 **	2.010	2.700
Hata1	30	14463.127	482.104			
Genel	47	86884.177				
Değişim katsayısı	CV= % 4.26					
2010-2011						
Varyasyon kaynakları	*Serbestlik derecesi*	*Kareler toplamı*	*Kareler ortalaması*	*Hesaplanan F değeri*	*Tablo değeri % 5*	*Tablo değeri % 1*
Tekerrür	2	266.948	133.474	0.143 öd	19.000	99.000
Yıl	1	251745.288	251745.288	270.076**	18.510	98.500
Hata1	15	1864.258	932.129			
Besin kaynakları	15	88247.618	5883.175	12.166 **	1.840	2.350
Yıl* besin kaynakları	62	59381.162	3958.744	8.186 **	1.840	2.350
Hata2	95	29015.488	483.591			
Genel		430520.762				
Değişim katsayısı	CV(a)= % 6.57, CV(b)= % 4.74					

*, 0.05'e göre önemli; **, 0.01'e göre önemli; öd, önemli değil

Çizelge 5- Farklı organik besin maddesi kaynaklarının cin mısırın tane verimine etkisi

Besin kaynakları	*2010*	*2011*	*Ortalama*
Geleneksel gübre	390.26 c-f*	571.76 a	481.01 B-E
Torf	362.09 f	480.45 bcd	421.27 F
Kompost	372.85 ef	488.78 bcd	430.82 F
Sığır gübresi	419.39 cd	472.57 cd	445.98 DEF
Tavuk gübresi	379.93 ef	466.44 d	423.18 F
At gübresi	462.24 ab	482.79 bcd	472.52 CDE
Koyun gübresi	408.65 cde	511.63 bc	460.14 C-F
Güvercin gübresi	475.93 a	491.04 bcd	483.49 BCD
Deniz yosunu + sığır gübresi	477.06 a	576.01 a	526.54 A
Solucan gübresi	381.66 def	500.60 bcd	441.13 EF
Kompost + humik asit	379.58 def	508.05 bcd	443.81 EF
Sığır gübresi + humik asit	433.04 bc	486.03 bcd	459.54 CF
Tavuk gübresi + humik asit	386.38 def	516.17 b	451.27 DEF
Koyun gübresi + humik asit	433.70 bc	560.44 a	497.07 ABC
Torf + humik asit	372.17 ef	578.28 a	475.22 CDE
At gübresi + humik asit	475.58 a	558.12 a	516.85 AB
Ortalama	413.16 B	515.57 A	
LSD	Yıl: 0.467 2010 Besin kaynakları: 36.721 2011 Besin kaynakları: 36.608 2010-2011 Ort. besin kaynakları: 2.011		

*, aynı harf grubuna giren ortalamalar arasında Duncan Testine göre 0.05 düzeyinde önemli farklılık yoktur

ortalamaları ele alındığında, farklı besin madde uygulamalarında tane verimi 421.27 kg da^{-1} ile 526.54 kg da^{-1} arasında değişmiştir. Tane verimi değeri en yüksek 526.54 kg da^{-1} ile deniz yosunu + sığır gübresi uygulamasından elde edilmiş ve daha sonra sırasıyla, at gübresi + humik asit (516.85 kg da^{-1}) ile koyun gübresi + humik asit (497.07 kg da^{-1}) parsellerinde belirlenmiştir (Çizelge 5). Birleştirilmiş ortalamalarda en düşük tane verimi ise 421.27 kg da^{-1} ile torf uygulamasından elde edilmiştir. Yukarıdaki sonuçlardan besin kaynakları içeriğinin, tane verimini olumlu bir şekilde etkilediği ve uygulamalar arasında fark görülmesine sebep olduğu söylenebilir.

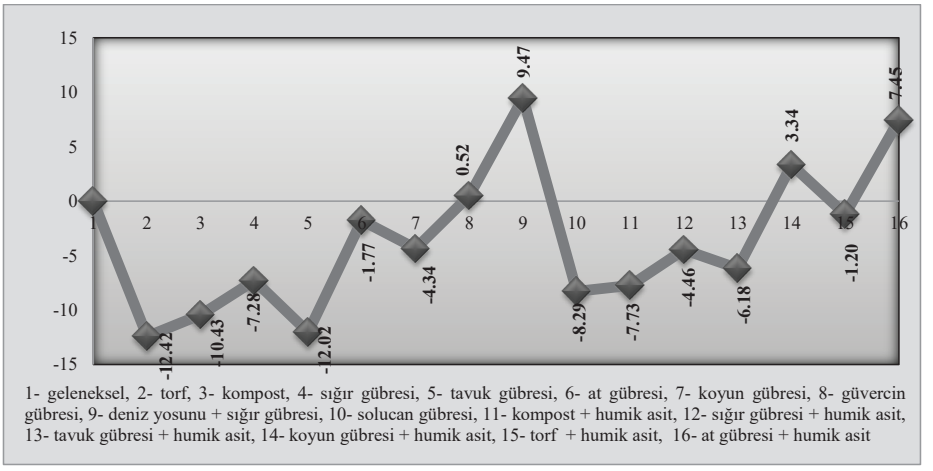
Deniz yosunu + sığır gübresi parselinde gübrelerin bir kısmının ekimde geri kalan kısmının ise özellikle yosun gübresinin yapraktan üç dönemde verilmesi, cin mısır koçan özelliklerini (koçan uzunluğu, koçan çapı, koçanda tane sayısı, koçanda tane ağırlığı) olumlu etkilemiş ve sonuçta geleneksel ve diğer organik üretim sistemlerine göre daha fazla tane verimi alınmıştır. Yazıcı & Kaynak (2001), deniz yosununun organik tarımda verim ve kaliteyi artırdığını belirterek çalışmamızı desteklemektedir. Warman & Munro-Warman (1993) ise deniz yosunu uygulamasının mısırda verime etkisinin bulunmadığını bildirmektedir. Beşirli et al (2009) domateste en fazla verimi koyun gübresinden aldığını, deniz yosununun tek başına kullanımının verime etkisinin olmadığını, ancak deniz yosunun sığır gübresi, koyun gübresi ve tavuk gübresi gibi materyallerle birlikte uygulanmasının daha uygun olacağını belirtmişlerdir. Çalışmamızda da deniz yosunu gübresi sığır gübresi ile birlikte uygulanmıştır.

Organik mısır yetiştiriciliğinde tane verimi ile ilgili farklı literatür sonuçları bildirilmiştir. Morris & Lathwell (2004), Efthimiadou et al (2010) ile Dordas et al (2008) sığır gübresinden; Khan et al (2008), Bamire & Amujoyegbe (2004) ile Şeker & Ersoy (2005) tavuk gübresinden; Amujoyegbe et al (2007) ile Mitchell & Tu (2005) tavuk gübresi + inorganik gübreden; Prasanna et al (2007) solucan gübresinden; Ashoka et al (2009) solucan gübre + kimyasal gübreden; Gürses (2010) yeşil gübreden; Leaungvutivirong et al (2004) organik gübre + kompost uygulamasından; Amanullah et al (2006) çiftlik gübresi, kümes gübresi ve bunların kombinasyonundan; Lee & Bartlett (1976) humik asit uygulamasından; Gerzabek & Ulah (1988) humik asit + çinko uygulamasından; Sharif et al (2004) organik gübre + inorganik gübre + humik asit uygulamasından; Gao et al (2003), Manju & Mukerji (1994) ve Kan (2005) çiftlik gübresinden; Thakur et al (2009), Wang et al (2003) ile Shafiq-ur-Rehman et al (2008) geleneksel üretim sisteminden; Liu (2003) ile Mahesh et al (2010) organik gübre + inorganik gübreden en yüksek mısır tane verimini aldıklarını bildirmişlerdir.

Organik besin kaynakları ile geleneksel yöntemden elde edilen tane verimi arasında meydana gelen % olarak artış ve azalış oranları hesaplanarak Şekil 1'de verilmiştir. Şekil 1'de yatay düzlem geleneksel üretim sistemi olarak kabul edilmiştir. Şekil 1'de görüldüğü gibi, torf uygulaması, tavuk gübresi ve kompost uygulamalarından elde edilen oransal tane verimi değerleri, geleneksel üretim sistemine göre sırasıyla, % 12.42, % 12.02 ve % 10.43 daha düşük bulunmuştur. Buna karşın deniz yosunu + sığır gübresi, at gübresi + humik asit, koyun gübresi + humik asit ve güvercin gübresi uygulamalarından elde edilen oransal tane verim değerleri ise geleneksel üretim sistemine göre sırasıyla, % 9.47, % 7.45, % 3.34 ve % 0.52 daha yüksek bulunmuştur. Diğer organik uygulamaların tane verimi değerleri bakımından beklenen etkiyi gösteremediği ve geleneksel üretim sisteminden daha düşük verim verdikleri görülmektedir.

3.2. Ekonomik analiz

Denemede 2010 yılında cin mısır üretimi için belirlenmiş olan dekara 179.98 TL üretim masrafı değeri, üretimde kullanılan organik besin kaynaklarının masraflarına ilave edilmiş ve böylece her bir organik gübre kaynağının toplam genel masrafı elde edilmiştir. Üretim masrafları yönünden uygulamalar karşılaştırıldığında, ortalama değerlere göre en fazla üretim masrafı torf (16454.98 TL da^{-1}) ve torf + humik asit (16504.98 TL da^{-1}) uygulamalarında yapılmıştır. Anılan bu gübrelerin verim değerlerinin geleneksel gübre sistemine yakın



1- geleneksel, 2- torf, 3- kompost, 4- sığır gübresi, 5- tavuk gübresi, 6- at gübresi, 7- koyun gübresi, 8- güvercin gübresi, 9- deniz yosunu + sığır gübresi, 10- solucan gübresi, 11- kompost + humik asit, 12- sığır gübresi + humik asit, 13- tavuk gübresi + humik asit, 14- koyun gübresi + humik asit, 15- torf + humik asit, 16- at gübresi + humik asit

Şekil 1- Farklı organik besin kaynakları ve geleneksel sistemden elde edilen tane veriminin değerlendirilmesi

olmasına karşılık, üretim masraflarının yüksek olması nedeniyle torf (-14282.44 TL da^{-1}) ve torf + humik asit (-14271.96 TL da^{-1}) uygulamalarında zarar edilmiştir. 2010 yılında üretim masrafı bakımından en ekonomik ve en az masraf olan uygulama 479.98 TL da^{-1} ile geleneksel gübre uygulamasında saptanmıştır.

Çizelge 6'ya göre cin mısırda 2010 yılı için bütçe analizi sonucunda ekonomik anlamda en fazla kârlılık 2280.64 TL da^{-1} ile at gübresi + humik asit uygulamasından, en az kârlılık ise 251.13 TL da^{-1} ile deniz yosunu + sığır gübresi uygulamasından elde edilmiştir. İlk yıl en fazla kârlılık gösteren at gübresi + humik asit (2280.64 TL da^{-1}) uygulamasını daha sonra sırasıyla, at gübresi (2250.60 TL da^{-1}), sığır gübresi + humik asit (1868.26 TL da^{-1}), sığır gübresi (1836.36 TL da^{-1}), tavuk gübresi (1528.18 TL da^{-1}), tavuk gübresi + humik asit (1516.88 TL da^{-1}) ve koyun gübresi + humik asit (1515.08 TL da^{-1}) uygulamaları takip etmiştir.

Çizelge 7'ye göre, 2011 yılı için cin mısır üretimi için belirlenmiş olan dekara 215.16 TL üretim masrafı değeri, üretimde kullanılan organik besin kaynaklarının masraflarına ilave edilmiş ve böylece her bir organik gübre kaynağının toplam genel masrafı elde edilmiştir. Üretim masrafları yönünden uygulamalar karşılaştırıldığında, ortalama değerlere göre en fazla üretim masrafı torf (17902.50 TL da^{-1}) ve torf + humik asit (17957.50 TL da^{-1}) uygulamalarında yapılmıştır. Anılan bu gübrelerin verim değerlerinin geleneksel gübre sistemine yakın olmasına karşılık, üretim masraflarının yüksek olması nedeniyle torf (-14994.74 TL da^{-1}) ve torf + humik asit (-14413.84 TL da^{-1}) uygulamalarında

Çizelge 6- 2010 Yılı cin mısır ekonomik kârlılık tablosu

2010 Yılı cin mısır ekonomik kârlılık tablosu						
	Besin kaynakları	*Genel masraflar*	*Besin kaynaklarının masrafı*	*Toplam genel masraf*	*Gelir**	*Kârlılık*
1	Geleneksel gübre	179.98 TL	300.00 TL	479.98 TL	390.26 kg da^{-1} x 3.5	885.93 TL
2	Torf	179.98 TL	16275.00 TL	16454.98 TL	362.09 kg da^{-1} x 6.0	-14282.44 TL
3	Kompost	179.98 TL	1357.14 TL	1537.12 TL	372.85 kg da^{-1} x 6.0	699.98 TL
4	Sığır gübresi	179.98 TL	500.00 TL	679.98 TL	419.39 kg da^{-1} x 6.0	1836.36 TL
5	Tavuk gübresi	179.98 TL	571.42 TL	751.40 TL	379.93 kg da^{-1} x 6.0	1528.18 TL
6	At gübresi	179.98 TL	342.86 TL	522.84 TL	462.24 kg da^{-1} x 6.0	2250.60 TL
7	Koyun gübresi	179.98 TL	857.14 TL	1037.12 TL	408.65 kg da^{-1} x 6.0	1414.78 TL
8	Güvercin gübresi	179.98 TL	1928.52 TL	2108.50 TL	475.93 kg da^{-1} x 6.0	747.08 TL
9	Deniz yosunu + sığır gübresi	179.98 TL	2431.25 TL	2611.23 TL	477.06 kg da^{-1} x 6.0	251.13 TL
10	Solucan gübresi	179.98 TL	1714.28 TL	1894.26 TL	381.66 kg da^{-1} x 6.0	395.70 TL
11	Kompost + humik asit	179.98 TL	1407.14 TL	1587.12 TL	379.58 kg da^{-1} x 6.0	690.36 TL
12	Sığır gübresi + humik asit	179.98 TL	550.00 TL	729.98 TL	433.04 kg da^{-1} x 6.0	1868.26 TL
13	Tavuk gübresi + humik asit	179.98 TL	621.42 TL	801.40 TL	386.38 kg da^{-1} x 6.0	1516.88 TL
14	Koyun gübresi + humik asit	179.98 TL	907.14 TL	1087.12 TL	433.70 kg da^{-1} x 6.0	1515.08 TL
15	Torf + humik asit	179.98 TL	16325.00 TL	16504.98 TL	372.17 kg da^{-1} x 6.0	-14271.96 TL
16	At gübresi + humik asit	179.98 TL	392.86 TL	572.84 TL	475.58 kg da^{-1} x 6.0	2280.64 TL

*, gelir dekardan alınan verim ile ürün bedelinin çarpılması sonucu elde edilmiştir

Çizelge 7- 2011 Yılı cin mısır ekonomik kârlılık tablosu

	2011 Yılı cin mısır ekonomik kârlılık tablosu					
	Besin kaynakları	*Genel masraflar*	*Besin kaynaklarının masrafı*	*Toplam genel masraf*	*Gelir**	*Kârlılık*
1	Geleneksel gübre	215.16 TL	330.00 TL	545.16 TL	571.76 kg da^{-1} x 4.0 0	1741.88 TL
2	Torf	215.16 TL	17902.50 TL	18117.66 TL	480.45 kg da^{-1} x 6.0 5	-14994.74 TL
3	Kompost	215.16 TL	1492.85 TL	1708.01 TL	488.78 kg da^{-1} x 6.0 5	1469.06 TL
4	Sığır gübresi	215.16 TL	550.00 TL	765.16 TL	472.57 kg da^{-1} x 6.0 5	2306.54 TL
5	Tavuk gübresi	215.16 TL	628.56 TL	843.72 TL	466.44 kg da^{-1} x 6.0 5	2188.14 TL
6	At gübresi	215.16 TL	377.15 TL	592.31 TL	482.79 kg da^{-1} x 6.0 5	2545.82 TL
7	Koyun gübresi	215.16 TL	942.85 TL	1158.01 TL	511.63 kg da^{-1} x 6.0 5	2167.58 TL
8	Güvercin gübresi	215.16 TL	2121.37 TL	2336.53 TL	491.04 kg da^{-1} x 6.0 5	855.23 TL
9	Deniz yosunu + sığır gübresi	215.16 TL	2674.38 TL	2889.54 TL	576.01 kg da^{-1} x 6.0 5	854.52 TL
10	Solucan gübresi	215.16 TL	1885.71 TL	2100.87 TL	500.60 kg da^{-1} x 6.0 5	1153.03 TL
11	Kompost + humik asit	215.16 TL	1547.85 TL	1763.01 TL	508.05 kg da^{-1} x 6.0 5	1539.31 TL
12	Sığır gübresi + humik asit	215.16 TL	605.00 TL	820.16 TL	486.03 kg da^{-1} x 6.0 5	2339.03 TL
13	Tavuk gübresi + humik asit	215.16 TL	683.56 TL	898.72 TL	516.17 kg da^{-1} x 6.0 5	2456.38 TL
14	Koyun gübresi + humik asit	215.16 TL	997.85 TL	1213.01 TL	560.44 kg da^{-1} x 6.0 5	2429.85 TL
15	Torf + humik asit	215.16 TL	17957.50 TL	18172.66 TL	578.28 kg da^{-1} x 6.0 5	-14413.84 TL
16	At gübresi + humik asit	215.16 TL	432.15 TL	647.31 TL	558.12 kg da^{-1} x 6.0 5	2280.64 TL

*, gelir dekardan alınan verim ile ürün bedelinin çarpılması sonucu elde edilmiştir

zarar edilmiştir. 2011 yılında üretim masrafı bakımından en ekonomik ve en az masraf olan uygulama 545.16 TL da^{-1} ile geleneksel gübre uygulamasında saptanmıştır.

Yapılan bütçe analizi sonucunda 2011 yılı için ekonomik anlamda en kârlı üretim 2545.82 TL da^{-1} ile at gübresi uygulamasından elde edilmiş olup bunu sırasıyla; tavuk gübresi + humik asit (2456.38 TL da^{-1}), koyun gübresi + humik asit (2429.85 TL da^{-1}), sığır gübresi + humik asit (2339.03 TL da^{-1}), sığır gübresi (2306.54 TL da^{-1}) ve at gübresi + humik asit (2280.64 TL da^{-1}) uygulamaları izlemiştir. Organik uygulamalar içerisinde en az net kârı (854.52 TL da^{-1}) denemenin tüm özelliklerinde en iyi performans gösteren deniz yosunu + sığır gübresi uygulamasında belirlenmiştir. Bunun nedeni bu uygulamanın toplam masrafı torf ve torf + humik asit dışında diğer organik gübrelerden daha yüksek çıkmasıdır. İkinci yıl uygulamalar arasında en düşük üretim masrafı ise at gübresi (592.31 TL da^{-1}) uygulamasında belirlenmiştir. 2011 yılında torf ve torf + humik asit dışındaki diğer organik gübre uygulamaların çoğu, geleneksel üretim sisteminden daha fazla kâr getirdiği görülmüştür. Denemenin ikinci yılında ilk yılda olduğu gibi ekonomik kârlılık sağlamayan uygulamalar, aynı zamanda en fazla üretim masrafına da sahip olan torf (-14994.74 TL da^{-1}) ve torf + humik asit (-14413.84 TL da^{-1}) uygulamalarında alınmıştır. Bu uygulamalardan yüksek verim alınması ve satış fiyatının geleneksel sisteme göre yüksek olması üretim masraflarını karşılayamamış ve torf ile torf + humik asit uygulamalarından zarar edilmesine neden olmuştur.

Çalışmanın ikinci yılında, uygulamalar arasında brüt kârı, organik cin mısır yetiştiriciliği uygulamalarının çoğunda geleneksel cin mısır

yetiştiriciliğinden (1741.88 TL da^{-1}) daha yüksek bulunmuştur. Bunda organik ürünlerin kg satış fiyatının geleneksel sisteme nazaran daha yüksek olmasından kaynaklanmaktadır. Araştırma sonucunda, Diyarbakır koşullarında verim, kalite ve net kârlılık kriterleri göz önüne alınarak, organik cin mısır yetiştiriciliği için özellikle sığır gübresi, at gübresi, tavuk gübresi, kompost, koyun gübresi ile humik asit uygulamalarının tavsiye edilebilir ve ekonomik anlamda en uygun uygulamalar olduğu söylenebilir. Ancak torf uygulaması için ekonomik anlamda bir uygulama olduğu söylenemez. Bulgularımız ekolojik tahıl üretiminde net kâr düzeyinin geleneksel tarıma göre düşük olduğunu bildiren Dobbs et al (1988), Shafiq-ur-Rehman et al (2008), Acar et al (2009)'ın bulguları ile çelişirken, Cengiz et al (2010) ve Şahin et al (2010)'ın organik uygulamalardan alınan verimlerin ticari gübreden daha karlı olduğu belirten tespitleri ile uyum içerisindedir.

4. Sonuç

Bu çalışma ile Diyarbakır koşullarında organik cin mısırın yetiştirilebileceği belirlenmiştir. Araştırmada en yüksek tane verimi veren deniz yosunu + sığır gübresi ile güvercin gübre uygulamaları ekonomik bulunmamıştır. Ancak verim, kalite ve net kârlılık kriterleri göz önüne alındığında, organik cin mısır yetiştiriciliğinde at gübresi, tavuk gübresi, kompost, sığır gübresi, koyun gübresi ile humik asit kullanılabilir ve ekonomik anlamda en uygun uygulamalar olduğu söylenebilir. Ayrıca cin mısırında organik gübreler ile humik asidin birlikte uygulanmasının, organik gübrelerin tek olarak uygulanmasına nazaran daha iyi sonuç verdiği görülmüştür.

Kaynaklar

Acar M, Dok M & Caner Y K (2009). Organik ve geleneksel tarım metodu ile üretilen nohut'un verim, maliyet ve kalite kriterleri bakımından karşılaştırılması. *I. GAP Organik Tarım Kongresi*, 17-20 Kasım 2009, Şanlıurfa, s. 38-46

Açıkgöz N, İlker E & GokcoL A (2004). Assessment of biological research on the computer. EU, TOTEM, No: 2, İzmir

Amanullah M M, Alagesan A, Vaiyapuri K, Pazhanivelan S & Sathyamoorthi K (2006). Intercropping and organic manures on the growth and yield of cassava (*Manihot esculenta* Crantz.). *Reseaerch Journal of Agriculture and Biological Sciences* **2**(5): 183-189

Amujoyegbe B J, Opabode J T & Olayinka A (2007). Effect of organic and inorganic fertilizier on yield and chlorophyll content of maize (*Zea mays* L.) and *Sorghum Bicolour* (L.) Moenc. *African Journal of Biotechnology* **6**(16): 1869-1873

Anaç D & Okur B (1996). Toprak verimliliğinin doğal yollar ile artırılması. *Ekolojik (Organik, Biyolojik) Tarım Organizasyon Derneği* (ETO), İzmir, s. 37-73

Anonim (2011). Diyarbakır iline ait 2010, 2011 ve uzun yıllar iklim verileri. Diyarbakır Meteoroloji Bölge Müdürlüğü, Diyarbakır

Ashoka P, Anand S R, Mudalagiriyappa P & Smitha R (2009). Effect of macro and micronutrients with organics on growth, quality, yield and economics of baby corn (*Zea mays* L.) in tungabhadra command area. *Crop Research (Hisar)* **37**(1-3): 15-18

Bamire A S & Amujoyegbe B J (2004). Economics of poultry manure utilization in land quality improvement among integrated poultry-maize holder farmers in Southwestern Nigeria. *Journal of Sustainable Agriculture* **23**(3): 21-37

Beşirli G, Sönmez İ, Keçeci M & Güçdemir İ H (2009). Organik domates yetiştiriciliğinde yeşil gübreleme ve bazı besin maddelerinin toprak yapısı üzerine etkisi. *1. GAP Organik Tarım Kongresi* 17-20 Kasım 2009, Şanlıurfa, s. 583-591

Cengiz R, Yanıkoğlu S & Sezer M C (2010). Sentetik ve organik gübrelerin mısırda (*Zea mays L.*) verim ve kaliteye etkisi. (Ed: A A Vural), *Organik Tarım Araştırma Sonuçları* 2005-2010. T.C. Tarım ve Köyişleri Bakanlığı, Ankara, s. 213-220

Delate K & Combardella C (2000). Comparison of organic and conventional crops at the nelly-kinyon long-term agroeciological research. 2000. Iova University. *Armstrong Research an Demonstraion Farm Bulletin.* **7**: 208-210

Dobbs T L, Leddy M G & Smolik J D (1988). Factor influencing the economic patential for alternative farming systems. Case Analyses in South Dakota. *American Journal of Alternative Agriculture* **3**: 26-34

Dordas C A, Lithourgidis A S, Matsi T & Barbayiannis N (2008). Application of liquid cattle manure and inorganic fertilizers affect dry matter, nitrogen

accumulation, and partitioning in maize. *Nutrient Cycling in Agroecosystems* **80**(3): 283-296

Efthimiadou A, Bilalis D, Karkanis A & Froud-Williams B (2010). Combined organic/inorganic fertilization enhance soil quality and increased yield, photosynthesis and sustainability of sweet maize crop. *Australian Journal of Crop Science* **4**(9): 722-729

Gao F, Cao L, Chen G & Shi K (2003). Applied test and study on biological organic fertilizer in glutinous corn. *Journal of Shanghai Jiaotong University-Agricultural Science* **21**(3): 237-241

Gerzabek M H & Ulah S M (1988). Influence of fulvic and humic acids on the Zn uptake by corn (*Zea mays* L.) from nutrient solution. *Mitteilungen der Deutschen Badenkundlichen Gesellschaft* **56**: 141-146

Gürses M A (2010). Mısır (*Zea mays indentata Sturt*.) yetiştiriciliğinde değişik yeşil gübre ve çiftlik gübresi uygulamalarının verim ve verim unsurlarına etkisi. Yüksek lisans tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Adana

Kan A (2005). Sert mısırda organik ve inorganik gübre dozlarının koçan püskülü verimi ve püsküldeki bitki besin elementi içerikleri üzerine etkileri. *Türkiye VI. Tarla Bitkileri Kongresi*, 5-9 Eylül 2005, Antalya (Araştırma Sunusu) Cilt I, s. 253-256

Khan H Z, Malik M A & Saleem M F (2008). Effect of rate and source of organic material on the production potential of spring maize (*Zea mays L.*). *Pakistan Journal of Agricultural Sciences* **45**(1): 40-43

Kün E (1997). Tahıllar II (Sıcak iklim tahılları), Ankara Üniversitesi Ziraat Fakültesi Yayın No: 1452, Ders Kitabı No: 432, Ankara

Leaungvutivirog C, Sunantapongsuk V, Limtong P, Nakapraves P & Piriyaprin S (2004). Effect of organic fertilizer on soil improvement in Mab Bon, Tha Yang, *Satuk and Renu Series for Corn Cultivation in Thailand. Symposium,* 14-21 August 2002, No: 57, Paper No: 1899

Lee Y S & Bartlett R J (1976). Stimulation of plant growth by humic substances. *Soil Science Society of American Journal* **40**: 876-879

Liu Y T (2003). A positioning study on applying organic fertilizer in maize field on dryland. *Journal of Maize Sciences* **11**(2): 86-88

Mahesh L C, Kalyanamurthy K N, Ramesha Y M, Yogeeshappa H, Shivakumar K M & Prakash H (2010). Effect of integrated nutrient management on growth and yield of maize (*Zea mays* L.). *International Journal of Agricultural Sciences* **6**(1): 275-277

Manju B & Mukerji K G (1994). Efficacy of root litter as a biofertilizer. *Biology and Fertility of Soils* **18**(3): 228-230

Mitchell C C & Tu S (2005). Long-term evaluation of pultry litter as a source of nitrogen for cotton and corn. *Agronomy J*ournal **97**: 399-407

Morris D R & Lathwell D J (2004). Anaerobically digested dairy manure as fertilizer for maize in acid and alkaline soils. C*ommunications in Soil Science and Plant Analysis* **35**(11-12): 1757-1771

Neill M & Robinson K (2001). NMSU testing organic alternative to chemical fertilizers. *New Mexica State University Research Bulletins* **6**: 2194-2195

Öktem A (1997). GAP bölgesinde mısır üretim olanakları. *Harran Üniversitesi Ziraat Fakültesi Dergisi* **1**(2): 113-122

Prasanna K, Halepyati A S, Desai B K & Pujari B T (2007). Effect of integrated nutrient management on the productivity and nutrient uptake by maize (*Zea mays* L.). *Karnataka Journal of Agricultural Sciences* **20**(4): 833-834

Resmi gazete (2010). Organik tarımın esasları ve uygulanmasına ilişkin yönetmelik. http://www.resmigazete.gov.tr/eskiler/2010/08/20100818-4.htm/ (Erişim tarihi: 18.08.2010)

Shafiq-ur-Rehman M A, Bukhsh A H A & Ishaque M (2008). Comparative performance and profitability of two corn hybrids with organic and inorganic fertilizers. *Pakistan Journal of Agricultural Sciences* **45**(3): 8-12

Sharif M, Ahmad M, Sarir M S & Khattak R A (2004). Effect of organic and ınorganic fertilizers on the yield and yield components of maize. *Pakistan Journal of Agriculture, Agricultural Engineering, Veterinary Sciences* **20**(1): 11-16

Şahin S, Atay S, Gökalp K, Çolak S, Demirtaş M N, Öztürk B, Yılmaz K U, Fidan Ş & Çelik B (2011). Malatya yöresinde organik kayısı yetiştiriciliği. *Organik Tarım Araştırma Sonuçları,* T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü, Ankara, Türkiye, s. 51-56

Şeker C & Ersoy İ (2005). Değişik organik gübreler ve leonarditin toprak özellikleri ve mısır bitkisinin (*Zea mays* L.) gelişimi üzerine etkileri. *Selçuk Üniversitesi Ziraat Fakültesi Dergisi* **19**(35): 46-50

Thakur G D, Bavalgave V G, Waghmare M S, Kesare B J & Khandekar B S (2009). Effect of fertilizer levels on growth and yield of sweet corn. *International Journal of Agricultural Sciences* **5**(1): 100-102

Ülger A C (1998). Farklı azot dozu ve sıra üzeri mesafelerinin patlak mısırda (*Zea mays everta* Sturt.) tane verimi ve bazı tarımsal özelliklere etkisi. *Çukurova Üniversitesi Ziraat Fakültesi Dergisi* **13**(1): 155-164

Vuruş H C, Çınar S & Akdemir Ş (2000). Çukurova bölgesinde makarnalık buğdayın azotlu gübre kullanımının ekonomik analizi. *Çukurova Üniversitesi Ziraat Fakültesi Dergisi* **15**(1): 85-90

Wang L H, Shi W D, Yang W Y & Lİ C S (2003). Effect of different ratios of fertilizer application on yield and growth of hybrid maize seeds. *Journal of Jilin Agricultural University* **25**(6): 643-648

Warman P R & Munro-Warman T R (1993). Do seaweed extracts improve vegetable production? optimization of plant nutrition. Eighth international colloquium for the optimization of plant nutrition, 31 August-8 September 1992, Lisbon, Portugal. Dordrecht: Kluwer Academic Publishers, 1993, pp. 403-407

Yazıcı K & Kaynak L (2001). Deniz yosunlarının organik tarımda kullanılma olanakları. *Türkiye 2. Ekolojik Sempozyumu*, 14-16 Kasım 2001, Antalya, s. 344-352

Yurtsever N (1984). Deneysel İstatistik Metotları. Toprak ve Gübre Araştırma Enstitüsü Genel Yayın No: 121, Ankara

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Effects of Heat Stress after Anthesis on PSII Photochemical Efficiency and the Antioxidant Activity of Wheat Cultivars

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ABSTRACT

This study was conducted to investigate the effects of heat stress after anthesis on the performance of Photosystem II (PSII) and the enzymatic activity of catalase and ascorbate peroxidase. Two treatments-normal and heat stress-were conducted on four bread wheat cultivars from 7 days after anthesis until maturity. Heat stress led to an acceleration of chlorosis, reduced the efficiency of electron transfer and increased concentrations of malondialdehyde; however, the level of susceptibility varied depending on the cultivars. On the 10th day of heat stress, reductions in fluorescence parameters, depending on the cultivar, were 6.9 to 18.9% for Fv/Fm, 9 to 21% for ΦPSII and 8.3 to 19.4% for F'v/F'm compared to normal conditions. Catalase activity increased after initial exposure to heat stress. However, after 10 days of treatment, catalase activity increased in the Chamran and Aflak cultivars by 32% and 45%, respectively, but it did not change in the Dez cultivar and decreased 22% in Darab2. Ascorbate peroxidase activity decreased in two treatments, while the amount of reduction in heat stress treatment was more than the normal treatment. The highest levels of enzymatic activity were observed in Chamran under heat stress conditions, whereas Darab2 and Dez showed the lowest activity of the enzymes. Chlorophyll fluorescence parameters and chlorophyll index had a significant negative correlation with the levels of malondialdehyde; however, they had a significant positive correlation with the antioxidant activity.

Keywords: Ascorbate peroxidase; Catalase; Chlorophyll fluorescence; High temperature

Çiçeklenme Sonrası Sıcaklık Stresinin Buğday Çeşitlerinin PSII Fotokimyasal Etkinliği ile Antioksidan Aktivitesine Etkisi

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ÖZET

Bu çalışmanın amacı çiçeklenme sonrası sıcaklık stresinin buğday çeşitlerinin fotosistem II (PSII) ile askorbat peroksidaz ve katalaz enzim aktivitelerine etkisini belirlemektir. Dört ekmeklik buğday çeşidine çiçeklenmeden 7 gün sonar

başlayıp olgunluğa kadar devam eden sıcaklık stresi uygulanmış, control olarak da sıcaklık stresi uygulanmayan bir grup oluşturulmuştur. Sıcaklık stresi klorozun hızlanmasına neden olmuş, elektron taşınım etkinliğini azaltmış ve malondialdehit konsantrasyonunu artırmış ancak duyarlılık düzeyi çeşitlere göre farklılık göstermiştir. Sıcaklık stresinin 10. gününde çeşitlere bağlı olarak floresans parametrelerindeki azalma kontrole göre Fv/Fm için % 6.9-18.9, ΦPSII için % 9-21 ve F'v/F'm için ise % 8.3-19.4 arasında değişmiştir. Sıcaklık stresinin başlmasıyla birlikte katalaz aktivitesi artış göstermiştir. Ancak sıcaklık stresi uygulmasından 10 gün sonra katalaz aktivitesi Chamran ve Aflak çeşitlerinde sırasıyla % 32 ve 45 oranında artmış, Dez çeşidinde değişmemiş ve Darab2 çeşidinde ise % 22 oranında azalmıştır. Askorbat peroksidaz aktivitesi hem kontrol grubu hem de sıcaklık stresi uygulanan bitkilerde azalmış ancak azalma sıcaklık stresi uygulananlarda daha fazla olmuştur. En yüksek enzimatik aktivite sıcaklık stresi uygulanan Chamran çeşidinde gözlenirken Darab ve Dez çeşitleri en düşük enzim aktivitesine sahip olmuşlardır. Klorofil floresans paramtresi ile klorofil indeksi malondialdehit düzeyleri ile önemli negative korelasyon gösterirken antioksidan aktivitesi ile önemli pozitif korelasyon göstermiştir.

Anahtar Kelimeler: Askorbat peroksidaz; Katalaz; Klorofil floreasns; Yüksek sıcaklık

1. Introduction

Heat stress during the grain filling period is one of the most important abiotic factors limiting wheat yields (*Triticum aestivum* L.). Due to the predicted 1.8 to 5.8 °C increase in global temperature by the end of this century due to global warming (Farooq et al 2011), the importance of this stress is increasing. Heat stress affects many cellular functions. Photosynthesis is one of the physiological processes most sensitive to heat. Under heat stress, decrease in photosynthetic activity leads to reductions in wheat growth (Harding et al 1990). Reduction of photosynthesis at high temperatures occurs due to the impairment of the structure and function of chloroplasts and decrease in chlorophyll content (Xu et al 1995). Several studies have shown that the inhibition of Rubisco activity directly leads to the inhibition of CO_2 assimilation under moderate heat stress (Salvucci & Crafts-Brander 2004), while under severe heat stress, the dissociation of the oxygen evolving complex, inhibition of electron transfer from quinone A to quinone B and general damage to the reaction centres of PSII also lead to the inhibition of photosynthesis (Harding et al 1990; Allakhverdiev et al 2008). Heat stress can impair enzyme functions because of the changes in the nature of the enzyme. Degradation of proteins and membranes caused by heat stress leads to an increase in the production of reactive oxygen species (ROS) and this is followed by oxidative stress (Sairam et al 2000). Plants have enzymatic and non-enzymatic refinement systems in order to suppress ROS and eliminate their destructive effects (Farooq et al 2011). Under normal thermal conditions, plants maintain a balance between the production and refinement of ROS. However, heat stress can disrupt this balance (either by increasing the production of activated oxygen species or reducing the quenching ability of ROS in the cell) and reinforce lipid peroxidation of cell membranes (Liu & Huang 2000; Allakhverdiev et al 2008).

This study was conducted to evaluate the effects of heat stress on the performance of PSII during the grain filling period in four wheat cultivars, each of which have different abilities of tolerating heat stress. Because the antioxidative system in plants can partially prevent oxidative damage, which is caused by the accumulation of electron acceptors and increasing ROS, the antioxidant activity of the four wheat varieties were evaluated.

2. Material and Methods

2.1. Plant material and growth conditions

Four spring bread wheat cultivars with similar phenology: Aflak (Debeira), Chamran (Attila), Dez (Kauz×2/Opatey//Kauz) and Darab2 (Maya's'/Nac) were used in this experiment. All cultivars have been released by the International Maize and Wheat Improvement Center (CIMMYT) and Chamran is recognised internationally for its ability to tolerate

heat. The cultivars were sown on November 28, 2011, in pots, 10 cm in diameter and 50 cm in height. All pots were kept at an experimental farm at Shahid Chamran University (Ahvaz, Iran) from the time of sowing to 7 days after anthesis. A total of 45 pots of each cultivar was sown for each replication. Each pot was filled with a mixture of loam soil (with 0.82% organic material) and 12 grams of rotten manure before planting. N, P and K were added to each pot; 36.8 mg N in the form of urea, 36.8 mg P in the form of triple superphosphate and 20 mg K in the form of potassium sulphate. Five seeds were sown in each pot and after three weeks, three seedlings were maintained per pot. At the end of tillering, stem elongation and also at the beginning of spike emergence, 36.8 mg N as urea was added to each pot. All pots were irrigated after every two days and as needed to avoid any water stress.

At 7 days after anthesis-defined as anthers extruded from 50% of main culm inflorescences-all pots of each cultivar were randomly divided into two groups and were transferred to two separate phytotrons with different thermal conditions and were kept there until maturity. One phytotron was maintained under normal temperature conditions; daytime temperatures were set at 25 °C for 4 h, 21 °C for 5 h before and after the 4 h period and 16 °C at night time for 10 h. The other phytotron was maintained under hotter conditions; daytime temperatures were set at 37 °C for 4 h, 31 °C for 5 h before and after the 4 h period and 25 °C at night time for 10 h. In both phytotrons, the relative humidity ranged between 50 and 70%, the photosynthetic photon flux density was set at 650 μmol m^{-2} s^{-1} at 10 cm above the plant spikes.

2.2. Measurements of chlorophyll fluorescence and SPAD values

Five main stems were selected among the tagged main stems (several main stems of similar age were tagged at the time of anthesis in each cultivar) and flag leaves of these stems were used for measuring parameters of chlorophyll fluorescence and SPAD values in both phytotrons. The fluorescence metre (Walz, Germany) was used for measuring these parameters (Fo, Fm, Ft, F'm) at 4, 7 and 10 days after temperature treatments. Fv/Fm, ΦPSII and F'v/F'm were calculated using equations 1 through 4 (Baker & Rosenqvist 2004).

$$Fv/Fm = (Fm - Fo)/Fm \quad (1)$$

$$\emptyset PSII = (F'm - Ft)/F'm \quad (2)$$

$$F'o = \frac{Fo}{(\frac{Fv}{Fm})+(\frac{Fo}{F'm})} \quad (3)$$

$$F'v/F'm = (F'm - F'o)/F'm \quad (4)$$

SPAD values of flag leaves were also measured at five-day intervals from 7 days after anthesis until the time of leaf yellowing by using a chlorophyll metre (Minolta Chlorophyll Meter SPAD-502).

2.3. Estimation of antioxidant enzymatic activity and malondialdehyde (MDA) concentrations

Three flag leaves from main stems of similar ages were sampled at four stages: 0, 4, 7 and 10 days after temperature treatments. Potassium phosphate extraction buffer (50 mM containing 1.0 mM EDTA, pH 7) was used to extract catalase (CAT), while ascorbate peroxidase (APX) was extracted using 50 mM potassium phosphate buffer (pH 7) containing 1.0 mM EDTA and 2 mM ascorbate (Almeselmani et al 2006). Each enzyme extract was prepared by first grinding 0.5 g leaf sample with liquid nitrogen and then with extraction buffer. Extracts were centrifuged at 10,000×*g* at 2 °C for 20 min. The supernatant was used to measure levels of enzyme activity.

CAT activity was assayed according to Beers & Sizer (1952). The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7), 15 mM H_2O_2 and 100 μL of enzyme extract. Each unit of CAT activity was determined by calculated the breakdown of H_2O_2 (mM) per minute by measuring the absorbance change at 240 nm and using the extinction coefficient of 39.4 M^{-1} cm^{-1}. APX activity was assayed according to Nakano & Asada (1981). The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7), 0.3 mM ascorbate, 0.07 mM EDTA, 0.1 mM H_2O_2 and 100 μL of enzyme extract. Each unit of APX activity was calculated by quantifying the amount of oxidized ascorbate (μM) per minute by

measuring the reduction of absorbance at 290 nm and using the extinction coefficient of 2.8 mM^{-1} cm^{-1}. Lipid peroxidation was assayed according to Heath & Packer (1969) by monitoring the formation of malionaldehyde-thiobarbituric. Concentrations of accumulated MDA were calculated by using an absorption coefficient of 155 mM^{-1} cm^{-1} and were expressed as µM per g of fresh weight.

2.4. Statistical analysis

The experiment was arranged in a completely randomized block design with three replicates in each phytotron. A combined analysis of variance was performed using GLM procedures across two phytotrons and genotypes for each measured trait. Also, comparisons of means were performed by Fisher's protected least significant difference at 5% probability. Associations between traits were examined by correlation analysis when appropriate.

3. Results and Discussion

3.1. Grain yield and its components

Heat stress significantly reduced the grain yields of the main spike. Maximum reductions in grain yield were observed in the Darab2 and Aflak cultivars, while the least amount of reductions in yields was observed in Dez and Chamran (Table 1). Under heat stress conditions, single grain weights decreased an average 34.4%. The largest percentage of grain weight reduction was observed in Darab2 (41.12%) whereas Dez had the lowest grain weight reduction (27.26%) among cultivars. Heat stress led to a significant reduction in the grain number per main spike (from 1.99 to 5.10%, depending on the cultivars) (Table 1). This indicates that the observed decline in main spike grain yields can mostly be attributed to the reduction in grain weights as has been observed in other studies (Tahir & Nakata 2005; Zamani et al 2014). The decrease in sink strength (either through reducing the number of endosperm cells (Feng et al 2000) or by reducing metabolic activity, which leads to the synthesis of starch in the grain (Keeling et al 1993)) and decrease in duration of starch accumulation in the grain (Hurkman et al 2003) can explain the sharp decline in grain weight under heat stress conditions. In this experiment, heat stress led to a significant reduction (on an average of 36.6%) in the grain filling duration of all cultivars (Table 2). The grain filling rate of Darab2 decreased

Table 1- Main spike grain yield and components in wheat cultivars grown under normal and heat stress temperature treatments and reduction (percent) yield under heat stress

Cultivar	*Grain yield (g main spike^{-1})*			*Grain number (per main spike)*		*Single grain weight (mg)*	
	N†	*H*‡	*R*§*(%)*	*N*	*H*	*N*	*H*
Chamran	1.27 b	0.94 bc	26.36 b	48.58 c	46.61 c	28.66 b	19.80 a
Aflak	1.29 b	0.86 c	33.09 a	50.81 c	48.19 c	30.01 b	18.49 a
Dez	1.32 b	1.03 a	21.96 b	61.20 a	59.97 a	24.65 c	17.93 a
Darab2	1.55 a	1.00 ab	35.22 a	56.82 b	54.05 b	33.49 a	19.72 a
Mean	1.36	0.96	29.16	54.35	52.21	29.20	18.98
Analysis of variance							
T¶	**			**		**	
G#	**			**		**	
T×G	**			ns		**	

†, normal conditions; ‡, heat stress conditions; §, reduction (percent) because of heat stress; ¶, temperature treatment; #, genotype; ns and **, non-significant and significant at 1% probability levels, respectively; means, followed by similar letter are not significantly different (P<0.05) according to LSD test

12% as a result of heat stress while that of Dez and Aflak increased 18% and 8%, respectively. There was no change in the grain filling rate observed in Chamran (Table 2). These results indicate that the reduction in grain filling duration (decrease in duration of starch accumulation in the grain) played an important role in the reduction in grain weight of all cultivars. In addition, the sharp decline in grain weight of Darab2 may be attributed to the reduction of grain filling rate as well as grain filling duration in this cultivar. Other reasons of reduction in grain weight under heat stress conditions include the impacts of heat stress on the photosynthetic system (Either through damage to PSII or by inhibiting of the activity of Rubisco activase) (Allakhverdiev et al 2008).

Table 2- Grain filling rate and duration in wheat cultivars grown under normal and heat stress temperature treatments

Cultivar	*Grain filling rate (mg grain^{-1} day^{-1})*		*Grain filling duration (day)*	
	N†	*H*‡	*N*	*H*
Chamran	1.09 b	1.09 b	26.55	18.17
Aflak	1.08 b	1.17 ab	27.98	15.85
Dez	0.90 a	1.06 b	27.46	16.96
Darab2	1.37 a	1.21 a	24.64	16.56
Mean	1.11	1.13	26.66	16.89
T¶	ns		**	
G#	**		ns	
T×G	**		ns	

†, normal conditions; ‡, heat stress conditions; ¶, temperature treatment; #, genotype; ns and **, non-significant and significant at 1% probability levels, respectively; Means, followed by similar letter are not significantly different (P<0.05) according to LSD test

3.2. Chlorophyll fluorescence and SPAD values

Heat stress led to a significant decrease in Fv/Fm, ΦPSII and F'v/F'm at 4, 7 and 10 days after exposure to high temperatures (P<0.01). The observed decline in Fv/Fm on the 7th day of heat stress varied from 3.3% in Chamran to 7% in Dez. The highest percentage of decline in ΦPSII was observed in Darab2 and Dez cultivars as 6.7% and 6.2%, respectively. The observed decline in F'v/F'm also varied from 2.8% in Chamran to 6.9% in Dez (Figure 1). Over time and under prolonged periods of stress 10 days after exposure to high temperatures, reductions in fluorescence values, depending on the cultivar, were 6.9 to 18.9% for Fv/Fm, 9 to 21% for ΦPSII and 8.3 to 19.4% for F'v/F'm compared to cultivars exposed to normal conditions. The lowest reduction in fluorescence was observed in Chamran, while the highest percentage was observed in Darab2 (Figure 1).

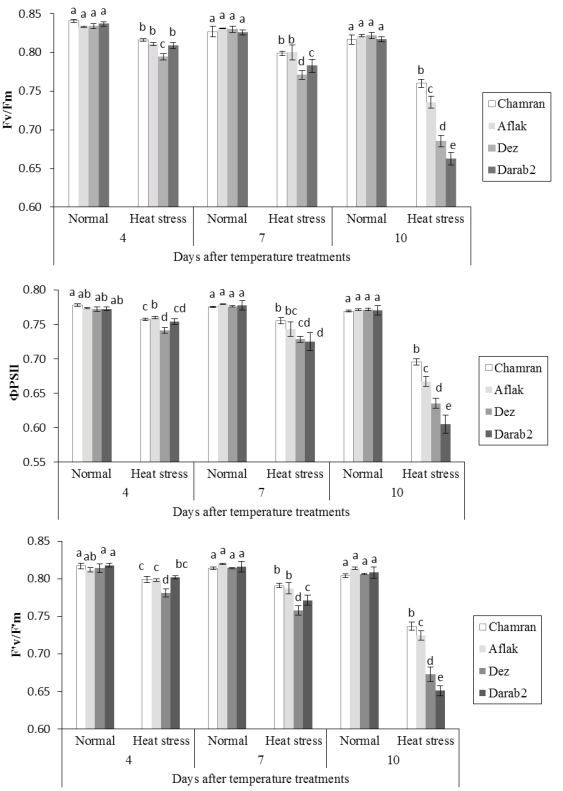

Figure 1- Changes of F_v/F_m , ΦPSII and F'_v/F'_m in flag leaves of wheat cultivars under normal and heat stress temperature treatments. The bars superscripted by different letters within the same "days after temperature treatments" are significantly different (P<0.05) according to LSD test

ΦPSII is an estimation of the quantum efficiency of linear electron transport through PSII and is an indicator of the amount of absorbed energy used in photochemical activity. Reduction in this parameter indicates a reduction in electron linear transmission in PSII and the slowing the flow of electrons due to a delay in the redox potential in the primary quinone acceptor of PSII (QA). F'v/F'm is also an estimation of the maximum photochemical efficiency of PSII at the given light intensity and reveals the efficiency of PSII when all reaction centres are open (Maxwell & Johnson 2000; Baker & Rosenqvist 2004). The reduction in ΦPSII and F'v/F'm under moderate heat stress is mainly associated with an increase in non-photochemical quenching (NPQ) of energy in the form of heat loss and is considered a mechanism of photoprotection. Due to an increase in NPQ and protection from photoinhibition, PSII centres remain intact under these conditions and Fv/Fm will not change (Maxwell & Johnson 2000; Zamani et al 2015). NPQ cannot remove excess excited electron energy under intense heat stress or prolonged exposure to heat stress. Therefore, photoinhibition damage occurs in PSII and Fv/Fm decreases (Dias et al 2011; Zamani et al 2015). In this study, a reduction in Fv/Fm, ΦPSII, and F'v/F'm under heat stress revealed that an important component of the reaction centres in PSII had been damaged. With an increase in the duration of heat stress, high temperatures accelerate the disruption of thylakoid components, separate light-harvesting pigments from the reaction centres of PSII and also accelerate the degradation of chlorophyll that is similar to conditions brought about by the ageing of plants (Harding et al 1990).

We observed a strong relationship between values of chlorophyll fluorescence and the relative content of chlorophyll (SPAD value) in the flag leaves (Table 4); this confirms the occurrence of disruption in chloroplasts, leaf damage and premature ageing under heat stress conditions. A reduction in chlorophyll content and chlorophyll fluorescence leads to a reduction in the photosynthetic capacity of the plant (Xu et al 1995). Plant yields decline if a plant fails to use alternative sources (stem reserves) to compensate for the reduction in photosynthetic capacity (Tahir & Nakata 2005). Chlorosis started earlier in Darab2 and Dez and these cultivars had the lowest chlorophyll index on the 10th day of heat stress. The evaluated parameters of chlorophyll fluorescence in these Darab2 and Dez were also low. Darab2 had the lowest values of Fv/Fm, ΦPSII and F'v/F'm and the highest reductions in these parameters under heat stress conditions combined with the highest reductions in grain yield. These observations confirm this cultivar is sensitive to heat stress. Despite a sharp decline of photosynthetic capacity in Dez, Dez is able to use its stem reserves for grain growth (Zamani et al 2014). Dez had the lowest reductions in grain yields under heat stress and can be classified as a semi-tolerant cultivar to heat stress.

3.3. Antioxidant enzymatic activity and MDA concentrations

Heat stress led to a significant increase in CAT activity at 4 and 7 days after temperature treatments. These increases ranged from 11% in Dez to 38% in Chamran on the 4th day of heat stress and 12% in Darab2 to 42% in Aflak on the 7th day of heat stress (Table 3). CAT activity in Aflak and Chamran was higher under stress conditions than normal conditions on the 10th day by 32% and 45%, respectively, while no changes in its activity were observed in Dez and it decreased in Darab2 by 22%.

An increase in CAT activity is related to an increase in stress tolerance (Sairam et al 2000). Increased CAT activity has been reported by Almeselmani et al (2006) in other tolerant wheat cultivars under heat stress conditions. Zhao et al (2007) reported that under heat stress conditions (34 °C/22 °C from 7 DAA to maturity), CAT activity was higher than normal conditions until 14 days after anthesis while its activity decreased after that period.

There was no significant difference in APX activity between the two temperature treatments until the 7th day of the experiment (Table 3). Over time, APX activity decreased in both thermal treatments. APX activity in Dez and Darab2 on the 10th day of heat stress decreased more than when under normal conditions by 27% and 24%, respectively.

Table 3- Antioxidant enzymes (CAT and APX) activity and MDA concentration in flag leaves of wheat cultivars under normal and heat stress temperature treatments

	CAT activity (µmol H_2O_2 dec min^{-1} mg^{-1} protein)					
Cultivar	*4 DAT*		*7 DAT*		*10 DAT*	
	N†	*H‡*	*N*	*H*	*N*	*H*
Chamran	145.43 a	191.77 a	121.83	166.87	110.13 a	146.93 a
Aflak	125.10 b	173.03 ab	112.13	159.13	84.20 b	121.70 a
Dez	141.60 ab	157.43 b	124.17	143.27	100.33 ab	92.50 b
Darab2	129.30 ab	157.50 b	127.70	143.53	111.50 a	86.50 b
Mean	135.36	169.93	121.46	153.20	101.54	111.91
Analysis of variance						
T¶	*		**		ns	
G#	**		ns		**	
T×G	ns		*		**	
	APX activity (nmol ascorbate dec min^{-1} mg^{-1} protein)					
Cultivar	*4 DAT*		*7 DAT*		*10 DAT*	
	N	*H*	*N*	*H*	*N*	*H*
Chamran	257.37 a	277.57 a	225.87	225.43	213.33 a	193.67 a
Aflak	188.00 c	210.43 c	239.30	179.73	177.23 b	160.77 b
Dez	228.47 b	242.20 b	213.57	227.53	191.20 b	139.60 c
Darab2	229.07 b	241.57 b	223.23	212.27	173.97 b	132.47 c
Mean	225.73	242.94	225.49	211.24	188.93	156.63
Analysis of variance						
T	ns		ns		**	
G	**		ns		**	
T×G	ns		*		*	
	MDA concentration (µmol g^{-1} FW)					
Cultivar	*4 DAT*		*7 DAT*		*10 DAT*	
	N	*H*	*N*	*H*	*N*	*H*
Chamran	1.50 b	1.57 b	1.55 a	1.78 c	1.91 a	2.34 c
Aflak	1.71 a	1.80 a	1.74 a	1.89 bc	2.02 a	2.53 c
Dez	1.50 b	1.88 a	1.55 a	2.05 ab	1.94 a	2.89 b
Darab2	1.72 a	1.94 a	1.54 a	2.17 a	2.00 a	3.21 a
Mean	1.61	1.79	1.60	1.97	1.97	2.74
Analysis of variance						
T	*		**		**	
G	*		*		**	
T×G	ns		*		**	

†, normal conditions; ‡, heat stress conditions; ¶, temperature treatment; #, genotype; ns, * and **, non-significant, significant at 5% and 1% probability levels, respectively; DAT, days after temperature treatments; Means, followed by similar letter are not significantly different (P<0.05) according to LSD test

Although, under heat stress, a reduction in APX activity was also observed in Chamran and Aflak but the difference between the two treatments was not significant (Table 3).

The observed decline in APX activity over the time was likely due to the oxidative degradation of protein molecules as a result of cellular ageing and increase in stress duration (Sairam et al 2000).

CAT and APX are two key enzymes in the active-oxygen scavenging system that can suppress the production of ROS (Zhao et al 2007). Kumar et al (2011) evaluated the isoenzymic profile of APX under a heat shock treatment in two tolerant and sensitive cultivars of wheat. They observed that the transcription levels and the number of different isoenzymes play very important roles in the removal of hydrogen peroxide and provide tolerance to thermal stress.

In this study, heat stress led to an increase in MDA, which is an indicator of peroxidation of phospholipids and unsaturated fatty acids. These increases ranged from 4% in Chamran and Aflak to 25% in Dez on the 4th day of heat stress and 8% in Aflak to 41% in Darab2 on the 7th day of heat stress. When the duration of heat stress was extended beyond 10 days, MDA concentrations increased from 23% in Chamran to 61% in Darab2 under heat stress conditions (Table 3).

With a reduction in efficiency of electron transfer in PSII and accumulation of reduced electron acceptors, oxygen acts as an alternative acceptor of electrons and the peroxidation of membrane fatty acids increases. A significant negative correlation between chlorophyll fluorescence parameters (indicators for the efficiency of PSII electron transfer) and the levels of MDA (Table 4) also represents an increase in the lipid peroxidation of the thylakoid membrane due to a reduction of PSII electron transport activity. Powerful antioxidant systems can partially control the production of toxic oxygen radicals and also balance the production and refining of these radicals. When the activities of antioxidants are reduced in plant tissues under heat stress conditions, the balance between ROS production and refining is disturbed and the peroxidation of unsaturated fatty acids of cell membranes increases (Liu & Huang 2000). This is corroborated by results obtained in this study; there was a significant negative correlation between MDA concentrations and activities of CAT and APX (Table 4). The negative relationship between MDA concentrations and activities of antioxidant enzymes, including CAT and APX, has been also reported by Asthir et al (2009). A significant negative correlation between the relative content of chlorophyll (SPAD value) and MDA concentrations of the flag leaves (Table 4) also demonstrates that the lipid peroxidation of membranes caused by ROS leads to the degradation of chlorophyll (chlorosis). Liu & Huang (2000) stated that reduced cell membrane stability, chlorophyll degradation and leaf senescence result from the lipid peroxidation of membranes caused by ROS. The significant positive correlation between the SPAD value and antioxidant activity observed in this study (Table 4) may also confirm the effect of ROS on chlorophyll degradation and acceleration of the ageing process.

Table 4- Correlation coefficient between chlorophyll fluorescence parameters, CAT activity, APX activity, MDA concentration and SPAD value under heat stress conditions on 7 and 10 days after temperature treatments in four wheat cultivars

	Fv/Fm	*ΦPSII*	*F'v/F'm*	*MDA*	*CAT*	*APX*
ΦPSII	0.97***					
F′v/F′m	0.99***	0.96***				
MDA	-0.95***	-0.97***	-0.94***			
CAT	0.92***	0.91***	0.91***	-0.94***		
APX	0.70**	0.77***	0.67**	-0.75***	0.65**	
SPAD value	0.82***	0.83***	0.80***	-0.82***	0.83***	0.69**

*** and **, significant at 0.1 and 1% probability levels, respectively

4. Conclusions

According to our results, the Chamran cultivar is semi-tolerant to heat stress; it demonstrated the lowest levels of MDA in all stages of this experiment and exhibited the highest activities of CAT and APX. It seems that an increase in CAT activity under heat stress in Chamran along with its higher activity of APX compared to the other cultivars, has been effective in combating heat stress. In Darab2 (which had the largest decreases in grain yield under heat stress conditions), the activities of APX and CAT decreased. The increase in MDA concentrations under heat stress confirms the sensitivity of Darab2 to hotter conditions. Dez is also semi-tolerant to

heat stress. There was a minimum reduction in yields of Dez under heat stress conditions, while the activities of CAT and APX were low in this cultivar. Reduction in electron transfer efficiency and the relative content of chlorophyll under hot conditions; as an indicator of damages to the photosynthetic system under heat stress, were also observed in this cultivar. Dez is probably able to compensate for a reduction in photosynthetic activity by using stem reserves and transferring those resources to growing grain (Zamani et al 2014). Also, an increase of the grain filling rate is contributed to the prevention of a sharp decline of grain weight in this cultivar.

It can be concluded that, in some cultivars tolerance to heat is related to the levels of antioxidant enzymatic activity. Heat-tolerant genotypes likely have more efficient refinement systems compared to sensitive genotypes.

Abbreviations and Symbols	
PSII	Photosystem II
QA	Primary quinone electron acceptor of PSII
Fv/Fm	Maximum quantum efficiency of PSII under given dark conditions
ΦPSII	Quantum efficiency of PSII under given light conditions
F′v/F′m	Maximum quantum efficiency of PSII under given light conditions
NPQ	Non-photochemical quenching
CAT	Catalase
APX	Ascorbate peroxidase
MDA	Malondialdehyde
DAA	Days after anthesis
ROS	Reactive oxygen species

References

Allakhverdiev S I, Kreslavski V D, Klimov V V, Los D A, Carpentier R & Mohanty P (2008). Heat stress: An overview of molecular responses in photosynthesis. *Photosynthesis Research* **98**: 541-550

Almeselmani M, Deshmukh P S, Sairam R K, Kushwaha S R & Singh T P (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Science* **171**: 382-388

Asthir B, Kaur S & Mann S K (2009). Effect of salicylic and abscisic acid administered through detached tillers on antioxidant system in developing wheat grains under heat stress. *Acta Physiologiae Plantarum* **31**: 1091-1096

Baker N R & Rosenqvist E (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* **55**(403): 1607-1621

Beers R F & Sizer I (1952). A spectrophotometric method for measuring the breakdown of hydrogen by catalase. *Journal of Biochemistry* **195**: 133-140

Dias A S, Semedo J, Ramalho J C & Lidon F C (2011). Bread and durum wheat under heat stress: A comparative study on the photosynthetic performance. *Journal of Agronomy and Crop Science* **197**: 50-56

Farooq M, Bramley H, Palta J A & Siddique H M (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences* **30**: 1-17

Feng C N, Guo W S, Shi J S, Peng Y X & Zhu X K (2000). Effect of high temperature after anthesis on endosperm cell development and grain weight in wheat. *Acta Agronomica Sinica* **26**: 399-405

Harding S A, Guikema J A & Paulsen G M (1990). Photosynthetic decline from high temperature stress during maturation of wheat. I. Interaction with senescence processes. *Plant Physiology* **92**: 648-653

Heath R L & Packer L (1969). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125**: 189-198

Hurkman W J, McCue K F, Altenbach S B, Korn A, Tanaka C K, Kothari K M, Johnson E L, Bechtel D B, Wilson J D, Anderson O D & DuPont F M (2003). Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Science* **164**: 873-881

Keeling P L, Bacon P J & Holt D C (1993). Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta* **191**: 342-348

Kumar R R, Goswami S, Kumar N, Pandey S K, Pandey V C, Sharma S K, Pathak H & Rai R.D (2011). Expression of novel ascorbate peroxidase isoenzymes of wheat (*Triticum aestivum* L.) in response to heat stress. *International Journal of Plant Physiology and Biochemistry* **3**(11): 188-194

Liu X & Huang B (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science* **40**: 503-510

Maxwell K & Johnson G N (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany* **51**(345): 659-668

Nakano Y & Asada K (1981). Hydrogen peroxidase is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* **22**: 867-880

Sairam R K, Srivastava G C & Saxena D C (2000). Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. *Biologia Plantarum* **43**(2): 245-251

Salvucci M E & Crafts-Brandner S J (2004). Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant Physiology* **134**: 1460-1470

Tahir I S A & Nakata N (2005). Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. *Journal of Agronomy and Crop Science* **191**: 106-115

Xu Q, Paulsen A Q, Guikema J A & Paulsen G M (1995). Functional and ultrastructural injury to photosynthesis in wheat by high temperature during maturation. *Environmental and Experimental Botany* **35**: 43-54

Zamani M M, Nabipour M & Meskarbashee M (2014). Stem water soluble carbohydrate remobilization in wheat under heat stress during the grain filling. *International Journal of Agriculture & Biology* **16**: 401-405

Zamani M M, Nabipour M & Meskarbashee M (2015). Effect of heat stress during grain filling on photosynthesis and grain yield of bread wheat (*Triticum aestivum L.*) genotypes. *Iranian Journal of Crop Sciences* **17**(1): 1-17

Zhao H, Dai T, Jing Q, Jiang D & Cao W (2007). Leaf senescence and grain filling affected by post-anthesis high temperatures in two different wheat cultivars. *Plant Growth Regulation* **51**: 149-158

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Assessment of Weed Competition Critical Period in Sugar Beet

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ABSTRACT

Weed control constitutes the most essential issue in cropping systems. The critical periods should especially be determined for each crop. Field experiments were conducted during the seasonal growing periods of sugar beet in 2012 and 2013 in Kayseri, Turkey to assess the critical weed control period (CPWC). A log-logistic model having four parameters was used to assist in monitoring and analyzing two sets of related, relative crop yield. Data was obtained during the periods of increased weed interference and as to compare, during the weed-free periods. In both years, the relative root yield of sugar beet decreased with a longer period of weed-interference and increased where there was a longer weed-free period. In 2012, the CPWC varied between 122-595 GDD (growing degree days) corresponding to 12 to 46 days after crop emergence (DAE). The following year, CPWC were found to be between 82-735 GDD, (8-54 DAE) based on 5% acceptable yield loss. Weed-free conditions are needed to be arranged as early as the first week after crop emergence and maintained up to and including nine weeks thereafter to avoid more than a 5% loss in sugar beet root yield. Those results could assist sugar beet producers through reducing the expenses significantly, as well as improving the efficacy of their weed management programs.

Keywords: Critical period; Nonlinear regression; Weeds; Competition; Sugar beet

Şeker Pancarında Yabancı Ot Rekabetinde Kritik Periyodun Belirlenmesi

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ÖZET

Yabancı ot kontrol dönemlerinin doğru olarak belirlenmesi, yabancı ot yönetimi planlarının önemli kıstaslarından birisidir. Şeker pancarında yabancı ot mücadelesinde kritik periyodun belirlenmesi amacıyla 2012 ve 2013 yıllarında Kayseri'de tarla denemeleri yürütülmüştür. İki set halindeki oransal verimin analizinde dört parametreli log-lojistik model kullanılmıştır. Veriler başlangıcı yabancı otlu ve başlangıcı yabancı otsuz olarak oluşturulan parsellerden elde edilmiştir. Her iki yılda da şeker pancarı verimi yabancı otlu kalma süresi arttıkça azalırken, yabancı otsuz kalma süresi arttıkça da artmıştır. % 5 kabul edilebilir verim kaybı seviyesinde 2012 yılında yabancı ot mücadelesinde kritik periyot

ürün çıkışından sonraki 12-46. günlere karşılık gelen 122 ila 595 GGD (gelişme gün derece) arasında değişmiştir. 2013 yılında ise 82-735 GGD olmuştur (çıkıştan sonraki 8 ile 54. günler). % 5'ten fazla ürün kaybını engellemek için, ürün çıkışından sonra ilk haftadan başlayarak 9. haftaya kadar ürünün yabancı otsuz tutulması gerekli olduğu belirlenmiştir. Bu sonuçlar şeker pancarı üreticilerinin giderlerini önemli ölçüde azaltmanın yanı sıra yabancı ot yönetim programlarının etkinliğinin artırılmasına da yardımcı olabilecek niteliktedir.

Anahtar Kelimeler: Kritik periyot; Doğrusal olmayan regresyon; Yabancı ot; Rekabet; Şeker pancarı

1. Introduction

Sugar is produced from sugar beet (*Beta vulgaris* L.) in Turkey as it was in majority of Europe because of climate conditions. Sugar beet prefer climates with warm days and cool nights. Thus, Central Anatolia is a favorable region for sugar beet culture. Irrigation is applied in sugar beet culture of Turkey and production is performed under a contract with a sugar company (Kiymaz & Ertek 2015). Recent annual world sugar beet production is around 247 million tons from 4.4 million hectares (FAO 2015). Sugar beets are primarily produced in France, United States, Germany, Russian Federation, Turkey, Poland, Ukraine, United Kingdom, and China (FAO 2015).

Ever-increasing world population and industrial developments always keep sugar production in the agenda of world countries. The primary goal of sugar beet producers is to achieve high yields and quality (Bakhshkandi et al 2013). Weeds are one of the major concerns in sugar beet culture. Sugar beet is extremely sensitive to weed competition during the initial growth stages, so effective weed control is critical at this stage (Jalali & Salehi 2013; Marwitz et al 2014). The presence of weeds can decrease sugar beet yield by 90%. While this yield loss is 45% in Asian countries, it is between 6-40% in Turkey (Güncan 2000). For example, a single barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) per 1.5 m^2 can reduce the yields around 5-15% (Norris 1996). To eliminate the damage caused by weeds or at least to reduce, weed control in agricultural fields are paid more attention. Such a case then leads labor requirements and great financial losses. Therefore, new methods are developed in time in order to identify the best weed control timing and to minimize costs (Malaslı 2010).

In Turkey, standard design procedures incorporate herbicides into weed control practices and cultivation is implemented strategically to manage weeds. Weed control essentially is done at the beginning of the growing season since young, newly emerged sugar beets are highly susceptible to weed competition at this critical stage. In fact, even before this stage, at the pre-sowing or before plant emergence, herbicides sprays are habitually applied for weed control in sugar beet fields. Following plant emergence, on the other hand, hoeing (either with machines or manual) is done for weed control (Jalali & Salehi 2013; Marwitz et al 2014).

For a successful integrated weed management (IWM), some key issues like the effect of control practices on weeds, growth and development stages of weed population and critical periods for applications should be taken into consideration (Young 2012). The criteria for critical period of weed control (CPWC) provide essential guidance on relevant time periods and growth stages during which crops ideally should be kept free of weeds to stop yield or quality reductions by weed interference (Evans et al 2003). Two separate weed interference scenarios are defined as; 1) the longest possible period from the time that crops are planted or from the time that the crops can live together side by side with the weeds without unreasonable yield loss (critical weed interfering period) and 2) the possible shortest period for crop to be retained weed-free prior to yield reduction effected by weed growth is no longer a problem (the critical weed-free period) (Evans et al 2003). Thus, the CPWC identifies the

most beneficial time periods for the best IWM program (Swanton & Weise 1991).

Weed-crop competition critical periods in sugar beet have been monitored and statistically analyzed only in a few environments and only for a limited variety of weed types. Sugar beet can tolerate weeds until 2-8 weeks after emergence, depending on the weeds, date of planting, weed emergence time and ecological factors (Salehi et al 2006). Irrigated sugar beet should be free from weeds for 10 to 12 weeks after planting, thereafter it could compete with weeds until the end of season and those weeds emerging later would be suppressed by sugar beet (Dawson 1977). Salehi et al (2006) carried out a study to identify the beginning and end of CPWC in sugar beet and identified the beginning of critical period as 25 days thereafter sowing for the first year and 4 days thereafter sowing for the following year; the end of critical period was identified as 78 days after sowing for the first year and 88 days thereafter sowing in the following year. The principle idea of this research was to determine the CPWC for sugar beet grown in Central Anatolian Region of Turkey, an area where there is little or no apparent knowledge on CPWC.

2. Material and Methods

2.1. Site description

Trials were carried out in 2012 and 2013 on arable lands of Yeşilhisar, Kayseri in Central Anatolian region of Turkey. The soil texture of the experimental site was clay with pH of 7.92, EC of 0.08 mS cm^{-1}, lime content of 20.19% and organic matter content of 1.24%. Available P and K concentrations of soil were 21.94 and 1390 kg ha^{-1}, respectively.

2.2. Experimental design

Experimental analyses were undertaken in accordance with local practices of the region. Initial tillage comprised of chisel plowing in spring and subsequent disking with a harrow. In general, pre-emergence and post-emergence herbicides are utilized in the region for weed control of sugar beet. In this study, however, weeding was done by hand hoeing. Valentina sugar beet cultivar was sown on 12 cm lines over rows 45 cm apart. The plots were fertilized in two stages. Initially, 500 kg ha^{-1} of compound fertilizers (13-24-12) were spread over the plots at the time of sowing. Then, the remaining fertilizers were spread over the plots in two parts with 300 kg of ammonium sulfate (AS) (21-0-0-24S) ha^{-1} and 300 kg of ammonium nitrate (AN) ha^{-1} (33% N) (2x300 kg= 600 kg ha^{-1}) and then the plants were irrigated. The crops were irrigated ten times during the growing period. The water level for each irrigation was calculated by taking crop water requirement, precipitation and temperature into consideration.

Experiments were designed in randomized blocks with 4 replicates. Two methods of weed interference treatments were used and these were initiated at crop emergence. To assess the start of CPWC, weeds were grown with the crop at 2 week intervals with sugar beet 0 to 12 WAE (week after emergence). To establish the end of a CPWC, plots were completely weeded at biweekly intervals for 0 to 12 WAE with occasional hand hoeing. Weed-free control and untreated weedy control plots were introduced into both parts of the experiment. All plots were 1.8 meters wide, 5 meters long and had four rows. All results were recorded from only the inner two rows of the plots.

2.3. Weed and crop measurements

Population density of weeds was determined from an arbitrarily placed 1x1 meter quadrat. At maturity, the sugar beet harvesting was made by hand from the inner two rows of each plot. Species composition and weed density were assessed through categorizing and counting the weeds from two 0.5 m^2 quadrate in each plot. Weeds were cut off from the ground and dried at 70 °C to determine aboveground dry matter. In both years, final crop harvests were carried out when the sugar beet had reached to full maturity. Samples for sugar beet yield determination from each plot were obtained by hand-harvesting from 4.5 m^2 areas of the middle two rows.

2.4. Growing degree days (GDDs) calculation

Total monthly rain fall (mm) and average temperatures (°C) throughout the experiments were recorded from the Yesilhisar Meteorological Station.

GDD values are commonly used as an independent variable for regression analysis and the relevant values were calculated in accordance with Gilmore & Rogers (1958) (Equation 1). The DAE (days after emergence) was preferred for the reference point for gathering of GDD.

$$GDD = \sum \left[\frac{T_{max} + T_{min}}{2}\right] - T_b \quad (1)$$

Where; T_{max} and T_{min}, daily maximum and daily minimum temperatures (°C), respectively; T_b, basis temperature (°C). For T_{max}, the values over 30 °C were assumed as 30 °C and for T_{min}, the values below 10 °C were taken as 10 °C. The base temperature was taken as 5 °C (Parthasarathi et al 2013).

2.5. Statistical analysis

Variance analysis (ANOVA) on all measured data was conducted by using R software (R Development Core Team 2006). Differences among years, treatments, replications and interactions were tested at significance level of $P<0.05$. The **r**elative yield of every plot was recorded as a percentage of the corresponding weed-free yield within each replication for each treatment. Statistical analysis was performed yearly, since the growing degree days were different in both years. Four-parameter log-logistic model were used for relative yield (% of weed-free). The data was analyzed by fixing the *D* term to 100 (Knezevic et al 2007) (Equation 2).

$$Y = \frac{C + (D - C)}{\{1 + exp[B\ (\log X - \log E)]\}} \quad (2)$$

Where; *Y*, response (e.g., relative yield); *C*, lower limit; *D*, upper limit; *X*, GDD found after crop emergence; *E*, GDD having a 50% response between the upper and lower limits (also known as the inflection point, I_{50}); *B*, slope of the line at the inflection point (the rate of change).

The regression analyses were carried out by using GDD as a quantitative variable because it is an established biological test measurement of time well suited for assessing the progress of growth and development (Gilmore & Rogers 1958). If one considers the curve fitting procedure, GDD is a more popular variable utilized for fitting regression models as appose to using a categorical parameters [e.g., crop growth stage (CGS)] as GDD ensures a constant and more accurate x-axis scale. It is a better indicator in comparing years and planting dates of different areas (Knezevic et al 2002). In addition, GDD can be used together with specific CGS to allow more expeditious field assessments thus, from a practical point of view; the essential data becomes more readily accessible to farmers, counselors and practitioners (Knezevic et al 2002).

Statistical analyses were carried out and graphs were drawn up with R software exploiting the DRC (dose response curves) statistical add-on package (Knezevic & Datta 2015). The rate of $YR_{2.5}$ (2.5% yield decrease), YR_5 (5% yield decrease) and YR_{10} (10% yield decrease) were obtained from the curves yielding an objective range for measuring the effects of increased periods of weed occurrence and weed-free treatments on crop yield. The 2.5%, 5% and 10% yield decrease were expressed in GDD indicating the impact of the length of weed interference. The estimation of GDD related to 90%, 95% and 97.5% relative yield was calculated from Equation 2 for each year and then the same was applied for the DAE. In the present study, the greatest yield decrease of 5% was randomly assigned as the value above which yield decrease was determined as being undesirable (Tursun et al 2012).

3. Results and Discussion

3.1. Weed density and dry matter

The weed populations were similar in both years (Table 1). The widespread weeds in the experimental area were identified as barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), European heliotrope (*Heliotropium europaeum* L.), common lambsquarters (*Chenopodium album* L.), spear

saltbush (*Atriplex patula* L.), round leaf cancer wort (*Kickxia spuria* L.), and redroot pigweed (*Amaranthus retroflexus* L.).

Table 1- The weed population densities (plants m^{-2}) in the season-long weedy treatment

Weed species	*Density (plants m^{-2})*	
	2012	*2013*
Echinochloa crus-galli L.	31	36
Heliotropium europaeum L.	14	17
Chenopodium album L.	13	16
Atriplex nitens L.	8	11
Kickxi aspuria L.	9	11
Amaranthus retroflexus L.	8	9
Euphorbia spp.	4	8
Anagallis foemina L.	5	8
Chondrilla juncea L.	5	7
Convolvulus arvensis L.	0.2	2
Sonchus arvensis L.	-	1
Tribulu sterrestris L.	1	0.5
Xanthium strumarium L.	1	0.4
Acroptilon repens L.	0.4	0.2
Sinapis arvensis L.	-	0.2
Stellaria media L.	0.3	-
Cuscuta spp.	-	1
Fumaria parviflora L.	-	0.25
Alhagi pseudalhagi L.	-	0.25
Total	99.9	128.8

These six species made up 83 and 78% of the total weed mass in 2012 and 2013, respectively. All these weeds are common in other summer grown crops in this location as well (Akça & Isik 2013). Some of these weeds (*E. crus-galli, A. retroflexus, C. album, X. strumarium,*) were the same type seen and analyzed in earlier studies carried out to find out the CPWC in other types of crops in Turkey (Isik et al 2006; Tursun et al 2012). Such weed species are abundant and important weeds in Turkey.

The total weed dry matter went up as the extent of weed interfering phase increased in the plots. The total dry matter was higher in 2013 than in 2012 (Figure 1, Table 2) and the weed acquisition weight increased more in 2013 than in 2012. These figures were established by assessment of the weed density in 2013 (Table 1). Our results were parallel with the reports of Kropff et al (1992), Salehi et al (2006), Jursik et al (2008) and Mobarak (2013) indicating increased weed biomass with increasing infestation durations.

3.2. Critical period for weed control

An interaction was seen between 2012 and 2013 and the treatments stages at the onset and at the end of the CPWC; so, all data of yield was additionally assessed separately for each year (Figure 2, Table 3).

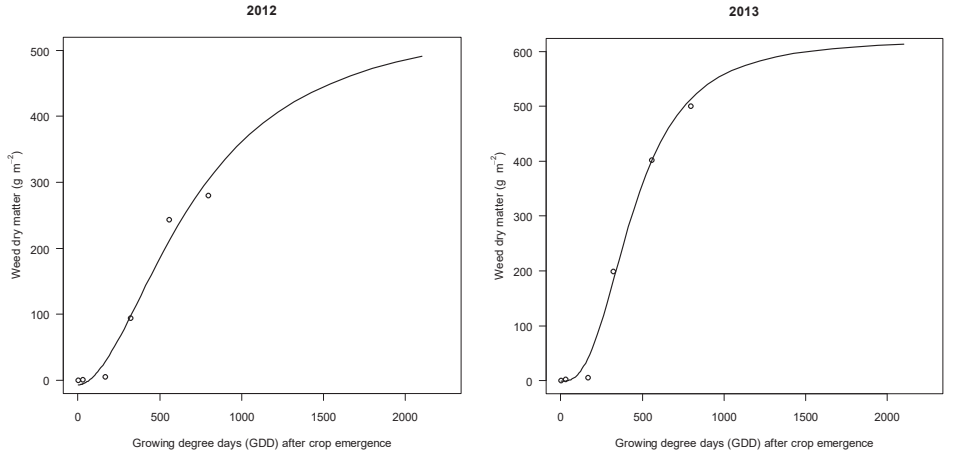

Figure 1- Weed dry biomass (g m^{-2}) response to duration of weed interference, shown by growing degree days (GDD); the regression lines were plotted using Equation 2; parameter values were given in Table 2

Table 2- Regression parameters (±SE) by year and practice for logistic model (Equation 2) describing the effects of weed interfering duration on weed dry biomass (g m^{-2})

Year	*Regression parameters (±SE)*			
	B	*C*	*D*	I_{50}
2012	-1.9 (0.6)	-6.8 (25.7)	557.9 (78)	713.5 (157.7)
2013	-2.7 (0.9)	-1.2 (40.3)	622.9 (68)	446.0 (74.1)

B, slope of the line at the inflection point; *C*, the lower limit; *D*, the upper limit; I_{50}, the GDD giving a 50% response between the upper and the lower limit

The length of weed-interference or weed-free period altered the sugar beet relative root yield (Figure 2). Increasing periods of weed interference noticeably reduced sugar beet root yields in both years. While the average sugar beet root yields from the weed free plots were 123583 kg ha^{-1} in 2012 and 125144 kg ha^{-1} in 2013; the whole season weed infested plots had root yields of 50027 kg ha^{-1} in 2012 and 2276 kg ha^{-1} in 2013. These results were again similar with the results of Salehi et al (2006) and Mobarak (2013) reporting lower root yields of sugar beet with rising weed interfering.

The CPWC varied in both years (Figure 2). The extent of the CPWC in sugar beet was 45, 34 and 24 days in 2012 and 60, 46, 32 days in 2013 with 2.5, 5 and 10% acceptable yield loss levels (AYL), respectively. A 5% yield loss is accepted as a rule for most crops in Turkey (Isik et al 2006; Tursun et al 2012). Based on 5% acceptable yield loss (AYL), the beginning of CPWC in sugar beet was identified

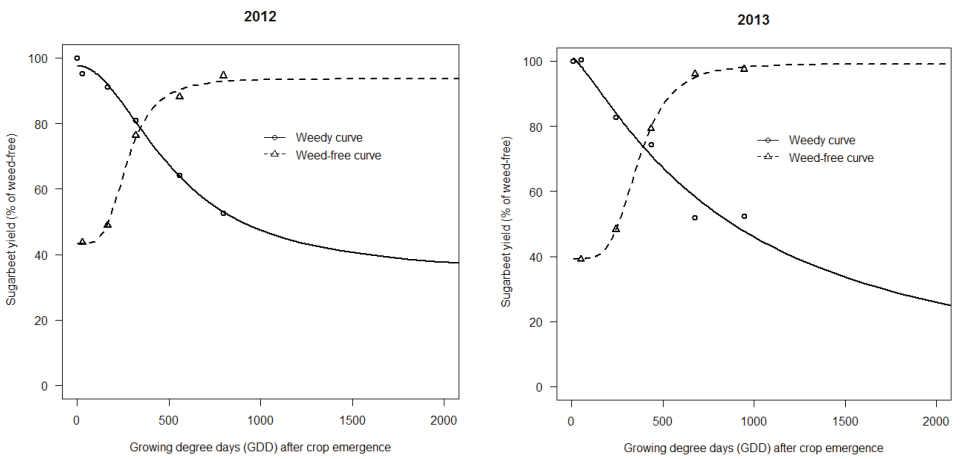

Figure 2- Effect of weed interference on sugar beet root yield (% of weed-free) as shown by growing degree days (GDD); the regression lines were plotted by using Equation 2; the parameter values were given in Table 3

Table 3- Parameter estimates (±SE) by year and application for logistic model (Equation 2) describing the influence of weed interfering duration on the relative yield of sugar beet

Year	*Treatments*	*Regression parameters (±SE)*			
		B	*C*	*D*	I_{50}
2012	Weedy	2.0 (0.3)	33.7 (2.8)	97.6 (2.3)	527.1 (35.3)
2012	Weed-free	-3.8 (1.0)	43.2 (2.4)	93.6 (2.8)	275.5 (19.1)
2013	Weedy	1.2 (0.4)	-1.1 (29.1)	101.3 (4.3)	877.9 (456.0)
2013	Weed-free	-4.2 (0.4)	39.2 (1.2)	99.2 (1.3)	366.7 (10.1)

B, slope of the line at the inflection point; C, lower limit; D, upper limit; I_{50}, the GDD giving a 50% response between the upper and the lower limit

as 122 GDD in 2012 and 82 GDD in 2013, which corresponds to 8-12 DAE (Table 4). Based on 2.5 and 10% AYL, onset of the CPWV was identified as between 86-177 GDD in 2012 (8-17 DAE) and as between 46-150 GDD in 2013 (4-15 DAE). The onset of CPWC was the same CGS (V1) in both years (Table 4).

The end of CPWC varied among the years (Figure 2). The end of CPWC in sugar beet was 595 GDD in 2012 and 735 GDD in 2013 corresponding to 46-54 DAE and V4-V5 CGS at 5% AYL (Table 4). The end of CPWC rose as the AYL decreasing from 10% to 2.5% (Figure 2). The differences between the end of CPWC in both years were possibly because of the differences in weed populations between the growing seasons (Table 1) and this might have been due to variations in sowing time and rainfall amounts in each respective years (Tursun et al 2012).

Weeds reduced sugar beet root yields by 60-82% when the weed interference was permitted throughout the growing season. Such findings comply with the results of Jursik et al (2008) and Mobarak (2013) indicating decreasing sugar beet root weight for each plant and root yield with increasing duration of weed presence. As stated by Salehi et al (2006) and Mobarak (2013), although sugar content did not show any significant difference between various treatments in both years, weed infestation decreased both root and sugar yields. It was observed that weed interference influenced both total production and quality. Parallel findings were indicated by Bukun (2004) in cotton and Isik et al (2006) in corn.

Present findings indicate that weed control measures in Central Anatolian Region of Turkey begin 4-8 days after sugar beet emergence. At early growth stages, sugar beet has a low competitive ability against weeds; as a result, critical period would start sooner (Salehi et al 2006). Dawson (1977) showed that weeds that germinated in the 2 and 4 weeks period after sugar beet sowing reduced yield between 26 and 100%, respectively. Therefore, effective control of weeds at early stages seems to be more important than that of later growth stages. A previous study indicated that critical weed control periods varied based on emergence periodicity and weed density (Bukun 2004).

Table 4- The critical weed control period (CPWC) for sugar beet highlighted in growing degree days (GDD), related crop growth stage (CGS) and days after crop emergence (DAE)

Year	*Yield reduction (%)*	*CPWC*		
		GDD	*CGS*	*DAE*
The beginning of CPWC				
2012	2.5	86	V1	8
	5	122	V1	12
	10	177	V1	17
2013	2.5	46	V1	4
	5	82	V1	8
	10	150	V1	15
The end of CPWC				
2012	2.5	718	V5	53
	5	595	V4	46
	10	489	V3	41
2013	2.5	871	V7	64
	5	735	V5	54
	10	616	V4	47

In Turkey, herbicide treatments and hoeing are primary methods used in weed control in sugar beet. As a result of early CPWC (1 week AE), producers are able to arrange the herbicide treatment and hoeing periods. Additional experiment should be carried out to find out the CPWC for other locations with different weed types and populations. Widespread information about CPWC in sugar beet could assist decision-makers in correct timing for herbicide application at post-emergence stage of crops. Enhanced knowledge and usage of CPWC would also lead to more effective and efficient ways of weed control. Reduced herbicide use would also reduce the risk of environmental pollution and the stress factors involved in trying to assess the most herbicide-resistant weeds (Hall et al 1992).

4. Conclusions

An effective integrated weed management system (IWM) relies on information on behaviors of the weeds and their impacts on yields. The CPWC is an essential issue in formulating strategies for IWM. The level weed interference is also influenced by light, water and plant nutrients. Sugar beet yield is therefore directly related and dependent on its ability to secure as much of these resources as possible throughout the growing season. Weeds should have an insignificant effect on sugar beet yield if they are controlled at the correct time. Based on 5% AYL figure, the present findings indicated that sugar beet tolerated weed interference up until 8 to 12 days after crop emergence (DAE**)**, therefore it was concluded that weed control practices should be performed right in this period. Plants must be free of weeds until 46 to 54 DAE to prevent yield loss over 5%. Weeds that emerge after 46 to 54 DAE grow in a competition with sugar beet.

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References

Akça A & Isik D (2013). Determination of weed species in sugar beet field (*Beta vulgaris* L.) in Kayseri Province in Turkey. In: *Proceedings of the 16th EWRS, European Weed Research Society Symposium.* 24-27 June, Samsun, Turkey, pp. 132-141

Bakhshkandi H, Mirshekari B, Pedram A & Alizadeh E (2013). Planting pattern could increase competitive power in sugar beet (*Beta vulgaris*) cultivars at interference with redroot pigweed (*Amaranthus retroflexus*). *Life Science Journal* **10**: 185-188

Bukun B (2004). Critical periods for weed control in cotton in Turkey. *Weed Research* **44**: 404-412

Dawson J H (1977). Competition of late emerging weeds with sugar beets. *Weed Science* **25**: 168-170

Evans S P, Knezevic S Z, Lindquist J L, Shapiro C A & Blankenship E E (2003). Nitrogen application influences the critical period for weed control in corn. *Weed Science* **51**: 408-417

FAO (2015). FAOSTAT Online Database, Available from: http://faostat.fao.org/ (Accessed September, 2015)

Gilmore E C & Rogers R S (1958). Heat units as a method of measuring maturity in corn. *Agronomy Journal* **50**: 611-615

Güncan A (2000). Şeker pancarlarında ekim öncesi yabancı ot mücadelesi. Pancar Ekicileri Eğitim ve Sağlık Vakfı Yayınları No: 5, Ankara, s. 143-148

Hall M R, Swanton C J & Anderson G W (1992). The critical period of weed control in grain corn (*Zea mays*). *Weed Science* **40**: 441-447

Isik D, Mennan H, Bukun B, Oz A & Ngouajio M (2006). The critical period for weed control in corn in Turkey. *Weed Technology* **20**: 867-872

Jalali A H & Salehi F (2013). Sugar beet yield as affected by seed priming and weed control. *Archives of Agronomy and Soil Science* **59**: 281-288

Jursik M, Holec J, Soukup J & Venclova V (2008). Competitive relationships between sugar beet and weeds in dependence on time of weed control. *Plant, Soil and Environment* **54**: 108-116

Kiymaz S & Ertek A (2015). Water use and yield of sugar beet (*Beta vulgaris* L.) under drip irrigation at different water regimes. *Agricultural Water Management* **158**: 225-234

Knezevic S Z & Datta A (2015). The critical period for weed control: Revisiting data analysis. *Weed Science* **63**(Special Issue): 188-202

Knezevic S Z, Evans S P, Blankenship E E, Van Acker R C & Lindquist J L (2002). Critical period for weed control: The concept and data analysis. *Weed Science* **50**: 773-786

Knezevic S Z, Streibig J C & Ritz C (2007). Utilizing R software package for dose-response studies: The concept and data analysis. *Weed Technology* **21**: 840-848

Kropff M J, Spitters C J T, Schneiders B J, Joenije W & Groot W D E (1992). An ecophysiological model for interspecific competition, applied to the influence of *Chenopodium album* L. on sugar beet. *Weed Research* **32**: 451-463

Malaslı Z M (2010). Şeker pancarı üretim alanlarında yabancı otla mücadele yöntemleri ve uygulama etkinliklerinin belirlenmesi. Yüksek lisans tezi, Harran Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Şanlıurfa

Marwitz A, Ladewig E & Marlander B (2014). Response of soil biological activity to common herbicide strategies in sugar beet cultivation. European Journal of Agronomy **54**: 97-106

Mobarak O M M A (2013). Determination of critical period of weed competition with sugar beet (*Beta vulgaris* L.) and weed control. PhD Thesis. Assiut University (Unpublished), Egypt

Norris R F (1996). Sugar beet integrated weed management. Publication UC IPM Pest Management Guidelines: Sugar beet, Veg Crops/Weed Science, UC Davis UC DANR Publication 3339

Parthasarathi T, Velu G & Jeyakumar P (2013). Impact of crop heat units on growth and developmental physiology of future crop production. *Research & Reviews: A Journal of Crop Science and Technology* **2**(1): 1-11

Salehi F, Esfandiari H & Mashhadi H R (2006). Critical period of weed control in sugar beet in Shahrekord Region. *Iranian Journal of Weed Science* **2**: 1-12

Swanton C J & Weise S F (1991). Integrated weed management: The rationale and approach. *Weed Technology* **5**: 657-663

Tursun N, Akinci I E, Uludag A, Pamukoglu Z & Gozcu D (2012). Critical period for weed control in direct seeded red pepper (*Capsicum annum* L.). *Weed Biology and Management* **12**: 109-115

Young S L (2012). True integrated weed management. *Weed Research* **52**: 107-111

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Kinematics Analysis and Simulation of A 5DOF Articulated Robotic Arm Applied to Heavy Products Harvesting

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ABSTRACT

Robotics can play a significant role to increase efficiency and lighten the farmer's load. Despite challenges in the agricultural robotic designs, robots are capable of performing various tasks and changing themselves accordingly, based on specific conditions. To address modern problems in the agricultural field, an agricultural robot is one of the key technologies. Although agricultural robotic is still in the development stage, robots have a bright future ahead. This paper proposes a new 5DOF articulated robotic arm design that would become a solution for heavy crop harvestings like pumpkin and cabbage. After the development stage, this robotic arm will be mounted on a robot tractor for real experimentation.

The main design process of this robotic arm was conceived using 6 stages of Shigley design process. All components were designed, assembled and analyzed by using Solidworks 2014 in compliance with Japanese Industrial Standards (JIS) standards. The parts of the system that had dynamic nature were analyzed manually using standard mechanical formulas. Calculations of the workspace required joint torque, and coordination of mass center position was done by using standard machine design methods. Denavit-Hartenberg method was used to calculate forward and inverse kinematics. To resolve the torque reduction, components were designed using different materials and mass centers and comparing their performance.

Results showed that total torque in Joints number 1, 2, 3, 4 and 5 were 6.15, 257.35, 103.4, 20.2 and 0.1 respectively with a rotational speed range of 15 ~ 60 rpm. Changes in the linkage material and servo motor location improved 29.7% ~ 47.7% and 29.7% ~ 68.9% of the total required torque for each joint. The maximum distance covered by the arm was 1421 mm from the and 2026 mm from the attachment point. According to the feedback received from a inverse kinematics equation algorithm, the fundamental operation of the robot arm had an optimal performance.

Keywords: Forward kinematics; Inverse kinematics; Torque; Workspace; Servo motor

Ağır Ürünlerin Hasadında Kullanılan Bir 5DOF Eklemli Robot Kolun Kinematik Analizi ve Simülasyonu

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ÖZET

Robotlar etkinliği artırmak ve çiftçilerin yükünü hafifletmekte önemli bir rol oynayabilir. Tarımsal amaçlı robot dizaynlarındaki zorluklara rağmen, robotlar çeşitli görevleri yerine getirmede ve kendilerini duruma göre değiştirmede belli koşullara göre kabiliyetlidirler. Tarım alanındaki modern problemlerin ifade edilmesinde, bir tarımsal robot anahtar teknolojilerden biridir. Tarımsal robotlar halen gelişme aşamasında olmalarına rağmen, robotlar parlak bir geleceğe sahiptirler. Bu yayın kabak ve lahana gibi ağır ürünlerin hasadına bir çözüm olabilecek yeni bir 5DOF eklemli robot kol dizaynını sunmaktadır.

Düzeneğin ana dizayn süreci altı aşamalı Shigley dizayn prosesi kullanılarak tasarlanmıştır. Tüm parçaların dizaynı, birleştirilmesi ve analizi JIS standartları ile uyumlu Solidworks 2014 kullanarak gerçekleştirilmiştir. Dinamik yapıda olan sistem parçaları standart mekanik formüllerin kullanılması ile manuel olarak analiz edilmişlerdir. İleri ve geri kinematiklerin hesaplanmasında Denavit-Hartenberg yöntemi kullanılmıştır. Tork azalma problemini ortadan kaldırmak için parçalar farklı materyallerle ve kütle merkezine göre dizayn edilmiştir ve beraber karşılaştırılmıştır.

Sonuçlar göstermiştir ki 1, 2, 3, 4 ve 5 numaralı eklemlerde toplam tork, 15 ~ 60 rpm rasyonel hız aralığı ile sırasıyla 6.15, 257.35, 103.4, 20.2 ve 0.1 olmuştur. Bağlantı materyalindeki ve servo motor lokasyonundaki değişiklikler her bir eklem için olan toplam gerekli torku % 29.7~ % 47.ve % 29.7% ~ % 68.9 aralıklarında iyileştirmiştir. Kol tarafından taranan maksimum mesafe J_2 den 1421 mm ve eklem noktasından da 2026 mm olmuştur. Ters kinematik eşitliği algoritmasından alınan geri beslemeye göre robot kolunun temel operasyonları optimum bir performans göstermiştir.

Anahtar Kelimeler: İleri yönlü kinematik; Ters kinematik; Tork; Çalışma alanı; Servo motor

1. Introduction

In the last three decades, the engineers had a new approach to design particular robots for the agriculture industry. Although it is an important industry, it faces several problems like the age distribution of farmers. The average age of a farmer is 65.9 years old in Japan (MAFF 2016) and 55.9 years old in USA (USDA 2015). With a declining farming population, the majority of the farming population is considered "too old" to handle the rigorous demands of the industry. Not to mention the work itself is susceptible to unpredictable weather conditions. Another issue is utilizing new agricultural technology. Learning how to operate new technology requires time and physical effort. According to the last report of Statistics Bureau of Japan, the number of workers decreased from 13.40 million in 1960 (30.2 percent of the total workforce) to 2.33 million in 2013 (3.7 percent), and the GDP share of the industries fell from 12.8 percent in 1960 to 1.2 percent in 2013. Japan's total agricultural output in 2013 was 8.47 trillion yen, down 0.7 percent from the previous year. Crops yielded 5.70 trillion yen, down 3.0 percent from the previous year. This was due to the rice output decreasing despite outputs of vegetables and fruits and nuts increasing. Based on the Global Agricultural Productivity report, in the next 40 years, agricultural output will need to increase by 100%. Consumer attitudes is changing to organic products and the total income per commercial farm household has decreased. In 2013, the total income per commercial farm household was 4.73 million yen, down 0.7 percent from the previous year. Of that amount, 1.32 million yen was from farming income, 1.53 million yen from non-farming income, and 1.87 million yen from pension benefits and other sources. Agricultural robotics can help address and solve these issues that farming communities encounter on a regular basis (Cassinis & Tampalini 2007). Some example include a multi-arm robotic harvester (Zion et al 2014), a strawberry-harvesting robot (Hayashi et al 2010), an apple harvesting robot (De-An et al 2011), an autonomous robot for white asparagus harvesting (Barawid Jr et al 2007), a cherry-harvesting robot (Tanigaki et al 2008), robots for tomato, petty-tomato, cucumber and grape harvesting (Kondo et al 1996) and Stationary robots that are used for sheep herding (Tanner et al 2001).

To design a robotic arm for agricultural applications, it is necessary to move the final point of a manipulator along some desired path at a prescribed speed (Angeles 1997). Furthermore, it is necessary for the system to be dynamically analyzed and modeled (Wang et al 2003). To reach this goal, it is essential to use forward and inverse kinematics (Karlik & Aydin 2000).

The motion takes place in the Cartesian space; but most of the industrial robots, especially the articulated robotic arm, are controlled in rotary joint spaces. Therefore, a kinematic transformation between the Cartesian space and joint space is needed (Balkan et al 2000). The most widely proposed methods for solving the inverse kinematic problem for redundant manipulators involve the use of the Jacobian pseudoinverse manipulator (Yahya et al 2011). Thanks to this method, many excellent types of research in the kinematics community had been done by the end of the 1980s and the beginning of 1990s. At the same time, resolving of inverse kinematics was considered to be the most difficult task in the field of kinematics. In 1988 Lee & Liang (1988) came up with a solution which was not very transparent, so most of the time the Raghavan & Roth (1990) solution is cited in the literature. There were many attempts to improve the controlling algorithm (Ghazvini 1993). As a result, there are many thousands of robots in the industry (Satoru 2011) but only a few are designed for agriculture application.

The promising results of laboratory investigations can be considered as a cornerstone for the development of models for farming robots. Currently, the agricultural robotic technology is in the development stage, and it is expected that the agricultural robots can cover all the needs of agriculture. However, researchers had not investigated the topic of heavy harvesting crops like cabbage, pumpkin, and watermelon as much as light crops. Since users intend to take advantage of fully automated processes in different aspects of agriculture through the use of robotic technology, further research; especially on harvesting agricultural heavy products is required.

This research presents a new type of 5DOF robotic arm mounted on a tractor for heavy crop harvestings like pumpkin and cabbage.

2. Material and Methods

In this study, a 5DOF (Degrees of Freedom) robotic arm for the harvesting the heavy agricultural products (RAVebots-1/Robotic Arm for Vehicle Robotic) was developed, which is shown schematically in Figure 1. The presented robotic arm is composed of serial links which are affixed to each other with revolute joints from the base frame to the end-effector. The RAVebots-1's structure was chosen to be manufactured for heavy product harvesting application. All components were designed, assembled and analyzed using Solidworks 2014. Dynamic components were analyzed by using standard mechanical formula. After finishing all component development, The RAVebots-1 was attached to a robot tractor.

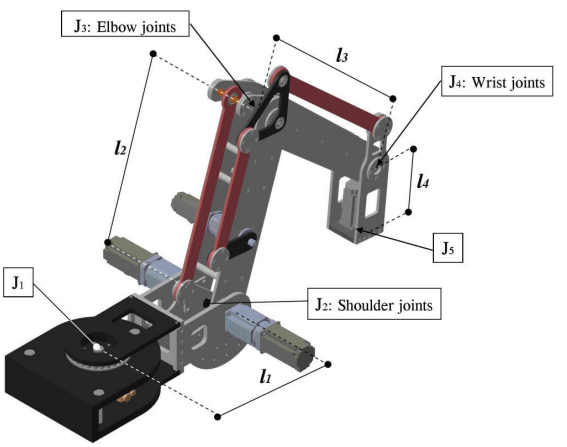

Figure 1- Assembled model of RAVebots-1

2.1. Description of the RAVebots-1 workspace

The robot workspace or reachable spaces consist of all the points in the Cartesian space that the end effector of the robotic arm can access. The workspace and a quick access to a certain point in all robotic arms are strongly dependent on linkages properties, joint properties (length, angles, angular velocity

and torque), DOF, angle/translation limitations, and robot configuration.

Consider the RAVebots-1 in Figure 2. The left side shows the complete work envelope of the robot from the side view. The right side of Figure 2 shows the whole workspace from the top view. The Maximum frontal distance covered by the arm is 1640 mm from and 1830 mm from the robotic arm and tractor's attachment point. The height limitation comes from the tractor height which was almost than 700 mm. All dimensions have a tolerance in the range of 2 ~ 5 mm. The maximum height of access point is 1430 mm. According to the accessible points in X and Z directions, the RAVebots-1 has enough range of motion to be used in horticulture applications. In addition, the RAVebots-1's workspace has a suitable reach to pick fruits; cut branches and perform precision farming.

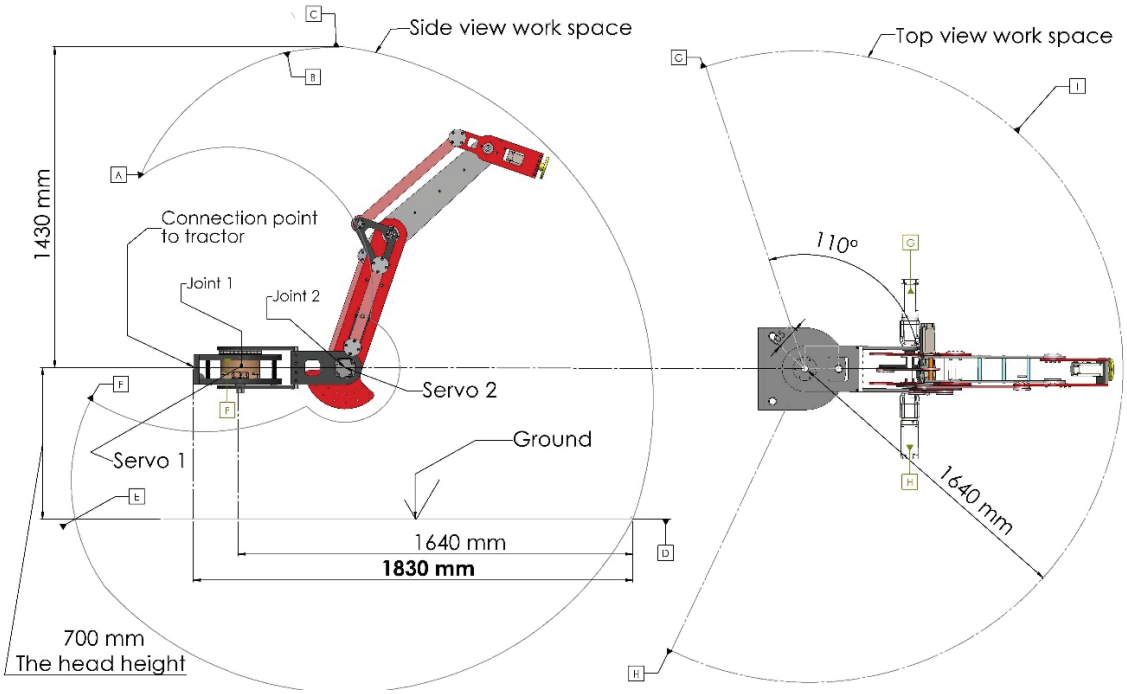

Figure 2- Workspace of the RAVebots-1

2.2. Structural design of RAVebots-1

2.2.1. Components analysis

Figure 3 shows a comparison between three design models of RAVebots-1 (A, B, and C) in terms of different material and structure. Overall, the main differences between A and B designs are related to the material; A used ASTM A36 steel and B used AL5202. The difference between A and B designs with design C relies not only on the material used but also in the servo motor position. A special alloy of Aluminum (AL5202) was used in design C, and the positions of the servo motors from joint 3 and 4 are closer to the position of joint 2.

The dynamic components were analyzed by using standard mechanical formulas. Due to the sensitivity of some parts such as the main chassis, a stress analysis was conducted on joint-1 structure and vertical plates using the

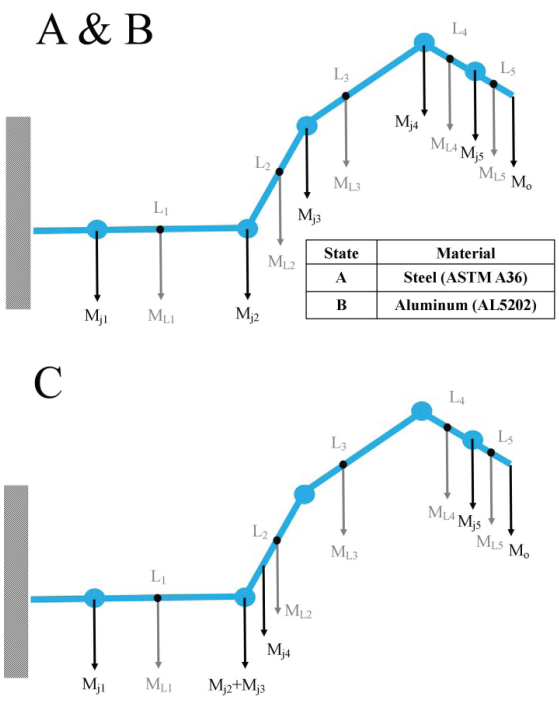

Figure 3- Components weight diagram in A, B, and C design

Solidworks 2014 simulation. The safety factor range for linkage and structure design was selected from 1.96 to 2.60; the range of joint and servo motor designs was selected from 1.1 to 3.00. All components were manufactured from steel (ASTM A36 steel) and aluminum (AL5205). The simulation type of Solidworks simulator was linear elastic isotropic (Richard & Keith 2006). Stress analysis results for the main stage showed that the maximum stress was less than the tensile strength (Figure 4).

2.2.2. Joints torque analysis

Selecting a proper motor and a motor driver to meet a specific application needs a torque calculation. At first, the inertia, friction, and load torque should be calculated. Then, it is possible to determine the required motor torque for the specific application. Finally, it is possible to select the proper motor and driver based on their speed-torque characteristics.

The load drive torque of the servo motor can be calculated by using Equation 1.

$$T_t = \left((I.\omega)+(N.K_i + T_{FM} + T_{FD}) + (T_g + T_s)\right) \times \frac{FOS}{\eta} \qquad (1)$$

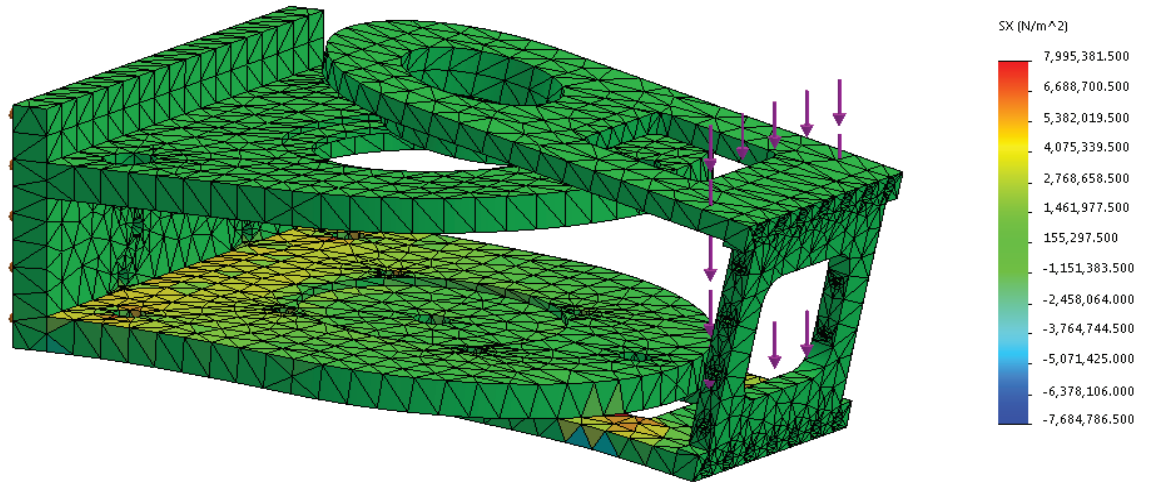

Figure 4- Stress analysis illustration of the main stage by using Solidworks

The symbols of Equation 1 are defined as follow:

Symbol	Meaning	Unit	Symbol	Meaning	Unit
I	Total moment of inertia in conversion into the motor's shaft	Nms^2	T_{FD}	Friction torque of the transmission system	N.m
ω	Motor shaft angular acceleration	$Rads^{-2}$	η	Efficiency of Servo motor	-
N	Motor usage	rpm	FOS	Factor of Safety	-
K_i	Braking constant	Nm/rpm	T_g	Gravity holding torque	N.m
T_{FM}	Motor static friction torque	N.m	T_s	Interference torque	N.m

2.3. Kinematics modeling

Robot kinematics refers to the analytical study of the motion of a robot manipulator. Denavit & Hartenberg (1955) showed that a general transformation between two joints requires four parameters. These parameters, known as the Denavit-Hartenberg (D-H) parameters which became a standard to describe robot kinematics (Funda et al 1990). The robot kinematics can be divided into forward and inverse kinematics (Siciliano & Khatib 2008). Forward kinematics problems are straightforward, with little complexity in driving their respective equations (Craig 1989). Inverse kinematics is more difficult to solve than forward kinematics (Serdar & Zafer 2006; Satoru 2011).

In this section, the analytical solution for the manipulator is examined using the D-H parameter into forward kinematics and inverse kinematics.

2.3.1. Forward kinematics

Forward kinematics problem involves finding the position and orientation of a robot end-effector as a function of its joint angles. Denavit-Hartenberg (D-H) method uses the four parameters including a_{i-1}, α_{i-1}, d_i and θ_i; which are the link length, link twist, link offset and joint angle, respectively. Figure 5 presents the coordinate frame assignment for a general manipulator (Serdar & Zafer 2006).

The matrix T_i^{i-1} is known as a D-H convention matrix given in Equation 2.

$$T_i^{i-1} = \begin{bmatrix} cos\,\theta_i & -cos\,\alpha_{i-1}\,sin\,\theta_i & sin\,\alpha_{i-1}\,sin\,\theta_i & a_{i-1}\,cos\,\theta_i \\ sin\,\theta_i & cos\,\alpha_{i-1}\,cos\,\theta_i & -sin\,\alpha_{i-1}\,cos\,\theta_i. & a_{i-1}\,sin\,\theta_i \\ 0 & sin\,\alpha_{i-1} & cos\,\alpha_{i-1} & d_i \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad (2)$$

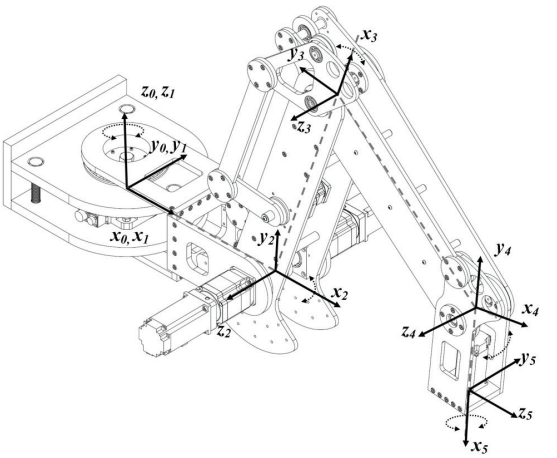

Figure 5- Axis's direction and angle parameters

In the matrix T_i^{i-1}, the quantities of $\alpha_{i-1}, a_{i-1}, d_i$ are constant for a given link while the parameter eter θ_i for a revolute joint is variable. The next step was determining the D-H parameters by using α_i. The completed D-H parameters for RAVebots-1 are listed in Table 1. Using the expression in Equation 2, the A-matrices of each joint can be built as shown in Equation 3.

Table 1- D-H Parameters of a RAVebots-1 Robot

Axis number	*Twist angle* α_{i-1}	*Link length* α_{i-1}	*Link offset* d_i	*Joint angle* θ_i
1	90°	l_1	0	$-105° < \theta_1 < 105°$
2	0	l_2	0	$0 < \theta_2 < 125°$
3	0	l_3	0	$-130° < \theta_3 < -10°$
4	−90°	l_4	0	$-115° < \theta_4 < 0°$
5	*0*	l_5	0	$0° < \theta_5 < 360°$

$$T_1^0 = \begin{bmatrix} cos\,\theta_1 & 0 & sin\,\theta_1 & l_1\,cos\,\theta_1 \\ sin\,\theta_1 & 0 & -cos\,\theta_1 & l_1\,sin\,\theta_1 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, T_2^1 = \begin{bmatrix} cos\,\theta_2 & -sin\,\theta_2 & 0 & l_2\,cos\,\theta_2 \\ sin\,\theta_2 & cos\,\theta_2 & 0 & l_2\,sin\,\theta_2 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

$$T_3^2 = \begin{bmatrix} cos\,\theta_3 & -sin\,\theta_3 & 0 & l_3\,cos\,\theta_3 \\ sin\,\theta_3 & cos\,\theta_3 & 0 & l_3\,sin\,\theta_3 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, T_4^3 = \begin{bmatrix} cos\,\theta_4 & 0 & -sin\,\theta_4 & l_4\,cos\,\theta_4 \\ sin\,\theta_4 & 0 & cos\,\theta_4 & l_4\,sin\,\theta_4 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad (3)$$

$$T_5^4 = \begin{bmatrix} cos\,\theta_5 & -sin\,\theta_5 & 0 & l_5\,cos\,\theta_5 \\ sin\,\theta_5 & cos\,\theta_5 & 0 & l_5\,sin\,\theta_5 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

The T-matrix is created by multiplying each T_5^0 matrix defined using Equation 3; the result is shown in Equation 4.

$$T_5^0 = \prod_{i=1}^{5} T_i^{i-1} = T_1^0\,T_2^1\,T\,{}_3^2\,T_4^3\,T_5^4 = \begin{bmatrix} r_{11} & r_{12} & r_{13} & r_{14} \\ r_{21} & r_{22} & r_{23} & r_{24} \\ r_{31} & r_{32} & r_{33} & r_{34} \\ r_{41} & r_{42} & r_{43} & r_{44} \end{bmatrix} \quad (4)$$

Where the matrix elements are defined in Equation 5 as:

$$\begin{aligned} &r_{11} = c_1c_{(2+3+4)}c_5 + s_1s_5, r_{12} = s_5\left(s_1 - c_1c_{(2+3+4)}\right), r_{13} = -c_1s_{(2+3+4)} \\ &r_{14} = c_1\left(l_1 + l_2c_2 + l_3c_{(2+3)} + l_4\,c_{(2+3+4)} + l_5c_5c_{(2+3+4)}\right) + l_5s_1s_5 \\ &r_{21} = s_1c_{(2+3+4)}c_5 - c_1s_5, r_{22} = -s_1c_{(2+3+4)}s_5 - c_1c_5, r_{23} = -s_1s_{(2+3+4)} \\ &r_{24} = s_1\left(l_1 + l_2c_2 + l_3c_{(2+3)} + l_4\,c_{(2+3+4)} + l_5c_5c_{(2+3+4)}\right) - l_5c_1c_5 \\ &r_{31} = s_{(2+3+4)}c_5, r_{32} = -s_{(2+3+4)}s_5, r_{33} = c_{(2+3+4)}, r_{34} = l_5s_{(2+3+4)}c_5 \\ &r_{14} = r_{24} = r_{34} = 0, r_{44} = 1 \end{aligned} \quad (5)$$

In the expressions of Equation 5, the variables are defined by Equation 6 as:

$$c_i = cos\,\theta_i,\ \ s_i = sin\,\theta_i\,,\ c_{ij} = cos\left(\theta_i + \theta_j\right),\ s_{ij} = sin(\theta_i + \theta_j) \quad (6)$$

By using the T-matrix, it is possible to calculate the values of (P_x, P_y, P_z) with respect to the fixed coordinate system. Then, the P_x, P_y and P_z obtained with direct kinematics are expressed as shown in Equation 7 as:

$$\begin{aligned} P_x = {} & cos\,\theta_1\,[l_1 + l_2\,cos\,\theta_2 + l_3\,cos(\theta_2 + \theta_3) + l_4\,cos(\theta_2 + \theta_3 + \theta_4) + \\ & l_5\,cos\,\theta_5\,cos(\theta_2 + \theta_3 + \theta_4)] + l_5sin\,\theta_1\,sin\,\theta_5] \end{aligned}$$

$$\begin{aligned} P_y = {} & sin\,\theta_1\,[l_1 + l_2\,cos\,\theta_2 + l_3\,cos(\theta_2 + \theta_3) + l_4\,cos(\theta_2 + \theta_3 + \theta_4) + \\ & l_5\,cos\,\theta_5\,cos(\theta_2 + \theta_3 + \theta_4)] - l_5cos\,\theta_1\,sin\,\theta_5] \end{aligned} \quad (7)$$

$$P_z = l_2\,sin\,\theta_2 + l_3\,sin(\theta_2 + \theta_3) + l_4\,sin(\theta_2 + \theta_3 + \theta_4) + l_5\,cos\,\theta_5\,sin(\theta_2 + \theta_3 + \theta_4)$$

The orientation of RAVebots-1 end-effector in space can be described by attaching a coordinate system to it and then describing the vector of its coordinate axes relative to reference frame. Figure 6 indicates the normal vector ($\vec{n}$), orientation vector ($\vec{o}$), approach vector ($\vec{a}$) and the resultant of all vectors ($\vec{D}$) of the end-effector which described in more detail in Equation 8.

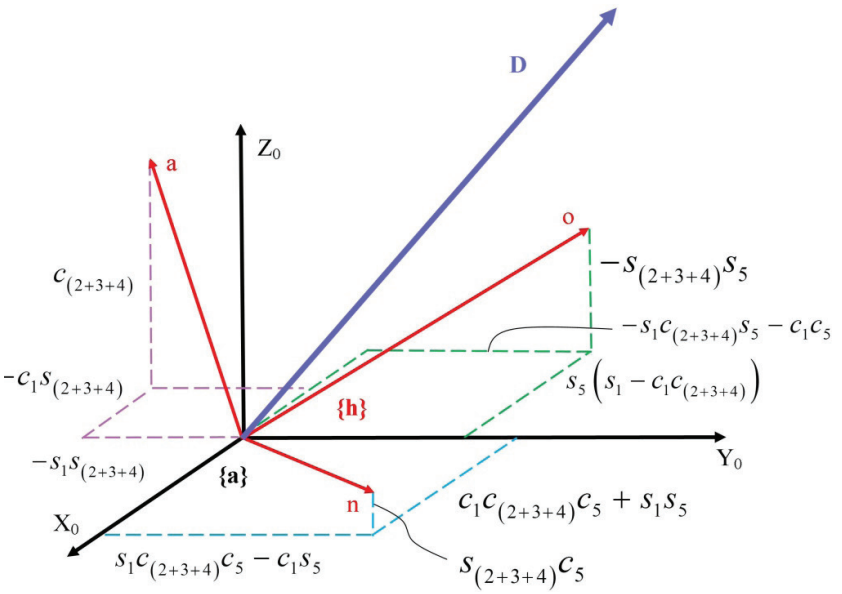

Figure 6- Rotation matrix elements

$$\vec{n} = \begin{bmatrix} c_1c_{(2+3+4)}c_5 + s_1s_5 \\ s_1c_{(2+3+4)}c_5 - c_1s_5 \\ s_{(2+3+4)}c_5 \end{bmatrix}, \vec{o} = \begin{bmatrix} s_5\left(s_1 - c_1c_{(2+3+4)}\right) \\ -s_1c_{(2+3+4)}s_5 - c_1c_5 \\ -s_{(2+3+4)}s_5 \end{bmatrix}, \vec{a} = \begin{bmatrix} -c_1s_{(2+3+4)} \\ -s_1s_{(2+3+4)} \\ c_{(2+3+4)} \end{bmatrix},$$

$$\vec{D} = (\vec{n} + \vec{o}) + \vec{a} = \begin{bmatrix} c_1\left(c_{(2+3+4)}(c_5 - s_5) - s_{(2+3+4)}\right) + 2s_1s_5 \\ s_1\left(c_{(2+3+4)}(c_5 - s_5) - s_{(2+3+4)}\right) + c_1c_4 \\ s_{(2+3+4)}(c_5 - s_5) + c_{(2+3+4)} \end{bmatrix} \quad (8)$$

2.3.2. Inverse kinematics

The conversion of the position and orientation of the manipulator's end-effector from Cartesian space to joint space is known as inverse kinematics. The inverse kinematics solution uses the position and orientation (p_x, p_y, p_z) of the robot's end-effector, which has been known to solve the joint angles (θ_1, θ_2, θ_3, θ_4, θ_5). In this study, the geometrical method was used.

In the RAVebots-1, the axes of the last two joints intersect at one point, which is referred to as point A. The position of point A is independent of the two joints of θ_4, and θ_5. Therefore, only the three previous joints should be considered when solving the position of point A. The position of point A is denoted as $P_a = [P_{ax}, P_{ay}, P_{az}]$ which showed by Equation 9.

$$P_{ax} = P_x - P_{x_5}^3, \; P_{ay} = P_y - P_{y_5}^3, \; P_{az} = P_z - P_{z_5}^3 \quad (9)$$

I. Solutions of the arm joint angles (θ_1, θ_2, θ_3).

The position of point A can be determined from the homogeneous transformation matrix, which is derived from T_1^0, T_2^1, T_3^2 as shown in Equation 10.

$$T_3^0 = \prod_{i=1}^{3} T_i^{i-1} = T_1^0 T_2^1 T_3^2$$

$$= \begin{bmatrix} c_1c_{23} & -c_1s_{23} & s_1 & c_1(l_3c_{23} + l_2c_2 + l_1) \\ s_1c_{23} & -s_1s_{23} & -c_1 & s_1(l_3c_{23} + l_2c_2 + l_1) \\ s_{23} & c_{23} & 0 & l_3s_{23} + l_2s_2 \\ 0 & 0 & 0 & 1 \end{bmatrix} \quad (10)$$

Elements of P_a can be described by Equation 11.

$$P_{ax} = c_1(l_3c_{23} + l_2c_2 + l_1), P_{ay} = s_1(l_3c_{23} + l_2c_2 + l_1), P_{az} = l_3s_{23} + l_2s_2 \quad (11)$$

From Equation 11, it is possible to obtain Equation 12 as:

$$\frac{P_{ay}}{P_{ax}} = \frac{s_1}{c_1} \rightarrow \theta_1 = Atan\,2(P_{ay}, P_{ax}) \quad (12)$$

From Equation 11, it is possible to obtain Equation 13.

$$P_{ax}.c_1 + P_{ay}.s_1 = ({c_1}^2 + {s_1}^2)(l_3c_{23} + l_2c_2 + l_1) = l_3c_{23} + l_2c_2 + l_1 = A \quad (13)$$

In the RAVebots-1, c_{23} can be obtained from Equation 12 as follows:

$$c_{23} = \frac{(P_{ax}.c_1 + P_{ay}.s_1) - l_2c_2 - l_1}{l_3} \quad (14)$$

It is possible to obtain s_{23} by solving Equation 11 as follows:

$$s_{23} = \frac{P_{az} + l_2s_2}{l_3} \quad (15)$$

Substituting Equation 14 and Equation 15 into the equation ${c_{23}}^2 + {s_{23}}^2 = 1$ yields Equation 16.

$$\left((P_{ax}.c_1 + P_{ay}.s_1 - l_1) - l_2c_2\right)^2 + (P_{az} + l_2s_2)^2 = {l_3}^2$$

$$P_{az}s_2 + (P_{ax}.c_1 + P_{ay}.s_1 - l_1)c_2 = \frac{(P_{ax}.c_1 + P_{ay}.s_1 - l_1)^2 + {l_2}^2 + {P_{az}}^2 - {l_3}^2}{2l_2} = A \quad (16)$$

Consider the variables *d*, *f*, and *g* as defined in Equation 17.

$$d = P_{az};\ f = P_{ax}.c_1 + P_{ay}.s_1 - l_1;\ g = \frac{(P_{ax}.c_1 + P_{ay}.s_1 - l_1)^2 + {l_2}^2 + {P_{az}}^2 - {l_3}^2}{2l_2} \quad (17)$$

Replacing the variables from Equation 17 in Equation 16 yields Equation 18:

$$d\,sin\,\theta_2 + f cos\theta_2 = g \quad (18)$$

Considering the approximations shown in Equation 19.

$$\begin{aligned} &f+g\neq 0, \quad d\sqrt{d^2+f^2-g^2}-d^2-f^2-fg\neq 0 \ \rightarrow \theta_2 \\ &\approx 2.\left(3.14159\,n+tan^{-1}\left(\frac{d-\sqrt{d^2+f^2-g^2}}{f+g}\right)\right), n\in z \\ &f+g\neq 0, \quad d\sqrt{d^2+f^2-g^2}+d^2+f^2+fg\neq 0 \ \rightarrow \theta_2 \\ &\approx 2.\left(3.14159\,n+tan^{-1}\left(\frac{d+\sqrt{d^2+f^2-g^2}}{f+g}\right)\right), n\in z \end{aligned} \tag{19}$$

$$d\neq 0,\ d^2+f^2\neq 0\,, g\approx -f\rightarrow \theta_2\approx 2.\left(3.14159\,n+tan^{-1}\left(\frac{f}{d}\right)\right), n\in z$$

$$g=-f\ \rightarrow \theta_2=2\pi n+\pi, n\in z$$

And if g= -f, x=$2n\pi$+π it is possible to obtain Equation 20.

$$\begin{aligned} \theta_2 &= Atan\,2\left(\frac{fg-\sqrt{d^4+d^2f^2-d^2g^2}}{d^2+f^2},\frac{1}{d}\left(\frac{f\sqrt{-d^2(-d^2-f^2+g^2)}-f^2g}{d^2+f^2}+g\right)\right) \\ \theta_2 &= Atan\,2\left(\frac{fg+\sqrt{d^4+d^2f^2-d^2g^2}}{d^2+f^2},\frac{1}{d}\left(\frac{-f\sqrt{-d^2(-d^2-f^2+g^2)}-f^2g}{d^2+f^2}+g\right)\right) \end{aligned} \tag{20}$$

The result of using Equation 14 and 15 yields Equation 21.

$$tan(\theta_2+\theta_3)=\frac{P_{az}+l_2s_2}{(P_{ax}.c_1+P_{ay}.s_1)-l_2c_2-l_1} \tag{21}$$

It is possible to solve Equation 21 as shown in Equation 22.

$$\theta_3=Atan\,2\left(P_{az}+l_2s_2,\ (P_{ax}.c_1+P_{ay}.s_1)-l_2c_2-l_1\right)-\theta_2 \tag{22}$$

II. Solutions of the wrist joint angles *(θ_4 and θ_5).*

The orientation of the robot is controlled by the rotation matrix, and the orientation of A is described by T_3^0. The orientation of end-effector is described by T_5^0. The relationship between T_3^0 and T_5^0 is $T_5^0=T_3^0\,T_5^3$. Matrix T_5^3 can be described as shown in Equation 23.

$$T_5^3=\prod_{i=3}^{5}T_i^{i-1}=T_4^3\,T_5^4=\begin{bmatrix} c_4c_5 & -c_4s_5 & -s_4 & (l_5c_5+l_4)c_4 \\ s_4c_5 & -s_4s_5 & c_4 & (l_5c_5+l_4)s_4 \\ s_5 & c_5 & 0 & l_5s_5 \\ 0 & 0 & 0 & 1 \end{bmatrix} \tag{23}$$

The elements of $P_{A_5}^{\ 3}$ come from the fourth column of the 4×4 matrix in Equation 23, which can be described by Equation 24.

$$P_{x_5}^{\ 3}=(l_5c_5+l_4)c_4,\ P_{y_5}^{\ 3}=(l_5c_5+l_4)s_4,\ P_{z_5}^{\ 3}=l_5s_5 \tag{24}$$

From Equation 24, it is possible to obtain Equation 25 as:

$$\frac{P_{y_5}^{\ 3}}{P_{x_5}^{\ 3}} = \frac{s_4}{c_4} \rightarrow \theta_4 = Atan\,2\left(P_{y_5}^{\ 3}, P_{x_5}^{\ 3}\right) \quad (25)$$

Also, Equation 25 yields Equation 26 and Equation 27.

$$sin\,\theta_5 = \frac{P_{z_5}^{\ 3}}{l_5} \quad (26)$$

$$cos\,\theta_5 = \frac{P_{x_5}^{\ 3} - l_4 c_4}{l_5 c_4} \quad (27)$$

Then from Equation 26 and Equation 27, it is possible to obtain Equation 28.

$$\theta_5 = Atan\,2\left(\frac{P_{z_5}^{\ 3}}{l_5}, \frac{P_{x_5}^{\ 3} - l_4 c_4}{l_5 c_4}\right) \quad (28)$$

3. Results and Discussion

3.1. Torque calculation

Table 2 presents the results of torque calculation explained in Equation 1. In agricultural robots, the speed is of secondary importance. The servo motor's speeds of RAVebots-1 is set to 15 rpm in J_1 and 60 rpm in J_5. Above this speed values, $T_{Dynamic}$ and the inertia increase dramatically. A bigger $T_{Dynamic}$ requires a more powerful power supply in order to control the servo motors. The T_{Static} in J_1 and J_5 is zero, because in the designing process the angle between the total force vector and the perpendicular length from pivot to force is 90°. In other words, the direction of the total force vector is not in the rotation direction. In general, because of the rotation speed, $T_{Dynamic}$ in each joint is not zero. Also, in J_2, J_3 and J_4, T_{Static} is bigger than $T_{Dynamic}$. It is shown, that the effect of T_{Static} is greater than $T_{Dynamic}$. As a conclusion, J_2 needs the most powerful servo motor for the highest torque, and J_5 needs the weakest one.

Table 2- Maximum joints specification in C design

Joint	*Speed (rpm)*	T_{Static} $(N.m)$	$T_{Dynamic}$ $(N.m)$	T_{Total} $(N.m)$	$T_{Total\ (include\ FOS)}$ $(N.m)$
J_1	15	0	5.15	6.15	18.5 (FOS= 3)
J_2	30	253	4.35	257.35	287.3 (FOS= 1.1)
J_3	30	101.9	1.5	103.4	173.5 (FOS= 1.7)
J_4	30	20.1	0.1	20.2	32.9 (FOS= 1.6)
J_5	60	0	0.1	0.1	0.2 (FOS= 2)

The results of the dynamic simulation in SOLIDWORKS showed in Figures 7 and 8 for joints 2 and 3, respectively. It can be seen that the value of total torque is not stable. According to Figure 7, the total torque value varies between 209.5 N.m and -30.2 N.m, and in Figure 8 this parameter changes in the range of 83.3 to 134.3 N.m. Therefore, this erratic parameter requires a logical *FOS* (Factor of Safety). In order to cover unpredictable circumstances like impact and fluctuation, in all design calculations, *FOS* was considered between 1.1 and 2.

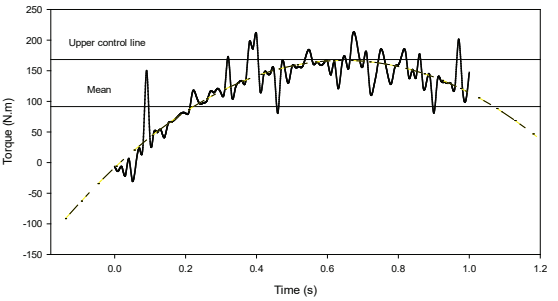

Figure 7- Torque calculation of Joint 2

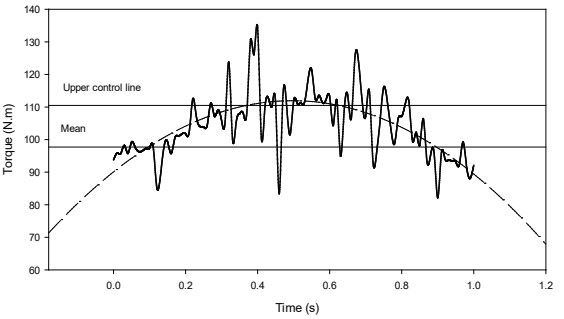

Figure 8- Torque calculation of Joint 3

3.2. The effect of material changes in the required torque

Table 3 illustrates the values of required torque in three different types of conditions (material and servo motor location) for the RAVebots-1. It is evident that the static, dynamic and total torque declines in different conditions, whereas the robot fundamental structure, remained unchanged. In condition A which is the reference condition, all the components were designed

and manufactured by using ASTM A36 steel and all servo motors were installed on their respective joint position. It means that the servo motor number 2 is set in the joint 2, the servo motor number 3 is set in the joint 3, and so on. In condition B, the components material was changed to AL5202. In condition C, not only the component materials were changed to AL5202 but also the positions of servomotors were improved. As shown in Figure 9, all torque values were reduced dramatically from condition A to C. It can be seen that the total torque in J_1, J_2, J_3 and J_4 was reduced from condition A to B due to the change of material. There is no rule for decreasing static torque because of the complex structure of the body. Also, the material changes were done on the main body components, and the joints, bolts and nuts material remained the same. The position of the servo motors changed for the conditions C; giving as a result reductions of total torque in J_1, J_2 and J_3 joints. Because of the RAVebots-1 special structure, the static torque in J_1 and J_5 is equal to zero for all conditions. As a conclusion, adjusting the material of the body and the servo motor location directly affects the torque values.

Table 3- Effect of linkage material changing and servo motor position improving joints torque

Condition	*Torque (N.m)*	I_1	I_2	I_3	I_4	I_5
A	Static	0	634	233.5	33.1	0
	Dynamic	14.48	14	3.1	0.17	0
	Total	15.48	648	236.6	33.27	0
B	Static	0	360.5	134.25	20.1	0
	Dynamic	7.57	8.5	1.95	0.1	0.1
	Total	8.57	369	136.20	20.2	0.1
C	Static	0	253	101.9	20.1	0
	Dynamic	5.15	4.35	1.5	0.1	0.1
	Total	6.15	257.35	103.4	20.2	0.1

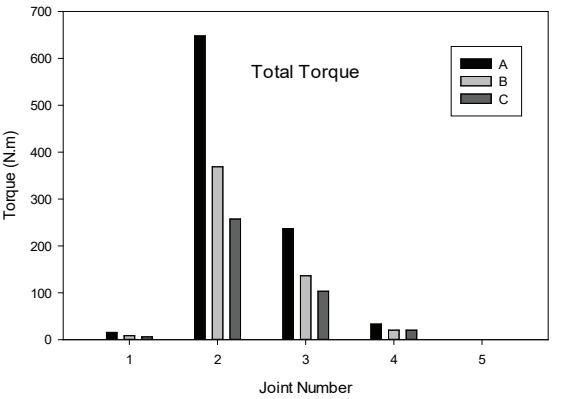

Figure 9- The impact of changing the type of material and the joint position in the total torque

3.3. Validation of forward kinematics

The length of the body links was I_1 = 484 mm; I_2 = 650 mm; I_3 = 600 mm; I_4 = 250 mm and I_5 = 250 mm. The forward kinematics can be used to find the end-effector coordinate of the robot movement by substituting the constant parameters values in Equation 7. The final equation of the end-effector's envelopment for the D-H convention of forward kinematics is listed as follows:

$$\begin{aligned} P_x &= \cos\theta_1\,[0.484 + 0.65\cos\theta_2 + 0.6\,\cos(\theta_2+\theta_3) + 0.25\cos(\theta_2+\theta_3+\theta_4) + \\ &\quad 0.25\cos\theta_5\cos(\theta_2+\theta_3+\theta_4)] + 0.25\sin\theta_1\sin\theta_5] \\ P_y &= \sin\theta_1\,[0.484 + 0.65\cos\theta_2 + 0.6\,\cos(\theta_2+\theta_3) + 0.25\cos(\theta_2+\theta_3+\theta_4) + \\ &\quad 0.25\,\cos\theta_5\cos(\theta_2+\theta_3+\theta_4)] - 0.25\cos\theta_1\sin\theta_5] \\ P_z &= 0.65\sin\theta_2 + 0.6\sin(\theta_2+\theta_3) + 0.25\sin(\theta_2+\theta_3+\theta_4) + \\ &\quad 0.25\cos\theta_5\sin(\theta_2+\theta_3+\theta_4) \end{aligned} \tag{29}$$

In the zero position, the orientation vectors are defined as follows in Equation 30.

$$Zero\ position \xrightarrow{\forall\theta_i,\ 1\le i\le 5,\ \theta_i=0} \vec{n} = \begin{bmatrix}1\\0\\0\end{bmatrix}, \vec{o} = \begin{bmatrix}0\\-1\\0\end{bmatrix}, \vec{a} = \begin{bmatrix}0\\0\\1\end{bmatrix}, \vec{D} = \begin{bmatrix}0\\0\\1\end{bmatrix} \tag{30}$$

Generally, the direction of the orientation vectors in the zero position proves the algorithm validity. It means, in the zero position, the normal vector ($\vec{n}$), the orientation vector ($\vec{o}$) and the approach vector ($\vec{a}$) have to be in the same direction of the axes *X*, *-Y* and *Z,* respectively. Therefore, all of the coordinate frames in Figure 5 were removed except the base, which is the reference coordinate frame for determining the link parameters in zero position. The zero position is necessary to choose a home position. The home position is the initial position of the arm and it can be any arbitrary position within the workspace. However, it is better to have a defined home position as a reference point to start the algorithm run.

4. Conclusions

Based on experimental results, one can conclude the factors that affected arm performance in the mentioned robotic design are the selection of material, torque optimization analysis (utilizing appropriate techniques), selecting optimized algorithms, and using adequate speed control for servo motors. Addressing design challenges and working through such challenges provides the opportunity to achieve the best possible produce. Findings based on experimental results are summarized below:

1. The results of kinematic calculation show that the final developed algorithm worked effectively. The presented algorithm establishes a smooth curve to move and reach the target point rapidly.
2. The presented strategy for material improvement and heavy components modification has positive results on maximum payload, mass center position, and total components weight. Also, it improved the servo motor's required torque more effectively. The Solidworks simulation results and the detailed mass effect on required torque for situation A, B, and C confirm this conclusion.
3. In the mentioned rotation speed for each join, the results show that RAVebots-1's reaction velocity varies from 1.18 to 1.68. This is the velocity between a specified home position and the maximum front position, located in 1640 mm from the main joint.
4. According to the RAVebots-1 workspace simulation, it is possible to expand the robot arm application for horticulture usage e.g. fruit picking, cutting the tree branches, cover the fruits and precision spraying.

Hopefully, in the future, the RAVebots-1 design will be produced and utilized in everyday agricultural practices, especially for harvesting heavy crops, as well as picking other crops. This robotic arm will be capable of harvesting by using a camera and a specially designed end-effector. The robot will be capable of collecting physical data of the crops (weight, volume, density, etc.), harvesting crops, and then depositing the crops into a designated location

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References

Angeles J (1997). Fundamentals of Robotic Mechanical Systems. Theory, Methods and Algorithms. Springer, New York

Balkan T, Özgören M K, Sahir Arıkan M A & Baykurt H M (2000). A method of inverse kinematics solution including singular and multiple configurations for a class of robotic manipulators. *Mechanism and Machine Theory* **35**(9): 1221-1237

Barawid Jr O C, Mizushima A, Ishii K & Noguchi N (2007). Development of an Autonomous Navigation System using a Two-dimensional Laser Scanner in an Orchard Application. *Biosystems Engineering* **96**(2): 139-149

Cassinis R & Tampalini F (2007). AMIRoLoS an active marker internet-based robot localization system. *Robotics and Autonomous Systems* **55**(4): 306-315

Craig J J (1989). Introduction to Robotics Mechanics and Control. USA:Addision-Wesley Publishing Company

De-An Z, Jidong L, Wei J, Ying Z & Yu C (2011). Design and control of an apple harvesting robot. *Biosystems Engineering* **110**(2): 112-122

Denavit J & Hartenberg R S (1955). A kinematic notation for lower-pair mechanisms based on matrices. *Journal of Applied Mechanics* **1**: 215-221

Funda J, Taylor R H & Paul R P (1990). On homogeneous transforms, quaternions, and computational efficiency. *IEEE Transactions on Robotics and Automation* **6**: 382-388

Ghazvini M (1993). Reducing the inverse kinematics of manipulators to the solution of a generalized eigenproblem. In: J Angeles, G Hommel, Kovács (Eds), *Computational Kinematics, Solid Mechanics and its Applications* **28**: 15-26

Hayashi S, Shigematsu K, Yamamoto S, Kobayashi K, Kohno Y, Kamata J & Kurita M (2010). Evaluation of a strawberry-harvesting robot in a field test. *Biosystems Engineering* **105**(2): 160-171

Karlik B & Aydin S (2000). An improved approach to the solution of inverse kinematics problems for robot manipulators. *Engineering Applications of Artificial Intelligence* **13**(2): 159-164

Kondo N, Monta M & Fujiura T (1996). Fruit harvesting robots in Japan. *Advances in Space Research* **18**(1-2): 181-184

Lee H-Y & Liang C-G (1988). Displacement analysis of the general spatial 7-link 7R mechanism. *Mechanism and Machine Theory* **23**(3): 219-226

MAFF (2016). TPP or no, aging farm sector needs true reform. [Online] Available: http://www.maff.go.jp/e/index.html.

Raghavan M & Roth B (1990). Inverse kinematics of the general 6R manipulator and related linkages. Transactions of the ASME, *Journal of Mechanical Design* **115**: 228-235

Richard B & Keith N (2006). Shigley's Mechanical Engineering Design. McGraw-Hill Publishing Company

Satoru G (2011). ROBOT ARMS. InTech publisher, ISBN 978-953-307-160-2

Serdar K & Zafer B (2006). Industrial robotics theory modelling and control. Pages 964 in C. Sam, ed

Siciliano B & Khatib O (2008). Handbook of Robotics, Springer publisher, ISBN 978-3-540-23957-4

Tanigaki K, Fujiura T, Akase A & Imagawa J (2008). Cherry-harvesting robot. *Computers and Electronics in Agriculture* **63**(1): 65-72

Tanner H G, Kyriakopoulos K J & Krikelis N I (2001). Advanced agricultural robots: kinematics and dynamics of multiple mobile manipulators handling non-rigid material. *Computers and Electronics in Agriculture* **31**(1): 91-105

USDA (2015). Farm Demographics - U.S. Farmers by Gender, Age, Race, Ethnicity, and More, United States Department of Agricultur (onile article), https://www.agcensus.usda.gov/Publications/2012/Online_Resources/Highlights/Farm_Demographics

Wang S-C, Hikita H, Kubo H, Zhao Y-S, Huang Z & Ifukube T (2003). Kinematics and dynamics of a 6 degree-of-freedom fully parallel manipulator with elastic joints. *Mechanism and Machine Theory* **38**(5): 439-461

Yahya S, Moghavvemi M & Mohamed H A F (2011). Geometrical approach of planar hyper-redundant manipulators: Inverse kinematics, path planning and workspace. *Simulation Modelling Practice and Theory* **19**(1): 406-422

Zion B, Mann M, Levin D, Shilo A, Rubinstein D & Shmulevich I (2014). Harvest-order planning for a multiarm robotic harvester. *Computers and Electronics in Agriculture* **103**: 75-81

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Developing a Machine Vision System to Detect Weeds from Potato Plant

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ABSTRACT

Potato is one of the widely used products all over the world that has numerous nutritional properties. Similar to other crops, different weeds grow along with potatoes in agricultural fields. These weeds reduce the performance of crops due to competing with them to absorb water, light, and nutrients from soil. Accordingly, in this study, a machine vision system with the hybrid artificial neural network-ant colony algorithm (ANN-ACO) classifier was developed for a site-specific spraying considering the weed type. Potato plant and three weed types including *Chenopodium album*, *Polygonum aviculare* L., and *Secale cereale* L. were used in this study. A digital camera (SAMSUNG WB151F (CCD, 14.2 MP, 30f/s) was placed in the center of the video acquisition system. The distance between plants and the digital camera was fixed at 40 cm. For video acquisition, only lamps of white LED with a light intensity of 327 lux were selected. For filming in order to evaluate the proposed system, a 4-hectare area of Agria potato fields in Kermanshah-Iran (longitude: 7.03°E; latitude: 4.22°N) was selected. Employing the Gamma test, among 31 features, 5 features (Luminance and Hue corresponding to YIQ color space, Autocorrelation, Contrast, and Correlation) were selected. The correct classification accuracy for testing and training data using three classifiers of the hybrid ANN-ACO, radial basis function (RBF) artificial neural network, and Discriminant analysis (DA) was 99.6% and 98.13%, 97.24% and 91.23%, and 69.8% and 70.8%, respectively. The results show that the accuracy of DA statistical method is much lower than that of the hybrid ANN-ACO classifier. Consequently, the results of the present study can be used in machine vision system for the optimum spraying of herbicides.

Keywords: Classification; Machine vision; Gamma test; Precision farming; Site-specific spraying

Patates Bitkisinde Yabani Otları Belirlemek için Yapay Görme Sisteminin Geliştirilmesi

ESER BİLGİSİ

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ÖZET

Patates, tüm dünyada yaygın olarak kullanılan ürünlerden birisi olup sayısız besleyici özelliklere sahiptir. Tarım arazilerinde diğer bitkilerde olduğu gibi pateteslerle birlikte farklı yabani otlar da yetişmektedir. Bu yabani otlar; su, ışık

ve topraktaki besin maddeleri için ana ürünle rekabete girerek bitkilerin büyüme performansını düşürürler. Bu nedenle çalışmada, yabani ot tipini göz önüne alarak bölgeye özel püskürtme yapan, yapay sinir ağı-karınca koloni algoritması (ANN-ACO)'ndan oluşan hibrit sınıflandırıcıya sahip bir yapay görme sistemi geliştirilmiştir. Bu çalışmada patates bitkisi ile *Chenopodium album*, *Polygonum aviculare* L. ve *Secale cereale* L. olmak üzere üç yabancı ot çeşidi kullanılmıştır. Video çekim sisteminin merkezine bir dijital kamera (SAMSUNG, WB151F (CCD, 14,2 MP, 30f/s) yerleştirilmiştir. Bitkiler ve dijital kamera arasındaki mesafe 40 cm olarak sabitlenmiştir. Video çekimi için yalnızca 327 lux ışık yoğunluğundaki beyaz LED lambaları seçilmiştir. Önerilen sistemi değerlendirmek üzere filme almak için Kermanshah-Iran'da (boylam: 7.03°E, enlem: 4.22°N), bir Agria patates tarlasının 4 hektarlık alanı seçilmiştir. Gamma testi uygulanarak, 31 özellik arasından 5 özellik (YIQ renk uzayına karşılık gelen parlaklık ve renk tonu, Otomatik korelasyon, Kontrast ve Korelasyon) seçilmiştir. Hibrit ANN-ACO, radyal esas fonksiyonlu (RBF) yapay sinir ağı ve Diskriminant analizi (DA) içeren üç sınıflandırıcı kullanılarak yapılan test ve eğitme verileri için düzgün sınıflandırma doğruluğu değerleri sırasıyla % 99.6 ve % 98.13, % 97.24 ve % 91.23, % 69.8 ve % 70.8'dir. Sonuçlar, DA istatistiksel yönteminin doğruluğunun, hibrit ANN-ACO sınıflandırıcısından çok daha düşük olduğunu göstermiştir. Sonuç olarak, sunulan çalışmanın sonuçları herbisitlerin en uygun şekilde püskürtülebilmesi için yapay görme sisteminde kullanılabilir.

Anahtar Kelimeler: Sınıflandırma; Yapay görme; Gamma testi; Hassas tarım; Bölgeye özel püskürtme

1. Introduction

The number and type of weeds increase in agricultural fields proportional to the increase in the area under cultivation of crops and crop diversity (Mursalin et al 2013). These weeds deteriorate the performance of crops due to competing with them to absorb water, light, and soil nutrients. Among the most important methods recently applied methods by farmers for weed exclusion weeds are the manual and mechanical methods and the use of herbicides. The time-consuming weeds control operations and presence of weeds and crops together are the most significant limitations of manual and mechanical methods, respectively (Mursalin et al 2013). Owing to the mentioned limitations, herbicides have been currently used in weeds exclusion. However, the use of herbicides as traditional uniform distribution in the whole of the agricultural field has led to groundwater pollution, crops poisoning, and environmental pollution (Mursalin et al 2013). Applying precision agriculture technology and herbicides with variable rates will provide the possibility of cutting down the costs and preventing these problems (Bossu et al 2008). The machine vision systems are among the technologies that can be used for this purpose. These systems usually have two main parts: 1) video acquisition, pre-processing, and features extraction and 2) the analysis and classification of extracted features. Generally, researchers have recently focused on identifying weeds using static images of the agricultural fields or greenhouses (Zhao et al 2009). The classification of different weed types and crops using computer vision and artificial neural networks has been considered among researchers (Zhao et al 2009). In this regard, Zhao et al (2009) classified three weed types, namely *Stellaria media*, *Celosia arentia*, and *Cephalanoplossetosum*, by applying the color principal component analysis (PCA). Their proposed method consists of two stages: 1) imaging of these three weed types in the laboratory (250 color images) and 2) dimensionality reduction using color PCA and classification. In the first step, the properties of three-dimensional color tensor were used to produce a vector space dimensionality reduction, and then the color PCA was utilized in dimensionality reduction. In the second step, the minimum Euclidean distance classifier was applied to recognize different weeds. The results showed that the accuracy of color PCA method is 4.4% higher than that of the conventional PCA that is 80.8%. Because this research was a laboratory-based work and, it did not yield practical results in the real conditions of agricultural fields. Mursalin et al (2013) classified five weed types including *Capsicum*, *Burcucumber*, *Cogongrass*, *Marsh herb*, and *Pigweed* using three Naïve Bayes, SVM, and C4.5 classifiers. They captured 400 images (80

images for every weed type) using a digital camera fixed 40 cm above ground and perpendicular to it. After pre-processing, nine features, namely Area, Perimeter, Convex Perimeter, Convex Area, Thickness, Solidity, Convexity, Form Factor, and Elongatedness were extracted from the weeds. The results indicated that Naïve Bayes classifier had the highest accuracy (99.3%) among these classifiers. Although the obtained accuracy was high for this database, it would not be achieved in weeds online identification in agricultural fields for two reasons: 1) a low number of samples in the database and 2) presence of frames with incomplete weeds and blurry frames in the provided videos.

Several researchers such as Bossu et al (2008) and Liu et al (2014) conducted some investigations to detect plant rows and weeds between these rows. Most of these systems used color characteristics to detect crop rows, assuming all plants between two neighboring rows as weeds. Montalvo et al (2013) demonstrated that automatic plant identification in agricultural fields based on imaging sensors is a big challenge. They proposed an automated expert system to identify weeds from corn in cornfields. The database utilized in their study consists of 230 images taken from the field in natural light conditions. Their proposed expert system has three stages: 1) the computation of vegetation indices and the use of the first threshold, 2) selecting black pixels and applying the second threshold, and 3) morphological operations and identification of masked and unmasked plants. In their research, the classification accuracy was achieved 89.9% using support vector machine. In this method, the plants placed between two corn rows were classified as the weed; thus, if the aim of weed recognition is spraying proportional to the type of weeds, this method is not practical. The major limitation of this method for weeds online identification is the small database size to train classification algorithm. Chowdhury et al (2015) presented a new texture feature based on the stable expert system to identify roadside vegetation. The database included 110 images in natural light conditions. From this database, 60 images corresponded to dense grass and the remaining 50 images with sparse grass. The proposed system included five steps of image pre-processing, feature extraction, training with classification, classification, and validation; and eventually statistical analysis to classify these two weed types. Applying the co-occurrence of binary pattern method, the extracted texture features corresponded to the images of vegetation. In the step of training and classification, three classifiers were exploited to combine the multiple decisions. These classifiers are supported vector machine, feed forward back-propagation neural network, and nearest neighbor. The overall classification accuracy after applying these three classifications was 92.72%. Complete training is one of the main conditions for the success of each classification algorithm. In their study, only 90 images for training and 20 images for testing were used to identify weeds in the field by the algorithm that would face significant challenges.

Wavelets were also utilized in the field of classification and segmentation by some researchers. For example, Chen et al (2011) presented a new method, using Gabor wavelets and lie group structure of region covariance to classify broadleaf weed images on Riemannian manifolds. In their study, 320 images were used from four different weed types, namely *Oxaliscorniculata* L., *Duchesneaindica (Andrews) Focke*, *Herba Glechomae* L., and *Ixerischinensis (Thunb.) Nakai* for analys. The images were captured under natural light conditions by a digital camera. The distance between the ground and the digital camera was fixed at 50 cm, and the viewing angle of the lens was approximately adjusted horizontally. The optimal multiresolution Gabor wavelets were employed to decompose images into texture features, and lie group structure of region covariance was used to extract the filtered image features on Riemannian manifolds. K-nearest neighbor method was applied to classify four above-mentioned weed types. The results showed that the total accuracy classification is 93.13%. Video camera must be used for the majority of weeds online recognition in the agriculture field. Although the machine vision algorithm must be able to recognize several weeds in each frame, there was only one object in every frame in their research. Consequently, the method proposed in their study

was not practical in online recognition application. As pointed out in this section, a great number of researchers have focused on the application of image processing for weeds classification, which is not practical in online identification based on the aforementioned reasons. In this regard, videos are analyzed to recognize weeds online identification using machine vision.

The aim of the current research is developing a machine vision system to classify potato plant and three weed types of *Chenopodium album*, *Polygonum aviculare* L., and *Secale cereale* L. based on video processing and the hybrid neural networks-ant colony (ANN-ACO) classifier.

2. Material and Methods

To design a machine vision system, several steps are necessary. Two parts in each machine vision system are most important; i.e., segmentation and classification. Hence, it is essential to find the suitable color space and threshold related to segmentation and suitable classifier related to classification. The methodology applied in this work is as follows:

1) collect the required data to train machine vision system; 2) find the best color space to segmentation; 3) extract different features from each object; 4) select effective features among extraction features to train of the classifier; and 5) apply different classifiers to select the best classifiers. After these steps, a machine vision system is proposed based on video processing to classify potato plant and three weed types of *Chenopodium album*, *Polygonum aviculare* L., and *Secale cereale* L.

2.1. Video acquisition

In the present research, some videos were taken from one Agria potato fields with four hectares area in controlled light conditions in Kermanshah–Iran (longitude: 7.03°E; latitude: 4.22°N). This field had three different weed types, tabulated with their corresponding names and varieties in Table 1. Figure 1 demonstrates images of potato plants and these three weed types. A chamber was designed and manufactured for video recordings with a speed of 0.145 ms^{-1} (Figure 2). The chamber light was provided by white LEDs with a light intensity of 327 lux. Videos were taken using SAMSUNG WB151F (CCD, 14.2 MP, 30f/s) camera. A computer with hardware of Intel Core i3 CFI i3 330M 2.13 GHz, RAM-4GB and 32 Bit equipped with MATLAB 2014 (a) and Microsoft office 2013 was used for analyzing. The distance between the camera and the plants was fixed as 40 cm. A tripod attached to the chassis along with rubber wheels was used to prevent the camera from shaking. Since the camera is moving in the field and each moment new details of plants are detected, the frames have to be analyzed individually.

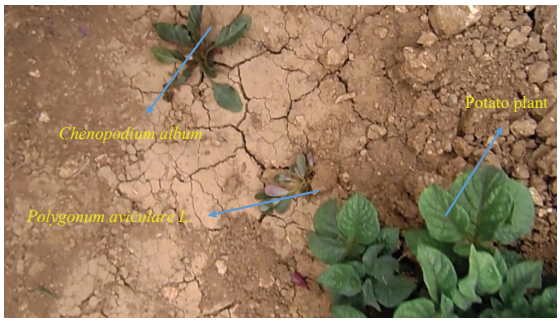
2.2. Pre-processing and segmentation

In the first stage of pre-processing and analysis of the taken films, they must be converted to their frames. This task was performed by a code written in Matlab software. The camera utilized in this study captured 30 frames per second, and videos were converted to their constructive frames for image analysis. The first and the most important step for image analysis is segmentation, which is generally divided into two parts. The first part associated with the background separation from green plants while the second part is related to separation parts of plants from each other. After surveying the different color models such as RGB, HSV, HIS, YIQ, CMY, and YCbCr, the RGB model were selected for the first part of segmentation. The main cause for selecting the RGB model is that after the first part of segmentation in RGB color space, the image noise

Table 1- Three different weed types in potato fields

Name	*Weeds*		
English name	Knot weed	Rye	Common lambsquarters
Scientific name	*Polygonum aviculare* L.	*Secale cereale* L.	Chenopodium album

(a)



(b)

Figure 1- Different weeds types and potato plant; (a), *Secale cereale* L. and potato plant; (b), *Polygonum aviculare* L., *Chenopodium album* and potato plant

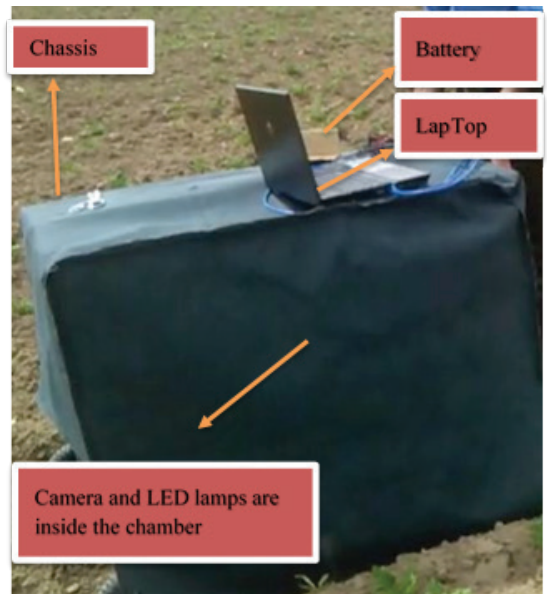


Figure 2- The specific chamber used for video recordings

was less compared to that of other color spaces. Because frames may include several green plants corresponding to different species, each object (integrated pixels in each frame are called object) must be analyzed separately. For this reason, the Bounding Box was used. Figure 3 shows a sample of the first part of segmentation. At the top of the frame, the number of detected objects (which is 4 in this frame) is shown. Each object is identified by the coordinates of the center of mass (x and y). In order to extract the shape features, the segmented images must be converted into binary images. Figure 4 depicts the frame conversion steps to improved binary image. Figure 4 (a) demonstrates an extracted frame from a video. Besides, Figure 4 (b) illustrates the segmented image corresponding to this frame that was obtained by Equation 1. In fact, Equation 1 classifies a pixel as plant if its Green component is greater than its Red or Blue components.

$$R(i,j)G(i,j) \mid B(i,j)G(i,j) \quad (1)$$

The binary image in Figure 4 (c) presents several noises and holes, which must be removed by morphological processing. The morphological filter

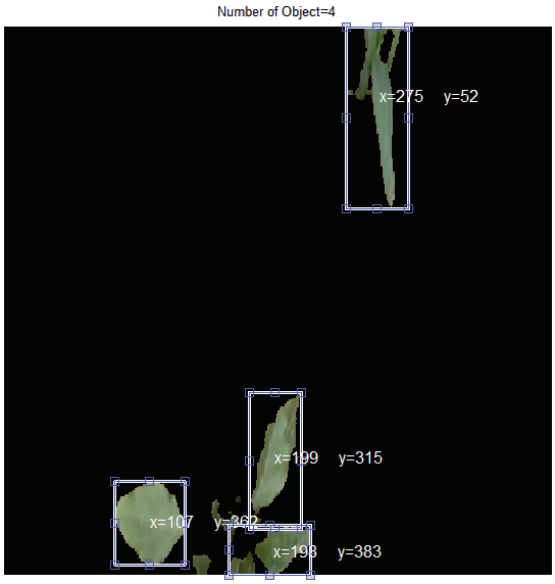


Figure 3- The second part of segmentation of green plants in a frame

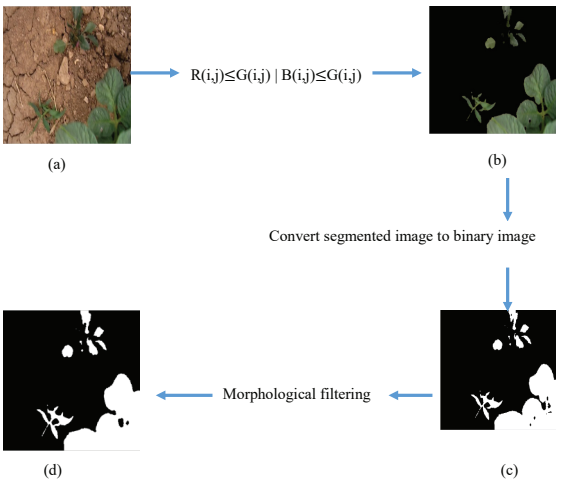


Figure 4- The proposed algorithm to convert color image to binary image; (a), original image; (b), segmented image; (c), binary image; (d), improved binary image

employed in this study was Closing. The Closing filter is a combination of two Dilation and Erosion operations that soften the contours of the object. This operation also leads to a connection between thin broken components and thin holes filling smaller than a structural member (Gonzalez et al 2004). Figure 4 (d) shows the improved binary image. After pre-processing operations on all frames, 3376 objects were extracted from the film taken. A total of 3376 objects corresponding to a 60-seconds video taken in the potato field were divided into two sets; training data with 2361 objects (all taken in 40 seconds) and testing data with 1015 objects (all taken in 20 seconds). Because the number of samples was not similar for all classes, all samples from each class were divided into two groups randomly: 1) training data (70% of all samples) and 2) testing data (30% of all samples). Thus, in the final training and testing data, there were 70% and 30% of samples related to each class.

2.3. Extracting features

Feature extraction is one of the most important tasks in machine vision. For this reason, 31 features were extracted from texture features based on the gray level co-occurrence matrix (GLCM), texture descriptors based on the histogram, color features, moment invariants, and shape features.

2.3.1. Texture features based on the gray level co-occurrence matrix (GLCM)

The gray level co-occurrence matrix includes information about the position of pixels with similar grayscale values. In this study, 10 features corresponding to the gray level co-occurrence matrix were extracted. Auto-correlation, contrast, correlation, difference entropy, difference variance, mean correlation 1, mean correlation 2, inverse difference, normalized inverse difference, and normalized inverse difference moment were textural extracting features. The gray level co-occurrence matrix must be normalized before introducing these features (Marques 2011).

2.3.2. Texture descriptors based on histogram

One of the most important methods to describe an area is the use of texture descriptors based on the histogram. Due to the textural difference among potato plants, *Secale cereale* L., *Polygonum aviculare* L., and *Chenopodium album*, these descriptors may be useful for classifying these four plant types. For this reason, two homogeneity and entropy features were used in this study (Gonzalez et al 2004).

2.3.3. Color features

The color models are used to determine color specifications in a standard, which is usually acceptable, method. In this study, three color spaces of YCbCr, HIS, and YIQ were utilized to extract their components (Gonzalez et al 2004).

YCbCr color model: This color model is used in digital images. In this space, the information of luminance is saved in Y, Cb, and Cr, which are the blue and red chroma components (Gonzalez et al 2004).

HSI color model: HIS color model was formed by applying Hue, Saturation, and Intensity components.

YIQ color model: YIQ color model consists of three Luminance, Hue, and Saturation components.

2.3.4. Moment invariants

Moment invariants are two-dimensional moments from the (p+q) degree that is applied to f(x,y) images with the dimensions of M×N. These moments are insensitive to transfer, congruency, reflection, and rotation. For this reason, moment invariants were employed in the present study. In the agricultural field, the leaves of potato plants and weeds have different sizes and orientations; therefore, moment invariants may be useful for classification purposes.

2.3.5. Shape features

Considering the difference in forms of the potato leaves, *Secale cereale* L., *Polygonum aviculare* L., and *Chenopodium album* plants, shape features may be useful parameters for classification purposes. Hence, in this study, eight features of Eccentricity, Orientation, ConvexArea, FilledArea, EulerNumber, EquivDiameter, Solidity, and Extent were extracted.

2.4. Selecting effective features

Selecting effective features is the main step for classification, which was done using Gamma test in the present work. Because Gamma test is a simple form of error deviation, it is applied to determine optimal inputs. This test indicates the estimated error, using direct data (Noori et al 2011). In this study, SPSS software was applied to perform Gamma test (Table 2). The results show that five variables including Auto-correlation, Contrast, Correlation, Luminance, and Hue corresponding to YIQ color space have lower Gamma values, and thus they are used as the best classification inputs.

2.5. Classification

In the present investigation, three classification methods of Discriminant analysis using SPSS statistical software, radial basis function (RBF) artificial neural network, and the hybrid of artificial neural network-ant colony (ANN-ACO) were tested for classification.

Table 2- The result of Gamma test performed on extracted features

Feature	*Gamma coefficient*	*Feature*	*Gamma coefficient*
Autocorrelation	-0.001	H	0.518
Contrast	-0.0012	S	0.291
Correlation	0.0015	I	-0.311
Difference variance	-0.234	Y	-0.0019
Difference entropy	-0.197	I	-0.0014
Mean correlation 1	-0.151	Q	-0.223
Mean correlation 2	0.137	Sixth moment invariant	0.186
Inverse difference	0.145	Seventh moment invariant	-0.018
Inverse difference normalized (INN)	0.18	Eccentricity	-0.002
Inverse difference moment normalized	0.231	Orientation	0.047
Homogeneity	0.085	ConvexArea	0.249
Entropy	0.05	FilledArea	0.234
Y	-0.281	EulerNumber	-0.71
Cb	-0.097	EquivDiameter	0.234
Cr	-0.305	Solidity	-0.338
Extent	-0.124		

2.5.1. The hybrid of artificial neural network-Ant Colony Algorithm

To optimize multilayer perceptron (MLP) artificial neural networks, five parameters, namely the number of neurons, the number of layers, transfer function, back-propagation network training function, and back-propagation weight/bias learning function must be optimized, which was done using ACO in this study (Sen & Mathur 2016). Table 3 also demonstrates the optimal values of MLP neural network. Figure 5 show the architecture of the ANN optimized using ACO and the criteria to stop learning process. Finally, Figure 6 presents a flow diagram for classification of four different types of plants using a video processing approach.

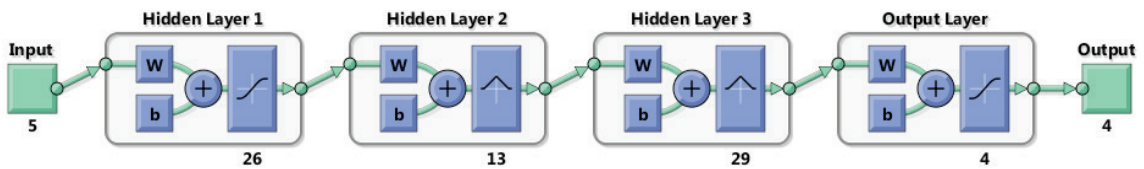
3. Results and Discussion

3.1. Image segmentation

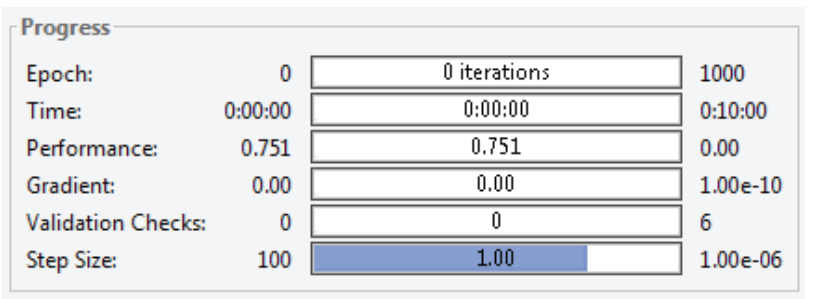
Figure 7 presents the segmentation results of RGB, YIQ, and HSI models and Figure 7 (b) shows the results of YIQ model. As one can see, some parts of plants were considered as background in this segmentation. The HSI model results were shown in Figure 7 (c). Due to the high noise shown in the figure for this model, it would not have the ability of segmentation. Finally, the results of RGB model are given in Figure 7 (d). Evidently, this model had done segmentation thoroughly; therefore, the rest of the analysis would be based on this model. This program performs the first segmentation part

Table 3- The optimized value to classify using ANN-ACO

The number of neuron:	The first layer: 26, the second layer: 13, the third layer: 29
The number of layer:	3
Transfer function:	The first layer: tansig, the second layer: tribas, the third layer: tribas
Backpropagation network training function:	Trainlm
Backpropagation weight/bias learning function:	Learngdm



(a)



(b)

Figure 5- (a), architecture of optimized ANN using ACO and (b), the criteria to stop learning process

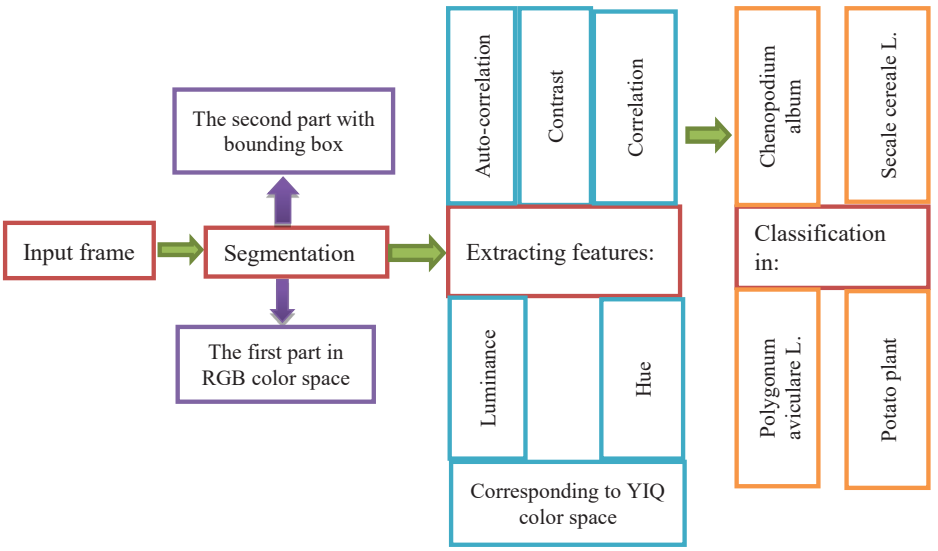


Figure 6- A flow diagram for classification four different types of plants

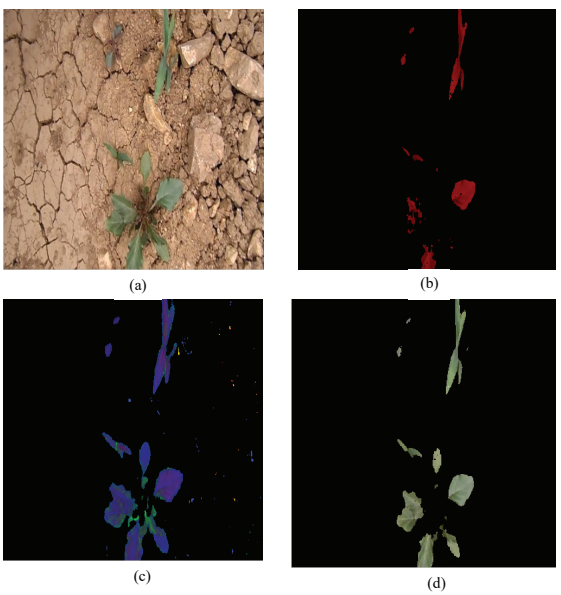


Figure 7- The different color models to segment; (a), original image; (b), YIQ color model; (c), HSI color model, and (d), RGB color model

using Equation 1; thus, it removes the parts pixel number less than 250 and considers the remaining parts as objects. Since the camera was moving in the agricultural field, each frame would present only a part of the plant. To reduce computing time and avoid possible errors, only the objects with pixel numbers higher than 250 were selected.

3.2. Classifying using the hybrid artificial neural network-Ant Colony Algorithm (ANN-ACO)

Figure 8 illustrates a comparison between the actual data and the predicted data, using the artificial neural network (ANN). The horizontal and vertical axes were consistent with the number of samples and the number of classes respectively. In this figure, the blue circles and the red stars correspond to actual data and the predicted data, respectively. Clearly, in most cases circles and stars had similar coordinates, implying that the ANN is trained correctly. Figure 8 (b) shows a magnified portion of Figure 8 (a). This finding proves that *Secale cereale* L. weed had more differences with other classes regarding Auto-correlation, Contrast, Correlation, Luminance, and Hue corresponding to YIQ color space features. Table 4 and Figure 9 present the classification of the testing data using the hybrid ANN-ACO. Each class was shown by a special color at the bottom of the diagram in Figure 9. This diagram demonstrates

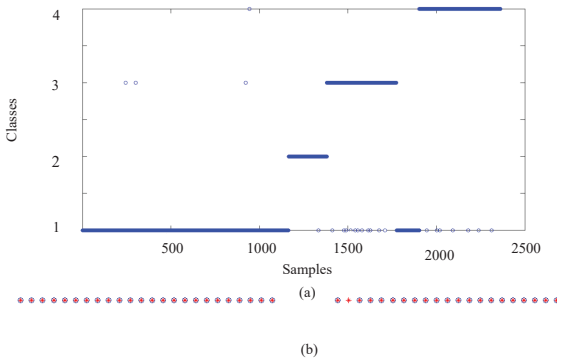


Figure 8- Comparison between real data and estimated data by ANN-ACO

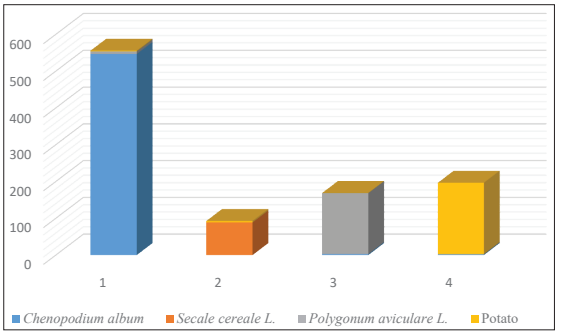


Figure 9- Classification of test data by ANN-ACO

that 547 samples corresponding with *Chenopodium album* were correctly classified while nine samples (seven samples as *Polygonum aviculare* L. and two samples as potato plant) are misclassified. The second diagram has only two colors: orange and yellow. The orange color matches *Secale cereale* L. plant (89 samples) that was classified accurately while the yellow color corresponds with potato plant (four samples) that was misclassified as *Secale cereale* L.. The third diagram represents *Polygonum aviculare* L. class. As it is evident, there are only two blue and yellow colors in this diagram, except for the gray color that matches *Polygonum aviculare* L. class. The blue color signifies *Chenopodium album* in which seven samples were misclassified as *Polygonum aviculare* L.. Moreover, one sample of potato plant was misclassified in this class. Ultimately, out 169 *Polygonum aviculare* L. samples, 166 ones were classified correctly. In the fourth diagram, which corresponds to potato plant class, there are three misclassified samples among 197 plants. Among these three samples, two samples represent *Chenopodium album* and one sample signifies *Polygonum aviculare* L.. Furthermore, in the testing data, all types of plants were incorrectly classified as *Secale cereale* L.. This finding proves that *Secale cereale* L. weed had more differences with other classes regarding Auto-correlation, Contrast, Correlation, Luminance, and Hue corresponding to YIQ color space features. Figure 10 shows the classification results of the hybrid ANN-ACO in six different frames. In this figure, Ch, pot, S, and polrefer to *Chenopodium* album, a potato plant, *Secale cereal*e L., and *Polygonum aviculare* L., respectively.

3.3. The classification using Radial Basis Function (RBF)

In addition to the hybrid ANN-ACO classifier, RBF neural network was also used to classify potato plant and three weed types, including *Chenopodium album*, *Polygonum aviculare* L., and *Secale cereale* L. based on the video processing applied in this research.

Table 4- The classification results related to testing data using ANN-ACO classifier. 1) *Polygonum aviculare* L.; 2) *Secale cereale* L., 3) *Chenopodium album*, and 4) potato plant

Classes	*1*	*2*	*3*	*4*	*All data*	*The percentage of incorrect classification (%)*	*The percentage of general classification (%)*
1	547	0	7	2	556	1.62	98.13
2	0	89	0	4	93	4.31	
3	3	0	166	0	169	1.77	
4	2	0	1	194	197	1.52	

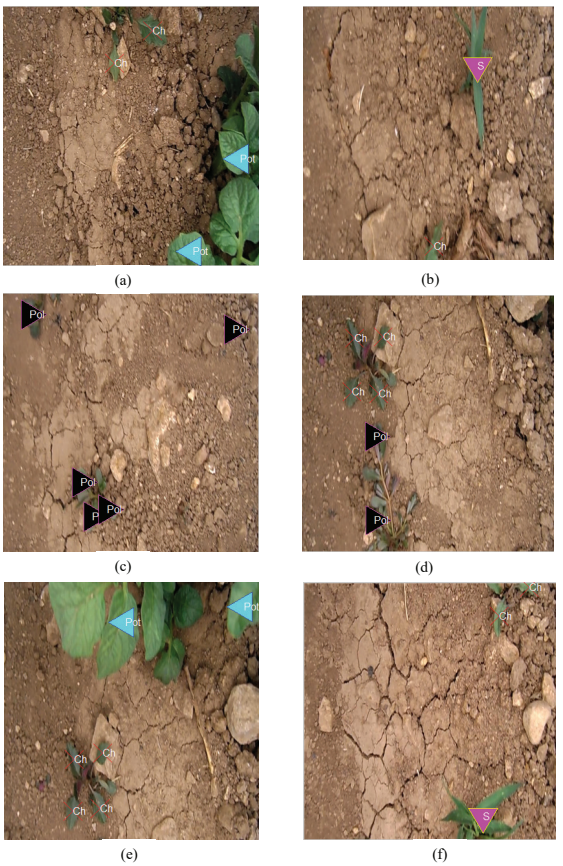


Figure 10- Identification of potato plant and three different weed types; (a), potato plant and *Chenopodium album*; (b), *Secale cereale* L. and *Chenopodium album*; (c), *Polygonum aviculare* L.; (d), *Chenopodium album* and *Polygonum aviculare* L.; (e), potato plant and *Chenopodium album*; (f), *Secale cereale* L. and *Chenopodium album*

Figure 11 indicates the testing data classification results using RBF. There are four classes shown as four diagrams in this figure. Each class in this figure is marked by a specific color. The first diagram corresponds to *Chenopodium album* class. There are different colors in this diagram, which means that the samples of other classes were misclassified in this class. Moreover, 33 samples were classified as *Chenopodium album* incorrectly. From these 33 samples, two samples correspond to *Secale cereale* L., 23 samples to *Polygonum aviculare* L., and eight samples to potato plant. The second diagram, which corresponds to *Secale cereale* L. class, show that the samples of *Chenopodium album* (12 samples) and *Polygonum aviculare* L. (one sample) were misclassified in this class. In addition, 22 samples were misclassified in *Polygonum aviculare* L. class. Among these 22 samples, 20 samples were corresponding to *Chenopodium album*, 1 sample to *Secale cereale* L., and the remaining one sample to potato plant. Finally, 15 samples correspond with *Chenopodium album*, two samples with *Secale cereale* L., and three samples to *Polygonum aviculare* L. were misclassified in potato plant class. By comparing the last two sections, it can be concluded that the various methods of artificial intelligence with same inputs provide different results, therefore, selecting an effective method for classification is of high significance.

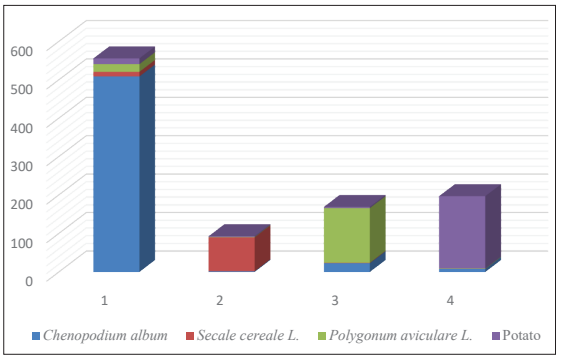


Figure 11- Classification of test data by RBF neural network classifier

3.4. Classification using discriminant analysis statistical method

Discriminant analysis statistical method was also applied to classify potato plant and *Chenopodium album, Polygonum aviculare* L. and *Secale cereale* L. in this research. This statistical method conducted to detect the accuracy performance of the hybrid ANN-ACO. Figure 12 illustrates the results of testing classification using Discriminant analysis method. The first diagram is the combination of four colors, implying that the data corresponding to *Chenopodium album* were misclassified using

Discriminant analysis method; as 31.7% of all samples corresponding to *Chenopodium album* were misclassified. The second diagram indicates that 10.7% of all samples were misclassified as *Secale cereale* L. weed. The third diagram shows that only 0.7% of all data were misclassified as *Polygonum aviculare* L. weed. Finally, the fourth diagram reveals that 83.8% of potato plant samples were classified correctly. The overall classification accuracy of testing data was 70.8%. The results confirmed that the accuracy of Discriminant analysis statistical method was lower than that of artificial intelligence methods. In most cases, statistical methods assume that the data have a normal distribution, and the truth or falsity of the results depends on this initial assumption. In contrast, machine-learning methods do not use any assumptions about data, and this is the case led to the differences between these two methods. Hence, as the results indicated, Discriminant analysis statistical is not a suitable method to classify the data used in this study.

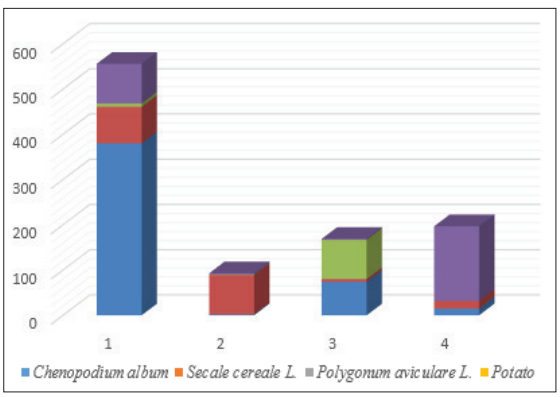


Figure 12- Classification of testing data by Discriminant analysis classifier

3.5. The performance comparison of two ANN-ACO and RBF classifiers

In general, classifiers were evaluated using three criteria of sensitivity, specificity, and accuracy was applied, where sensitivity is a fraction of the samples that are correctly classified; specificity is a fraction of the samples that are classified by the system; and accuracy is the total classification rate of the classifier. These three criteria are calculated using Equations 2-4 (Liu et al 2015).

$$Sensitivity = \frac{TP}{TP+FN} \quad (2)$$

$$Specificity = \frac{TN}{FP+TN} \quad (3)$$

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (4)$$

Where; TP is the number of studied class samples correctly identified as studied class; TN is the number of other classes samples correctly identified as other classes; FN is the number of studied class samples incorrectly identified as other classes; and FP is the number of other classes samples incorrectly identified as studied class (Wisaeng 2013). Table 5 presents the results corresponding to these three criteria. The table also demonstrates that the sensitivity of all classified classes using ANN-ACO is over 90%. For example, the sensitivity of *Chenopodium album* is 99.77%, suggesting that the system has correctly identified *Chenopodium album* weeds by 99.77%. The accuracy of ANN-ACO classifier for all classes is over 99.6%. For instance, the accuracy of *Secale cereale* L. is 99.96%, denoting that this classifier system has correctly classified other plants in addition to *Secale cereale* L. weeds. The criterion of specificity for all classes is over 99.2%. *Secale cereale* L. weed has the highest value of specificity, namely 100%; implying that the classification system did not classify any samples in this class incorrectly. The results shown in Table 5 prove the superiority ANN-ACO to RBF. For example, the accuracy corresponding to *Polygonum aviculare* L. in RBF classifier is 98.41%, whereas in ANN-ACO classifier it is 99.76%, being 1.38% more than RBF classifier. As previously mentioned, the database is the same for both classifiers, and this difference is only due to classifiers classification method. The classifier is of the main parts of machine vision system, especially for classifying weeds and crops to the optimal spraying of herbicides. As stated earlier, there is no possibility of direct comparison

of the employed method in this study with those of other researchers. However, Table 6 compares the correct classification rate of the present study and those of two other studies. In the first study (Hlaing & Khaing 2014), the authors classified four weed types, including Rape plant, Lanchon, Pigweed, and Kyautkut using Area Thresholding Algorithm. As shown in Table 6, from 35 samples, six samples were misclassified; therefore, the correct classification rate of the system was 82.85%. The second study is consistent with the study by Arribas et al (2011) who identified sunflower from non-sunflower, using the generalized softmax perceptron (GSP) neural network. This classifier system misclassified 25 samples from 192 samples, thus the correct classification rate of the system being 85%. By comparing the above-mentioned results and the results of the present study, the superiority of the method applied in this study is demonstrated. Hence, by employing this method, the possibility of making a machine vision system to real-time detection of *Chenopodium album*, *Polygonum aviculare* L., and *Secale cereale* L. weeds in potato agricultural field is raised up.

Table 5- Criteria of confusion matrix performance

	RBF			*The hybrid ANN-ACO*		
Class	*Sensitivity*	*Accuracy*	*Specificity*	*Sensitivity*	*Accuracy*	*Specificity*
Chenopodium album	99.30	97.37	96.03	99.77	99.66	99.61
Secale cereale L.	96.31	99.57	99.05	99.54	99.96	100
Polygonum aviculare L.	92.37	98.41	98.12	99.49	99.79	99.24
Potato plant	96.07	99.09	99.13	99.12	99.74	99.56

Table 6- A comparison between correct classification rate of present study and those of two other studies

Method	*The number of samples*	*The number of misclassified samples*	*Accuracy rate (%)*
Proposed model	1015 (testing data)	19	98.128
Hlaing & Khaing (2014)	35	6	82.85
Arribas et al (2011)	192	29	85

4. Conclusions

A novel method based on machine vision was applied to classify potato plant and three weed types of *Chenopodium album*, *Polygonum aviculare* L., and *Secalec ereale* L.. In order to survey the performance of the proposed classification method, a novel classifier known as the hybrid artificial neural network-Ant Colony (ANN-ACO) was presented. The findings confirmed that this classifier had the highest classification accuracy. In this work, the low computation speed in some moment was observed. Such a low speed is due to the excessive computations required to classify the weeds. Since the Matlab is not a compiled programming environment, the algorithm can be recorded using a compiled programming language like C for raising the speed of computations.

References

Arribas J I, Sánchez-Ferrero G V, Ruiz-Ruiz G & Gómez-Gil J (2011). Leaf classification in sunflower crops by computer vision and neural networks. *Computers and Electronics in Agriculture* **78**: 9-18

Bossu J, Gee C & Truchetet F (2008). Development of a machine vision system for a real time precision sprayer. *Electronic Letters on Computer Vision and Image Analysis* **7**(3): 54-66

Chen Y, Lin P, He Y & Xu Z (2011). Classification of broadleaf weed images using Gabor wavelets and Lie group structure of region covariance on Riemannian manifolds. *Biosystems Engineering* **109**: 220-227

Chowdhury S, Verma B & Stockwell D (2015). A novel texture feature based multiple classifier technique for roadside vegetation classification. *Expert Systems with Applications* **42**: 5047-5055

Gonzalez R C, Woods R E & Eddins S L (2004). Digital Image Processing Using MATLAB. Prentice Hall, New York

Hlaing S H & Khaing A S (2014). Weed and crop segmentation and classification using area thresholding. *International Journal of Research in Engineering and Technology* **3**: 375-382

Liu H, Lee S H & Saunders C (2014). Development of a machine vision system for weed detection during both of off-season and in-season in broadacre no-tillage cropping lands. *American Journal of Agricultural and Biological Sciences* **9**(2): 174-193

Liu X, Du H, Wang G, Zhou S & Zhang H (2015). Automatic diagnosis of premature ventricular contraction based on Lyapunov exponents and LVQ neural network. *Computer Methods and Programs in Biomedicine* **122**: 47-55

Marques O (2011). Practical image and video processing using matlab. John Wiley & Sons, Inc, Hoboken, New Jersey

Montalvo M, Guerrero J M, Romeo J, Emmi L, Guijarro M & Pajares G (2013). Automatic expert system for weeds/crops identification in images from maize fields. *Expert Systems with Applications* **40**: 75-82

Mursalin M, Hossain M M, Noman M K & Azam M S (2013). Performance analysis among different classifier including naive bayes, support vector machine and C4.5 for utomatic weeds classification. *Global Journal of Computer Science and Technology Graphics & Vision* **8**(3): 11-16

Noori R, Karbassi A R, Moghaddamnia A, Han D, Zokaei-Ashtiani M H, Farokhnia A & Gousheh M G (2011). Assessment of input variables determination on the SVM model performance using PCA, Gamma test, and forward selection techniques for monthly stream flow prediction. *Journal of Hydrology* **401**: 177-189

Sen T & Mathur H D (2016). A new approach to solve Economic Dispatch problem using a Hybrid ACO-ABC-HS optimization algorithm. *Electrical Power and Energy Systems* **78**: 735-744

Wisaeng K (2013). A Comparison of decision tree algorithms for UCI repository classification. *International Journal of Engineering Trends and Technology* **4**: 3393-3397

Zhao F, Cai C, Huang S, He D & Zhu J (2009). Weed Seeds Recognition Using Locally Linear Embedding. In: 2009 *International Conference on Test and Measurement,* 5-6 December, Hong Kong, China, pp. 59-62

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Effect of Environment and Genotype on the Protein Quality Attributes and Baking Characteristic of Newly Developed Wheat Inbred Lines

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ABSTRACT

The present work examined the effect of genotype and environment on protein content and fractions, gluten and starch fraction, SSL (sodium stearoyl-2-lactylate) and DMG (distilled mono glyceride) binding ability of starch and specific loaf volume (SLV) of six wheat genotypes grown in three different environment. Genotype and environment significantly affected all quality attributes under investigation. However, protein content and fractions showed differences in relative effects of genotype and environment. Most of the protein quality characteristics were influenced more by genotype than environment. Size distribution of gluten subunits was significantly affected by genotype and environment. It was observed that as the flour protein content increased, the magnitudes of monomeric proteins appeared to rise, but glutenin decreased. Flour protein content was expressively associated with gliadin and dough making characteristics. Environment influenced both the amounts of total protein and the quantities of different protein fractions, which, in turn, influenced baking quality.

Keywords: Wheat genotypes; Loaf volume; Electrophoresis; Molecular weight; Protein; Starch

Kendileme ile Geliştirilmiş Yeni Buğday Hatlarının Protein Kalitesi ve Ekmeklik Özelliklerine Çevre ve Genotipin Etkisi

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ÖZET

Bu çalışmada üç farklı çevrede yetiştirilmiş olan altı buğday genotipinin protein içeriği ve fraksiyonlarına, nişasta fraksiyonlarına, nişastanın SSL (sodium stearol-2-laktilat) ve DMG (distilled mono gliserit) bağlama gücüne ve spesifik ekmek hacmine (SLV) çevre ve genotip etkisi araştırılmıştır. Genotip ve çevre incelenen tüm kalite özelliklerini önemli düzeyde etkilemiştir. Ancak protein içeriği ve fraksiyonları genotip ve çevreye bağlı olarak farklılıklar göstermiştir.

Protein kalite özelliklerinin çoğunda çevreye göre genotip daha etkili olmuştur. Glutenin alt ünitelerinin boyut dağılımları da çevre ve genotip tarafından önemli düzeyde etkilenmiştir. Un protein kapsamı arttıkça monomeric proteinlerin boyutlarının da artma eğilimi gösterirken glutenin içeriğinin azaldığı gözlenmiştir. Un protein içeriği, gliadin ve hamur yapım özellikleri ile yüksek derecede ilişkili olmuştur. Çevre, hem toplam protein hem de ekmek yapım kalitesini belirleyen protein fraksiyonları miktarı üzerine etkili olmuştur.

Anahtar Kelimeler: Buğday genotipleri; Ekmek hacmi; Elektroforez; Moleküler ağırlık; Protein; Nişasta

1. Introduction

Wheat (*Triticum aestivum* L.) is an important and most widely cultivated crop in the world, providing 20% of all calories consumed globally. The primary objective of wheat breeders is to develop varieties with improved agronomic and technological properties, as well as increased amounts of beneficial nutrients and fewer amounts of anti-nutritional factors (Welch 2005). Knowing the biochemical characteristics responsible for the end-use quality features of wheat grains could have major impacts on wheat breeding programs and would lead to develop breeds with overall grain quality like handling, milling, and baking. Wheat flour protein composition or molecular size distribution and solubility have been reported as important variables influencing the processing quality of wheat (Graybosch et al 1996). Differences in both protein quantity and quality meaningfully alter flour quality and bread making. Environmental conditions can induce changes in the secondary, tertiary, or quaternary structure of proteins and thereby affect its structural and functional properties (Kim et al 2000). Protein characteristics in terms of its quantity and quality are the major determinants of the end use quality of wheat flour. The measurable expression of the crude protein in wheat flour is connected with the total organic nitrogen, while protein quality relate precisely to physiochemical features of the gluten constituent at the molecular level (Johansson et al 2003). Following the sequential Osborne extraction procedure, albumins and globulins of wheat endosperm represent 20% to 25% of total grain proteins (Merlino et al 2009). Nutritionally, albumins and globulins (non-glutens) have an exceptional amino acid balance (Žilić et al 2011). Further, the same authors reported that numerous of these proteins are enzymes involved in the metabolic activity. Triboi et al (2000) reported that proportions of gliadins in flour increased at higher protein content and lower proportions of glutenin. Glutenin and gliadin are the main constituents of the storage protein in wheat grains and they make a significant influence on the dough rheology and baking features (Panozzo & Eagles 2000). Differences in both protein content and composition meaningfully alter the bread quality (Panozzo & Eagles 2000). However, grain protein content and composition depends principally on wheat genotype, but it is also considerably affected by environmental conditions and the interaction between genotype and environment (Zhu & Khan 2001). Zhang et al (2007) reported that genotype, the environment, and the interaction of these factors expressively affected greatest number of the quality characters and a number of protein fractions. Genotype primarily determined the amount of the protein fractions and bread making factors, whereas the environment was the most significant source of difference in the noodle quality parameters. Further the same authors stated that higher bread scoress with $r= 0.70$ ($P<0.001$) and $r= -0.74$ ($P<0.001$) were achieved when using flours with higher quantity of glutenin and lower ratio of gliadin. Though, protein content and composition have a moderate unwanted influence on sensory quality of final products. Consequently, when breeding cultivars with enhanced bread making quality both allelic variation and quantity of storage protein fractions should be considered. A comprehensive understanding of the variability in protein content and composition among newly developed wheat

inbred lines could assist our continuing efforts to enhance both quantity and quality of wheat proteins and could thus motivate better selection of excellent wheat varieties. Therefore, this study was carried out to examine the effect of genotype and growing environment on protein quality attributes of newly developed wheat genotypes grown in three different environments (Madani, Hudeiba and Dongola) in the Sudan.

2. Material and Methods

2.1. Materials

Six genotypes of bread wheat (*Triticum aestivum* L.) (G1, RGO/SERI/TRAP//Bow; G2, KAU2*CHEN// BCN.CMB; G3, PYT#23 (DWR39xCONDOR "S") 14PxT; G4, TEVEE "S"/SHUHA "S"; G5, CONDOR "S"/14PYT//DWR39; G6; IHSGE#20) were grown in three different environments. The three environments are; 1, Hudeiba Research Station Farm, longitude 33° 92` E, latitude 17° 56` N and, with annual temperature of 29.7 °C, rainfall of 117 mm and relative humidity of 37%; 2, Wad Medani Research Station Farm, longitude 33° 29` E, latitude 14° 24` N and, with annual temperature of 28.3°C, rainfall of 362 mm and relative humidity of 42%; 3, Dongola, North Sudan, latitude 19° 10' N and longitude 30° 29' E and, with annual temperature of 27.6 °C, rainfall of 21 mm and relative humidity of 35%.

The genotypes were developed through extensive wheat breeding programs at the Agricultural Research Corporation (ARC), Gazira, Sudan. Sodium stearoyl-2-lactylate, distilled mono glyceride, petroleum ether, dithiothreitol, and dimethyl sulfoxide were obtained from Sigma-Aldrich Co., St. Louis, USA. Unless otherwise stated all the reagents used in the current study were of analytical grade. Three independent replicates of each sample were used for the analysis.

2.2. Protein content and fractionation

The protein content was determined according to AOAC (2000). The protein fractions were extracted according to their solubility using the method of Landry & Moureaux (1970).

2.3. Binding strength of sodium stearoyl-2-lactylate (SSL) and distilled mono glyceride (DMG) to starch

Two-tenths of SSL or DMG was suspended in 100 mL distilled water at 54 °C in 400 mL beaker. About 20 g of starch were added to the suspension and mixed. The suspension was placed in a preheated oven at 100 °C for 25 minutes. The mixture was occasionally shaken without stirring. After 25 min, the mixture was taken out and immediately frozen and then freeze dried. The dried sample was then powdered using a laboratory mill and stored at 4 °C. About 10 g of the freeze-dried starch-surfactant was weighed and extracted in a Gold Fish Apparatus using petroleum ether for 12 h. Petroleum ether was evaporated over boiling water bath, the extracted surfactant was re-dissolved in 50 mL of SSL or DMG solvent and transferred quantitatively to a volumetric flask and the volume was completed to 100 mL. A standard surfactant solution was prepared by dissolving 0.20 g of surfactant in exactly 100 mL of the corresponding surfactant solvent. The binding strength of SSL and DMG to different starches was measured using gas chromatography-mass spectrophotometry (GC-MS, Shimadzu GC 17A, QP-5000, Japan).

2.4. Baking test

The baking test was carried out following the procedure defined previously (Badi et al 1978).

2.5. Starch and gluten fractionation

Wheat flour starch and gluten were separated by dough-washing method (Wolf 1964).

2.6. Molecular weight characterization

The molecular weight characterization was carried out using microchip capillary electrophoresis according to method of Uthayakumaran et al (2006).

2.7. Statistical analysis

The data of three independent experiments were examined by ANOVA and Duncan's multiple range test (DMRT). Correlation coefficients among all quality traits were evaluated based on the means of all genotypes in the individual environment using Stat View software. Significance was accepted at $P<0.05$, $P<0.01$, and $P<0.001$.

3. Results and Discussion

3.1. Protein content and fractions

As shown in Table 1, the protein content (PRC) varied between genotypes and location (Madani, Hudeiba, and Dongola). Among the lines grown in Madani, Hudeiba and Dongola, lines G4, G2, and G2, exhibited 13.4, 14.06, and 13.87% PRC, respectively. Among the locations, Dongola gave higher average PRC value (13.38%) followed by Hudeiba (13.10%) and Madani (11.82%). The PRC of G2 grown at Hudieba is comparable to that of the Canadian cultivar. The variation in PRC among location could be attributed to changes in environmental conditions, cultivation practices as well as genotypes. The results revealed that the PRC of wheat has been connected more with environmental factors, such as rainfall, temperature, soil fertility, and fertilizer application practices, than genetic makeup. Bouacha et al (2014) reported that the grains PRC depend on agronomical practices, genotypes, soil nitrogen content, heat and drought stresses. The environment was the most powerful variable, though some authors reported that genotype and genotype-by-environment had superior influence on flour protein than the environment (Grausgruber et al 2000). The mean values of the protein fractions of the genotypes grown at the three locations are presented in Table 2. The average value of globulins for each of the

Table 1- Protein content (g 100 g^{-1}) and fractions (% of total protein), starch and gluten fractions (g), SSL and DMG (mg g^{-1}) and specific loaf volume (cm^3 g^{-1}) of six local wheat genotypes grown at three different locations and Canadian wheat flour

Location	*Lines*	*PRC*	*GLB*	*ALB*	*GLI*	*GLT*	*INSP*	*STF*	*GTF*	*SSL binding*	*DMG binding*	*SLV*	*GLT/GLI*
Madani	G1	11.15^{b*}	26.32^{a}	12.39^{c}	39.85^{c}	22.26^{a}	0.49^{a}	49.38^{c}	17.00^{c}	178.62^{b}	200.00^{a}	2.40^{c}	0.56
	G2	10.94^{c}	21.33^{b}	14.00^{b}	40.63^{cb}	21.97^{a}	0.49^{a}	55.18^{b}	19.86^{a}	166.95^{c}	187.12^{b}	2.92^{b}	0.54
	G3	10.36^{c}	21.61^{b}	15.89^{a}	41.38^{b}	22.94^{a}	0.46^{b}	54.97^{b}	16.86^{c}	108.97^{d}	200.00^{a}	2.91^{b}	0.55
	G4	13.40^{a}	19.57^{b}	9.36^{d}	50.58^{a}	20.74^{b}	0.42^{c}	43.31^{d}	18.83^{b}	200.00^{a}	160.90^{c}	3.44^{a}	0.41
	G5	12.25^{b}	21.40^{b}	12.99^{c}	49.93^{a}	17.91^{c}	0.42^{c}	60.15^{a}	16.05^{c}	200.00^{a}	200.00^{a}	2.92^{b}	0.36
	G6	12.81^{b}	18.59^{c}	14.46^{b}	48.95^{a}	19.91^{b}	0.42^{c}	55.62^{ab}	19.24^{ab}	200.00^{a}	200.00^{a}	3.35^{a}	0.41
Location Mean		11.82^{C}	21.47^{B}	13.18^{A}	45.22^{B}	20.95^{A}	0.45^{A}	53.10^{A}	17.97^{B}	175.75^{C}	191.30^{C}	2.99^{C}	0.46
Hudeiba	G1	13.60^{a}	25.48^{c}	9.28^{d}	42.32^{d}	21.23^{a}	0.40^{c}	50.60^{c}	16.23^{c}	200.00^{a}	200.00^{a}	2.45^{b}	0.50
	G2	14.06^{a}	22.02	12.41^{b}	47.60^{b}	18.89^{d}	0.42^{c}	37.21^{e}	19.23^{a}	200.00^{a}	200.00^{a}	2.56^{b}	0.40
	G3	12.63^{b}	31.24^{a}	11.46^{c}	37.81^{e}	20.36^{b}	0.45^{b}	43.30^{d}	11.89^{e}	200.00^{a}	200.00^{a}	2.53^{b}	0.54
	G4	13.13^{a}	25.42^{c}	12.66^{b}	45.16^{c}	19.44^{c}	0.40	55.84^{a}	17.85^{b}	200.00^{a}	189.00^{c}	2.91^{a}	0.43
	G5	12.63^{b}	26.51^{b}	12.71^{b}	43.25^{d}	19.37^{c}	0.48^{a}	34.62^{f}	13.85^{d}	198.30^{b}	200.00^{a}	2.91^{a}	0.45
	G6	12.55^{b}	20.28^{d}	13.32^{a}	50.02^{a}	18.57^{d}	0.41^{c}	53.00^{b}	18.00^{ab}	200.00^{a}	192.58^{b}	3.30^{a}	0.37
Location Mean		13.10^{B}	25.15^{A}	11.97^{B}	44.36^{C}	19.64^{B}	0.42^{A}	45.76^{B}	16.17^{C}	199.71^{A}	196.93^{A}	2.78^{C}	0.44
Dongla	G1	12.36^{b}	30.48^{a}	11.56^{c}	37.19^{e}	20.74^{b}	0.49^{a}	38.75^{c}	12.43^{c}	200.00^{a}	191.85^{a}	2.79^{b}	0.56
	G2	13.87^{a}	25.04^{b}	12.74^{b}	42.92^{d}	21.39^{a}	0.49^{a}	30.29^{d}	14.43^{b}	200.00^{a}	188.00^{b}	2.77^{b}	0.50
	G3	13.55^{a}	23.13^{c}	10.74^{c}	45.41^{c}	21.46^{a}	0.41^{b}	44.34^{a}	19.13^{a}	198.60^{b}	178.60^{d}	2.81^{b}	0.47
	G4	13.76^{a}	25.01^{b}	6.09^{d}	51.39^{a}	19.46^{c}	0.41^{b}	41.89^{b}	18.60^{a}	200.00^{a}	180.30^{c}	3.51^{a}	0.38
	G5	13.68^{a}	21.93^{d}	14.69^{a}	42.39^{d}	18.37^{d}	0.41^{b}	44.93^{a}	18.23^{a}	195.40^{c}	106.10^{f}	2.83^{b}	0.43
	G6	13.11^{a}	19.37^{e}	12.05^{b}	46.86^{b}	19.51^{c}	0.41^{b}	45.16^{a}	19.30^{a}	195.70^{c}	127.60^{e}	3.54^{a}	0.42
Location Mean		13.38^{B}	24.16^{A}	11.31^{B}	44.36^{C}	20.15^{A}	0.43^{A}	40.89^{D}	17.02^{B}	198.28^{B}	162.07^{D}	3.04^{B}	0.45
Canadian wheat		14.60^{A}	21.82^{B}	13.75^{A}	46.94^{A}	18.37^{B}	0.43^{B}	43.61^{C}	18.5^{A}	166.40^{D}	195.24^{B}	4.00^{A}	0.39

*, values are means of duplicate samples; means not sharing a common superscript letter of a, b or c in a column for genotypes in each location or A, B, C or D in a column for locations are significantly different at P<0.01 as assessed by Duncan's Multiple Range Test; PRC, protein content; GLB, globulin; ALB, albumin; GLI, gliadin; GLT, glutenin; INSP, insoluble protein; GTF, gluten fraction; STF, starch fraction; SSL, sodium stearoyl-2-lactylate; DMG, distilled mono glyceride; SLV, specific loaf volume

three locations genotypes ranged from 18.59 to 26.32% for Madani location, 20.28 to 31.24% for Hudeiba, and 19.37 to 30.48% for Dongola, while the Canadian wheat exhibited 21.82%. Albumin ranged from 9.36 to 15.89% for Madani and from 9.28 to 13.32% for Hudeiba and from 6.09 to 16.69% for Dongola while 13.75% was reported for the Canadian. Gliadin is a dominant fraction of all genotypes grown in all locations. Among genotypes grown in Madani, the highest gliadin value (50.58%) was recorded in G5 compared to 39.85% for G1. A mean value of 50.02% gliadin as a highest value in

Table 2- Correlation coefficient among the protein quality attributes of wheat genotypes grown at three different locations

	PRC	*GLB*	*ALB*	*GLI*	*GLT*	*INSP*	*GTF*	*STF*	*SSL*	*DMG*
Wad Madani										
GLB	-0.58									
ALB	-0.68	0.01								
GLI	0.91**	-0.70	-0.47							
GLT	-0.67	0.41	0.25	-0.82*						
INSP	-0.81*	0.73	0.29	-0.97***	0.76					
GTF	0.26	-0.50	-0.12	0.03	0.20	0.07				
STF	-0.34	-0.09	0.75	-0.04	-0.39	-0.04	-0.29			
SSL	0.84*	-0.24	-0.65	0.68	-0.74	-0.50	0.22	-0.15		
DMG	-0.54	0.36	0.79	-0.38	-0.03	0.24	-0.48	0.77	-0.30	
SLV	0.73	-0.96***	-0.25	0.76	-0.37	-0.76	0.53	-0.19	0.34	-0.57
Hudeiba										
GLB	-0.31									
ALB	-0.39	-0.39								
GLI	0.20	-0.98***	0.57							
GLT	0.15	0.64	-0.94**	-0.77						
INSP	-0.48	0.49	0.24	-0.43	-0.08					
GTF	0.59	-0.89*	0.26	0.87*	-0.50	-0.72				
STF	-0.11	-0.21	-0.11	0.21	0.13	-0.80	0.33			
SSL	0.37	-0.17	-0.25	0.13	0.14	-0.82*	0.41	0.63		
DMG	0.25	0.36	-0.49	-0.49	0.42	0.53	-0.47	-0.77	-0.31	
SLV	-0.60	-0.53	0.77	0.65	-0.73	-0.02	0.28	0.32	-0.20	-0.68
Dongola										
GLB	-0.49									
ALB	-0.10	-0.22								
GLI	0.60	-0.55	-0.67							
GLT	-0.11	0.43	-0.10	-0.28						
INSP	-0.37	0.743	0.23	-0.70	0.57					
GTF	0.50	-0.85*	-0.21	0.78	-0.44	-0.97***				
STF	-0.12	-0.51	-0.07	0.32	-0.58	-0.86*	0.74			
SSL	-0.06	0.79	-0.57	-0.06	0.68	0.61	-0.58	-0.67		
DMG	-0.15	0.74	-0.53	-0.09	0.81*	0.60	-0.55	-0.62	0.97***	
SLV	0.06	-0.44	-0.58	0.77	-0.45	-0.54	0.55	0.41	-0.21	-0.22

PRC, protein content; GLB, globulin; ALB, albumin; GLI, gliadin; GLT, glutenin; INSP, insoluble protein; GTF, gluten fraction; STF, starch fraction; SSL, sodium stearoyl lactylate; DMG, distilled mono glyceride; SLV, specific loaf volume; *, P<0.05; **P<0.01; *** P<0.001

Hudeiba was observed for G6 compared to 37.81% as the lowest observed for G3. For genotypes grown in Dongola, G4 had higher gliadin value (51.39 %) where G1 scored the lowest (37.19%). Canadian wheat recorded 46.95% of gliadin. The results showed that some genotypes among locations have higher gliadin content compared to the other gluten fraction. Generally, gliadins have been reported for its contribution to dough viscosity (Uthayakumaran et al 1999). Glutenin fraction of G3 (22.94%) of Madani was the highest relative to lowest exhibited by G5 (17.91%), while at Hudeiba the values for G2 and G6 were 21.23% and 18.57%, respectively. Dongola data showed 21.46% for G3 and 18.37% for G5 as the highest and lowest values, respectively. Canadian wheat exhibited 18.37% of glutenin. The glutenin content of the investigated genotypes was superior to that of the Canadian, which indicated that the dough elasticity will be improved because glutenins contribute to dough elasticity (Uthayakumaran et al 1999).

The insoluble protein fraction for all genotypes that grown in all locations range from 0.40 to 0.49% while that of Canadian wheat was 0.43%. Regarding the sites, the mean value of globulin of genotypes grown in Hudeiba (25.15%) was the highest followed by Dongola (24.16%) and Madani (21.47%) while that of albumin Madani recorded higher value (13.18%) followed by Hudeiba (11.97%) and Dongola (11.31%). The average value of gliadin and glutenin fractions was greater in Madani and were found to be 45.22 and 20.95%, respectively, followed by Dongola which gave 44.36 and 20.15%, respectively, and Hudeiba scored 44.36 and 19.64%, respectively. The mean value of insoluble protein of genotypes was found to be higher for lines grown in Madani (0.45%) followed by those grown in Dongola (0.43%) and Hudeiba (0.42%) while Canadian cultivar showed 0.43%. Zhu & Khan (2001) reported that the environment not only influenced total flour protein content but it also affected the amounts of various protein fractions and the molecular size distributions of glutenin subunits. Panozzo & Eagles (2000) reported that the proportion of glutenin in flour protein was extremely dependent on cultivar, whereas, the effect of environmental difference was greater than cultivar variation for gliadin, though cultivar selection was still necessary. Environmental variation influence was higher than cultivar variation for the dough rheological properties. Further, Panozzo & Eagles (2000) reported that throughout the environments, the amount of gliadin improved with increasing the flour protein content, while the share of glutenin declined. Zhang et al (2007) declared that genotype, environment, and their interaction meaningfully affected most of the wheat quality characters and the quantity of protein fractions. Our study revealed that genotype mainly determined the quantity of gluten protein fractions while the environment was the greatest cause of differences in other quality factors. Johansson et al (2003) reported that cultivar and environmental inspirations elevated the discrepancy in protein content and they also increased the differences in most of the examined protein constituents.

3.2. Starch and gluten fractions

Genotypes, G5 (60.15 g), G4 (55.84 g) and G6 (54.16 g) grown in Madani, Hudeiba and Dongola, respectively gave significantly ($P<0.01$) higher values of starch fraction (Table 1). Regarding gluten fraction, G2 (19.86 g), G2 (19.23 g) and G6 (19.30 g) for the locations, respectively gave significantly ($P<0.01$) higher values. Genotypes grown in Madani gave significantly ($P<0.01$) higher average value of starch fraction (53.10 g) followed by Hudeiba (45.76 g) and Dongola (40.89 g) while Canadian cultivar had a value of 43.61 g. The mean value of gluten fraction of all genotypes for each location was differed from each other and slightly lower than that of the Canadian cultivar. Significant variations in starch and gluten fractions were observed ($P<0.01$) among genotypes. However, among locations, the starch fraction was significantly varied but gluten fraction did not vary significantly indicating that genotype and location had have affected starch and gluten yield. The results obtained are in accordance with those of Wilson et al (2008) who stated that the environment impact on wheat properties seemed

to have a significant effect on bread baking quality and various starch properties than genetic control. In addition, our results are close to that obtained by Sayaslan et al (2006) who found a range of 54.50-63.40% for starch yield and 3.70-18.20% for gluten yield. The excess gluten recovery of wheat flour protein could be attributed to the fact that the isolated gluten contains lipid, carbohydrate and other minor components in addition to protein (Van Der Borght et al 2005). Significant differences in isolated starch and gluten are of great importance for the starch/gluten industry and they could thus be used for selection of wheat cultivars with appropriate characteristics to meet specific requirements.

3.3. Binding ability of sodium stearoyl-2-lactylate (SSL) and distilled mono glyceride (DMG)

The binding strength of SSL and DMG to different starches was measured using gas chromatography-mass spectrophotometry and the results are presented in Table 1. The binding strength of SSL of G4, G5 and G6 (200.00 mg g^{-1}) significantly ($P<0.01$) varied among genotypes grown in Madani and no significant variation in SSL binding ability of genotypes grown in Hudeiba which exhibited a range of 198.30 to 200.00 mg g^{-1}. For genotype grown in Dongola G1-G4 showed significantly ($P<0.01$) higher values compared to other genotypes G5 and G6. Among locations, the average value of genotypes grown in Hudeiba gave significantly higher SSL binding ability followed by Dongola while those grown in Madani gave the least mean value but still greater than that obtained from Canadian cultivar (166.40 mg g^{-1}). DMG binding ability of the starch of genotypes grown in Madani varied between genotypes with G1, G3, G5 and G6 showed considerably ($P<0.01$) higher value (200.00 mg g^{-1}) while those grown in Hudeiba, G1, G2, G3, and G5 recorded significantly ($P<0.01$) higher values. However, for genotypes grown in Dongola only G1 scored considerably ($P<0.01$) higher value (191.85 mg g^{-1}). Among locations, the average value of DMG binding ability of the starch of genotypes grown in Hudeiba was significantly ($P<0.01$) higher than those grown in Madani and Dongola as well as that of Canadian cultivar. It has been reported that sodium stearoyl-2-lactylate (SSL) is an anionic oil-in-water emulsifier that is used to enhance the quality of bread. When added to the dough, SSL could improve mixing tolerance, gas retention, and dough resistance and it is thus known as dough strengther (Ribotta et al 2010). Nonionic emulsifiers such as distilled mono glyceride (DMG) play a major role in bread volume and crumb texture (Gray & Bemiller 2003). The results revealed that the effect of surfactants differed with the type of starch. High significant differences for genotypes and locations were found to affect the starch binding ability. SSL and distilled monodiglyceride (as dough strengtheners) their binding capacity of the newly developed lines is comparable to those of Canadian cultivar which indicated that the end-use of such genotypes will be highly improved.

3.4. Specific loaf volume

The mean specific loaf volume (SLV) of the newly developed genotypes is shown in Table 1. Among genotypes grown in Madani, genotype G4 scored significantly higher value of SLV (3.44 cm^3 g^{-1}) than the other genotypes while G6 gave higher value (3.30 cm^3 g^{-1}) and among those grown in Hudeiba and those grown in Dongola (3.54 cm^3 g^{-1}). Among the locations, the average SLV of genotypes grown in Dongola gave higher value (3.05 cm^3 g^{-1}) than those grown in Madani and Hudeiba but lower than that of the Canadian cultivar. It has been reported that the protein content and GLT/GLI ratio have different influences on dough and bread quality parameters. Rises in the protein content at constant GLT/GLI ratio improved extensibility, mixograph peak resistance, maximum resistance to extension, mixing time, and loaf volume. Increases in the GLT/GLI ratio at constant protein content increased mixing time, mixograph peak resistance, maximum resistance to extension and loaf volume. Rises in GLT/GLI ratio decreased resistance breakdown and extensibility (Huang & Khan 1997). However, in the present study a negative correlation ($r= -0.64$, -0.71 and -0.74 for the genotypes grown in Madani, Hudieba and Dongola, respectively) was observed

between GLT/GLI ratio and the loaf volume which indicated that as GLT decreases the loaf volume increases. Although there was an improvement in most of the quality attributes such as glutenin and insoluble (aggregated) gluten fraction, that responsible for the improvement of the baking quality of the flour of the investigated genotypes compared to that of Canadian, but the loaf volume of the investigated genotypes was found to be lower than that of the Canadian cultivar. The variation in a specific loaf volume of the investigated genotypes and Canadian may be due to the differences in genetic, as well as environmental conditions among the locations, the genotype, and it's interaction with the environment. In addition, differences in the chemical nature of gluten and gluten fractions between the investigated genotypes and the Canadian cultivar may exist. Similar results have been reported by Wilson et al (2008) who reported that loaf volume was correlated with protein quantity and quality. The dough forming ability of wheat flour during fermentation is the main determinants of its baking properties. Therefore, dough strength contributes to the quality of both flour and bread.

3.5. Correlation among quality attributes

Correlation analysis of wheat flour end-use quality standards is one of the striking approaches that can yield sufficient evidence of association of qualitative characteristics and their interaction effects that provide a proper evaluation of the overall quality of wheat flour. Table 2 shows the correlation between the protein content, protein factions, starch and gluten fractions, and sodium stearoyl lactylate (SSL) and distilled monodiglyceride (DMG) binding properties of the six wheat genotypes grown in different locations in Sudan (Madani, Hudeiba, and Dongola). The results showed that the correlations were varied between attributes (positive, negative, weak) of genotypes. The protein content (PRC) was considerably ($P<0.01$) and positively associated ($r= 0.91$) with gliadin (GLI) and significantly ($P<0.05$) correlated with SSL ($r= 0.84$) but negatively interrelated ($P<0.05$, $r= -0.81$) with insoluble protein (INSP) for genotypes grown in Madani, while the PRC of those grown in Hudeiba and Dongola showed weak correlation (either +ve or -ve) with the quality attributes. Similarly, previous reports showed a high correlation between wheat flour protein and gliadin fraction (Saint Pierre et al 2008). In addition, Johansson et al (2003) reported a positive correlation between protein concentration and the total quantities of glutenins, gliadins, and mono- and polymeric proteins. They also reported that the correlation between the quantity of gliadins and sodium dodecyl suphate (SDS)-soluble mono- and polymeric proteins were frequently greater than the association between glutenins and the SDS-insoluble mono- and polymeric proteins. The globulin (GLB) content of genotypes grown in Madani was significantly ($P<0.001$) and negatively interrelated ($r= -0.96$) with specific loaf volume (SLV) while for those grown in Hudieba it was significantly ($P<0.001$) and negatively interrelated ($r= -0.98$) with GLI and considerably ($P<0.05$) and negatively correlated ($r= -0.89$) with gluten fraction (GTF). For those grown in Dongola, GLB significantly ($P<0.05$) and negatively correlated ($r= -0.85$) with gluten fraction (GTF). Similarly, Whitney et al (2014) reported a highly negative association between loaf volume and globulin and albumin fractions. The albumin (ALB) fraction of genotypes grown in Madani and Dongola showed weak correlation (either +ve or -ve) with the quality attributes but for those grown at Hudeiba was significantly ($P<0.01$) and negatively correlated ($r= -0.94$) with glutenin (GLT). GLI of genotypes grown at Madani was significantly ($P<0.05$) and negatively correlated ($r= -0.82$) with GLT and INSP ($P<0.001$, $r= -0.97$) while that of those grown in Hudeiba was significantly ($P<0.05$) and positively correlated ($r= 0.87$) with GTF. However, genotypes grown at Dongola showed a weak correlation (either +ve or -ve) with the quality attributes. A weak correlation (either +ve or –ve) of GLT with the quality characteristics of genotypes grown at Madani and Hudeiba was observed but significantly ($P<0.05$) and positively correlated ($r= 0.81$) with distilled monodiglyceride (DMG) binding property for those grown at Dongola. The INSP of genotype grown at Madani showed weak correlation

(either +ve or -ve) with the quality attributes but significantly ($P<0.05$) and negatively correlated ($r= -0.82$) with SSL for those grown at Hudeiba and significantly ($P<0.001$) and negatively correlated ($r= 0.97$) with GTF and STF ($P<0.05$, $r= 0.86$). STF and DMG showed a weak correlation (either +ve or -ve) with the quality attributes for genotypes grown in all location. However, SSL showed only significant ($P<0.001$) and positive correlation ($r= 0.97$) with DMG for genotypes grown in Dongola.

3.6. Molecular weight characterization

Microchip capillary electrophoresis was used to characterize the page pattern of the proteins of the six genotypes (Figure 1). As shown in Figure 1, there were variations in molecular weight of genotypes grown in Madani which ranged from 14.30 to 224.70, 13.60 to 220.90, 14.30 to 223.60, 13.90 to 220.10, 13.90 to 181.20 and 13.80 to 211.70 kDa for genotypes G1, G2, G3, G4, G5 and G6, respectively. For those grown in Hudeiba also showed variations in genotypes molecular weight which ranged from 14.30 to 225.70, 14.30 to 222.70, 14.30 to 217.90, 14.20 to 219.90, 14.00 to 181.80 and 14.90 to 212.30 kDa for genotypes G1, G2, G3, G4, G5 and G6, respectively. Whereas those grown in Dongola gave a range of 14.20-224.20, 14.20-222.10, 13.80-235.00, 14.20-221.80, 13.70-183.70 and 14.30-213.20 kDa for the genotypes, respectively. The Canadian cultivar protein molecular weight ranged from 14.20 to 217.60 kDa. The results revealed significant variations among locations and among genotypes in high molecular weight subunits. The highest molecular weight subunit was obtained for genotype grown in Dongola (235 kDa) while the lowest one was obtained for that grown in Madani (181.20 kDa). Among genotypes grown in Madani, the highest molecular weight was scored by G3 and the lowest was for G5. Regarding those genotype grown at Hudeiba, G1 recorded higher molecular weight (225.70 kDa), and G5 recorded the lower one (181.80 kDa). The genotypes grown in Dongola characterized by high molecular weight subunits with a higher one recorded for G3 and lower one recorded for G5. It was observed that G5 at all locations gave a lower value among higher molecular weight subunits. The justification for this variation is not known, but it might be due to the differences in HMW subunits between the genotypes as well as locations. These molecular variances might lead to variations in response to the environment, but which are not observable in other genotypes. Compared to Canadian cultivar, most of the genotypes had higher molecular weight subunits than that of the Canadian. The high molecular weight glutenin subunits (HMW-GS) which has the highest molecular size are shown in the upper part of the measuring range clearly revealed that, the HMW subunits appeared in the range of 100-240 kDa with Lab-on-a-chip. Well distinguished from the other proteins, above 100 kDa. At the middle of the measuring range, the applied procedure provides the patterns of the LMW-GS and gliadin fractions, in the range of 40 and 50 kDa, which are entirely overlapping. Below 30 kDa there are mainly albumins and globulins. The results, however, indicate that the effect of the environmental conditions on the protein profile is detectable with Lab-on-a-chip. Other studies confirmed that the environmental conditions such as fertilizer and temperature mainly affect the quantity, composition and/or polymerization of gluten proteins. For example under increased nitrogen fertilizer an increase in gliadin/glutenin and HMW-GS/LMW-GS ratios were observed (Dupont et al 2006). Analysis of variance demonstrated an important influence of environmental conditions on molar masses of the polymeric fraction of wheat flours. The six selected local wheat cultivars used in the present study had very diverse allelic compositions of their high molecular weight, these differences in properties presumably arise due to the effects of varying climate, soil type, and agronomic practices on the quantities' variation in gluten protein composition, i.e. relative amounts of gliadin and glutenin proteins and the molecular size distribution of glutenin. Southan & MacRitchie (1999) reported that wheat proteins molecular weight distribution was known as the primary determinant of physical features of dough. Pevious reports have shown that properties such as stretchy strength are associated with a fraction of polymer having molecular weight

above a critical value and the molecular weight distribution of this fraction (Southan & MacRitchie 1999). The proportion of monomeric and polymeric proteins and the molecular weight distribution of the polymeric proteins determine the molecular weight distribution of wheat proteins (Rhazi et al 2014). The molecular weight distribution of the polymeric proteins depends mainly on HMW-GS/ LMW-GS ratio, allelic variation of HMW-GS, and the occurrence of altered gliadins that act as chain terminators (Rhazi et al 2014).

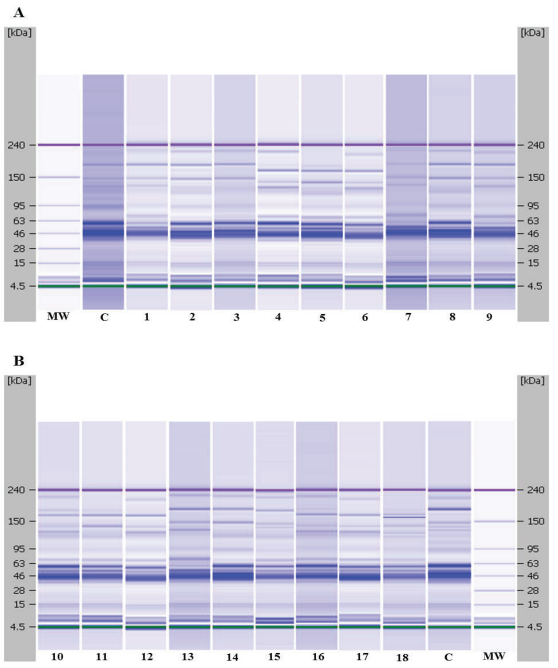

Figure 1 (A, B)- Molecular weight characterization of wheat genotypes proteins by microchip capillary electrophoresis; lane 1-6 (G1-G6, Madani); lane 7-12 (G7-G12, Hudeiba); lane 13-18 (G13-G18, Dongola); MW, molecular marker; C, Canadian wheat (control)

4. Conclusions

In conclusion, the results obtained clearly indicate that both the genotype and environment significantly affected the protein and starch quality attributes. Among genotypes grown in Madani and Hudieba, G6 gave the highest loaf volume, SSL, and DMG binding ability and therefore the flour of this genotype is potentially suitable for breadmaking. The remaining genotypes presented little breadmaking characteristics and thus their flours might be used for other bakery products. The low breadmaking quality of such genotypes make alarm for the needs of breeding wheat genotypes with high yield and excellent bread making quality suitable for the environment of the Sudan.

References

AOAC (2000). Association of Official Analytical Chemists 14th ed. Washington, DC. Official Methods of Analysis

Badi S M, Elfaki H A & Perten H (1978). Evaluation of Sudanese wheat varieties. *Sudan Journal of Food Science and Technology* **10**: 5-11

Bouacha O D, Nouaigui S & Rezgui S (2014). Effects of N and K fertilizers on durum wheat quality in different environments. *Journal of Cereal Science* **59**: 9-14

Dupont F M, Hurkman W J, Vensel W H, Tanaka C, Kothari K M, Chung O K & Altenbach S B (2006). Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. *European Journal of Agronomy* **25**: 96-107

Grausgruber H, Oberforster M, Werteker M, Ruckenbauer P & Vollmann J (2000). Stability of quality traits in Austrian-grown winter wheats. *Field Crops Research* **66**: 257-267

Gray J A & Bemiller J N (2003). Bread staling: Molecular basis and control. *Comprehensive Review in Food Science & Food Safety* **2**: 1-21

Graybosch R A, Peterson C J, Shelton D R & Baenziger P S (1996). Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. *Crop Science* **36**: 296-300

Huang D Y & Khan K (1997). Quantitative determination of high molecular weight glutenin subunits of hard red spring wheat by SDS-PAGE. I. Quantitative effects of total amounts on bread-making quality characteristics. *Cereal Chemistry* **74**: 781-785

Johansson E, Prieto-Linde M L, Svensson G & Jönsson J Ö (2003). Influences of cultivar, cultivation year and fertilizer rate on amount of protein groups and amount and size distribution of mono-and polymeric proteins

in wheat. *The Journal of Agricultural Sciences* **140**: 275-284

Kim T D, Ryu H J, Cho H I, Yang C H & Kim J (2000). Thermal behavior of proteins: Heat-resistant proteins and their heat-induced secondary structural changes. *Biochemistry* **39**: 14839-14846

Landry J & Moureaux T (1970). Heterogeneite des glutelines du grain de mais: Extraction selective et composition en acides amines des trios fractions isolees. *Bulletin De La Societe De Chimie Biologique* **52**: 1021–1037

Merlino M, Leroy P, Chambon C & Branlard G (2009). Mapping and proteomic analysis of albumin and globulin proteins in hexaploid wheat kernels (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **18**: 1321-1337

Panozzo J F & Eagles H A (2000). Cultivar and environmental effects on quality characters in wheat. II. Protein. *Crop Pasture Science* **51**: 629-636

Rhazi L, Graybosch R A & Aussenac T (2014). Molecular characterization of three transgenic high molecular weight glutenin subunit events in winter wheat. *Journal of Cereal Science* **60**: 631-638

Ribotta P D, Pérez G T, Añón M C & León A E (2010). Optimization of additive combination for improved soy-wheat bread quality. *Food Bioprocess Technology* **3**: 395-405

Saint Pierre C, Peterson C J, Ross A S, Ohm J B, Verhoeven M C, Larson M & Hoefer B (2008). Winter wheat genotypes under different levels of nitrogen and water stress: Changes in grain protein composition. *Journal of Cereal Science* **47**: 407-416

Sayaslan A, Seib P A & Chung O K (2006). Wet-milling properties of waxy wheat flours by two laboratory methods. *Journal of Food Engineering* **72**: 167-178

Southan M & MacRitchie F (1999). Molecular weight distribution of wheat proteins. *Cereal Chemistry* **76**: 827-836

Triboi E, Abad A, Michelena A, Lloveras J, Ollier J L & Daniel C (2000). Environmental effects on the quality of two wheat genotypes: 1.Quantitative and qualitative variation of storage proteins. *European Journal of Agronomy* **13**: 47-64

Uthayakumaran S, Gras P W, Stoddard F L & Bekes F (1999). Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. *Cereal Chemistry* **76**: 389-394

Uthayakumaran S, Listiohadi Y, Baratta M, Batey I L & Wrigley C W (2006). Rapid identification and quantitation of high-molecular-weight glutenin subunits. *Journal of Cereal Science* **44**: 34-39

Van Der Borght A, Goesaert H, Veraverbeke W S & Delcour J A (2005). Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *Journal of Cereal Science* **41**: 221-237

Welch R M (2005). Biotechnology, biofortification and global health. *Food & Nutrition Bulletin* **26**: 304-306

Whitney K, Ohm J B & Simsek S (2014). Addition of glucose oxidase for the improvement of refrigerated dough quality. *Cereal Chemistry* **91**: 548 - 553

Wilson J D, Bechtel D B, Wilson G W T & Seib P A (2008). Bread quality of spelt wheat and its starch. *Cereal Chemistry* **85**: 629-638

Wolf M J (1964). Wheat Starch Isolation. In: R Whistler (Ed), *Methods in Carbohydrate Chemistry*, Academic Press, London, pp. 6-9

Zhang P, He Z, Zhang Y, Xia X, Liu J, Yan J & Zhang Y (2007). Pan bread and Chinese white salted noodle qualities of Chinese winter wheat cultivars and their relationship with gluten protein fractions. *Cereal chemistry* **84**: 370-378

Zhu J & Khan K (2001). Effects of genotype and environment on glutenin polymers and breadmaking quality. *Cereal Chemistry* **78**: 125-130

Žilić S, Barać M, Pešić M, Dodig D & Ignjatović-Micić D (2011). Characterization of proteins from grain of different bread and durum wheat genotypes. *International Journal of Molecular Sciences* **12**: 5878-5894

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The Effect of Diflufenican and Its Mixture with S-metolachlor and Metribuzin on Nitrogenase and Microbial Activity of Soil under Yellow Lupine (*Lupinus luteus* L.)

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ABSTRACT

The aim of the study was to evaluate the effect of the active substance of diflufenican and its combination with s-metolachlor or metribuzin, applied to yellow lupine, on the nitrogenase activity, the population size of selected groups of microorganisms, the activity of soil enzymes and their sensitivity to the tested preparations. All analysed preparations caused a reduction in the total number of bacteria and the number of actinobacteria and oligotrophic bacteria at the beginning of the vegetation period of yellow lupine. In the combination where diflufenican was used separately a stimulatory effect on nitrogenase activity was observed. The research revealed very high sensitivity of dehydrogenases and acid phosphatase to the soil contamination caused by application of all the tested herbicides. The dehydrogenases activity values were closely correlated with reduced populations of the groups of microorganisms. Diflufenican applied separately caused a relatively small negative effect on biological soil properties and consequently could have a smaller negative effect on soil environment contamination in comparison to other variants.

Keywords: Active substances; Herbicides; Number of microorganisms; Leguminous plants; Soil enzyme activity

Diflufenican ve Diflufenican'ın S-metolachlor ve Metribuzin ile Karışımlarının Sarı Acı Bakla (*Lupinus luteus* L.) Bitkisinde Nitrojenaz Enzimi ve Topraktaki Mikrobiyal Aktivite Üzerine Etkisi

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ÖZET

Bu çalışmanın amacı diflufenican ve diflufenicanın s-metolachlor veya metribuzin ile birlikte aktif madde bazında sarı acı bakla bitkisine uygulanmasının nitrogenaz aktivitesi, kimi mikroorganizma gruplarının sayıları ve toprak enzim aktivitesine etkisi ile enzimlerin uygulanan herbisitlere duyarlılıklarını belirlemektir. Tüm uygulamalar sarı acı baklanın vejetasyon periyodunun başlangıcında toplam bakteri sayısının, aktinobakteri sayısının ve oligotrofik bakteri sayısının azalmasına neden olmuştur. Diflufenicanın tek başına uygulanması durumunda nitrogenaz aktivitesinin arttığı tespit edilmiştir. Bu araştırma sonuçları toğrağa denenen herbisitlerin bulaşmasının dehidrogenaz ve asit fosfataz aktivitelerinde yüksek düzeyde duyarlılığa yol açtığını göstermiştir. Dehidrogenaz aktivitesi ile sayıları azalan mikroorganizma grupları arasında yakın ilişki bulunduğu tespit edilmiştir. Diflufenicanın tek başına uygulanmasının biyolojik toprak özellikleri üzerine çok düşük düzeyde olumsuz etkide bulunması sonucunda kirletici unsurlara diğer uygulamalara göre toprak çevresine de düşük düzeyde olumsuz etkilerinin olacağı sonucuna ulaşılmıştır.

Anahtar Kelimeler: Aktif maddeler; Herbisitler; Mikroorganizma sayıları; Baklagil bitkileri; Toprak enzim aktivitesi

1. Introduction

The cultivation of leguminous plants has a wide range of advantages, including the production of atmospheric nitrogen, the recovery of nutrients washed out into deeper soil layers by means of the long root system, improvement of the soil structure by leaving air tracts, and increasing the sorption capacity of the soil complex and the content of humus by leaving a large mass of crop residues (Galbally et al 2010). Soil nitrogen-fixing bacteria play an important role in leguminous plant productivity (Angelini et al 2013) as well as for other crops. In spite of many advantages of leguminous plants, there is not much interest in the production of these species in Poland, as the area where these crops are sown amounts to only 1% of the total crop area (GUS 2014). Leguminous plants tend to increase the number of weeds, due to their slow initial growth. Therefore, agronomists tend to use chemical methods of controlling weeds, and they continue to extend the range of herbicides added to the soil. Consequently, the accumulation of various contaminants in soil often deteriorates its properties, especially its bioactivity, including the process of biological nitrogen fixation by bacteria symbiotic with leguminous plants.

Herbicides are chemical compounds which affect life in the soil (Singh & Ghoshal 2010). Being substances with high bioactivity, they may cause an imbalance in the quantity and quality of soil microorganisms. When herbicides are applied in recommended doses the inhibitory effect on microorganisms is transient (Adhikary et al 2014). Zain et al (2013) found a transient negative effect on soil microorganisms in an oil palm plantation even when a double dose of herbicides was applied. The biodegradation of pesticides can take place along different metabolic pathways. It is connected with the structure and properties of the preparation applied, and the environmental conditions. Regarding the latter, the availability of oxygen, chemical properties of soil and the enzymatic potential from individual microorganisms inhabiting a particular soil play a vital role in the biodegradation of plant protection compounds (Arbeli & Fuentes 2007). Investigations revealed that adaptation of microorganisms to herbicide treatment occurs after several weeks of application, which appeared as fluctuations of soil enzyme activities (Sebiomo et al 2011).

Crop protection products applied to soil may have direct and indirect effects on the functioning of soil enzymes. Bielińska et al (2014) indicate that the direct effect consists in the influence on the extracellular enzyme activity. On the other hand, the indirect effect consists in the impact of pesticides on enzyme biosynthesis by soil microorganisms, as well as on the composition of the population of soil microorganisms (increasing/decreasing the population of microorganisms producing

extracellular enzymes) and on the plant root systems (the state of symbiosis, production of root secretions, releasing enzymes from dead roots).

In order to assess the influence of the degree of soil contamination with herbicides, biological tests are used, including the number of soil microorganisms and enzyme activity (Sharma & Suri 2011).

The aim of the study was to assess how selected active ingredients of herbicides, applied to yellow lupine, affect the nitrogenase activity, the populations of selected groups of microorganisms (total number of bacteria, copiotrophic bacteria, oligotrophic bacteria, actinobacteria and fungi), the activity of soil enzymes (dehydrogenases activity (DHA), acid phosphatase activity (PAC)) and their sensitivity (RS-soil resistance) to the tested preparations.

2. Material and Methods

2.1. Experimental design

Studies were conducted in 2011 and 2012 on plots of Brody Agricultural Experimental Station of the Department of Agronomy, which belongs to Poznań University of Life Sciences (52.25895° N, 16.17874° E, 93 m a.s.l.). The yellow lupine (*Lupinus luteus* L.) cultivar of Mister was sown in plots of 28 m^2 (4×7 m). The experimental design was split block with four replications. Soil experimental plots, according to the FAO/WRB classification, were haplic luvisols. The granulometric composition of the soil ranged from light clayey sands to strong sands deposited on light clays. Parts smaller than 0.002 mm diameter make up 6% (loamy sand). The groundwater level rose in the period of excessive waterlogging. However, the groundwater usually occurred at a depth of 2 m. The content of organic substance in the soil used in the experiment was 1.6%, and the soil pH was 6.0. The content of macroelements in amounts of pure components were as follows; N, 98.9 kg ha^{-1}, P, 65.8 mg kg^{-1}, K, 226.6 mg kg^{-1}, Mg, 69.0 mg kg^{-1}. In the spring, mineral NPK fertilisation was applied at an amount of 40-60-60 kg ha^{-1} (188 kg ha^{-1} ammonium nitrate and 250 kg ha^{-1} Agrafoska P-K 24-24). Some local climatic data for experimental area are presented in Table 1.

Soil samples were collected from four variants. The samples differed in the type of herbicides used for yellow lupine cultivation. The doses of herbicides were optimal and recommended for these crops. One of the plots was the control sample, where no pesticide was used, while the other three differed in the type of active substances (Table 2). Diflufenican (2',4'-difluoro-2-[α,α,α-trifluoro-m-tolyloxy] nicotinanilide) is an active ingredient of Legato 500

Table 1- Meteorological conditions in 2011 and 2012 at experimental site

Month	*Temperature (°C)*			*Precipitation (mm)*		
	1961-2010	*2011*	*2012*	*1961-2010*	*2011*	*2012*
January	-1.6	0.9	-1.9	40.3	73.9	42.6
February	-0.6	-3.5	-0.2	32.7	47.9	26.1
March	2.8	-5.7	-2.5	39.1	20.0	12.0
April	8.0	8.8	8.0	37.2	22.9	15.4
May	13.2	14.8	14.4	57.1	77.2	69.8
June	16.5	16.0	17.3	64.1	163.0	125.3
July	18.2	19.2	20.1	81.2	194.6	67.3
August	17.6	18.7	19.1	66.0	60.1	51.5
September	13.3	14.3	12.9	48.9	30.0	33.7
October	8.6	8.2	10.3	42.0	47.6	10.9
November	3.6	4.8	5	45.3	54.8	34.1
December	0.0	-1.5	2.7	48.4	16.5	27.8
Average/sum	8.3	7.9	8.8	602.3	808.5	516.5

Table 2- Scheme of field experiment

Experimental variants	*Doses of active substances (g ha^{-1})*	*Trade name of the herbicide*
Control (no herbicide)	0	-
Diflufenican	100	Legato 500 SC
Diflufenican + s-metolachlor	100+1152	Legato 500 SC + Dual Gold 960 EC
Diflufenican + metribuzin	100+140	Legato 500 SC + Sencor 70 WG

SC herbicide manufactured by Adama. Diflufenican is an active substance that belongs to the group of anilide herbicides. The half-life degradation is 15-30 weeks, which seems relatively fast (Bending et al 2006). S-metolachlor (2-chloro-*N*-2-ethyl-6-methylphenyl-N-[(1S)-2-methoxy-1-methylethyl] acetamide) is an active substance in Dual Gold 960 EC herbicide manufactured by Syngenta. Metribuzin (4-amino-6-*tert*-butyl-3-(methylthio)-as-triazin-5(4*H*)-one) is an active substance in Sencor 70 WG herbicide manufactured by Bayer Crop Science. Metribuzin has been found to be a substance with short half-life degradation in the surface soil layer, but high mobility into deeper soil layers and consequently a high potential to transfer into groundwater were also observed (Pot et al 2011). S-metolachlor is characterized by movement in and out of the soil matrix, and can reach ground and surface water (Zemolin et al 2014).

Each time 14-18 individual samples were collected from a particular experimental variant, and they were used for a representative mixed sample. Soil samples were collected to analyse four terms, which were related to the consecutive stages of yellow lupine growth and doses of herbicides applied. The samples were collected at the following terms: before sowing (BBCH 0), during emergence (BBCH 10-13), at the beginning of flowering (BBCH 51-69), and after harvest.

2.2. Nitrogen fixation measurements

The activity of nitrogenase was assessed as acetylene reduction activity (ARA) at the beginning of flowering of plants. The methodology of nitrogenase activity measurements was based on Sawicka (1983), Niewiadomska & Sawicka (2002) and Lorenc-Plucińska et al (2013). The results of nitrogenase activity were expressed in nmol C_2H_4 plant h^{-1}.

2.3. Soil enzymatic activity analyses

The analyses of the enzymatic activity of soil in different variants were based on the colorimetric method applied to measure the dehydrogenase activity (DHA), where 1% TTC (triphenyl tetrazolium chloride) was used as a substrate. The measurement took place after 24-hour incubation at 30 ºC and a wavelength of 485 nm, and it was expressed in mmol TPF (triphenyl formazan) kg^{-1} DM 24 h^{-1} (Thalmann 1968).

The biochemical analyses of soil involved the determination of activities of acid (EC 3.1.3.2) phosphomonoesterases (PAC) with the method developed by Tabatabai & Bremner (1969). The activities were determined with disodium p-nitrophenyl phosphate tetrahydrate used as a substrate after 1 h of incubation at 37 °C at a wavelength of 400 nm. The results were converted into mmol PNP (p-nitrophenol) kg^{-1} DM h^{-1} (Lorenc-Plucińska et al 2013).

2.4. Microbial analyses

The samples were collected from the soil under the plants, from inter-rows, at a depth of 15-20 cm. The number of microorganisms was measured with the method of pour plate dilution developed by Koch on appropriate agar medium in five replications. The mean number of colonies was calculated per dry weight of soil in the following way; the total number of bacteria was measured on a ready Merck-Standard agar after 72 hours of incubation at 25 °C; the number of fungi was measured on Martin (1950) medium after five days of incubation at 24 °C; the number of copiotrophs was measured on NB medium after five days of incubation at 25 °C; the number of oligotrophs was measured on DNB medium (Ohta & Hattori 1980) after five days of

incubation at 25 °C; the number of actinobacteria was measured on Pochon medium after five days of incubation at 25 °C (Grabińska-Łoniewska 1999).

The level of contamination of the herbicides in the soil was analysed based on the soil resistance (RS) index (Equation 1).

$$RS = 1 - \frac{2 \mid D_0 \mid}{C_0 + \mid D_0 \mid} \quad (1)$$

Where; C_0, soil resistance under natural conditions over time t_0; P_0, resistance of soil subjected to pressure over time; $D_0 = C_0 - P_0$ (Orwin & Wardle 2004).

2.5. Statistical analysis

The results were statistically analysed with the aid of Statistica 10.0 software. In order to compare the mean values of the biological parameters at individual terms of analyses and the influence of selected herbicides, two-way analysis of variance was used (α= 0.05). It was followed by detailed Tukey's test (post-hoc Tukey HSD). Principal component analysis (PCA) was used to analyse the biological activity data resulting from the effect of herbicides at individual stages of yellow lupine development.

3. Results and Discussion

3.1. Soil microorganisms

The mean values of the results of the two-year study on the influence of different active substances of herbicides on the number of selected groups of soil microorganisms in yellow lupine cultivation were analysed statistically. The two-way analysis of variance revealed that the analysed factors had a highly significant influence (α= 0.001) on the dynamics of variation in the number of selected groups of soil microorganisms, i.e. total number of bacteria, actinobacteria, and oligotrophic bacteria. The analysis did not reveal an influence of the factors on the number of fungi or copiotrophic bacteria (Table 3).

The results showed that the crop protection products applied in the experiment disturbed the microbial balance of all the microorganisms. Variations in the populations of the microorganisms (total number of bacteria, actinobacteria, fungi, oligotrophic and copiotrophic bacteria) depended on the preparation applied and the term of analysis.

Table 3- F test statistics and significance levels of two-way analysis of variance for the number of selected groups of microorganisms and enzymes with type of herbicide and term of analysis fixed factors (*, P= 0.001; **, P= 0.01; *, P= 0.05; ns, not statistically significant)**

Parameter	*Herbicides*	*Term*	*Interaction*
Microorganisms			
Total number bacteria	1.01^{ns}	3.06^{ns}	2.31^{ns}
Actinobacteria	16.72^{***}	133.0^{***}	14.68^{***}
Fungi	0.9^{ns}	8.3^{***}	0.44^{**}
Copiotrophic bacteria	1.16^{ns}	2.21^{ns}	1.55^{ns}
Oligotrophic bacteria	1.17^{ns}	42.02^{***}	2.4*
Enzymes			
Dehydrogenases (DHA)	5.7^{**}	96.14^{***}	1.7^{ns}
Acid phosphatase (PAC)	5.3^{***}	74.60^{***}	4.7^{***}
Nitrogenase	43.16^{***}	-	-

The analysis of the influence of the applied crop protection products on the total number of bacteria revealed a significant decrease in the population of these groups of soil microorganisms during plant emergence. In the variant where diflufenican and s-metolachlor were simultaneously used, the number of bacteria dropped by 73% as compared with the control sample. There was a similar decrease in the population of this group of microorganisms in the variant where diflufenican was combined with metribuzin. The smallest response in the total number of bacteria was observed when diflufenican was applied separately; the population dropped by 27% in comparison with the non-herbicide samples (Table 4).

The application of diflufenican and its combination with the other active substances significantly inhibited the proliferation of another group of microorganisms, i.e. actinobacteria. There was a highly significant inhibitory effect of the pesticides before sowing, immediately after their application. The use of the herbicide

containing diflufenican as the active ingredient caused a decrease in the population of this group of microorganisms by 92%. On the other hand, diflufenican combined with s-metolachlor and diflufenican combined with the herbicide containing metribuzin reduced the population of this group of microorganisms by 82% and 46%, respectively, as compared with the control sample. In all the variants there was a noticeable increase in the population of actinobacteria during plant flowering. However, in variants where herbicides had been applied the population of actinobacteria was lower than in the control, uncontaminated soil sample (Table 4).

The actinobacteria were the most sensitive to the products applied, because during the whole vegetation the pesticides caused the number of actinobacteria to drop below the level noted in the uncontaminated control soil sample. Moreover, the decrease in the number of actinobacteria caused by the application of the herbicides may have resulted from the high toxicity of metabolites formed by the transformation of diflufenican, s-metolachlor and metribuzin. According to Niewiadomska et al (2011), both actinobacteria and oligotrophic bacteria belong to the 'eco-group' of microorganisms, which indicate soil fertility. When crop protection

Table 4- The number of selected groups of microorganisms in soil under yellow lupine analysed in different experimental variants and terms (mean values ± SE; different letters denote significant differences at level α= 0.05 among experimental variants within certain parameters and terms)

Experimental variants	*Term of analysis*			
	Before sowing	*Emergence*	*Beginning of flowering*	*After harvesting*
Total number of bacteria (cfu 10^5 g^{-1} DM)				
Control	12.88±3.4^{f}	33.79±9.4^{b}	42.56±8.7^{a}	7.98±1.1^{e}
Diflufenican	21.41±3.8^{de}	24.57±2.2^{d}	28.25±10.3^{cd}	15.20±7.7^{de}
Diflufenican + s-metolachlor	18.97±3.9^{e}	8.80±2.9^{g}	28.00±3.4^{cd}	19.21±3.3^{de}
Diflufenican + metribuzin	34.42±1.7^{b}	14.89±1.6^{f}	26.60±3.8^{cd}	30.40±8.8^{c}
Actinobacteria (cfu 10^5 g^{-1} DM)				
Control	103.08±6.6^{d}	155.83±7.8^{c}	459.54±15.6^{a}	35.72±2.6^{f}
Diflufenican	8.65±0.6^{g}	83.57±7.4^{de}	308.11±11.3^{b}	13.68±3.0^{g}
Diflufenican + s-metolachlor	18.22±4.3^{g}	40.3±6.7^{f}	439.04±36.3^{a}	26.74±2.9f^{g}
Diflufenican + metribuzin	51.29±6.1^{e}	67.27±7.7^{e}	80.67±4.6^{de}	12.54±2.9^{g}
Fungi (cfu 10^4 g^{-1} DM)				
Control	0.37±0.6^{d}	1.40±0.4^{d}	6.40±0.6^{a}	2.60±0.7^{cd}
Diflufenican	1.12±1.1^{d}	3.30±1.3^{c}	1.10±0.1^{d}	3.40±0.1^{c}
Diflufenican + s-metolachlor	1.86±0.8^{d}	2.30±1.3^{d}	3.40±0.8^{c}	4.80±0.3^{b}
Diflufenican + metribuzin	2.66±0.6^{cd}	5.40±1.1^{ab}	3.70±0.2^{c}	3.80±0.7c
Oligotrophic bacteria (cfu 10^5 g^{-1} DM)				
Control	48.35±9.8^{d}	5.08±1.4^{hi}	76.46±11.6^{ab}	21.28±6.2^{fg}
Diflufenican	12.39±9.7^{h}	5.13±1.7^{h}	85.12±12.4^{a}	17.86±9.9^{h}
Diflufenican + s-metolachlor	21.21±2.8^{fg}	1.46±0.6^{i}	73.92±9.1^{ab}	24.11±8.5^{f}
Diflufenican + metribuzin	29.94±4.4^{e}	4.36±1.2^{i}	63.27±12.2^{c}	60.05±10.9^{c}
Copiotrophic bacteria (cfu 10^5 g^{-1} DM)				
Control	29.23±9.6^{b}	20.71±9.1^{bc}	53.11±7.5^{a}	8.74±1.2^{bc}
Diflufenican	12.76±5.2^{bc}	28.23±7.7^{b}	66.67±4.0^{a}	23.94±4.3^{b}
Diflufenican + s-metolachlor	17.85±4.8^{bc}	10.26±1.3^{c}	21.28±5.1^{b}	28.62±5.2b
Diflufenican + metribuzin	27.70±4.3^{b}	16.35±2.8^{bc}	12.58±1.2^{bc}	22.80±2.5^{bc}

cfu, colony form unit

products are applied, their metabolites may disturb the growth of actinobacteria and thus reduce the fertility of the soil environment. The negative influence of herbicides on the proliferation of soil bacteria, including actinobacteria, was also observed by Sebiomo et al (2011), who studied atrazine, s-metolachlor, paraquat and glyphosate. Kucharski & Wyszkowska (2008) presented similar conclusions when they observed that the number of soil microorganisms decreased significantly after sulfosulfuron herbicide had been applied.

In the present study, no statistically significant effect of the tested substances on the growth of fungi was observed. On the other hand, when the yellow lupine was flowering, the use of herbicides caused a decrease in the fungal population. Lipşa et al (2010) reported that metolachlor caused a larger reduction of number of fungi in the soil. On the other hand, Anastasi et al (2012) stated that fungi play a major role in all ecosystems as decomposers. They are important participants in degradation of the major organic pollutants in the soil, such as herbicides. According to Baćmaga et al (2014) and Cycoń & Piotrowska-Seget (2009), some species of microorganisms use herbicides as a source of carbon and nutrients. The properties of microorganisms to degrade pesticides partly reduce toxic substances. In consequence, this may not only have a positive effect on the yield but may also improve the biological properties of soil. It is noteworthy that the preparations applied in our research additionally stimulated the number of fungi during the emergence of yellow lupine. The stimulating effect of herbicides on the proliferation of fungi was also observed by Crouzet et al (2010), who studied the effect of mesotrione applied to soil at doses of 0.45-45 mg kg^{-1}, and by Zabaloy et al (2010), who researched the influence of dichlorophenoxyacetic acid applied to soil at doses of 1 to 10 mg kg^{-1}. On the other hand, Martinez et al (2008) found that sulfentrazone did not affect the number of fungi in soil. Meanwhile, the study by Giri et al (2011) suggested that application of pesticides may create a great problem for beneficial soil microorganisms and microbial transformation of several primary and secondary nutrients. They observed that pesticides such as endosulfan and mancozeb exerted a detrimental effect on transformation of sulphur, whereas 2,4-D created a favourable beneficial effect on sulphur transformation in the soil environment.

Similarly to other groups of microorganisms, oligotrophic bacteria also responded with variation in their population in the soil, which had been induced by the application of crop protection products. The negative influence of the xenobiotics before sowing, immediately after their application and during the plants' emergence was observed. However, at the stage of yellow lupine flowering there was a noticeable increase in this group of microorganisms in the variant where diflufenican and the mixture diflufenican with s-metolachlor had been applied. On the other hand, after yellow lupine had been harvested, there was a significant increase in this group of microorganisms in the variant where diflufenican had been applied in a mixture with metribuzin (Table 4).

The field doses of herbicides also disturbed the balance of copiotrophic bacteria in the soil. Although the results were not statistically confirmed, the population of these microorganisms decreased before the plants' emergence and while they were flowering, especially in the variants where diflufenican had been applied in combination with s-metolachlor or metribuzin. When diflufenican was applied separately, it proved to be the least toxic, as during the emergence and flowering of yellow lupine the population of copiotrophs increased by 40% and 28%, respectively, as compared with the uncontaminated soil sample (Table 4). The results of the study presented by Sahoo et al (2016) indicated that pretilachlor at the recommended dose can be safely used for controlling grassy weeds in rice fields, but application of pretilachlor at a higher dose, in general, significantly reduced the number of bacteria, actinobacteria, fungi, nitrogen fixers, and microbial biomass carbon.

3.2. Nitrogen fixation

A statistically significant influence of plant protection chemicals on nitrogenase activity was noted in

all treatments of the field experiment in which herbicides were applied. The highest nitrogenase activity was observed for application of diflufenican in comparison to the control, while the application in combination with metribuzin and s-metolachlor inhibited the analysed enzyme (Figure 1). Khan et al (2006) also recorded a reduction of nitrogenase activity after the pre-emergent application of methabenzthiazuron (MBT), terbutryn, and linuron to chickpea cultivation. Similar results were noted by Angelini et al (2013), who revealed a negative effect of a series of active ingredients (including s-metolachlor) on the diazotrophic bacterial community and soil nitrogenase activity. Moreover, they also noted a prolonged negative effect on the next culture, even at one year after application.

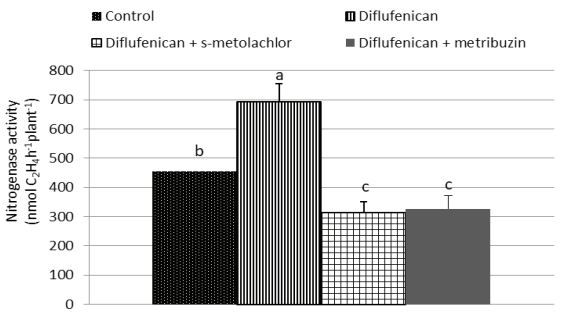

Figure 1- The effect of herbicides on nitrogenase activity (means ± SE; different letters denote significant differences at level α= 0.05)

3.3. Soil enzymes

When diflufenican and the mixture of diflufenican with s-metolachlor or with metribuzin were used, they significantly affected the activity of the soil enzymes, i.e. dehydrogenases and acid phosphatase (Table 3). Soil enzymes can be treated as indicators of changes in the soil environment caused by application of several xenobiotics. These enzymes are involved in many processes of biodegradation of natural and anthropogenic compounds, such as herbicides (Wang et al 2009; Baćmaga et al 2012; Baćmaga et al 2014).

The crop protection products applied in the experiment had a negative influence on the dehydrogenases activity (DHA) at all the terms of analyses. The results revealed that when the plants were at the full flowering stage and after the harvest, this parameter was most negatively influenced by the crop protection products combining diflufenican with s-metolachlor or with metribuzin (Figure 2). The activity of soil dehydrogenases was previously found to be the most sensitive detector of the soil microorganisms' condition, due to their occurrence in all living cells (Gomez et al 2009). Dehydrogenases are intercellular enzymes whose activity is closely correlated with microbial activity (Bello et al 2013). Our results indicate very high sensitivity of dehydrogenases to the soil contamination caused by the application of diflufenican, the mixture of diflufenican with s-metolachlor or the mixture with metribuzin. The highest inhibition of DHA was noted in the variant when diflufenican with metribuzin was applied. Baćmaga et al (2014) obtained similar results in their research on the activity of these enzymes in soil contaminated with metazachlor. Kucharski et al (2008) noted the negative effect of herbicides (Harpun 500SC, Faworyt 300, Akord 180OF) on the dehydrogenase activity. Cycoń et al (2013) also described the reduced activity of these enzymes caused by the application of napropamide.

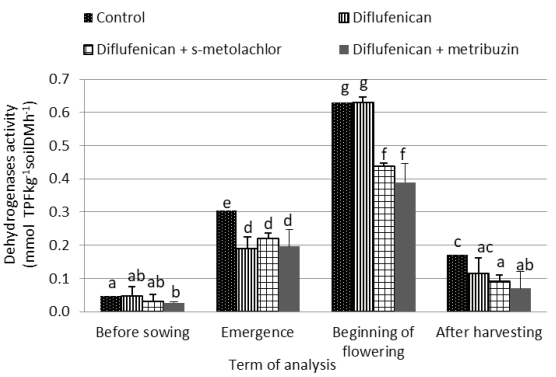

Figure 2- The effect of herbicides on dehydrogenases activity in different terms (means ± SE; different letters denote significant differences at level α= 0.05)

Physiological processes in microorganisms can be affected by pesticides and their metabolites, and in turn modify the activity of soil enzymes (Hussain

et al 2009; Romero et al 2010). However, the separate application of glyphosate and diflufenican in loam and sandy loam did not change the soil microbial activity, while inhibition was noted for the combination of the above-mentioned substances (Tejada 2009).

Phosphatase was another enzyme used for testing the soil quality. It is responsible for the mineralisation of organic phosphorus compounds (Rahmansyah et al 2009). In our experiment, acid phosphatase also reacted negatively to the crop protection products applied in the experiment. However, its activity decreased at the stage of the plants' emergence only in the variant where diflufenican had been applied separately and in the combination with s-metolachlor. At the other terms of analyses the herbicides stimulated the activity of the enzyme. The application of the preparations increased the acid phosphatase activity by 14% at the flowering stage and by 31% after the harvest, as compared with the control sample (Figure 3). Baćmaga et al (2012) obtained similar results in their research on the influence of carfentrazone and a mixture of diflufenican + mesosulfuron-methyl + iodosulfuron-methyl-sodium.

The ratio of sensitivity (RS) values (Table 5) varied depending on whether diflufenican had been applied separately or in combination with s-metolachlor or in combination with metribuzin.

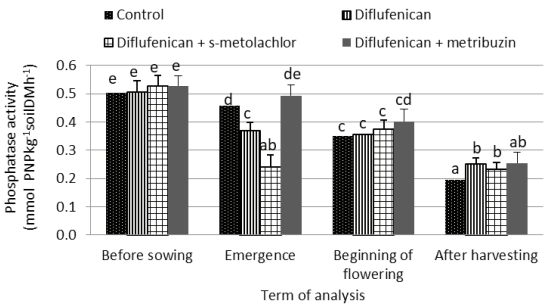

Figure 3- The effect of herbicides on acid phosphatase activity in different terms (means ± SE; different letters denote significant differences at level α= 0.05)

The highest RS level was observed for dehydrogenases (0.959) when the plants were flowering in the variant where diflufenican had been applied in combination with s-metolachlor, whereas the lowest RS value was observed for acid phosphatase (0.022) immediately after the application of diflufenican in combination with metolachlor.

Regardless of the herbicides applied, dehydrogenases exhibited greater sensitivity to contamination than acid phosphatases, and it did not decrease during the vegetation of yellow lupine. On the other hand, the sensitivity of acid phosphatases depended on the variant applied and the term of application (Table 5). When diflufenican was applied separately, the enzymes exhibited low sensitivity

Table 5- Resistance of soil (RS) enzymes to contamination with herbicides (PAC, acid phosphatase activity; DAH, dehydrogenases activity; different letters denote significant differences at level α= 0.05 among experimental variants within certain parameters and terms)

Experimental variants	*Term of analysis*			
	Before sowing	*Emergence*	*Beginning of flowering*	*After harvesting*
RS index of PAC				
Diflufenican	0.358^{ab}	0.531^{ab}	0.817^{a}	0.519^{ab}
Diflufenican + s-metolachlor	0.022^{b}	0.568^{c}	0.568^{ab}	0.486^{ab}
Diflufenican + metribuzin	0.347^{ab}	0.529^{ab}	0.488^{ab}	0.137^{b}
RS index of DAH				
Diflufenican	0.939^{a}	0.704^{ab}	0.954^{a}	0.534^{ab}
Diflufenican + s-metolachlor	0.792^{a}	0.564^{ab}	0.959^{a}	0.810^{a}
Diflufenican + metribuzin	0.911^{a}	0.295^{c}	0.787^{ab}	0.723^{ab}

immediately after the treatment, before the plants were sown (0.358). However, later, when the plants were flowering, the sensitivity increased (0.817). On the other hand, the contamination of the soil with diflufenican combined with s-metolachlor did not cause such high sensitivity of acid phosphatase as when diflufenican was applied separately. The RS reached the highest value during the plants' emergence (0.580), but later the sensitivity decreased. In the variant where diflufenican and metribuzin were applied, the highest value of the ratio was observed during the plants' emergence and flowering, i.e. 0.529 and 0.488, respectively, but later it dropped to 0.137 (Table 5).

The soil ecosystem is considered to be stable if it can oppose stress-inducing factors and quickly recovers the state of balance (Orwin & Wardle 2004; Griffiths & Philippot 2013). Soil stability is usually estimated according to resistance and resilience. In our study the RS value was calculated by estimating the resistance of the soil contaminated with diflufenican separately and diflufenican combined with s-metolachlor as well as diflufenican combined with metribuzin.

The imbalance in the dehydrogenases activity proved to be more sensitive to the effect of the herbicides than phosphatase. Griffiths & Philippot (2013) emphasised that long-term application of xenobiotics can have an impact on defensive mechanisms of the soil and furthermore cause an imbalance of the maintenance of soil biological stability.

The results of the influence of the herbicides on the bioactivity of soil were illustrated by means of principal component analysis (PCA). The analysis highly (58.55%) explained the dependences occurring in the soil environment under yellow lupine due to the application of diflufenican as well as diflufenican combined with s-metolachlor and diflufenican combined with metribuzin. At all the terms of analyses, there was a strong correlation between the number of microorganisms and soil enzyme activities. Moreover, there was a positive correlation between the effect of the crop protection products and the bioactivity of soil when yellow lupine was flowering. At the other terms of analyses, there were negative correlations (Figure 4).

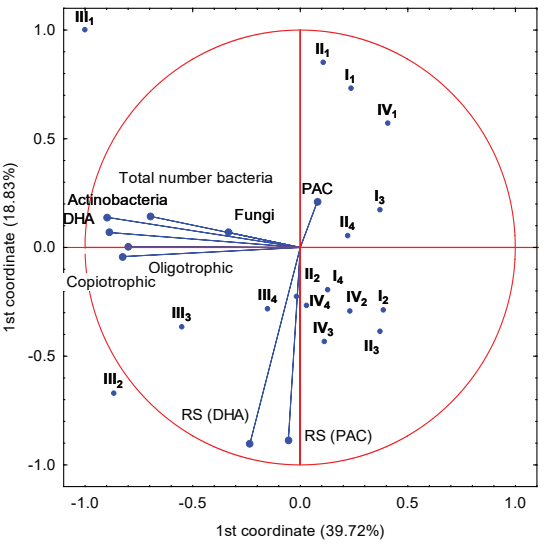

Figure 4- The dependence between the population of microorganisms and soil enzymatic activity for all variants with herbicides at the terms of analyses; I, before sowing; II, emergence; III, beginning of flowering; IV, after harvesting; 1, control; 2, diflufenican; 3, diflufenican + s-metolachlor; 4, diflufenican + metribuzin; PAC, acid phosphatase; DHA, dehydrogenases; RS, soil resistance

Overall, soil fertility can be affected by active substances of herbicides, due to changes of the number of soil bacteria. The quality of soil is inextricably associated with the microbial transformations. Therefore, in order to better understand their effect, it is necessary to make a detailed analysis of the microbial processes they manage (Bello et al 2008; Saha et al 2012; Baćmaga et al 2014). Our results indicate that the crop protection products applied in the experiment, diflufenican and the product in combination with s-metolachlor or metribuzin, applied to yellow lupine had various effects on the groups of soil microorganisms, and the effects depended on the product applied and on the term of analyses. S-metolachlor herbicide is

one of the three most used herbicides in the world in the chloroacetanilide class. This herbicide has high toxicity and can be leached, representing a powerful source of groundwater pollution (Liu et al 2001). Martins et al (2007) stated that only four microorganisms in soil have the ability to degrade this xenobiotic.

4. Conclusions

Diflufenican and its combinations with s-metolachlor or metribuzin applied to yellow lupine caused a statistically significant biological imbalance in the soil, reducing the total number of bacteria as well as the number of actinobacteria and oligotrophic bacteria at the beginning of vegetation, immediately after the treatment. The experiment revealed a stimulatory role of diflufenican used separately on nitrogenase activity, while in combination with other products an inhibitory effect was observed. The research revealed very high sensitivity of dehydrogenases and acid phosphatase to the soil contamination caused by the application of diflufenican, the mixture with s-metolachlor as well as the mixture with metribuzin. The field doses of the xenobiotics used in the research significantly reduced the dehydrogenase activity during the whole vegetation season. The dehydrogenase activity values were closely correlated with reduced populations of the groups of microorganisms.

In summary, the application of certain active ingredients alone or as a mixture should be considered according to the potential effect on soil microbiological activity and further soil fertility.

References

Adhikary P, Shil S & Patra P S (2014). Effect of herbicides on soil microorganisms in transplanted chilli. *Global Journal of Biology, Agriculture and Health Science* **3**(1): 236-238

Anastasi A, Tigini V & Varese G C (2012). The Bioremediation Potential of Different Ecophysiological Groups of Fungi. *Soil Biology* **32**: 29-49

Angelini J, Silvina G, Taurian T, Ibáñez F, Tonelli L M, Valetti L, Soledad Anzuay M, Ludueña L, Muñoz V & Fabra A (2013). The effects of pesticides on bacterial nitrogen fixers in peanut-growing area. *Archives of Microbiology* **195**: 683-692

Arbeli Z & Fuentes C L (2007). Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions and a discussion on the tropical dimension. *Crop Protection* **26**: 17-33

Baćmaga M, Boros E, Kucharski J & Wyszkowska J (2012). Enzymatic activity in soil contaminated with the Aurora 40WG herbicide. *Environmental Protection and Engeneering* **38**(1): 91-102

Baćmaga M, Kucharski J, Wyszkowska J, Borowik A & Tomkiel M (2014). Responses of microorganisms and enzymes to soil contamination with metazachlor. *Environmental Earth Science* **72**: 2251-2262

Bello D, Trasar-Capeda C, Leirós M C & Gill-Sotres F (2008). Evaluation of various tests for diagnosis of soil contamination by 2,4,5-trichlorophenol (2,4,5-TCP). *Environmental Pollution* **156**: 611-617

Bello D, Trasar-Capeda C, Leirós M C & Gill-Sotres F (2013). Modification of enzymatic activity in soils of contrasting pH contaminated with 2,4-dichlorophenol and 2,4,5-trichlorophenol. *Soil Biology and Biochemistry* **56**: 80-86

Bending G D, Lincoln S D & Edmondson R N (2006). Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. *Environmental Pollution* **139**: 279-287

Bielińska J, Futa B & Mocek-Płóciniak A (2014). Soil enzymes as bio-indicators of soil health and quality. Libropolis Press, Lublin

Crouzet O, Batisson I, Besse-Hoggan P, Bonneomoy F, Bardot C, Poly F, Bohatier J & Mallet C (2010). Response of soil microbial communities to the herbicide mesotrione: A dose-effect microcosm approach. *Soil Biology and Biochemistry* **42**: 193-202

Cycoń M & Piotrowska-Seget Z (2009). Changes in bacterial diversity and community structure following pesticides addition to soil estimated by cultivation technique. *Ecotoxicology* **18**(5): 632-642

Cycoń M, Wójcik M, Borymski S & Piotrowska-Seget Z (2013). Short-term effects of the herbicide napropamide on the activity and structure of the soil microbial community assessed by the multi approach analysis. *Applied Soil Ecology* **66**: 8-18

Galbally I E, Meyer M C P, Wang Y P, Smith C J & Weeks I A (2010). Nitrous oxide emissions from a legume pasture and the influences of liming and urine addition. *Agriculture, Ecosystems and Environment* **136**: 262-272

Giri P K, Saha M, Halder M P & Mukherjee D (2011). Effect of pesticides on microbial transformation of sulphur in soil. *International Journal of Plant Animal and Environmental Science* **1**(2): 115-121

Gomez E, Ferreras L, Lovotti L & Fernandez E (2009). Impact of glyphosate application on microbial biomass and metabolic activity in Vertic Arggiudoll from Argentina. *European Journal of Soil Biology* **45**: 163-167

Grabińska-Łoniewska A (1999). Laboratory classes in general microbiology. Warsaw Technology University Press, Warsaw

Griffiths B S & Philippot L (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiological Review* **37**: 112-129

GUS (2014). Central Statistical Office of Poland. Agriculture in 2014. Retrieved in March, 24, 2015 from http://www.stat.gov.pl

Hussain S, Siddique T, Saleem M, Arshad M & Khalid A (2009). Impact of pesticides on soil microbial diversity, enzymes and biochemical reactions. *Advances in Agronomy* **102**: 159-200

Khan M S, Zaidia A & Rizvib P Q (2006). Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity, and seed production in chickpeas. *Communications in Soil Science and Plant Analysis* **37**(11-12): 1783-1793

Kucharski J & Wyszkowska J (2008). Biological properties of soil contaminated with the herbicide Apyros 75 WG. *Journal of Elementology* **13**(3): 357-371

Lipşa F D, Ulea E, Chiriac I P & Coroi I G (2010). Effect of herbicide s-metolachlor on soil microorganisms. *Lucrări Ştiinţifice Seria Agronomie* **53**(2): 110-113

Liu W P, Liu H J, Zheng W & Lu J H (2001). Adsorption of chloroacetanilide herbicides on soil structural influence of chloroacetanilide herbicidefor their adsorption on soils its components. *Journal of Environmental Science* **13**(1): 37-45

Lorenc-Plucińska G, Walentynowicz M & Niewiadomska A (2013). Capabilities of alders (*Alnus incana* and *A. glutinosa*) to grow in metal-contaminated soil. *Ecological Engineering* **58**: 214-227

Martin J P (1950). Use of acid, rose Bengal and streptomycin in the plate method for estimating soil fungi. *Soil Science* **69**: 215-233

Martinez C O, Silva C M M S, Fay E F, Maia A H N, Abakerli R B & Durrant L R (2008). Degradation of the herbicide sulfentrazone in a Brazilian typic hapludox soil. *Soil Biology and Biochemistry* **40**: 879-886

Martins P F, Martinez C O, De Carvalho G, Irajara P, Azevedo A A, Pileggi S A, De Melo I S & Pileegi M (2007). Selection of microorganisms degrading S-metolachlor herbicide. *Brazilian Archives of Biology and Technology* **50**(1): 153-159

Niewiadomska A & Sawicka A (2002). Effect of carbendazim, imazethapyr and thiram on nitrogenase activity, number of microorganisms in soil and yield of hybrid lucerne (*Medicago media*). *Polish Journal of Environmental Studies* **6**: 737-744

Niewiadomska A, Sawińska Z & Wolna-Maruwka A (2011). Impact of selected seed dressing on soil microbiological activity in spring barley cultivation. *Fresenius Environmental Bulletin* **20**(5a): 1252-1261

Ohta A & Hattori T (1980). Bacteria sensitive to nutrient broth medium in terrestrial environments. *Soil Science of Plant Nutrition* **26**: 99-107

Orwin K H & Wardle D H (2004). New indices for quantifying the resistance and resilience of soil biota to exogenous disturbance. *Soil Biology and Biochemistry* **36**: 1907-1912

Pot V, Benoit P, Le Menn M, Eklo O-M, Sveistrup T & Kvaerner J (2011). Metribuzin transport in undisturbed soil cores under controlled water potential conditions: experiments and modelling to evaluate the risk of leaching in a sandy loam soil profile. *Pest Management Science* **67**: 397-407

Rahmansyah M, Antonius S & Sulistinah N (2009). Phosphatase and urease instability caused by pesticides present in soil improved by grounded rice straw. *Journal of Agricultural and Biological Sciences* **4**(2): 56-62

Romero E, Fernández-Bayo J, Díaz J M C & Nogale R (2010). Enzyme activities and diuron persistence in soil amended with vermicompost derived from spent grape marc and treated with urea. *Applied Soil Ecology* **44**: 198-204

Saha S, Dutta D, Karmakar R & Ray D P (2012). Structure-toxicity relationship of chloroacetanilide herbicides: relative impact on soil microorganisms. *Environmental Toxicology and Pharmacology* **34**: 307-314

Sahoo S, Adak T, Bagchi T B, Kumar U, Munda S, Saha S, Berliner J, Jena M & Mishra B B (2016). Non-target effects of pretilachlor on microbial properties in tropical rice soil. *Environmental Science and Pollution Research* **23**(8): 7595-7602

Sawicka A (1983). The ecological aspects of dinitrogen fixation. Poznań Agricultural University Annals, *Scientific Dissertations* **134**: 1-57

Sebiomo A, Ogundero V W & Bankole S A (2011). Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology* **10**: 770-778

Sharma P & Suri C R (2011). Biotransformation and biomonitoring of phenylurea herbicide diuron. *Bioresources Technology* **102**: 3119-3125

Singh P & Ghoshal N (2010). Variation in total biological productivity and soil microbial biomass in rainfed agroecosystems: Impact of application of herbicide and soil amendments. *Agriculture, Ecosystem and Environment* **137**: 241-250

Tabatabai V & Bremner J (1969). Use of p-nitrophenyl phosphate for assays of soil phosphatase activity. *Soil Biology and Biochemistry* **1**: 301-307

Tejada M (2009). Evaluation of soil biological properties after addition of glyphosate, diflufenican and glyphosate+diflufenican herbicides. *Chemosphere* **76**: 365-373

Thalmann A (1968). Zur methodik der bestimmung der dehydrogenase aktivität in boden mittels triphenyltetrazoliumchlorid (TTC). *Landwirtschaftliche Forschung* **21**: 3-4

Wang Q, Zhou D & Cang L (2009). Microbial and physicochemical properties of soil contaminated with herbicide Triflurotox 250 EC. *Polish Journal of Environmental Studies* **13**: 223-231

Zabaloy M C, Garland J L & Gomez M A (2010). Assessment of the impact of 2,4-dichlorophenoxyacetic acid (2,4-D) on indigenous herbicide degrading bacteria and microbial community function in an agricultural soil. *Applied Soil Ecology* **46**: 240-246

Zain N M M, Mohamad R B, Sijam K, Morshed M M & Awang Y (2013). Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. *African Journal of Microbiology Research* **7**(5): 367-374

Zemolin S R, Avila L A, Cassol G V, Massey J H & Camargo E R (2014). Environmental fate of s-metolachlor-a review. *Planta Daninha* **32**(3): 655-664

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Jasmonic Acid Induced Systemic Resistance in Infected Cucumber by *Pythium aphanidermatum*

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ABSTRACT

Damping-off disease of cucumber is one of the most destructive diseases of cucumber in worldwide. In this work, the potential of jasmonic acid (JA) for induce resistant against damping off disease was investigated. The effect of JA on activity of Polyphenol oxidase (PPO), Peroxidase (PO) and Catalase (CAT) enzymes and total phenol was assayed by spectrophotometric method. Expression level of three plant defense genes as *Lipoxygenase, Cupi4* and *Phenylalanine ammonia-lyase* genes was analyzed using qRT-PCR method. Drop-plate method was used to assay inhibitory effect of JA on radial growth of fungi. Exogenic application of JA decreased disease severity in the infected plants but did not inhibit mycelia growth on solid medium compared to control. Our results showed that JA application substantially increased the activity of oxidative enzymes at different concentration. The highest enzyme activity was recorded after 48 hours post infection (hpi) at a concentration of 400 mg L^{-1} of JA. Gene expression analysis revealed that JA is differentially able to increase the mRNA transcripts of all tested genes at 48 hpi. The expression level of *Cupi4* gene was higher than the other genes in treated plants. Induced systemic resistance by JA was mediated through an enhanced expression of ISR marker genes and increase of antioxidant enzymes activity. Based on these results, we suggest that exogenic application of JA could be considered as plant resistance inducer.

Keywords: Real-time PCR; Defensive enzymes; Elicitors; Damping-off; Gene expression; *Cpui4* gene

Pythium aphanidermatum ile Bulaşık Hıyarlarda Jasmonik Asitin Uyardığı Sistemik Dayanıklılık

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ÖZET

Çökerten hastalığı dünyanın her yerinde hıyarlarda en tahripkâr hastalıklarından biridir. Bu çalışmada, çökerten hastalığına karşı jasmonik asitin (JA) uyarılmış dayanıklılığı teşvik etme potansiyeli incelenmiştir. JA'nın polifenoloksidaz (PPO),

peroxidaz (PO) ve katalaz (CAT) enzimlerinin aktiviteleri ve toplam fenollere etkisi spektrofotometrik yöntemle belirlenmiştir. Üç bitki savunma geninin, *Lipoxygenase, Cupi4* ve *Phenylalanine ammonia-lyase* ifade seviyeleri qRT-PCR yöntemi kullanılarak analiz edilmiştir. Mantarın dairevi gelişimine JA'nın engelleyici etkisi damlatma yöntemi kullanılarak tespit edilmiştir. JA'nın dıştan uygulanması bulaşık bitkilerdeki hastalık şiddetini azaltmasına rağmen katı ortamda misel gelişimini engellememiştir. Sonuçlarımız, farklı yoğunluklarda JA uygulamasının oksidatif enzim faaliyetini büyük oranda artırdığını göstermiştir. En yüksek enzim faaliyeti 400 mg L^{-1} JA yoğunluğunda bulaştırmadan 48 saat sonra kaydedilmiştir. Gen ifadesi analizleri bulaştırmadan 48 saat sonra JA'nın bütün test edilen genlerin mRNA kopyalarını farklı da olsa artırabildiğini ortaya koymuştur. *Cupi4* geninin ifade edilme seviyesi, uygulama yapılan bitkilerde, diğer incelenen genlerinkinden daha fazla bulunmuştur. JA tarafından uyarılmış sistemik dayanıklılık, ISR işaretleyici genlerinin ifade edilmesi ve antioksidan enzim faaliyetinin artışı vasıtasıyla olmuştur. Sonuçlar, JA'nın dıştan uygulanmasının bitki dayanıklılığını uyarıcı olarak kabul edilebileceğini göstermiştir.

Anahtar Kelimeler: Real-time PCR; Savunma enzimleri; Elisitörler; Çökerten hastalığı; Gen ifadesi; *Cpui4* gene

1. Introduction

Damping-off disease of cucumber caused by *P. aphanidermatum* is one of the most destructive diseases of cucumber under greenhouse condition (Chaube & Pundhir 2005). The activation of resistance responses, such as hypersensitive resistance and expression of relative-defense genes during systemic acquired resistance mechanism are mediated by a signal transduction pathway (Edreva 2004). Plant hormones play an important role in molecular signaling to enhance resistance mechanisms (Vallad & Goodman 2004). Different antioxidant enzymes such as polyphenol oxidase (PPO) and peroxidase (PO) are involved in phenol oxidation and are correlated with mechanisms of plant defense against microbial pathogens (Zhang & Klessig 2001; Maffei et al 2007). Jasmonic acid (JA) as a member of plant hormones, regulates plant responses to various biotic and abiotic stresses (Gundlach et al 1992). The increase of JA in infected plants with some plant pathogenic fungi has been reported, whereas in mutant plans for JA signaling, fungal growth and development were increased (Trusov et al 2009). Rhizome and seeds priming of ginger plants with synthetic analogue of phytohormones resulted in induced systemic protection against *P. aphanidermatum* (Ghosh & Purkayastha 2003). The role of lipoxygenase (LOX) genes in plant defense responses indifferent plants has been investigated. Increase of Lox gene expression has been reported in several infected plants with pathogenic agents as *Pseudomonas syringae* in Arabidopsis (Melan et al 1993) and in compatible and incompatible tomato lines infected with *Pseudomonas syringae* (Koch et al 1992). Plant defense against pathogenic agents will be affected by lignin content of plant cell wall. Phenylalanine ammonia-lyase (PAL) enzyme is involved in plant cell wall lignifications (Miedes et al 2015). Expression level of *PAL* gene in resistant line of tomato was suppressed compared to susceptible line (Lee et al 1992). Volatile plant secondary metabolites could directly suppress growth and development of pathogens in attacked tissues. This suppression could be resulted from Lox-catalyzed reactions (Croft et al 1993). A new cDNA molecule named cucumber pathogen-induced 4 (*Cupi4*) has been characterized in infected cucumbers with different pathogens and after treatment with three biochemical plant resistance inducers. A high level of *Cupi4* transcripts was accumulated in infected tissues and was systemically spread throughout plant (Phuntumart et al 2006). In this study the effect of JA on the radial growth of *P. aphanidermatum,* antioxidant enzymes activities, expression level of some defense related genes and disease severity in inoculated cucumber with *P. aphanidermatum* was investigated.

2. Material and Methods

2.1. Infection of cucumber with P. aphanidermatum

Seeds of the susceptible cucumber plant (cv. Es2862) were surface sterilized in 3% chloramines

T (Sigma) for 3 minutes and washed three times in sterile distilled water. Surface sterilized seeds were planted in plastic pots (16.5 cm deep × 5.5 cm top diameter) containing autoclaved sand-loam-clay soil (1:2:1). After the seedling emergence, the seedlings were irrigated every three to five days, or as necessary for normal growth, with full-strength Hoagland's solution. A standard isolate of *P. aphanidermatum* was used in this study. A piece of 7 day-old cultures from *P. aphanidermatum* growth on Corn Meal Agar (CMA, Merck) medium was used for plant inoculation at 4- to 6-leaf-stage (Chamswarng & Cook 1985). A plague of medium colonized by the fungus was placed in the vicinity of cucumber roots. Disease severity assay was evaluated based on a 0-5 scale (Table1) (Sunwoo et al 1996).

Table1- Infection degree based on disease severity in infected cucumbers with *Pythium aphanidermatum*

Infection degree	*Symptoms*	*Percentage*
0	No visible disease symptoms	0
1	Leaves slightly wilted	0-30
2	30-50% of entire plant diseased	30-50
3	0-70% of entire plant diseased	50-70
4	70-90% of entire plant diseased	70-90
5	Plant dead	100

2.2. JA treatment

Chemical treatment of plants was done with a 5% JA solution (J-2500, Sigma-Aldrich St. Louis) dissolved in 100% ethanol. Ethanol 0.1% (w v^{-1}) was applied to control plants. Solution was neutralized by NaOH to pH 7.A concentration of 0, 100, 200 and 400 mg L^{-1} (1 mL) were used for the treatment of plants by dropping on the surface of all leaves of 21 old-day cucumber plants (at 4- to 6-leaf stage) (Anderson et al 2004). In control plants, a 10% (v v^{-1}) solution of ethanol in water was applied. The three replications, each consisting of six pots, were performed for each treatment (Traw & Bergelson 2003).

2.3. Antifungal activity assay

Antifungal activity was assayed on PDA solid medium using drop-plate method. For this reason, 9-cm Petri dishes were inoculated with a 5 mm agar plug cut from the edge of one week old *P. aphanidermatum* cultures. One hundred micro liters (three drops) of JA in different concentrations were then added to the Petri dishes and then were incubated at 25±2 ℃. Cultures were observed daily for a week (Gavin 1957).

2.4. Biochemical assay

To protein extraction, a fine powder of leaf tissues prepared in liquid nitrogen was homogenized with 1mL of sodium phosphate buffer (0.1 M, pH 6.5). The homogenate was filtered and centrifuged at 6800 rpm for 15 min at 4 °C (Rahman et al 2006; Abkhoo & Sabbagh 2016). The supernatant liquids were used for the enzyme activity assay. To phenolic compound extraction, a 70% aqueous ethanol (v v^{-1}) solvent was used. Folin-Ciocalteu (Sigma) reagent was used to determine total phenolic content (Singleton & Rossi 1965). An aliquot (1 mL) of extracts was added to 9 mL of distilled water. One mL of Folin-Ciocalteu phenol reagent was added to the mixture and was shaken. After 3 minutes incubation, 10 mL of saturated Na_2CO_3 solution was added to the mixture. The reaction was incubated at room temperature for 60 minutes and then, the absorbance was measured at 765 nm using an UV-Vis spectrophotometer (Unico, USA). The Microsoft excel software was used to calculate the standard deviations and mean values. To PO enzyme activity assay, 10 µL of protein extracts and 2 mL pyrogallol (0.05 M) were added to 0.5 mL of H_2O_2 (1%) and were incubated at room temperature (Hammerschmidt et al 1982). To investigate PPO enzyme activity, 200 µL of the protein extract was added to 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and then 200 µL of catechol (0.01 M) was added (Donovan et al 1998; Doan et al 2014) and for catalase activity assay, crude leaf extract (10 µL) was added to the reaction mixture (1 mL) contain sodium phosphate buffer (0.05 M, pH 7.0) and H_2O_2 in the final concentration of 10 mM (Barka 2001).

Absorbance changes were monitored at 240, 420 and 470 nm wavelengths for catalase, PO and PPO enzyme activity assay, respectively.

2.5. qRT-PCR analysis

Expression level of *Lox (F:* GAGAGCGTAAG GAATGGGATAGAA; R: CACCGGGTTCGGAA AGG), *PAL* (F: GGTGTTCTGCGAGGTGATGA, R: AGGGTGGTGCTTCAGCTTGT) and *Cupi4* (F: TCACTGTGGTGTGTGCTCTC; R: ACTCAA GCCATTGCCTTCCA) genes was analyzed using qRT-PCR method. The A*ctin* gene (F: GAAGG AATAACCACGCTCAG; R: ACACAGTTCCCAT CTACGAG) was used as reference gene. Quantitative RT-PCR was done by using a light cycler (Corbett 3000, Australia) at 95 °C for 4 minutes, followed by denaturation at 95 °C for 20 seconds, 20 second primer annealing at 61 °C, and elongation at 72 °C for 30 seconds in 40 cycles, with a five-minute extension at 72 °C after the final cycle. Each gene amplification was prepared in triplicate.

2.6. Statistical analysis

The SPSS statistical software (version 16.0) was used for data analysis. Values were expressed as mean ± standard deviation (SD). Gene expression analysis was calculated using the Representational State Transfer (REST) method (Pfaffl 2001).

3. Results and Discussion

3.1. Fungal inhibitory and disease severity

The disease severity was reduced three days after treatment of plants with 400 mg L^{-1} concentration of JA in the presence of *P. aphenidermatum,* when compared to control. Disease reduction was not significantly different from the first to fourth days after application of 100 mg L^{-1} JA compared to control plants (Table 1). The results of inhibitory growth test showed that radial growth of *P. aphenidermatum* on solid medium had not significantly affect from all concentrations of jasmonic acid (Table 2). A positive effect of methyl jasmonate (MeJA) on mycelial growth and spore germination of different necrotrophic fungi as Colletotrichum acutatum (Cao et al 2008), *Alternaria alternate* (Kępczyńska & Kępczyńska 2005), or *Erysiphe graminis* fsp. *Hordei* (Schweizer et al 1993) has been reported. But, the effect of methyl jasmonate on mycelial growth of *Aspergillus flavus* showed that this compound did not affect mycelial growth or colony appearance (Goodrich-Tanrikulu et al 1995). The inhibitory effect of exogenous application of methyl jasmonate and JA on the mycelial growth and spore germination of soil-born fungi has not been well elucidated. These results indicate that reduce of disease severity cannot be related to direct effect of JA on fungi growth at the root surface of cucumber. Disease severity test in cucumber seedlings infected with *P. aphanidermatum* was assayed at three week after inoculation. In the control (non-treated infected cucumber), disease incidence was higher than that treated plants. The maximum reduction was recorded at a concentration of 400 mg L^{-1} of JA. Also, disease severity was significantly reduced at concentration of 100 mg L^{-1} and 200 mg L^{-1} of JA compared to control but this reduction did not significantly differ at 200 mg L^{-1} compared to 100 mg L^{-1} of JA (Table 2). Activation of jasmonate-dependent defense

Table 2- The effect of JA on disease severity (three week after inoculation) and growth rate inhibition of *Pythium aphanidermatum*on solid culture medium

JA concentration (mg L^{-1})	*Disease severity** (%)*	*Inhibition percentage**
0	4^{a}*	1.93 (±0.02)
100	2.86^{b}	1.95 (±0.04)
200	2.85^{b}	1.85 (±0.06)
400	1.57^{d}	1.65 (±0.05)

*, each value is the mean (±SD) based on 48 measurements in duplicate experiments; **, in a column means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range tests (DMRT)

pathways in *Arabidopsis thaliana* plants infected with *Pseudomonas syringae* pv. *Tomato* (Pst) lead to significant reduction in disease severity (Van et al 2000). Pre-harvest application of sweet cherry fruit with methyl jasmonate (2 mM) significantly reduced soft rot disease compared to control (Yao & Tian 2005). Based on these data, we suggested that JA application at a high concentration (up to 400 mg L^{-1}) could decrease disease incidence via systemic acquired resistance mechanism.

3.2. Total phenolic compounds

Total phenolic compounds assay showed a significant increase of these compound 48 hpi upon treatment of JA at 400 mg L^{-1} concentration and then was slowly decreased at 72 hpi for all concentration of JA. For 24 hpi, there was not significant increase of phenolic compounds between JA concentrations while compared to control (Figure 1). Phenolic compounds in plant are necessary for growth and defense responses to invading pathogens. In plants, a broad range of secondary metabolites with toxic activity against pathogens are produced (Lattanzio & Cardinali 2006). These defensive compounds can reduce disease damage only after initial time after inoculation which leads to an effective defense response (Wittstock & Gershenzon 2002). A direct and positive relevancy between the quantity and quality of phenolic compounds and induced systemic resistance has been demonstrated in infected plants with different pathogens in the presence of resistance inducers (Elliot 1999; Amzad Hossain & Shah 2015).

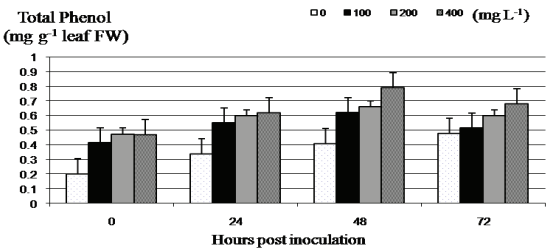

Figure 1- The effect of JA on the phenol component in infected cucumber plant with *Pythium aphanidermatum*

3.3. Oxidative enzyme activity

The polyphenol oxidase activity was increased within 3d after inoculation for all JA concentrations (Figure 2), but the highest level was observed with 400 mg L^{-1}. JA is as classes of plant hormones modulates oxidative enzyme activities which result to plant resistance and subsequently protect plant tissues against pathogens (Gundlach et al 1992). As shown in Figure 2, during the first day after inoculation (24 hpi), PPO activity was not increase for all JA applications. The highest level of enzyme activity was observed at a concentration of 400 mg L^{-1} two days after inoculation. At 72 hpi this rate was significantly reduced when compared to 48 hpi (Figure 2).

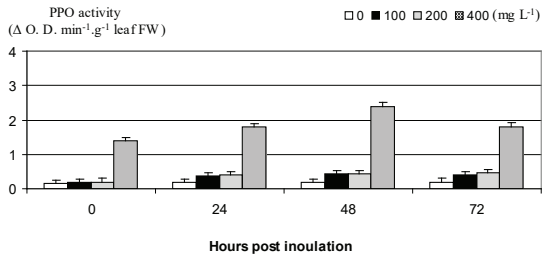

Figure 2- Change of polyphenol oxidase activity in infected cucumber with *Pythium aphanidermatum* under JA treatments

These results indicate the effect of JA timing to trigger defense mechanisms in infected cucumbers. Relation between PPO enzymes to wounding and enzymatic browning has been studied in a number of plants (Demir & Kocacaliskan 2001). This enzyme plays an important role in biosynthesis of alkaloids under biotic and abiotic stress (Bilková at al 2005). Like PPO enzyme, a significant level of PO enzyme activity was recorded at a concentration of 400 mg L^{-1} during the 48 hpi. A significant reduction was observed after 72 hpi in all JA concentrations but this reduction was not lower than the early days after inoculation (Figure 3). These results indicate long-term effects of JA on systemic acquired resistance. The antioxidant PO enzyme is essential for induced systemic resistance and could be used as a biomarker of induced resistance in plants for

cell wall lignifications (Kuć 2001). In this work, JA application on infected plants showed a direct relation between disease reduction and enzyme activity. Our data showed that PO activity is enhanced in treated plants at different concentration of JA.

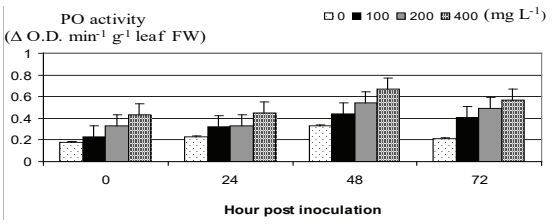

Figure 3- Peroxidase activity in infected cucumber with *Pythium aphanidermatum* under different JA treatment

Increase of PO activity has been resulted in number of plants associated with phytopathogenic fungi (Kruger et al 2003; Silva et al 2008) and also other biotic-elicitor as bacteria, nematode and virus. In the infected Palm trees with *Fusarium oxysporum* sp. *albedinis,* application of JA at a concentration of 50 µM resulted in increase of PPO and PO activity, 1.3-2.2 times, respectively compared to non-treated plants (Jaiti et al 2009). The results of this study are in agreement with related works. A significant increase of catalase level was observed within 48 hpi for all JA concentration and then was returned to control treatment at 72 hpi. Unlike other enzyme, the highest effect of JA was noted at 200 ppm of JA concentration resulted to increase of catalase level in infected cucumber (Figure 4). These observations indicate that low concentrations of JA could play an important role in enhancement of induced defense mechanisms. Change in oxidative enzyme activities induced by silicon application has been demonstrated in infected cucumbers with *Phytophthora meloni* and they showed a positive correlation between silicon concentrations and CAT enzyme activity compared to control plants (Mohaghegh et al 2011). Increase of antioxidant enzymes activity have been shown in infected wheat with *Blumeria graminis* f. sp. *tritici*, (Elliott & Snyder 1991). The present results here are consistent with our previous study in which change in PPO and PO enzymes were increased in infected wheat with *Fusarium graminearum* induced by silicon (Ghazimohseni et al 2014) and infected cucumber plans with *Phytopthora melonis* induce by commercial extract of alga (Abkhoo & Sabbagh 2016). A high level of induced systemic resistance in inoculated cucumbers with *Pseudomonas syringe* pv. *syringe* (a hypersensitive reaction inducer) at 24 hpi has been demonstrated (Smith et al 1991) which is in agreement with our results. Based on these results, we could conclude that JA can leads to induced systemic resistance through increase of oxidative enzymes activity especially at the initial time after infection.

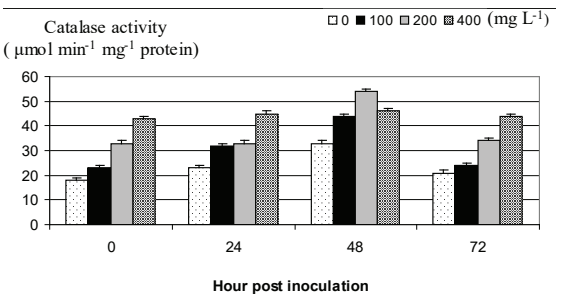

Figure 4- Catalase activity in infected cucumber by *Pythium aphanidermatum* treated by different JA concentration

3.4. Gene expression analysis

Gene expression analysis showed that the level of *Lox*gene in response to damping-off disease reached to a maximum level at 48 h post inoculation at all concentrations of JA. Quantitative real-time-RT PCR confirmed a 21-fold change of *Lox* gene expression in infected plants treated with JA at concentration of 400 mg L^{-1} at 48 h post inoculation when compared to the control treatment (Figure 5).These results indicate that over expression of *Lox* gene is directly dependent on pathogen stimulation at the first days post inoculation. These results indicate that a low concentration of JA is able to induce systemic acquired resistance. The decretive role of *Lox* genes in systemic acquired resistance and the plant defense responses to abiotic stresses such as pathogenic fungi has been demonstrated

(Feussner & Wasternack 2002). It has been reported that *Lox* genes are expressed in Arabidopsis, tomato and cucumber plants in response to exogenous application of phytohormones (Feussner & Wasternack 2002; Liu et al 2010).

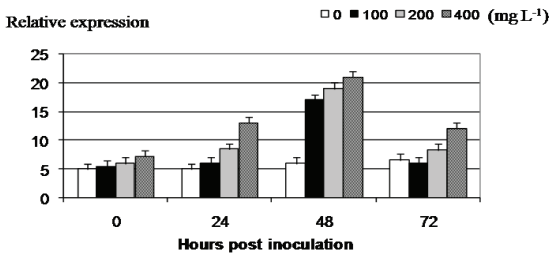

Figure 5- Expression change of *Lox* gene in cucumber plants under JA treatment

Expression pattern of *Pal* gene showed a high expression level at 48 h post inoculation for 400 mg L^{-1} concentration (Figure 6). These observations confirm that JA plays an important role in PAL enzyme mediation which leads to increase cell wall lignifications as a physical barrier to phytopathogenic agents. *Pal* genes over-expression (11 putative genes for PAL synthesis) has been reported in rice plants against *Magnaporthe oryzae* (Giberti et al 2012). A low concentration of salicylic acid and benzothiadiazole (BTH) resistance inducers, activated the phenylalanine ammonia-lyase (PAL) defense gene in tissue culture of parsley (Thulke & Conrath 1998). In our study, control plants (non-treated) showed no significant increase in PAL activity while an increase of transcription rate of *Pal* gene was found at different JA concentration during all times-points after inoculation (Figure 6). These results suggest an essential role of JA on activation and up-regulation of defense-relates genes.

The levels of *Cupi4* transcripts were higher than the other tested genes in infected cucumber. Unlike other genes, high expression of *Cupi4* gene was recorded at 72 h post inoculation (25 times) (Figure 7). These results suggest that Cupi4 might not play an important role in defense mechanisms at the early time-points of plant inoculation. The role of *Cupi4* gene to induce resistance of cucumber against plant bacterial agents has been demonstrated. The mRNA transcripts of the *Cupi4* gene accumulate locally in the infected cucumber leaves with *Pseudomonas lachrymans* at 12 hpi and spread systemically throughout the plant leaves after 48 hpi. The antibacterial properties of *Cupi4* transcripts have been suggested by finding of bacterial host cells death and high accumulation of *Cupi4* mRNA in these cells (Phuntumartet al 2006). The effect of different bi-fertilizers on expression of defense-related genes in infected cucumbers with *P. aphanidermatum* showed a high level expression of *Cupi4* mRNA transcripts (Sabbagh & Valizadeh 2016) that is in agreement with our finding in this study. So, based on our results, *Cupi4* transcripts might acts as hypersensitive reaction inducer in infected tissues of plants but not at early time points of inoculation.

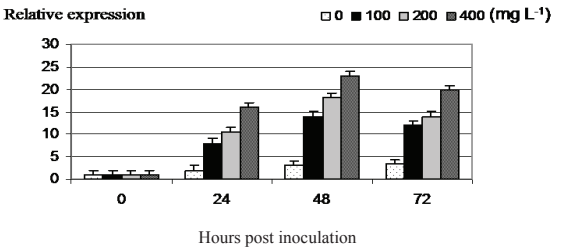

Figure 6- Expression level of *PAL* gene in infected cucumber with *Pythium aphanidermatum* under JA treatments

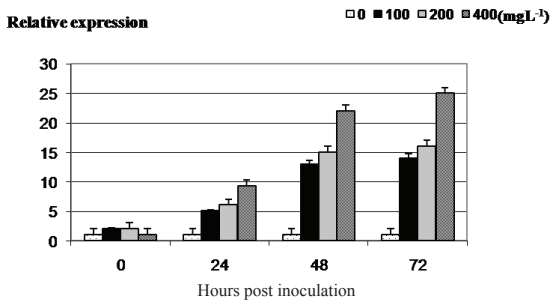

Figure 7- The effect of JA on *Cupi4* gene expression at three time interval after inocuation with *Pythium aphanidermatum*

4. Conclusions

In conclusion, the change of antioxidant enzymes due to using phyto-hormones as jasmonic acid can plays an important role to decrease of plant diseases. Improvement of plant growth under disease stress by application of phyto-hormones at the first time of inoculation could lead to disease suppression followed by systemic resistance through increase of antioxidant enzymes activities and pathogenesis related proteins. Study of different aspects of gene expression analysis such as microarray and DNA hybridization will increase insight into plant defensive mechanisms.

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Abbreviations and Symbols	
JA	Jasmonic acid
PPO	Polyphenol oxidase
PO	Proxidase
Cat	Catalase
PAL	Phenylalanine ammonia-lyase
Lox	Lipoxygenase
Cupi4	Cucumber pathogen-induced 4
hpi	Hour post inoculation
FW	Fresh weight

References

Abkhoo J & Sabbagh S K (2016). Control of *Phytophthoramelonis* damping-off, induction of defense responses, and gene expression of cucumber treated with commercial extract from *Ascophyllum nodosum*. *Journal of Applied Phycology* **28**(20): 1332-1342

Amzad Hossain M & Shah M D (2015). A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. *Arabian Journal of Chemistry* **8**(1): 66-71

Anderson J P, Badruzsaufari E, Schenk P M, Manners J M, Desmond O J & Ehlert C (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *The Plant Cell* **16**(12): 3460-3479

Barka E A (2001). Protective enzymes against reactive oxygen species during ripening of tomato (*Lycopersicon esculentum*) fruits in response to low amounts of UV-C. *Functional Plant Biology* **28**(8): 785-791

Bilková A, Bezakova L, Bilka F & Psenak M (2005). An amine oxidase in seedlings of *Papaver somniferum* L. *Biologia Plantarum* **49**(3): 389-394

Cao S, Zheng Y, Yang Z, Tang S, Jin P, Wang K & Wang X (2008). Effect of methyl jasmonate on the inhibition of *Colletotrichum acutatum* infection in loquat fruit and the possible mechanisms. *Postharvest Biology and Technology* **49**(2): 301-307

Chamswarng C & Cook R J (1985). Identification and comparative pathogenicity of Pythium species from wheat roots and wheat-field soils in the Pacific Northwest. *Phytopathology* **75**(7): 821-827

Chaube H & Pundhir V (2005). *Crop diseases and their management*: PHI Learning Pvt. Ltd Publisher, New Delhi

Croft K P C, Juttner F & Slusarenko A J (1993). Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv. *phaseolicola*. *Plant Physiology* **101**(1): 13-24

Edreva A (2004). A novel strategy for plant protection: Induced resistance. *Journal of Cell and Molecular Biology* **3**(2): 61-69

Elliot J (1999). Application of antioxidant vitamins in foods and beverages. *Food Technology* **53**: 46-48

Elliott C & Snyder G H (1991). Autoclave-induced digestion for the colorimetric determination of silicon in rice straw. *Journal of Agricultural and Food Chemistry* **39**(6): 1118-1119

Feussner I & Wasternack C (2002). The lipoxygenase pathway. *Annual Review of Plant Biology* **53**(1): 275-297

Gavin J J (1957). Analytical Microbiology. II. The Diffusion Methods. *Applied Microbiology* **5**(1): 25-33

Ghazimohseni V, Sabbagh S K, Esmaeilzadeh Bahabadi S & Ghorbani M (2014). Application of silicon in induction of systemic resistance against Fusarium wheat head blight diseas. *Biological Control of Pest and Plant Disease* **2**(3): 128-137

Ghosh R & Purkayastha R (2003). Molecular diagnosis and induced systemic protection against rhizome rot disease of ginger caused by *Pythium aphanidermatum*. *Current Science* **85**(12): 1782-1786

Giberti S, Bertea C M, Narayana R, Maffei M E & Forlani G (2012). Two phenylalanine ammonia lyase isoforms are involved in the elicitor-induced response of rice to the fungal pathogen *Magnaporthe oryzae*. *Journal of Plant Physiology* **169**(3): 249-254

Goodrich-Tanrikulu M, Mahoney N E & Rodriguez S B (1995). The plant growth regulator methyl jasmonate inhibits aflatoxin production by *Aspergillus flavus*. *Microbiology* **141**(11): 2831-2837

Gundlach H, Muller M, Kutchan T & Zenk M (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceding Natlonal Academic Science USA* **89**: 2389-2393

Hammerschmidt R, Nuckles E & Kuć J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* **20**(1): 73-82

Jaiti F, Verdeil J L & Hadrami I (2009). Effect of jasmonic acid on the induction of polyphenoloxidase and peroxidase activities in relation to date palm resistance against *Fusarium oxysporum* f. sp. *albedinis*. *Physiological and Molecular Plant Pathology* **74**(1): 84-90

Kępczyńska E & Kępczyńska J (2005). Inhibitory effect of methyl jasmonate on development of phytopathogen *Alternaria alternata* (Fr.) Keissl. and its reversal by ethephon and ACC. *Acta Physiologiae Plantarum* **27**: 491-496

Koch E, Meier B M, Eiben H-G & Slusarenko A (1992). A lipoxygenase from leaves of tomato (*Lycopersicon esculentum* Mill.) is induced in response to plant pathogenic pseudomonads. *Plant Physiology* **99**(2): 571-576

Kruger W, Szabo L & Zeyen R (2003). Transcription of the defense response genes chitinase IIb, PAL and peroxidase is induced by the barley powdery mildew fungus and is only indirectly modulated by R genes. *Physiological and Molecular Plant Pathology* **63**(3): 167-178

Kuć J (2001). Concepts and direction of induced systemic resistance in plants and its application. *European Journal of Plant Pathology* **107**(1): 7-12

Lattanzio V M & Cardinali A (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: *Advances in Research* **661**(2): 23-67

Lee S, Nazar R N, Powell D A & Robb J (1992). Reduced PAL gene suppression in Verticillium-infected resistant tomatoes. *Plant Molecular Biology* **18**(2): 345-352

Liu S, Liu X & Jiang L (2010). Genome-wide identification, phylogeny and expression analysis of the lipoxygenase gene family in cucumber. *Genetics and Molecular Research* **10**(4): 2613-2636

Maffei M E, Mithöfer A & Boland W (2007). Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry* **68**(22): 2946-2959

Melan M A, Dong X, Endara M E, Davis K R & Ausubel F M (1993). An Arabidopsis thaliana lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. *Plant Physiology* **101**(2): 441-450

Miedes E, Vanholme R, Boerjan W & Molina A (2015). The role of the secondary cell wall in plant resistance to pathogens. *Frontier in Plant Science* **5**(358): 1-12

Mohaghegh P, Khoshgoftarmanesh A, Shirvani M, Sharifnabi B & Nili N (2011). Effect of silicon nutrition on oxidative stress induced by P*hytophthora melonis* infection in cucumber. *Plant Disease* **95**(4): 455-460

Pfaffl M W (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**(9): e45, doi: 10.1093/nar/29.9.e45

Phuntumart V, Marro P, Métraux J P & Sticher L (2006). A novel cucumber gene associated with systemic acquired resistance. *Plant Science* **171**(5): 555-564

Rahman M, Sayem S, Alam M, Islam M & Mondol A (2006). Influence of sulphur on nutrient content and uptake by rice and its balance in old Brahmapura floodplanin soil. *Journal of Soil Natural* **1**(3): 1-10

Sabbagh S K & Valizadeh S (2016). Effect of bio-fertilizers on greenhouse cucumber resistant to damping-off disease caused by *Pythium aphanidermatum* and increase of yield component. *Biological Control of Pests and Plant Diseases* **5**(1): 111-122

Schweizer P, Gees R & Mosinger E (1993). Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f.sp. *hordei*. *Plant Physiology* **102**(2): 503-511

Silva M, Guerra-Guimarães L, Loureiro A & Nicole M (2008). Involvement of peroxidases in the coffee resistance to orange rust (*Hemileiavastatrix*). *Physiological and Molecular Plant Pathology* **72**(1): 29-38

Singleton V L & Rossi J A (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**(3): 144-158

Smith J A, Hammerschmidt R & Fulbright D W (1991). Rapid induction of systemic resistance in cucumber by Pseudomonas syringae pv. syringae. *Physiological and Molecular Plant Pathology* **38**(3): 223-235

Sunwoo J Y, Lee Y K & Hwang B K (1996). Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-n-butyric acid. *European Journal of Plant Pathology* **102**(7): 663-670

Thulke O & Conrath U (1998). Salicylic acid has a dual role in the activation of defence□related genes in parsley. *The Plant Journal* **14**(1): 35-42

Traw M B & Bergelson J (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in Arabidopsis. *Plant Physiology* **133**(3): 1367-1375

Trusov Y, Sewelam N, Rookes J E, Kunkel M, Nowak E & Schenk P M (2009). Heterotrimeric G proteins mediated resistance to necrotrophic pathogens includes mechanisms independent of salicylic acid, jasmonic acid/ethylene- and abscisic acid- mediated defense signaling. *The Plant Journal* **58**(1): 69-81

Vallad G E & Goodman R M (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* **44**(6): 1920-1934

Wittstock U & Gershenzon J (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology* **5**: 300-307

Yao H & Tian S (2005). Effects of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biology and Technology* **35**: 253-262

Zhang S & Klessig D F (2001). MAPK cascades in plant defense signaling. *Trends in Plant Science* **6**(11): 520-527

TARIM BİLİMLERİ DERGİSİ-JOURNAL OF AGRICULTURAL SCIENCES
YAZIM KURALLARI

Genel

Tarım Bilimleri Dergisi, tarım bilimleri alanında ve yazım dili İngilizce olan özgün araştırma makaleleri yayınlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayanan çalışmalar derginin kapsamı dışındadır. Basılacak makalelerin daha önce hiçbir yerde yayınlanmamış olması ve yayın haklarının verilmemiş olması gerekir. Dergide yayımlanacak makalelerin her türlü sorumluluğu yazarına/yazarlarına aittir.

Yayımlanması için gönderilen eser, yayın ilkeleri doğrultusunda Başeditör tarafından ön incelemeye alınır. Başeditör, dergide yayınlanabilecek nitelikte bulmadığı makaleleri editörlere/hakemlere göndermeden yazara/yazarlara iade kararı verme hakkına sahiptir. Ayrıca yazım kurallarına uymayan veya anlatım dili yetersiz olan makaleler, düzeltilmek üzere yazara/yazarlara iade edilir. Değerlendirmeye alınan makaleler, incelenmek üzere en az 2 hakeme gönderilir. Hakem değerlendirmesinden geçen makalelere ait düzeltmeler, düzeltmeler listesiyle birlikte en fazla 30 gün içerisinde sisteme yüklenerek gönderilmelidir. Bu süreden sonraki gönderimler kabul edilmez. Yazarlara makaleleri hakkındaki editör ve hakem görüşleri ve önerileri 8 hafta içerisinde bildirilir. Başeditör, hakem raporlarını ve/veya istenilen düzeltmelerin yeterli olup olmamasını dikkate alarak makalenin yayımlanıp yayımlanmamasına yönelik nihai karar vericidir.

Makalede isimleri yer alan tüm yazarlar, yayım haklarını Tarım Bilimleri Dergisine verdiklerine dair **Makale Gönderme ve Telif Hakkı Devir Sözleşmesini** imzalamalıdır. Makalenin yayımlanması kabul edildikten sonra makale metninde, yazarlarında ve yazarların sıralamasında değişiklik yapılamaz. Makale sunum ya da işlem ücreti alınmamakla birlikte, yayıma kabul edilen makaleler için, sorumlu yazar Ankara Üniversitesi adına açılmış banka hesabına 400 TL yatırmalıdır. Makaleden sorumlu yazara banka hesap numarası, makalenin basıma kabul edilmesinden sonra bildirilir.

Makale Yükleme

Hazırlanan makaleler; sadece makaleden sorumlu yazar (makalenin yayım başlangıcından basım sonrasındaki her türlü yazışmalarda sorumluluğu bulunan) tarafından Tarım Bilimleri Dergisi web sayfasındaki çevrimiçi **Makale Gönderme ve Değerlendirme Sistemi** kullanılarak elektronik ortama yüklenmelidir. Makale yükleme bölümünün **"Başvuruyu Yükle"** bölümünde pdf formatındaki makale dosyasına ilave olarak "**Ek Dosyalar**" bölümüne aşağıdaki dosyaların da yüklenmesi gerekir.

- ✓ Makalenin pdf ve Word (2003 veya daha üst versiyonları) formatındaki dosyası. Sisteme yüklenen makalenin hem pdf formatında ve hem de Word formatında iletişim, ad-soyad, kurum gibi yazarları tanıtıcı bilgiler bulunmamalıdır.
- ✓ Tüm yazarlar tarafından imzalanmış ve pdf formatında taranmış olan "Makale Gönderme ve Telif Hakkı Devir Sözleşmesi". Yayıma kabul edilmesi durumunda bu formların aslı da posta ile editöre gönderilmelidir.
- ✓ Yazar Makale Kontrol Listesi (pdf formatında),
- ✓ Yazarların ad-soyad, kurum ve iletişim bilgilerini içeren Word dosyası,
- ✓ Gerekiyorsa Etik Kurul Raporu eklenmelidir.

Derginin Kapsamı

Tarım Bilimleri Dergisi, tarım bilimleri alanında yapılan özgün araştırmaları ve yeni bulguları içeren makaleleri yayımlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayana çalışmalar derginin kapsamı dışındadır. Derleme makaleler, yayın komisyonunun çağrısı üzerine hazırlanmışsa normal inceleme ve değerlendirme sürecinden geçirilerek yayınlanır.

Makale Hazırlama

Makaleler, A4 boyutundaki kâğıdın tek yüzüne 12 punto Times New Roman yazı tipinde ve çift satır aralıklı yazılmalıdır. Sayfanın sağında, solunda, altında ve üstünde 3'er cm boşluk bırakılmalıdır. Makalenin her sayfası ve satırları numaralandırılmalıdır. Yazar ad(lar)ı açık olarak yazılmalı ve herhangi bir akademik unvan belirtilmemelidir. Editörler kurulu, anlatım dili yeterli olmayan makaleleri değerlendirme dışı tutabilir. Yazar(lar)ın makale göndermeden önce eseri dil yönünden bir dil bilimciye incelettirmesi tavsiye olunur. Sıralama olarak, İngilizce özet ve peşinden Türkçe özet verilir. Bu durum şekil ve çizelge başlıkları için de geçerlidir.

Makale; Türkçe Başlık, Türkçe Özet, Anahtar Kelimeler, İngilizce Başlık, İngilizce Özet, Keywords, 1.Giriş, 2.Materyal ve Yöntem, 3.Bulgular ve Tartışma, 4.Sonuçlar, Teşekkür (varsa), Kısaltmalar ve/veya Semboller (varsa), Kaynaklar bölümleri ile Şekil ve Çizelgelerden oluşmalıdır. Bölüm adları koyu yazılmalıdır.

Makale, "Kaynaklar" bölümü dâhil 16 sayfayı geçmemelidir. Yazar(lar), bu kısımların oluşturulmasında derginin web sayfasındaki **Makale Hazırlama Şablonunu** kullanmalıdır.

Başlık: Kısa ve açıklayıcı olmalı, 14 punto ve koyu, kelimelerin ilk harfi büyük olmalı, ortalanarak yazılmalı ve 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 13 punto ve koyu yazılmalıdır.

Özet ve Anahtar Kelimeler: Türkçe ve İngilizce özetlerin her biri 300 kelimeyi geçmemelidir. Türkçe ve İngilizce özetlerde sırasıyla "Özet" ve "Abstract" kelimeleri kullanılmalıdır. Özet, çalışmanın amacını, nasıl yapıldığını, sonuçları ve sonuçlar üzerine yazar(lar)ın yaptığı değerlendirmeleri içermelidir. Özetlerin 1 satır altına, her anahtar kelimenin ilk harfi büyük diğerleri küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi biçimde tanımlayacak ve aralarında noktalı virgül (;) olacak şekilde en fazla 6 anahtar kelime yazılmalıdır.

1. Giriş: Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir.

2. Materyal ve Yöntem: Kullanılan materyal ve yöntem aynı başlıkta verilmelidir. Alt başlık verilecekse bölüm numarası ile birlikte numaralandırılmalı (2.1. gibi) ve italik yazılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır.

3. Bulgular ve Tartışma: Elde edilen bulgular verilmeli, gerekirse çizelge, şekil ve grafiklerle desteklenerek bulgular açıklanmalıdır. Elde edilen bulgular tekrardan kaçınılması amacıyla ya çizelge ya da grafik olarak verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. İstatistiki analiz yönteminin doğru seçilmediği ve/ya analizin gereği gibi yapılmadığı durumlarda Başeditör makaleyi değerlendirme dışında tutabilir. Bulgular tartışılmalı ancak gereksiz tekrarlardan kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri açıklanmalıdır.

4. Sonuçlar: Elde edilen sonuçlar, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir. Giriş ile Bulgular ve Tartışma bölümünde verilen ifadeler bu kısımda aynı şekilde tekrar edilmemelidir.

Teşekkür: Gerekli ise mümkün olduğunca kısa olmalı ve yapılan katkı ifade edilerek verilmelidir.

Kısaltmalar ve/veya Semboller: Makalede kısaltmalardan mümkün olduğunca kaçınılmalıdır. Semboller Makale Hazırlama Şablonunda belirtildiği gibi verilmelidir. Kısaltma ve semboller metin içinde ilk kez kullanıldığı yerde açıklanmalıdır. Uluslararası geçerliliği olan ve yerleşik kısaltmalar tercih edilmelidir. Kısaltmalar makalenin başlığında kullanılmamalıdır. Semboller SI sistemine göre verilmelidir.

Kaynaklar: Eserde yararlanılan kaynaklara ilişkin atıf metin içinde "(Yazarın soyadı yıl)" yöntemine göre yapılmalıdır. Örnek: (Doymaz 2003), (Basunia & Abe 2001). Yazara atıf yapılırsa sadece yayının yılı parantez içine alınmalıdır. Örnek: Doymaz (2003)'e göre ya da Basunia & Abe (2001). Üç ya da daha fazla yazar için makale içindeki atıfta "et al" kullanılmalıdır. Örnek: (Lawrence et al 2001) veya Lawrence et al (2001)'e göre. Aynı yazarın aynı yıl içinde 1'den fazla yayını varsa, yıldan sonra küçük harfler verilmelidir. Örnek: (Akpınar et al 2003a). Aynı yazarın birden fazla yayınına atıf yapılacaksa yıldan sonra noktalı virgül (;) işareti ile ayırt edilmelidir. Örnek: (Akpınar 2007; 2009; 2013). Birden fazla atıf yapılırsa atıflar arasında noktalı virgül (;) kullanılmalı ve eskiden yeniye doğru yıl sırasına göre verilmelidir. Örnek: (Perl et al 1987; Bailly et al 1996; Copeland & McDonald 2001; Goel & Sheoran 2003). Eğer bilginin, kaynağın belirli bir sayfasından ya da sayfalarından alındığı belirtilmek istenirse (Hardeman & Jochemsen 2012, s 657-674; Naess 1991, s 34) biçiminde gösterilmelidir. Kaynaklarda Anonim ya da Anonymous şeklinde gösterim yapılmamalıdır.

Kaynaklar bölümünde metin içinde atıfı yapılan tüm kaynaklar alfabetik olarak (yazarların soyadlarına göre) ve orijinal dilinde verilir. Aynı yazara birden çok atıf yapılıyorsa önce tek isim, sonra iki isim ve sonra da üç ve daha fazla yazarlı kaynak sırasına göre hepsi kendi içinde eskiden yeniye yıl sırasına göre verilmelidir. İki veya daha fazla yazarlı eserlerin bildiriminde son yazardan önce "&" kullanılmalıdır. Örnek: Lawrence K C, Funk D B & Windham W R (2001). Dergi isimleri kısaltma yapılmadan tam adı ile ve italik yazılmalıdır. Kongre kitaplarında Türkçe ya da yabancı dilde özeti yayınlanmış çalışmalara atıf yapılamaz. Makaledeki yanlış atıf ve kaynak gösterimlerine ait sorumluluk yazar(lar)a aittir. Kaynaklar bölümündeki her bir kaynağın sonuna nokta (.) konmamalıdır.

Dergi:

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Bicer Y (2003a). Single layer drying behaviour of potato slices in a convective cyclone dryer and mathematical modelling. *Energy Conversion and Management* **44**(10): 1689-1705

Kitap:

Yıldırım O (1996). Bahçe Bitkileri Sulama Tekniği. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1438, Ders Kitabı: 420, Ankara

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Kitapta Bölüm:

Fıratlı Ç (1993). Arı yetiştirme. (Ed: M Ertuğrul), *Hayvan Yetiştirme*, Baran Ofset, Ankara, s. 30-34

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Yazarı Belirtilmeyen Kurum Yayınları:

TUİK (2012). Tarım İstatistikleri Özeti. Türkiye İstatistik Kurumu, Yayın No: 3877, Ankara

ASAE (2002). Standards S352.2, 2002, Moisture measurement-unground grain and seeds. ASAE, St. Joseph, MI

İnternetten Alınan Bilgi:

FAO (2013). Classifications and standards. http://www.fao.org/economic/ess/ess-standards/en/ (Erişim tarihi:10.02.2013)

Tez:

Koyuncu T (1992). Tarım arabalarında kullanılan çarpma etkili frenlerin araştırılması. Yüksek lisans tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Tam Metin Kongre/Sempozyum Kitabı:

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, Turkey, pp. 565–569

Kara Z & Beyoğlu N (1995). Konya ili Beyşehir yöresinde yetiştirilen üzüm çeşitlerinin göz verimliliklerinin belirlenmesi üzerine bir araştırma. *Türkiye II. Ulusal Bahçe Bitkileri Kongresi. Bildiriler (II)*: 3-6 Ekim, Adana, s. 524-528

Şekiller ve Çizelgeler: Şekil, grafik, fotoğraf ve benzerleri "Şekil", sayısal değerler ise "Çizelge" olarak belirtilmelidir. Tüm şekil ve çizelgeler makalenin sonuna yerleştirilmelidir. Şekil ve çizelgelerin boyu tek sayfa düzeninde en fazla 16x20 cm ve çift sütun düzeninde ise genişliği en fazla 8 cm olmalıdır. Şekil ve çizelgelerin boyutu baskıda çıkabilecek çözünürlükte olmalıdır. Araştırma sonuçlarını destekleyici nitelikteki resimler 600 dpi çözünürlüğünde "jpg" formatında olmalıdır. Renkli resimler yerine gri ya da siyah tonlu resimler tercih edilmelidir. Çizelgelerde düşey çizgi kullanılmamalı ve makale hazırlama şablonunda belirtildiği gibi hazırlanmalıdır. Her çizelge ve şekle metin içerisinde atıf yapılmalıdır. Tüm çizelge ve şekiller makale boyunca sırayla sadece **Table 1** ve **Figure 1** kısmı koyu olacak şekilde numaralandırılmalıdır. Çizelge ve şekil başlıkları ve açıklamaları kısa ve öz olmalıdır. Çizelge ve şekillerin İngilizce başlıkları, Türkçe başlığın hemen altına italik olarak yazılmalı, ilk yazılan Türkçe başlık yazısı koyu olmalıdır. Şekil ve çizelge başlık yazıları 9.5 punto, şekil ve çizelgelerin içindeki yazılar 9 punto, çizelge altı yazılar 8 punto Times New Roman yazı karakterinde olmalıdır. Şekillerde yatay ve düşey kılavuz çizgiler ve rakamlar bulunmamalıdır. Ancak istatistiksel karşılaştırmalar yapılıyorsa küçük harfler bulunabilir. Çizelge ve şekillerde kısaltmalar kullanılmış ise hemen altına bu kısaltmalar açıklanmalıdır. Şekil ve çizelge başlıkları ile çizelge altı yazılarının sonuna nokta (.) konmamalıdır.

Birimler: Tüm makalelerde SI (Systeme International d'Units) ölçüm birimleri kullanılmalıdır. Ondalık kesir olarak nokta kullanılmalıdır (1,25 yerine 1.25 gibi). Birimlerde "/" kullanılmamalı ve birimler arasında bir boşluk verilmelidir (m/s yerine m s^{-1}, J/s yerine J s^{-1}, kg m/s^2 yerine kg m s^{-2} gibi). Sayı ile sembol arasında bir boşluk bırakılmalıdır (4 kg N ha^{-1}, 3 kg m^{-1} s^{-2}, 20 N m, 1000 s^{-1}, 100 kPa, 22 °C ve % 29 gibi). Bu kuralın istisnaları düzlemsel açılar için kullanılan derece, dakika ve saniye sembolleridir (°, ′ ve ″). Bunlar sayıdan hemen sonra konmalıdır (10°, 45', 60" gibi). Litrenin kısaltması "l" değil "L" olarak belirtilmelidir. Cümle sonunda değillerse sembollerin sonuna nokta konulmamalıdır (kg. değil kg).

Formüller ve Eşitlikler: Formüller numaralandırılmalı ve formül numarası formülün yanına sağa dayalı olarak parantez içinde gösterilmelidir. Formüllerin yazılmasında Word programı matematik işlemcisi kullanılmalı, ana karakterler 12 punto, değişkenler italik, rakamlar ve matematiksel ifadeler düz olarak verilmelidir. Metin içerisinde atıf yapılacaksa "Eşitlik 1" biçiminde verilmelidir (…ilişkin model, Eşitlik 1' de verilmiştir).

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Manuscript Submission

Manuscripts should be submitted to journal's online submission system by the corresponding author. All submissions should include following documents:

- ✓ **Title page** with author names, titles, addresses and contact information (in Word format).
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Manuscripts should be typed using Times New Roman font 12 pt. with numbered lines, in the left-hand margin and double spacing throughout, i.e. also for abstracts, footnotes and references. The pages of the manuscript, including the title page, abstract, references, tables, etc. should be numbered consecutively. Make the width at 3 cm for all margins. Place tables and figures with captions after the text. Each figure and table should be referred to in the text. Avoid excessive use of italics to emphasize part of the text.

Manuscripts should include the following sections; **Title** (short, specific and informative), **Keywords** (indexing terms, up to 6 items), **1. Introduction**, **2. Material and Methods**, **3. Results and Discussion**, **4. Conclusions**, **Acknowledgements** (if needed), **Abbreviations and Symbols** (if needed), **References**, **Figures and Tables** with captions not exceeding 16 pages (with references). All headings and titles should be written in bold.

Acknowledgements

Acknowledgements should be a brief statement at the end of the text and may include source of financial support. The contract number should be provided.

References

Cite references in the text as author's family name should be followed by the year of the publication in parentheses (Peter 2010; Basunia & Abe 2001). Use et al after the first author's family name for citations with three or more authors (Lawrence et al 2001). For citations of the same authors published on the same year, use letters after the year (Dawson 2009a).

References cited in the text should be arranged chronologically. The references should be listed alphabetically on author's surnames, and chronological per author. Names of journals should be in full titles rather than the abbreviations. Avoid using citations of abstract proceedings. The following examples are for guidance.

Journal Articles

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Biçer Y (2003a). Single layer drying behavior of potato slices in a convective cyclone dryer and mathematical modeling. *Energy Conversion and Management* **44**(10): 1689-1705

Books

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Book Chapter

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds.), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Publications of Institutions / Standard Books

ASAE (2002). Standards S352.2, 2002, Moisture measurement - unground grain and seeds. ASAE, St. Joseph, MI

Internet Sources

FAO (2013). Classifications and standards. Retrieved in April, 12, 2011 from http://www.fao.org/economic/ess/ess-standards/en/

Thesis and Dissertations

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Conference Proceedings (Full papers)

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, pp. 565–569

Tables and Figures

Tables and Figures should be numbered consecutively and accompanied by a title at the top. All tables and figures should not exceed 16x20 cm size. Figures should have high resolution, minimum 600dpi in jpg format. For publication purposes use grayscale images. Avoid using vertical lines in tables.

Illustrations

Do not use figures that duplicate matter in tables. Figures can be supplied in digital format, or photographs and drawings, which can be suitable for reproduction. Label each figure with figure number consecutively.

Units

Units of measurement should all be in SI units. Use a period in decimal fractions (1.24 rather than 1,24). Avoid using "/". Include a space between the units (m s^{-1} rather than m/s,J s^{-1} rather than J/s, kg m s^{-2} rather thankg m/s^2). Units should have a single space between the number and the unit (4 kg N ha^{-1}, 3 kg m^{-1} s^{-2}, 20 N m, 1000 s^{-1}, 100 kPa, 22 °C). The only exceptions are for angular definitions, minutes, seconds and percentage; do not include a space (10°, 45›, 60», 29%). The abbreviation of liter is "L".

Formulas and Equations

Number each formula with the reference number placed in parentheses at the end. Use Word mathematical processor for formulas with 12pt., variances in Italics, numbers and mathematical definitions in plain text. If needed, refer as "Equation 1" in the text (….the model, as given in Equation 1).

Note: Title and Abstract, Tables and Figures Captions are translated into Turkish by editor for authors whose native language is not Turkish.