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Research Article

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Effect of economase replacing vitamin E and selenium on growth performance and meat quality of broilers

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Abstract

One hundred, one-day-old male broiler chicks (Ross 308) were allocated in five treatment groups; control (100 mg/kg Vit E), 20 mg/kg Vit E, 20 mg/kg Vit E +Economase; 40 mg/kg Vit E, 40 mg/kg Vit E+Economase. The birds were fed *ad libitum* for 42 days in individual cages. Body weight gain, food intake, food efficiency (gain/food intake) and carcass weight were measured. Breast and thigh meats were sampled for TBARS evaluation, blood samples were taken for biochemical parameters. No significant differences between growth performances of treatment groups were observed. Drip loss was not affected significantly ($P>0.05$), but water holding capacity was lower ($P<0.05$) at 20 mg/kg Vit E in contrast to the control and the other treatments groups receiving 20 or 40 mg/kg vit E with Economase. Blood total antioxidant capacity in control groups was significantly ($P<0.05$) higher than the treatment groups. TBARS values of the groups receiving 100 mg/kg vitamin E or 40 mg/kg Vitamin E plus Economase found to be lower. The results suggest that vitamin E level in the commercial broiler diets could be reduced to 40 mg/kg using Economase at 200 mg/kg without loss in the growth performance, meat quality and also oxidative stability.

Keywords: Vitamin E, Selenium, Broiler, Meat, Performance

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Introduction

It is well known that any molecule with an atom that contains a single unpaired electron in its outer orbit is termed an oxidant. These atoms are unstable and have a strong attraction for the electrons of other atoms or molecules in order to regain their resting state. The process of transferring electrons to the oxidant is termed oxidation, and a new free radical is formed in the process (Surai, 2002). In fact, oxidation is a very general process, which affects lipids, pigments, proteins, DNA, carbohydrates, and vitamins (Kanner, 1994). In muscle and fat tissue, oxidation continues post-mortem and affects the shelf-life of meat and meat products. It is generally accepted that lipid oxidation is one of the primary mechanisms of quality deterioration in foods, especially in meat products (Kanner, 1994; Morrissey et al., 1998). The latter becomes more important because of a trend toward increasing the polyunsaturated fatty acids (PUFA long-chain) content (Smet et al., 2008) due to the nutritionists' recommendations to reduce intake of saturated fatty acids, as a high degree of polyunsaturation accelerates oxidative processes leading to deterioration in physical and chemical characteristics, such as meat flavour, colour, texture, water holding capacity besides nutritional value (O'Neill et al., 1998; Coetzee and Hoffman, 2001). The major strategies for preventing lipid oxidation are, therefore, the use of antioxidants. In fact, antioxidants can be organic or inorganic and nutrient or non-nutrient in nature. They function to protect animal tissue against highly reactive oxygen containing products produced chemically and by metabolism. These so-called reactive oxygen species (ROS) can be organic or inorganic compounds in which oxygen is a critical component. Their production is linked to the use of oxygen as the primary electron acceptor in aerobic metabolism. Compounds such as superoxide anion ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), alkoxyl radical (RO) and peroxyl radical (ROO) attack cellular lipid, protein, DNA and carbohydrate. Chemical attacks on the unsaturated fatty acids of cellular membranes produce products such as the peroxyl radical (ROO), which initiates a chain reaction that can lead to compromised cell membranes and eventually cell death, unless a more reactive electron donor, an antioxidant such as alpha-tocopheryl acetate, is introduced, whereupon the chain reaction is blocked (Liebler, 1993).

It is well known that when lipid hydroperoxides are oxidized to peroxyl radicals, the peroxyl radicals react with α-tocopherol much faster than with other polyunsaturated fatty acids. The result is a corresponding organic hydroperoxide and α-tocopheroxyl radical.

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The α-tocopheroxyl radical leaves the cell membrane lipid bilayer and enters the surrounding aqueous medium where glutathione peroxidase can use an electron from glutathione to restore α-tocopherol to its active or reduced state. Thus, although Se and vitamin E fulfil distinct functions, an inadequate supply of either can exacerbate the metabolic demand for the other (Burk and Levander, 1999). It is well documented that vitamin E and selenium are the vital nutrient sources to maintain health, growth and product quality in farm animal production. Vitamin E is a fat-soluble nutrient found in body fat depots, plasma lipoproteins, and cell membrane phospholipids, where it serves as an important antioxidant. Selenium (Se) fulfils an antioxidant role as a component of glutathione peroxidases (GSHPx). Se is widely distributed in the body, but the most labile reservoir is in the liver. It is found in body tissue principally as selenomethionine (SeMet) or as selenocysteine (SeCys), the latter found in GSHPx. Both nutrients are should be examined together because of their related functions and similar deficiency signs. However, Se or vitamin E each has unique metabolic roles, and the factors that alter the oxidative state of an animal may differentially affect their dietary needs.

In poultry nutrition, vitamin E and selenium requirements meet through vitamin and trace mineral premixes in diets. As the production and consumption of poultry meat has increased continuously during the last decades in many parts of the world (Pettersen et al., 2004), the increased production with high PUFA contents has demanded considerable quantities of antioxidants, mainly alpha-tocopheryl acetate in order to meet animals' need alive and also consumer satisfaction for meat quality. It has been known that a higher level of natural antioxidants in broiler diets provides a simple method for improving oxidative stability, sensory quality, self life and acceptability of poultry meat and meat products (Sheehy et al., 1993). However, current market evaluation has shown that price of Vitamin E (dl-alpha-tocopheryl acetate) sharply increased, as its level in broiler diets and total needs of poultry industry have increased. Few attempts have been made to replace or spare vitamin E with natural extracts for oxidative stability in meat with varying degrees of success (Papageorgiou et al., 2003; Basmacioglu et al., 2004; Haak et al., 2006). Smet et al. (2008) found dietary natural antioxidant extracts being less effective than the combination of α-tocopheryl acetate and synthetic antioxidants. They also reported marked differences between different natural antioxidant extracts for protecting broiler meat against oxidation.

Recently, a product called "Economase" has been introduced to the market. The product has been developed through nutrogenomic studies and claimed to replace 80% of Vitamin E and also selenium in order to reduce cost of broiler diets. On the other hand either vitamin E deficiencies or considerable excesses have been shown to have adverse effects on immune function in birds. Paradoxically, vitamin E in low-density lipoproteins oxidized *in vitro* in the absence of aqueous antioxidants may act as a prooxidant. However, prooxidant activity of high intakes of vitamin E has not yet been confirmed *in vivo* (Traber, 1999). It could be speculated that not only because of the cost reason, in order to establish and maintain the physiological equilibrium by preventing any possible negative effect of high level, replacement of dietary supplemental vitamin E could be of benefit in broiler production.

The proposed project is aimed to determine whether vitamin E (dl-alpha-tocopheryl acetate) level in the diet could be reduced to 20 or 40 mg/kg by using Economase at 200 g/ton feed without any negative effects on performance, oxidative stability, meat quality and storage stability in countries where vitamin E used in broiler diets at about 100 mg/kg.

Materials and Methods

The present study was carried out in a fully (light-moisture-temperature) controlled experimental unit for broilers in Department of Animal Science, Faculty of Agriculture, University of Çukurova, Adana-TURKEY. One hundred, one-day-old male broiler chicks (Ross 308) were divided into five treatment groups (group 1: control (100 mg/kg Vit E; dl-alpha-tocopheryl acetate), group 2: 20 mg/kg Vit E, group 3: 20 mg/kg Vit E +Economase; group 4: 40 mg/kg Vit E, group 5: 40 mg/kg Vit E+Economase, Table 1) of similar mean body weight, comprising 20 birds each. Economase® developed by Alltech's Nutrigenomics Center, Kentucky-USA, is a a mix of vitamin E, Sel-Plex and some other antioxidants. The product is claimed to reduce the costs of vitamin E supplementation with 80%, as the special blend makes more vitamin E available on cell lever, which means that less vitamin E and selenium have to be supplemented via the feed.

Experimental diets based on maize and soya were used in a-three stage feeding regime (starter diet 0-10 days, grower diet 11-21 days, finisher diet 22-42 days, Table 2). Experimental diets were formulated to have 0.3 ppm selenium, except the diets containing Economase, which is known to have selenium to meet broilers' need. All the animals were housed in individual cages of a fully controlled experimental room. The birds were fed ad libitum for a period of 42 days under conventional raising conditions. Thermoneutral (from 30 reducing to 24 ^{0}C by 3 ^{0}C/week) temperature regimes were applied. Light was provided for 24 hours each day and water was continuously available.

Broiler growth performance was assessed by measuring body weight gain, feed intake, feed efficiency (gain/feed), water consumption, carcass weight. Body weight was recorded weekly by weighing individual birds. Weights of feed issued were recorded and feed residues were weighed daily for individual birds. The feed efficiency was determined on weekly basis for individual birds.

At the end of the experiment when the chicks were 6 weeks of age all the birds were slaughtered for carcass evaluation (yield, drip loss, water holding capacity). The carcasses were immediately plucked, eviscerated, scalded and then chilled for 4 hours. Six carcasses per group close to the average group weight were selected and divided into right and left portions to analyse meat quality. The right portion of the breast and thigh meats (without skin) were sampled for tissue oxidation status by thiobarbituric acid-reactive substances (TBARS) evaluation on 0, 3, 7 days of storage at +4 ^{0}C. The left portions were shock-freezed at -75 ^{0}C for 12 hours then stored 30 days at -25 ^{0}C. TBARS analyses of fridge and deep-freeze samples were based on Tarladgis et al. (1960) and is expressed as micrograms of malondialdehyde (MDA) per gram of tissue.

During slaughter process blood samples of the representative animals of each group were also taken to obtain serum in order to analyze for glucose, triglyceride,

and also total antioxidant capacity, total oxidant status. Serum glucose, cholesterol and triglyceride concentrations were measured with commercial kits (glucose GOD-PAP; Roche Diagnostics, GmbH, Germany), cholesterol (cholesterol CHOD-PAD; Roche Diagnostics, GmbH, Germany), or triglyceride (triglycerides GPO-PAP; Roche Diagnostics, GmbH, Germany) on an automated KEYLAB LiquiVet Analyzer. Total antioxidant capacity (mmol Trolox Equal./L) and total oxidant status (μmol H_2O_2 Equiv/L) were analysed using commercial kits (Rel-Assay-Diagnostics, Mega Tıp San. Tic. Ltd., Gaziantep-Turkey) by automated colorimetric methods (Erel, 2004, 2005).

Determination of drip loss was assayed using fresh breast fillets which were weighed then placed on polystyrene trays (1/tray), underplayed with an absorbent pad, overwrapped with an oxygen permeable PVC film (6000–8000 $ml/cm^3/m^2/24$ h), and stored at 4°C under fluorescent light for up to 8 d. Fillets were weighed at 48 h intervals and the drip loss at each time (2, 4, 6, 8 days) point was calculated as the percentage reduction in weight relative (%) to the initial (day 0) weight (O'Neill et al., 1998).

Water holding capacity of breast samples was measured by the press method of Grau and Hamm (1956). A 25-gram of fresh breast fillet sample for each replicate was weighed then minced. A 5-gram-minced sample was taken then placed between double filter paper (Schleicher and Schuell 595 150 mm, ref no. 311812) then pressed over a glass vitriol by 2.5 kg weight about 5 minutes. At the end of the time the sample was weighed to calculate water holding capacity using the formula (Water Holding Capacity, % =100-[(first weight-last weight)/first weight*100)] of Barton-Gade et al. (1993).

The data obtained in the study were analysed using GLM (General Linear Model) procedure of the Statistical Analysis System (SAS, 1996), and Duncan's New Multiple Range Test in SAS was used to identify significant differences among treatments means. Results obtained in this study were presented as means per bird with standard errors of the difference between means (SED).

Table 1. Treatment groups and their vitamin E + selenium and Economase level in each kg of feed.

Treatment Groups	Vitamin E (mg/kg)	Selenium (ppm)	Economase (mg/kg)
1*	100	0.3	-
2	20	0.3	-
3	20	-	200
4	40	0.3	-
5	40	-	200

*** control**

Table 2. Composition and nutrient contents of the experimental diets.

Ingredients (g/kg)	Starter Diet (0-11 days)	Grower Diet (12-21 days)	Finisher Diet (22-42 days)
Yellow corn	427.00	520.17	496.14
Fullfat soya	222.00	277.00	300.00
Wheat middlings (15% CP)	75.00	36.00	60.00
Corn gluten meal (60% CP)	15.00	15.00	-
Soybean meal (47.5% CP)	159.00	59.00	35.00
Poultry offal meal (%&% CP)	40.00	35.00	35.00
Meat and bone meal (34% CP)	40.00	40.00	33.00
Vegetable oil	-	-	20.00
Dicalcium phosphate (18% P)	1.20	-	-
Limestone, ground	3.61	1.58	3.57
Common salt	1.30	1.59	1.70
Sodium bicarbonate	1.50	1.30	1.50
Lysine	3.14	2.81	1.70
DL-Methionin	3.28	2.58	2.42
Thronine	0.87	0.87	0.87
Choline Chloride (60%)	0.50	0.50	0.50
Organic acids	1.00	1.00	1.00
Enzyme	1.00	1.00	1.00
Toxin binder (Mycosorb)	1.00	1.00	1.00
Anticoccidial	0.60	0.60	0.60
Pellet binder	-	-	2.00
Vitamin Premix*	2.00	2.00	2.00
Trace mineral premix**	1.00	1.00	1.00
Total	**1000.00**	**1000.00**	**1000.00**
Calculated Analyses (%)			
Dry matter	87.30	87.20	87.46
Crude protein	23.61	21.40	18.57
Ether extract	6.51	8.71	11.53
Crude fiber	3.04	3.43	3.38
Crude ash	5.94	5.44	5.25
Lysine	1.46	1.28	1.06
Methionine	0.68	0.59	0.46
Methionine + Cystine	1.09	0.96	0.79
Calcium	1.07	0.90	0.88
Available Phosphorus	0.50	0.45	0.42
Sodium	0.17	0.17	0.17
Metabolizabl Energy (Mj/kg)	12.8	13.2	13.8

* two kg of vitamin premix contained 15.000.000 IU Vitamin A, 5.000.000 IU Vitamin D_3, 3.000 mg Vitamin K_3, 3.000 mg Vitamin B_1, 8.000 mg Vitamin B_2, 60.000 mg Niacin, 15.000 mg Ca-D-Pantothenate, 5.000 mg Vitamin B_6, 20 mg Vitamin B_{12}, 2.000 mg Folic Acid, 200 mg D-Biotin, 100.000 mg Vitamin C (not containing vitamin E, which was used as a supplement according to the experimental design)
** each kg of trace mineral premix contains 120.000 mg Mangan, 80.000 mg Iron, 80.000 mg Zinc, 16.000 mg Copper, 1.250 mg Iodine, 200 mg Cobalt (not containing selenium, which was used as a supplement according to the experimental design).

Results

The results with respect to growth performance, feed intake and feed conversion efficiency are summarised in Tables 3. At the beginning of the trial all the groups had similar body weight. The results obtained at 6 weeks of age showed that feeding diets containing vitamin E at 20 or 40 mg/kg with or without Economase did not affect body weight significantly; however, the groups receiving 20 mg/kg vitamin E had numerically lower body weight gain than the control and 40 g/kg vitamin E groups. The difference was about 100-120 grams per bird. The groups receiving 40 g/kg vitamin with Economase exhibited the highest weight gain at 42 days old. However, dietary treatments affected feed intake significantly (P<0.05). The control group consumed significantly lower amount of feed than the groups receiving 20 or 40 mg/kg Vitamin E alone or 40 mg/kg vitamin E with Economase. Results with respect to feed conversion efficiency showed a significant deterioration as the vitamin E levels in the diet reduce. The best conversion efficiency was obtained in the control group. Carcass weight was not affected by the treatment groups but the birds receiving 20 mg/kg vitamin E has almost 60-80 grams less carcass weight than the groups receiving 100 (control) or 40 mg/kg vitamin E with Economase groups. The results showed that dietary treatments had no significant (P>0.05) effects on carcass yield and the groups had similar values.

The results with respect to meat quality showed that dietary treatment did not affect drip loss significantly (P>0.05). Drip loss values obtained on day 2, 4, 6 or 8 increased as the day passed without no significant (P>0.05) difference between treatment groups for the same day. However, water holding capacity was affected significantly (P<0.001) by dietary treatments. The group receiving 20 mg/kg Vit E had significantly lower water holding capacity than the group receiving 100 mg/kg vitamin E or vitamin E (20 or 40 mg/kg) with Economase (Table 4).

The results with respect to tissue oxidation status by thiobarbituric acid-reactive substances (TBARS, mg/kg MDA) obtained on 0, 3, 7 days for fridge (+4C) samples and on 30 days for defreeze (-25C) samples for breast and thigh meats showed significant changes. The both meat samples (breast and thigh) were affected in a similar manner, in which the TBARS values increased (P<0.05) in all groups as they wait +4°C in the fridge. The groups receiving vitamin E at 100 mg/kg or 40 mg/kg with Economase had significantly lower (P<0.05) TBARS values than the group receiving vitamin E at 20 mg/kg alone. The frozen samples analyzed on day 30 had similar TBARS values in all groups, which were lower than their fridge counterparts on day 0.

The results with respect to blood metabolites revealed that dietary treatments had a significant (P<0.001) effect on total antioxidant capacity (TAC). The groups receiving 100 mg/kg vitamin E had significantly higher TAC than the groups receiving 20 or 40 mg/kg vitamin E with or without Economase (Table 6). However, dietary treatment had no significant effect on total oxidant status (TOS); all treatment groups had similar TOS values. Oxidative stress Index, which was calculated by TOS/TAC, was not affected by dietary treatments but the groups receiving 100 mg/kg or 40 mg/kg vitamin E with or without Economase exhibited numerically lower oxidative stress index than the groups receiving 20 mg/kg vitamin E with or without Economase. The results also showed that serum glucose, total cholesterol, VLDL cholesterol and triglyceride concentrations were not affected (P>0.05) by dietary treatments. All the groups had similar values for each parameter (Table 6).

Table 3. Growth performances of broilers receiving vitamin E and Economase

Parameters	Dietary Treatment Groups (Vitamin E with or without Economase)						
	100 (control)	20-	20+	40-	40+	P	SED
Initial Body weight (g/bird)	45.7	45.0	44.4	45.1	44.5	0.873	0.93
Body weight Gain (BWG, g/bird/42 days)	2941	2843	2870	2930	2960	0.266	44.82
Feed Intake (FI, g/bird/42 days)	4396 b*	4646 a	4524 ab	4680 a	4656 a	0.050	78.68
Feed Conversion Efficiency (FI/BWG)	1.50 c	1.63 a	1.58 b	1.60 ab	1.57 b	0.001	0.017
Carcass Weight (CW, g/bird)	2165	2080	2111	2148	2177	0.196	34.15
Carcass Yield (CW/BW, %)	72.5	72.0	72.4	72.1	72.5	0.715	0.309

*:[a, b, c]; means in same row with different superscript letters are significantly different (P<0.05).
SED; standard error of difference between means

Table 4. Drip loss and water holding capacity of breast meat of broilers receiving vitamin E and Economase.

Parameters	Dietary Treatment Groups (Vitamin E with or without Economase)					P	SED
	100 (control)	20-	20+	40-	40+		
Drip loss on Day 2 (%)	1.99	2.67	2.3	2.54	2.04	0.249	0.26
on Day 4 (%)	3.59	4.15	4.14	4.1	4.57	0.600	0.46
on Day 6 (%)	5.17	5.88	5.66	6.16	6.19	0.651	0.55
on Day 8 (%)	7.31	8.55	8.32	8.03	7.86	0.618	0.63
Water holding capacity (%)	87.22 a	83.85 b	86.39 a	85.71 a	86.25 a	0.002	3.70

*:[a, b]; means in same row with different superscript letters are significantly different (P<0.05).
SED; standard error of difference between means

Table 5. TBARS values of thigh and breast meats of broilers receiving vitamin E and Economase.

Parameters	Dietary Treatment Groups (Vitamin E with or without Economase) 100 (control)	20-	20+	40-	40+	P	SED
Thigh meat							
on day 0	0.24	0.35	0.33	0.29	0.29	0.223	0.035
on day 3 (kept at +4°C)	0.52 b	0.66 a	0.56 ab	0.55 ab	0.53 b	0.063	0.037
on day 7 (kept at +4°C)	1.09 b	1.50 a	1.28 ab	1.31 ab	1.21 b	0.033	0.091
on day 30 (kept at -25°C)	0.17	0.17	0.20	0.16	0.16	0.614	0.021
Breast meat							
on day 0	0.26 c	0.40 a	0.36 ab	0.30 bc	0.28 bc	0.012	0.032
on day 3 (kept at +4°C)	0.60 ab	0.70 a	0.64 ab	0.56 b	0.53 b	0.049	0.043
on day 7 (kept at +4°C)	1.15 b	1.53 a	1.35 ab	1.31 ab	1.22 b	0.025	0.082
on day 30 (kept at -25°C)	0.16	0.16	0.20	0.19	0.18	0.777	0.026

*:[a, b,]; means in same row with different superscript letters are significantly different (P<0.05).
SED; standard error of difference between means

Table 6. Blood metabolites of broilers receiving vitamin E and Economase.

Parameters	Dietary Treatment Groups (Vitamin E with or without Economase) 100 (control)	20-	20+	40-	40+	P	SED
Total Antioxidant Capacity (TAC, mmol Trolox Equal./L)	1.11 a	0.70 b	0.79 b	0.71 b	0.85 b	0.001	0.074
Total Oxidant Status (TOS, µmol H_2O_2 Equiv./L)	3.07	2.72	2.85	2.64	2.76	0.816	0.280
Oxidative Stress Index (TOS/TAC)	3.27	4.25	4.07	3.96	3.39	0.670	0.586
Serum Glucose Level (mg/dl)	226.0	229.7	248.3	235.3	229.0	0.273	7.576
Serum Total Cholesterol Level (mg/dl)	138.8	140.8	144.5	135.0	144.5	0.878	7.365
Serum VLDL Cholesterol Level (mg/dl)	6.17	6.33	7.00	6.50	6.17	0.792	0.532
Serum triglyceride Level (mg/dl)	31.50	31.33	34.33	32.67	30.83	0.905	2.786

*:[a, b,]; means in same row with different superscript letters are significantly different (P<0.05).
SED; standard error of difference between means

Discussion

The results of the experiment reported here demonstrate a considerable advantage of Economase by sparing vitamin E in broiler diets by about 60 mg/kg under our experimental condition. The results obtained in the experiment showed that the groups receiving 40 mg/kg vitamin E with Economase at 200 mg/kg has similar growth and meat quality performance at 6 weeks of age. However it was claimed that Economase could spare 80% of vitamin E in broiler diets. Our results could not support this view, as lower performance with respect to growth and meat quality were observed with the diet containing 20 mg/kg vitamin E plus Economase. Our experiment, however, could provide substantial information about Economase and its vitamin E sparing activity about 60%. Our results with respect to performance of broilers receiving vitamin E alone at 20, 40 or 100 mg/kg showed that increasing vitamin E level results in better performance in terms of body growth and also feed efficiency. This observation support the findings of Kennedy et al. (1991), who reported broilers receiving supplemental vitamin E attained 1.4% higher weigh and 0.8% better feed efficiency.

In fact, compared with other meats, broiler meat is relatively abundant in PUFA, as broilers are fast growing animals and their diets are highly nitrogenous and rich in energy, especially with PUFA (e.g. Rhee et al., 1996). An increase in PUFA content influence lipid oxidation and can affect colour, flavour, and, subsequently, oxidative stability during storage, especially under suboptimal storage condition (Basmacioglu et al., 2004). However, it has long been known that lipid oxidation can be retarded by the use dietary antioxidant. It has also been showed that dietary antioxidant supplementation result in good oxidative stability (e.g. Cortinas et al., 2005), even longer and better stability dependent upon antioxidant level and also application length in the diet (Yucelt, 1998; Coetzee and Hoffman, 2001). Vitamin E has been recognised one of the most powerful antioxidant and its commercial source dl-alpha-tocopheryl acetate has been mostly used in animal diets. The beneficial effect of dietary α-tocopheryl acetate to meet vitamin E needs and subsequent enhanced stability of lipids in muscle foods has been extensively reported for poultry, beef, veal and pigs (e.g. Gray et al., 1996).

However, as the production and consumption of poultry meat has been increased continuously during the last decades in many parts of the world, this increased has demanded considerable quantities of α-tocopheryl acetate. It has recently been noted that price of α-tocopherol increased and scientist has been focused on natural alternatives (e.g. Smet et al., 2008) or sparing agents for α-tocopherol.

Nutrogenomic studies through nanotechnology could also provide some other alternatives to α-tocopherol. Such a product called "Economase" has been developed and claimed 80% sparing activity of Vitamin E in broiler diets without loss of oxidative stability. The results obtained in the present study show that Economase could have 60% sparing activity of vitamin E in terms of growth and also meat quality with respect to TBARS and other relevant values. The groups receiving Economase with 40 mg/kg vitamin E produce acceptable TBARS values in tight and breast meats on day slaughter and 3 and 7 days storage at 4°C in the fridge. Frigg (1992) reported approximate scale for interpretation of TBARS values in meat and meat products. According to his scale, TBARS values; x 0.2 means good quality, 0.2-0.5 means limited, tolerable, 0.5-1.5 means somewhat oxidised, 1.5-5.0 means oxidised, >5 means rancid, non-edible. Our results showed that feeding a diet with 40 mg/kg Vitamin E with Economase produce limited, tolerable quality of meat up to 3 days storage at 4°C in fridge, similar to the control receiving 100 mg/kg vitamin E. Their values are lower than those obtained with 20 or 40 mg/kg vitamin E levels. These findings may suggest that Economase could provide a great synergetic potential for supporting dietary α-tocopherol for oxidative stability. However, previous studies, testing sparing effect or synergistic effects of natural extracts containing antioxidant on the oxidative stability of meat had gained limited success (e.g. Smet et al., 2008). Our results with respect to meat quality also revealed that use of Economase with 40 mg/kg vitamin E produce breast meat having similar drip loss and water holding capacity to the control group receiving 100 mg/kg vitamin E. All these results may suggest that Economase helps vitamin E in maintain oxidative stability and cell integrity in muscle food. Our results also revealed that Economase support broiler growth at 40 mg/kg vitamin E level by stimulating feed intake, but not enough to maintain feed conversion efficiency in comparison to the control receiving 100 mg/kg vitamin E. This might be attributed to intestinal epithelium and its integrity, as Economase might not support intestinal epithelium integrity for nutrient absorption. This requires further investigations.

Our results with respect to blood metabolites showed that Economase had no significant effects on serum glucose, cholesterol, VLDL cholesterol and triglyceride levels, but increased total antioxidant capacity without significant changes in total oxidant status, meaning that Economase support blood oxidant constituents. It is well known that blood contains primary, secondary and tertiary antioxidants. Primary antioxidants, which prevent formation of free radicals, are SOD, GSHPx, transferin seruloplazmin, etc. The secondary antioxidants, which remove free radicals and prevent their reactions in the body, are vitamin C, vitamin E, beta-carotene, uric acid, bilirubin, albumin, etc. Tertiary antioxidants, which repairs damages given by free radicals, are methionine reductase and DNA repairing enzymes (Mutlu et al., 2009). Economase seem to have poverty to support total body antioxidant pool by enhancing primary, secondary or tertiary antioxidant activity in the body.

The present study was conducted to evaluate sparing effect of Economase for vitamin E. Such an effect of Economase could be of benefit in economical and also immunological point of views. It has been speculated that vitamin E deficiencies or considerable excesses could have adverse effects on immune function in birds. Paradoxically, vitamin E in low-density lipoproteins oxidized in vitro in the absence of aqueous antioxidants may act as a prooxidant. However, prooxidant activity of high intakes of vitamin E has not yet been confirmed *in vivo* (Traber, 1999). The results of the present study could, therefore, be value for not only reduction in feeding cost, it is also value for establishing and maintaining the physiological equilibrium by preventing any possible negative effect of high level of vitamin E.

In conclusion, the results obtained in the present experiment suggest that vitamin E level in the commercial broiler diets could be reduced to 40 mg/kg with using Economase at 200 mg/kg without loss in growth performance and meat quality.

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Research Article

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Effect of boron treatments on boron distribution and fresh leaf yield of tea plant

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Abstract

A two-year fixed field experiment was designed in Artvin, Turkey, with the aim of determining the effect of soil and foliar boron treatment on fresh leaf yield, shoot length, and also the transport and distribution of boron in the shoots of the tea plant. The experiment was conducted in a domestic producer's tea garden indicating boron deficiency in Arhavi district of Artvin. In the experiment, 400 g B da^{-1} to the soil and 400 mg B L^{-1} to the leaves of the tea plant were applied in a liquid form. DOT (Disodium Octaborate Tetrahydrate, $Na_2B_8O_{13}.4H_2O$) with 20.8 % B was used as a boron source. At the end of the experiment, it was determined that soil and foliar boron treatment caused a substantial increase in the fresh leaf yield, the shoot length, and also the boron concentration of the shoots of the tea plant. However, the boron concentration of the leaves at the tip of the shoots was still under the critical level.

Keywords: Boron transport, boron distribution, tea plant, boron fertilization

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Introduction

Tea plant has a substantial importance for the world and Turkey in terms of consumption and economic aspects. Global tea consumption increasingly reached approximately 5 million tonnes with China (33 %), India (21 %) and Turkey (5 %) taking up the top places. Total tea production in the world has exceeded 5 million tonnes a year of which about 38 % is produced only in China while 24 % in India and 9% in Kenya (FAO, 2015). Tea consumption in Turkey tripled between 1945 and 1950; therefore, the land cultivated with tea plant reached from 3.000 ha to 76.000 ha (Kacar, 2010). As reported in CAYKUR 2015 tea sector report, Turkey ranks 8th in the world in the extent of land for cultivating tea, 6th in tea production, 3rd in tea consumption and 1st in tea yield obtained from per unit of harvested area.

Apart from being a popular beverage worldwide, ingredients of tea boosts the body's vitality, strengthens bones and teeth, reduces heart disease and cancer risk, has a positive effect on weight problems and diabetes (Naito and Yoshikawa, 2009; Yang and Wang, 2010; Goenka et al., 2013; Kim and Kim, 2013). Researches show that boron is effective in yield as well as taste and smell of black tea and, moreover, it is effective on quality of tea as it increases the tannin content of tea leaves (Pethiyagoda and Krishnapillai, 1971; Kacar, 2010). Boron deficiency in plants is frequently observed in acid-reactive soils where rainfall is abundant. In the Eastern Black Sea region where tea cultivation is intensively carried out, the research results, showing 97 % boron deficiency detected in the soil cultivated with tea plants and 98 % boron deficiency in Artvin province (Taban et al., 2015), have helped to shape the subject and area of this research. In order to raise the quality of tea leaf prevent severe environmental and health problems, it is necessary to know the concentration of essential plant nutrients of the tea plant and its soil as well as the optimal doses of the nutrients to be applied, their application methods, optimum forms, application times and frequency.

Within the frame of the topics mentioned above, the aim of this research is to determine the effect of the foliar and soil boron treatment on a) yield, b) shoot height, c) boron concentration of shoot leaves, d) general distribution of boron in shoots of the tea plant.

Materials and Methods

The Establishment and Implementation of the Experiment

A two-year fixed field experiment was carried out in a tea garden run by a domestic producer in Yemişli village of Arhavi district in Artvin province (primary coordinates: 37T0692339 E, 4577909 N, secondary coordinates: 41,32837 N, 41,29641 E, altitude: 22 m). The experiment was conducted on 3 April 2014 in the first year and on 25 March 2015 in the second year. The field experiment was laid out with randomized block design with 5 replications. The experimental field was divided into twenty 2x1 m plots with 0.5 m and 1 m buffer area between the plots and the blocks, respectively.

A soil sample was collected from a few points (0-20 cm in depth) of the experimental field according to the productivity principle as reported by Jackson (1962). The soil samples collected from the experimental field were air-dried at room temperature, crushed and passed through 2 mm sieve to be prepared for the analysis.

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The available boron analysis was carried out with hot water method developed by Bingham (1982). Soil pH and EC was determined in a 1:2.5 ratio of soil:water suspension (SSDF, 1951; Grewelling and Peech, 1960); soil texture by using Bouyoucos hydrometer (Bouyoucos, 1951); organic C by using modified Walkey-Black method (Jackson, 1962); the total N by using Kjheldahl method (Bremner, 1965); available P by using Bray Kurtz No. 1 method mainly developed for acidic soils (Bray and Kurtz, 1945); exchangable K^+, Ca^{++} and Mg^{++} by extracting in 1 N NH_4OAc (pH 7,0) (Pratt, 1965); available Zn, Fe, Cu ve Mn 0,005 M DTPA+0,01 M $CaCl_2$+0,1 M TEA (pH 7,3) (Lindsay and Norvell 1978). As a result of the analysis, the available boron concentration was very low (0.35 mg kg^{-1})and the reaction of the soil was very acidic (pH 4,39). Some other characteristics of the soil were as follows: texture clay; EC 568 µS cm^{-1} (without salt); organic matter 57.0 g kg^{-1} (excess); available P 55.6 mg kg^{-1} (too much); total N 8.20 g kg^{-1} (excess); NH4OAc extractable K^+, Ca^{++} and Mg^{++} (mg kg^{-1}) 298 (medium), 1224 (sufficient) and 434 (sufficient), respectively; DTPA extractable Fe, Cu, Zn , Mn (mg kg^{-1}) 211 (good), 0.72 (sufficient), 4.11 (excess) and 73.6 (excess), respectively. The concentration of the elements extracted in solution was read by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry, Perkin Emler Model DV 2100) (Boss and Fredeen 2014).

Fertilizing

During the experimental process, boron was applied to the soil and leaves in the form of DOT (Disodium Octaborate Tetrahydrate, $Na_2B_8O_{13}.4H_2O$) according to the application plan (Table 1). Trademark Etidot-67 (% 20,8 B) was used as a boron source during the preparation of the treatments.

Soil boron fertilization was carried out on 4 April 2014 in the first year and on 26 March 2015 in the second year. Boron fertilizers in liquid form were homogeneously sprayed onto the soil surface and also mixed thoroughly with the soil using a hoe. Foliar boron fertilization was carried out in each year a) at the stage of the conduction of the experiment and b) after the first two harvest. Foliar boron fertilizers were applied on 4 April, 16 May, 10 July in 2014; and on 26 March, 31 May and 25 July in 2015. During the application process, fertilizers were meticulously applied to the soil and plant by taking any precautions against contamination; furthermore, spreader-adhesive was used in the solution aiming to prevent flow and washing. Basic fertilization was planned considering the soil analysis results; therefore, 12.5 kg N da^{-1}; 2.5 kg of P_2O_5 da^{-1}; 5 kg K_2O da^{-1} were applied in the form of compound NPK (25:5:10) on 25 March 2014 in the first year and on 1 March 2015 in the second year.

Sampling of the Leaves

Ten branches of the tea plant were randomly chosen from each plot and marked. The leaves of the 10 of the 8 branches marked with white tags were sampled considering their location in the stem while the other 2, marked with red tags, were evaluated generally (Figure 1).

The leaves above the harvest base were collected starting from the top of the shoots (from the leaves under the apical bud) considering their location on the stem in the 8 of the 10 marked branches and coded as “L1”, “L2”, “L3”, “L4”, “L5”. The shoot leaves of the other 2 branches were randomly collected without considering their location with the aim of determining the general boron concentration of the shoot leaves and coded as “G”, short for general (Figure 1).

Leaf sampling was carried out before harvesting on 14 May, 8 July, 1 September in 2014; 29 May, 23 July and 10 September in 2015.

Harvesting

Harvesting was carried out within the campaign season declared by General Directorate of Tea Enterprises (CAYKUR) on 15 May, 9 July and 2 September in 2014; 30 May, 24 July and 11 September in 2015. Tea plucker preferred by the farmers in the region was used for harvesting which was meticulously performed following the directions of CAYKUR. The leaves harvested from each parcel were weighed while the shoot length of the marked branches was measured before sampling.

The Analysis of the Leaf Samples

Total boron concentration of the samples was determined with the use of microwave wet digestion method and the solution extracted from the leaves was read by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry, Perkin Emler Model DV 2100) (Boss and Fredenn, 2014).

Statistical Analysis

Fisher's least significant difference (LSD) test was used to determine the significant differences among the means. JMP Statistical Software (ver: 9.0.2) was used for all statistical computations.

Meteorological data of the experimental field

Previous research suggests that the annual average temperature should not be below 14 °C, rainfall should be over 2000 mm and relative humidity should be 70% in order to be able to grow tea plant in an economic sense (Tanton, 1982; Kacar, 2010). According to the data gathered from Turkish State Meteorological Service (TSMS), the average annual temperature is 14.51 °C, the average monthly rainfall is 2253 mm and the average annual relative humidity is 72.07 % in the area where the experiment was conducted (Table 2).

Results

The Effect of Boron Treatment on Fresh Tea Yield

Boron treatments had a positive effect on fresh tea yield in all three harvest season in both years, and this effect was found statistically significant at $p < 0.01$. When examined considering harvest seasons, it was seen that fresh tea yield was increased in the 2nd harvest in comparison to the 1st harvest and decreased in the 3rd harvest in both years. Except for the control group, each of the boron treatments increased the fresh tea yield (Table 3).

In the experimental field, boron deficiency symptoms were observed in the control group. Growth failure and deformed leaves were the first manifestations of the symptoms (Pethiyagoda and Krishnapillai, 1971) in the shoots of the control group (Figure 2). Not surprisingly, the least fresh tea yield was obtained from control groups (S0L0) in all three harvest season in both years (Table 3).

Considering statistical analysis, it is not possible to comment on a treatment coming to the forefront; however, when it is compared to the yield obtained from S0L0, the treatments were generally lined as S1L1> S0L1> S1L0 in all three harvest season in both years. Boron treatments had a positive effect on total fresh tea yield in both years, and this effect was found statistically significant ($p < 0.05$). In the first year, the effect of S1L1 on the total tea yield was different from other treatments and control group.

Even though their effect on the yield is different from control group, none of the treatments differed from each other considering their effect on the fresh tea yield in the second year (Table 3).

Table 1. Boron doses of the soil and foliar treatments.

Soil Boron Treatments	Doses	Foliar Boron Treatments	Doses
S0	0 g B da^{-1}	L0	0 mg B L^{-1}
		L1	400 mg B L^{-1}
S1	400 g B da^{-1}	L0	0 mg B L^{-1}
		L1	400 mg B L^{-1}

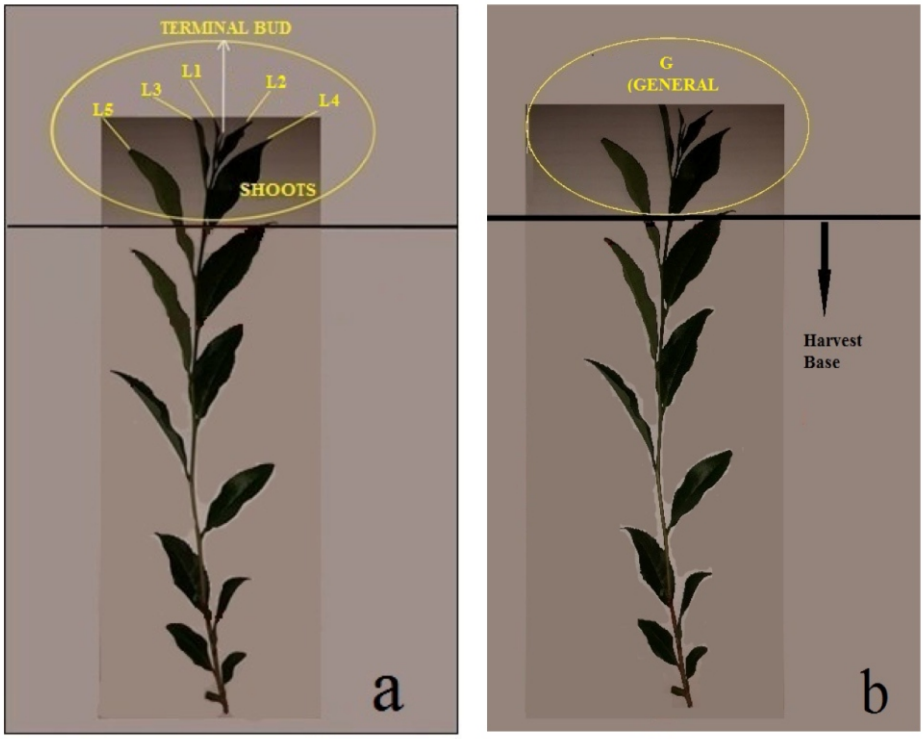

Figure 1. Leaf sampling a) considering the location b) without considering the location.

Table 2. Meteorological data of Arhavi-Artvin during the harvest season.

MONTHS	Temperature °C			Total Precipitation (mm)	Average Humidity (%)
	Average	Lowest	Highest		
			2014		
May	16.9	10.3	21.3	23.2	80.2
June	19.7	12.7	23.8	114	80.2
July	22.2	16.4	26.4	75.2	83.4
August	23.2	17.8	27.1	121	84.0
September	19.3	10.6	23.5	323	81.8
			2015		
May	15.4	6.4	19.5	12.8	80.4
June	19.0	13.6	22.6	57.4	89.9
July	21.0	15.5	24.5	18.0	85.5
August	22.9	15.6	27.3	326	87.5
September	21.6	15.7	25.8	80.6	82.2

Table 3. Effect of boron treatments on fresh tea yield (kg da^{-1}) in the first and second year of the experiment.

YEAR I								
Treatments	**1st Harvest**	**Increase, %**	**2nd Harvest**	**Increase, %**	**3rd Harvest**	**Increase, %**	**Total**	**Increase, %**
S0L0	492b	-	505c	-	190c	-	1187c	-
S0L1	523b	6	644a	28	271b	43	1438b	21
S1L0	507b	3	581b	15	271b	43	1359b	14
S1L1	639a	30	654a	29	322a	69	1614a	36
F Value	24.6***		54.3***		18.4***		12.5*	
LSD	42		29		39		142	
Treatments				**YEAR II**				
S0L0	476c	-	534c	-	314c	-	1324c	-
S0L1	575b	21	693a	30	348b	11	1616ab	22
S1L0	552b	16	643b	20	370a	18	1566b	18
S1L1	666a	40	689a	29	390a	24	1745a	32
F Value	25.8***		28.3***		22.7***		8.81*	
LSD	48		43		21		167	

*: p<0.05, ***: p<0.001 a, b, c ↓ : Different lower cases in the same column represent statistically significant differences among the treatments

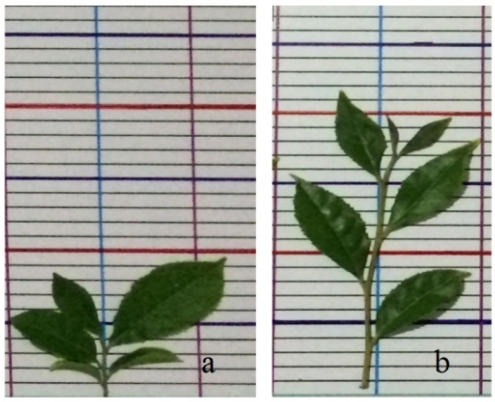

Figure 2. a. Boron deficiency in the control group (S0L0) (leaves: darker and thicker than normal, terminal bud: in hibernate), b. a healthy tea-shoot grown in the plots on which S1L1 was applied.

YEAR I						
Treatments	**1st Harvest**	**Increase, %**	**2nd Harvest**	**Increase, %**	**3rd Harvest**	**Increase, %**
S0L0	12.2c	-	14.2c	-	11.9b	-
S0L1	15.5ab	27	17.8b	25	14.6a	23
S1L0	14.6b	20	18.0b	27	14.6a	23
S1L1	15.7a	29	19.6a	38	14.9a	25
F Value	23.0***		63.5***		35.5***	
LSD	1.04		0.88		0.72	
Treatments				**YEAR II**		
S0L0	16.2c	-	17.2c	-	12.8b	-
S0L1	19.4a	20	19.5b	14	15.8a	23
S1L0	19.4a	20	20.3ab	18	16.5a	29
S1L1	18.7b	15	20.5a	19	16.1a	26
F Value	45.2***		24.8***		20.0***	
LSD	0.70		0.94		1.16	

***: p<0.001 a, b, c ↓ : Different lower cases in the same column represent statistically significant differences among the treatments

Table 4. Effect of boron treatments on shoot length (cm) of the tea plant in the first and second year of the experiment.

The Effect of Boron Treatment on Shoot Length

Boron treatments had a positive effect on the shoot length of the tea plant in all three harvest seasons in both years and this effect was found statistically significant at $p < 0.01$. The shortest shoot lengths were determined in the control groups in all three harvest seasons in both years. Except for the control group, each of the boron treatments increased the shoot length of the tea plant. Considering statistical analysis, it is not possible to comment on an treatment coming to the forefront in both years (Table 4).

The Effect of Boron Treatment on Boron Concentration of the Tea Shoots

In both year and each harvest season, individual and co-effects (interactions both boron treatment and leaf) of the boron treatments and each leaf of the tea shoots were found to be important on boron concentration and this effect was found to be statistically significant at $p < 0.001$. The boron concentration of the shoot leaves increased significantly after the soil and foliar boron treatment no matter whether applied separately or together. The highest boron concentration was identified in the 5th leaves (L5) while the least boron concentration was found in the 1st leaves (L1) since the data show a steady decrease starting from the L5 to the L1. Except for control groups, each of the treatment increased the boron concentration of the each leaf of the shoot. In all three harvest seasons in both years, the highest level of boron concentration was generally determined in the shoots on which S1L1 was applied, additionally, S1L1 treatment was followed by S1L0 and S0L1, respectively. On the other hand, when assessed statistically, it is not possible to mention an treatment coming to the forefront in terms of its effect on the boron concentration of the shoot leaves. The S0L1 treatment did not elevate the boron concentration of the shoots to the desired level in any harvest season during both years of the experiment. Except for the 1st harvest of the first year, none of the treatments could manage to raise the boron concentration above the critical limit in L1 and L2 in any harvest season in both years (Table 5; Figure 3, 4).

Discussion

As a result of the analysis, it is concluded that boron treatments had a positive effect on the yield in each harvest season in both years. This situation might suggest that the lack of boron nutrition of the experimental field and tea plant cultivated in this land might be the reason of this positive outcome. The deficiency of boron concentration in tea cultivated lands has been reported by a number of different researchers who conducted experimentation on the issue in Eastern Black Sea region (Kacar et al., 1979; Ozyazıcı et al. 2015; Taban et al., 2015; Ozkutlu et al., 2016). The treatments caused an increase in the boron concentration of the shoots (Table 5) and it is considered that this increase had a positive effect on the yield of the tea plant. By means of soil and foliar treatment, Taban et al. (2015) managed to increase the fresh tea yield of 21.87 % in a tea cultivated land detected boron deficiency at 96.62% in the Eastern Black Sea region.

Boron helps feed the plant by transporting the photosynthesis products to the necessary organs when there is a need. Boron forms boron-sugar complexes in plants and plays an important role in the short and long-distance transport of sugars (Matoh et al., 1996; Kaneko et al. 1997; Jackson, 1991). Blaser et al. (1967) reported that the lack of boron is sensed by the plant more acutely especially in the meristematic development stages. There is no evidence to indicate the direct effects of boron on nitrogen metabolism; however, in the absence of it, protein synthesis is reduced in the plant (Amberger, 1975). Likewise, boron is also effective in pectin synthesis and fat metabolism. In case of boron deficiency, the thickening, brittleness, and breakage of cell walls are explained by the inadequacy of pectin synthesis (Spurs, 1957; Kacar, 2010). One of the theories about the role of boron in plant metabolism is its effect on the stability of plant hormone level. Dyar and Webb (1961) found that boron plays an important role in auxin and IAA biosynthesis of the plants at the cambium tips. In the presence of boron, the increase in intake of some plant nutrients also might be related to the increase in yield. Pollard et al. (1977) found that the P uptake of plants fed with boron was higher than the ones grown in the boron-deficient environment. Marscher (1995) reported that the loss of K through washing was more common in boron deficient leaves. Similarly, the loss of sugar, amino acids, and phenols through washing is much more in case of boron deficiency and can be removed by addition of boron.

Table 5. The boron concentration (mg kg^{-1}) of each leaf of the shoots (L1, L2, L3, L4, and L5) and general boron concentration of the shoot leaves (G) in all three harvest season of the first and second year of the experiment.

Treatments	1st Harvest						2nd Harvest						3rd Harvest					
	L1	L2	L3	L4	L5	G	L1	L2	L3	L4	L5	G	L1	L2	L3	L4	L5	G
YEAR I																		
S0L0	15.9Db	16.3Db	16.5Db	17.0Db	20.8Da	17.2D	11.7Bb	11.9Bb	12.3Cab	12.7Ba	12.9Ba	11.9B	9.10Cc	9.55Dbc	9.63Dbc	10.6Cab	11.4Da	9.90C
S0L1	22.5Cb	23.2Cb	23.1Cb	24.8Cab	28.4Ca	25.9C	14.0Bb	14.1Bb	14.9Cab	15.4Bab	16.3Ba	15.3B	11.6Bc	12.5Cc	12.4Cc	14.6Cb	15.8Ca	13.4B
S1L0	30.9Bd	40.7Bc	48.9Bb	53.4Bb	60.8Ba	47.2B	22.3Ac	22.6Ac	29.1Bb	35.1Ab	42.1Aa	29.6A	18.3Ad	19.8Bcd	23.0Bbc	25.3Bb	31.2Ba	23.5A
S1L1	40.8Ae	50.0Ad	57.5Ac	66.9Ab	73.6Aa	54.3A	22.4Ac	25.0Ac	35.8Ab	39.9Aab	49.2Aa	33.1A	20.6Ae	23.9Ad	28.0Ac	31.8Ab	36.7Aa	26.6A
Treatment (T)			***			***			***			***			***			***
Leaves (L)			***						***						***			
T X L int			***						***						***			
YEAR II																		
S0L0	10.3Cc	10.3Cc	11.8Cb	11.8Db	13.8Ca	11.8C	9.63Cb	11.1Db	11.2Cb	12.9Ca	13.1Da	11.3C	9.02Dc	9.21Cbc	9.56Bbc	10.1Db	11.7Da	10.4C
S0L1	15.8Bc	17.2Bc	22.1Bb	23.5Cb	27.0Ba	20.3B	13.4Bc	16.0Cbc	18.8Bb	24.5Ba	24.7Ca	20.0B	11.0Cc	13.2Bb	12.8Bb	13.6Cb	17.9Ca	14.2B
S1L0	20.4Ac	21.1Ac	25.1ABbc	28.9Bab	30.6Ba	26.0A	16.0Bd	18.2Bd	22.2Bc	26.5Bb	31.0Ba	23.6B	12.2Bd	17.2Ac	21.5Ab	24.0Bab	27.2Ba	22.5A
S1L1	19.6Ac	23.4Abc	27.3Ab	38.3Aa	41.0Aa	30.2A	20.5Ad	25.2Ac	28.4Ac	35.3Ab	43.5Aa	33.1A	14.0Ad	15.1ABd	21.3Ac	28.6Ab	35.4Aa	23.4A
Treatment (T)			***			***			***			***			***			***
Leaves (L)			***						***						***			
T X L int			***						***						***			

***: $p<0.001$; A, B, C, D ↓ : Different upper cases in the same column represent statistically significant differences among the treatments; a, b, c →: Different lower cases in the same row represent statistically significant differences among the leaves

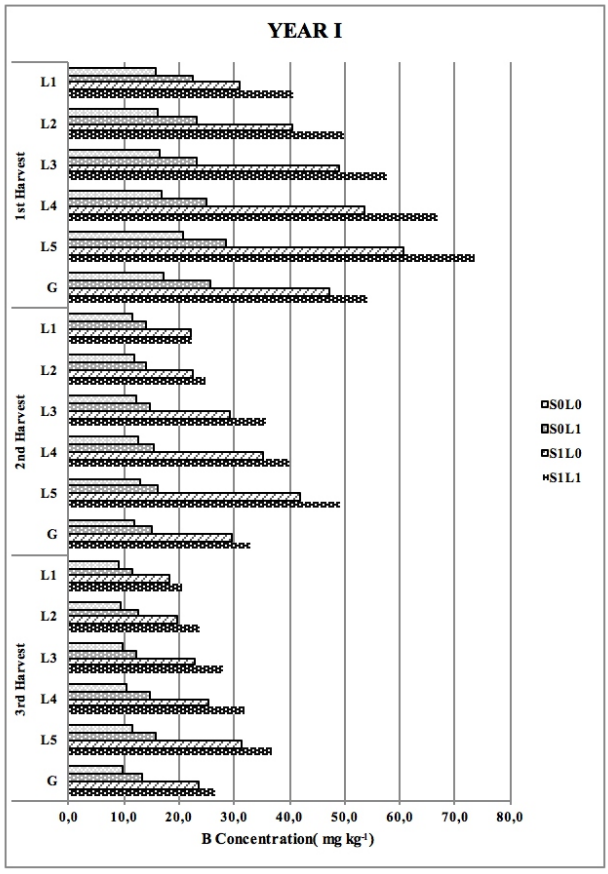

Figure 3. The effect of boron treatment on the boron concentration of each leaf of the shoots (L1, L2, L3, L4 and L5) and general boron concentration of the shoot leaves (G) in all three harvest season of the first year of the experiment.

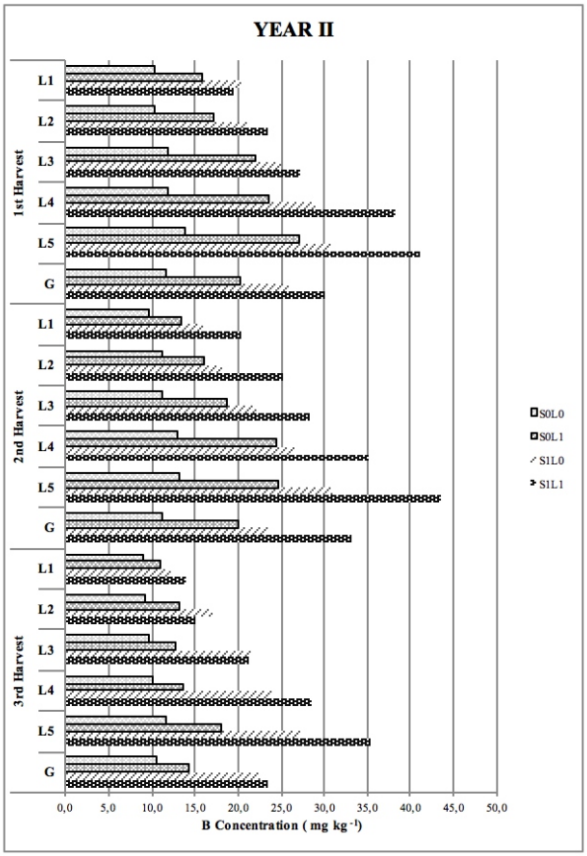

Figure 4. The effect of boron treatment on the boron concentration of each leaf of the shoots (L1, L2, L3, L4, and L5) and general boron concentration of the shoot leaves (G) in all three harvest season of the second year of the experiment.

When examined considering harvest seasons, it was seen that fresh tea yield (kg da^{-1}) was increased in the 2nd harvest in comparison to the 1st harvest and decreased in the 3rd harvest, in both years (Table 3). According to the meteorological conditions during the experiment, the average temperature recorded in the 2nd harvest in July showed a significant rise in comparison to the records in the 1st harvest in May (Table 2). Due to the fact that the tea is a tropical plant, the fresh tea yield is more likely to be affected by the increase in the temperature in July as well as the application of boron. Depending on the increase in temperature, the soil temperature also rises, thus ion movement in the soil also increases with the increasing kinetic energy. The 2nd harvest precipitation averages in June, are also relatively higher than the 1st harvest. Plant nutrients in soil increasing in activity with the rising temperature can more easily reach the plant roots with the help of increasing precipitation, so that they can be more available for the plant (Gunes et al. 2010).

According to Urs and Fischer (1994), nitrogen metabolism changes from assimilation to remobilization, nitrite reducing enzymes decrease, catabolic enzymes increase and chloroplasts begin to degrade due to the harvest seasons. Researchers noted that the plant growth was rapid in the early stages of the harvest season due to the availability of nutritions reserved in vegetative storage organs. However, the mineral content of the plant drops during the ripening and young plant tissues contains more NPK since the nutrient uptake of the plant decreases in time and dry matter formation continues. The changing inclines of the plants in mineral uptake depending on the age are seen as another reason for the difference between the vegetation periods (Korkmaz et al., 1993; Aktas, 1995). In the experiment, 25-5-10 was applied as a base fertilizer and did not repeated during the harvest season in both years. The plant nutrients applied to the soil are quickly lost due to precipitation, fixation, and evaporation.

The average fresh tea yield obtained from per unit of the harvested area varies between 1300 and 1700 kg da^{-1} (CAYKUR 2015). In the experiment, the average yield obtained from the control group (S0L0) remained below the average of Turkey in the first year with 1187 kg da^{-1} and slightly above in the second year with 1324 kg da^{-1} while the yield obtained from the plots especially from the ones soil and foliar fertilizers applied in together (S1L1) was between or above the average values of Turkey.

It is believed that the factors causing the increase of fresh tea yield are also valid for the increase of the shoot length of the tea plant. As a result of the boron deficiency, the distance of internodes shortens and dwarfism is seen since the development of the plant is regressed and apical meristem hibernates; therefore the shoot length of the plant become shorter than usual (Neales, 1960; Pethiyagoda and Krishnapillai, 1971; Lovatt and Dugger, 1984; Lukaszewski and Blevins, 1996) (Figure 2). Gohain et al. (2000) found that the distance of internodes lined up as 2.43; 2.47; 2.43 and 2.48 cm as a result of the boron treatment in the boric acid form in 0; 0.5; 1 ve 1.5 kg ha^{-1}, respectively.

When all three harvest seasons in two years are generally evaluated, none of the soil and foliar treatments used in the experiment could bring the boron concentration of the first two leaves (L1 and L2) of the shoots above the critical level needed for a healthy growth of tea plant. The S0L1 treatment could not bring the boron concentration of the shoots to the desired level in any harvest season of both years. In order for the foliar treatment to be successful, the fertilizer must be able to be absorbed by the leaf and transported to the necessary organs (Bukovak and Wittwer, 1957; Haslett et al., 2001), and in this case, phloem transport comes into question. There is no evidence for the presence of the polyol-complexes in tea plant which help to transport boron in the phloem. The plant needs water uptake and boron was carried to the upper organs with the help of it as a result of the water loss which occurs in the plant organs during the day . According to Pate (1975), inside the dead cells of xylem transport occurs from the roots to the greens of the plant. Bell (2016) noted that the highest accumulation of boron in the plant occurs in the old leaves; more specifically, on their tips and edges.

The age of the plants is also one of the important factors affecting the uptake and transport of boron since the upward concentration gradient decreases with the age (Shelp et al., 1987; 1992). It is expressed in many local and foreign academic sources that the biological lifespan of a tea plant (*Camellia sinensis var. sinensis*) is 100 years and its economic lifespan is 70-80 years. If the 1940s is based on for the Eastern Black Sea, today's tea plantations in Turkey is about 80 years old; in other words, they are about to complete their biological lifespan as well as appearing to be at the boundary of their economic lifespan (Kacar, 2010).

As a result of the research, it was found that the foliar boron treatment also significantly increased the yield, shoot length and boron concentration of the tea plant in addition to the soil treatment (Table 3, 4, 5). This result shows that during the times when the soil treatment is difficult to conduct, boron deficiency can be eliminated with a foliar treatment. Besides being more economical, the foliar treatment gives the opportunity to apply the fertilizer more homogeneously and also removes the deficiency in a shorter time than the soil treatment. On the other hand, using soil treatment, the nutrient can be kept in the soil for a longer time and the repetition of fertilization is less frequently needed (Amiri et al., 2008).

Conclusion

As a result of the analysis, the boron concentration of the soil of the experimental field and also tea plant cultivated in this area were found insufficient for a healthy growth of the tea plant. After the soil and foliar boron treatment, fresh tea yield, shoot length and boron concentration of the tea plant increased; in other words, tea plant deprived of boron gave a positive reaction to the boron fertilization. When evaluated in general, it is seen that the boron concentration of the each leaves of the shoots was raised with the help of S1L1 and S1L0 treatment. Although foliar treatment alone (S0L1) did not bring the boron concentration of any of the shoot leaves above the desired value, it could significantly increase the yield and shoot length compared to the control group (S0L0) in most harvest season. This result shows that during the times when the soil treatment is difficult to conduct, boron deficiency can be eliminated with a foliar treatment.

One of the important findings of the research that the changing values of the yield, shoot length and boron concentration of the shoots depend on the vegetation period. This result shows that the plant nutrients accumulated in the storage organs was used by the plant and consumed up until the 3rd harvest.

Moreover, the nutrients, even though added to the soil by fertilization, are quickly lost due to precipitation, fixation, and evaporation. In order to prevent such losses, it is needed to keep it ready in the soil when it is most needed; furthermore, it is necessary not to give all of the base fertilizer at once but divided into pieces to be able to apply during the different stages of the plant development.

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Research Article

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A cost-effective approach for chicken egg weight estimation through computer vision

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Abstract

Egg weighing and classification are among the most significant phases done in egg processing by industries which are tedious if done manually by poultry owners, and egg inspectors and graders. This study presented an alternative way of estimating chicken egg weight through computer vision minimizing human interaction during the process. In this study, 15 eggs of white leghorn chicken layers of different sizes were tested. The eggs' image was captured using an inexpensive yet reliable webcam which was then loaded onto the Matlab workspace for image processing and further image analysis. The center of gravity of the image was determined, and the extraction of minor axis length and major axis length followed. The obtained values were used to compute the egg's weight mathematically. Through the different image processing methods, image dimensions were extracted and used to calculate the desired output. The results of this study showed 96.31% accuracy in estimating the egg's weight and classification validated by manual egg weighing and classification procedure.

Keywords: Egg weight, webcam, image processing, image analysis

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Introduction

Poultry egg production has indeed provided excellent opportunities to poultry farmers in the Philippines. According to the report of the Philippine Bureau of Agricultural Statistics, for five consecutive years starting 2009, egg production had the following increase percentage: 1.53%, 4.01%, 4.52%, 4.61%, and 5.11% (CountrySTAT-Philippines, 2014). However, poultry owners are burdened by hiring additional workers for their poultry farms to do the different routinary tasks viz. egg harvesting and collection, cleaning and washing of eggs, segregation of cracked and non-cracked eggs, egg grading and classification, and sorting and packaging. Moreover, these are apparently tedious works, especially when done manually.

Egg weighing is one of the essential phases of egg processing. Egg inspectors and graders must carefully examine and check the eggs before it will be packed for market distribution. The problem is that it takes a lot of human effort to do this work which necessitates the search for a more convenient yet less expensive alternative. This is the reason why researchers are developing an alternative way of doing this through computer vision.

As of now, there are already existing egg sorter and packaging machines that are available globally, and there are even existing researches on egg weight estimation using computer vision involving more components (Dangphonthong and Pinate, 2016). Their ultimate goal is to help those poultry farm owners, egg graders, and inspectors to do their jobs right. However, although there are already studies developed in this domain such as the study of Abdanan Mehdizadeh et al., (2014), Soltani et al. (2015), and Waranusast et al. (2016), one of the main concerns is the cost of developing the set-up.

An existing study of egg weight estimation was presented using a machine vision system combined with artificial neural network technique (Asadi and Raoufat, 2010). In this research study, an alternative method for estimating chicken egg weight through computer vision was developed. This computerized method of egg weight estimation used a simple and less expensive but reliable webcam aided with software. This system featured the following image processing method: image acquisition, pre-processing, detection, and analysis of an egg image to acquire the desired result. Consequently, this system will determine the egg's weight and classification.

This study aims to show that even an inexpensive webcam with the aid of software can be used to estimate an egg's weight from a distance without human interaction through computer vision. Furthermore, this study aims to develop a less expensive automated chicken egg weight estimation method which is indeed economically beneficial in the long run.

Materials and Methods

Physical Setup

The system's hardware specifications were suitably chosen based on the compatibility of the programming language used for image processing and the computer vision device (webcam). It only used a low-cost webcam for image acquisition.

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Figure 1 below shows the physical setup of the egg weight estimation system. The prototype housing was made out of a ply board to support the webcam on its top which was positioned perpendicular to the inspected egg. The egg was placed in a hole of a candling booth so that the light would penetrate to the egg and making it possible to observe the egg's shape and edges.

The indicated dimensions of the system's prototype shown in Figure 2 were very well-observed because of its effect on the image processing result when not done correctly. The working distance between the front of the webcam lens and the object was set to 14 cm. When the object's height parameter was set to 15 cm or below, the nonlinearity has less impact on the system accuracy. The error variation by this setting is acceptable (Chmelař and Dobrovolný, 2012). Though a working distance of 15 cm to 1 cm could have a fair result, the researcher opted to choose 14 cm so that the image of a bigger size egg could still fit on the frame. Bigger eggs appear larger than the frame if the working distance is 13 cm. According to Euclid's law, the farther away an object is, the smaller it appears; consequently, the closer the object from the viewer, the bigger it seems.

The interior portion of the box was themed black to darken the area for candling purposes. Egg's defect detection using candling method could be easily observed in a dark room (Broyde, 2000). This candling booth contained a lighting system (5W softone standard bulb with its receptacle). Once it was lighted on, the light penetrates into the egg's shell which then enabled the camera to detect its shape.

This research used eggs of a white leghorn chicken layers since it is the common chicken layer breed used in medium- to large-scale poultry egg production in the Philippines. The egg's shape of this breed of chicken is typically axial symmetric concerning its major axis. It was assumed that the eggs to be inspected using this system are fresh so that the density which is 1.031 g cm^{-3} will be applicable (Paganelli et al., 1974). It was also assumed that before the eggs are placed onto the system; they were already washed and cleaned, so dirt like blood, feather, feces or mud and even those cracked eggs if any were already removed. For demonstration, this system only does the pick and drop method in placing the eggs horizontally beneath the camera.

The block diagram of the egg weight estimation system as shown in Figure 3 consisted of 5 steps: (1) Image acquisition, (2) Image pre-processing step, (3) Image enhancement technique, (4) Image segmentation and (5) Image analysis.

System Design

To achieve the desired output by estimating the egg's weight, this system had gone through the following processes:

a. Image acquisition

Image acquisition is the process of capturing the egg's image to be processed through a low-cost webcam. To get the best image the webcam could capture, it was set to its highest capable resolution which is 640 x 480, and the returned color space was set to grayscale. It carries the intensity information where black have the low or weakest intensity, and white have the high or strongest intensity. The boundary between these two regions forms the edge. Edge detection operator was then applied to detect the object boundaries and edges (Al-amri et al., 2010).

b. Image pre-processing

The grayscale image was converted to a binary image using a threshold value determined by the program. That is when the image intensity is less than the threshold value; it would be replaced with black, otherwise, a white pixel if the image intensity is greater than that value. This grayscale transformation helps to make the image clearer for easy image boundary detection.

c. Image enhancement technique

This step increases the signal-to-noise ratio and accentuates image features by modifying the colors or intensities of an image. It involved image noise removal with linear, median, or adaptive filtering.

d. Image augmentation algorithm

In this step, the program determined the region boundaries of the image. This system used edge-based methods. Once the boundary was already identified, the entire area of the image was filled with white pixels to make the image look solid.

e. Image analysis

This is the phase of extracting the image's meaningful information such as determining the region of interest, finding the center of gravity and measuring object properties. Since egg image formed an elliptical shape, there were 2 axis lengths present in the image, the minor axis length and the major axis length concerning its center of gravity or centroid. Figure 4 shows the egg image's minor axis (breadth) and the major axis (length). This image property was measured (in pixel).

The calculated distance in the pixel value of the minor and major axis length, converting pixel to the real-world unit (in cm) followed. It was accomplished by multiplying the distance value (in pixel) of the minor and major axis length with the spatial calibration factor, 0.0111 cm per pixel. A constant value of 0.0111 cm per pixel was being used during the calculation. This spatial calibration factor is only for this setting. Spatial calibration factor allows one to translate a measurement from pixel units into real-world units (National Instruments Corporation). It is dependent on some parameters that were applied to this setup like the resolution of the webcam, the image acquisition software, and the distance and environment of the working area.

The formula in Eq. 1 was used to calculate the egg's volume, where, *L* is the egg's length (converted in cm) obtained through measuring the image major axis length and *B* is the egg's breadth (converted in cm) obtained through measuring the image minor axis length (Altuntas and Sekeroglu, 2010).

$$V_{egg} = \left(\frac{\pi}{6}\right) \times LB^2 \quad \text{(Eq. 1)}$$

With the obtained value of the egg's volume (in cubic cm), computation of the egg's weight using the mass formula follows. The formula to determine egg's mass was shown in Eq. 2, where V_{egg} value was replaced with the calculated egg volume value (in cubic cm) taken from the previous step and 1.031 g cm^{-3} was the density of the fresh chicken egg (Paganelli et al., 1974).

$$m_{egg} = V_{egg} * 1.031 \quad \text{(Eq. 2)}$$

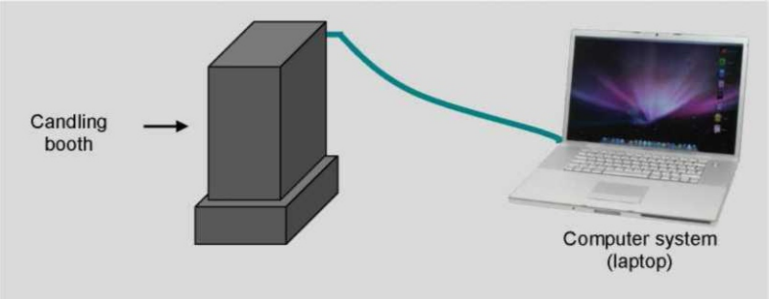

Figure 1. Physical Setup of the Egg Weight Estimation System

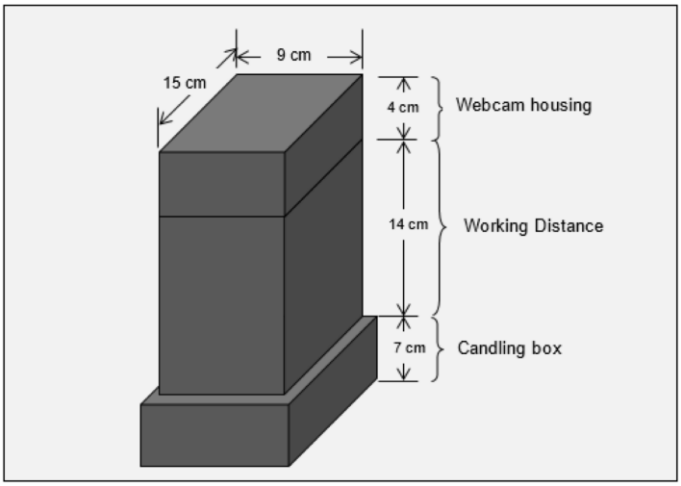

Figure 2. Parts and Dimensions of the System Prototype

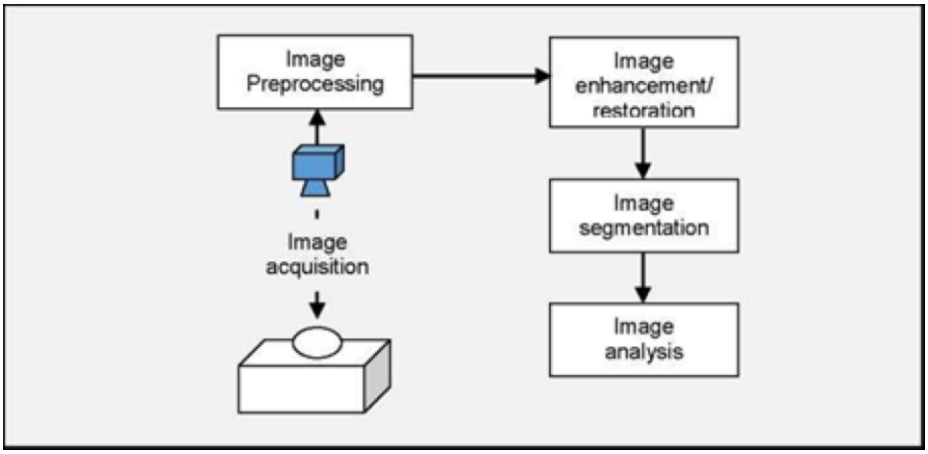

Figure 3. Block Diagram of the Egg Weight Estimation System

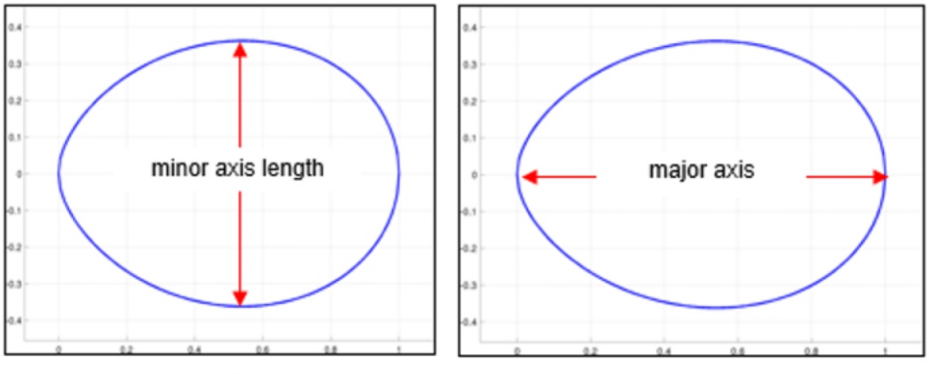

Figure 4. (a) Minor Axis (Breadth) and (b) Major Axis (Length) of an Egg

With the computed egg's weight (in gram) from the previous step, egg size classification followed by evaluating the egg's mass value based on the categories given by the Philippine National Standard for Table eggs (PNS/BAFPS, 2005).

User Interface Design

The programming language used in this system is Matlab R2012b. It had the following image processing related toolbox: computer vision system, image acquisition, image processing and GUI. Using the guide Matlab command, the algorithm for chicken egg weight's estimation is shown in Figure 5.

Evaluation of the System

To assess the effectiveness of chicken egg weight estimation system, it was tested to 15 fresh chicken egg samples that come in different sizes. The 15 egg samples were first weighed manually using an analytical balance. The measured values were then compared to the result of the egg weight estimation system.

After the manual weighing of the 15 fresh egg samples, they were loaded individually to the developed system for inspection. Each egg was being inspected 12 times or in 12 trials. These 12 trials were divided into four positions. These four (4) positions thought to be the possible positions of the eggs when loaded in a conveyor or egg holder during the inspection. Each position had 3 trials to test the system accuracy. In the first position (Position 1), the egg was positioned horizontally, parallel to the camera's orientation. In the second position (Position 2), the egg's position was changed, rotating it 10-15° from its original position (Position 1). While in the third position (Position 3), the egg was rotated up to 180° from its original position (Position 1). And finally, for the fourth position (Position 4), the egg was held vertically 10-15° inclination point from its original position (Position 3). Three trials (Trial 1, Trial 2 and Trial 3) were conducted for each position. Figure 6 shows the captured image of different egg positions.

Table 1. Table of egg classification with their corresponding weight

Weight class	Weight range (in grams/egg)
Jumbo	70 and above
Extra Large	65 – 70
Large	60 – 65
Medium	55 – 60
Small	50 – 55
Pullets	45 – 50
Peewee	40 – 45
Too light	< 40

PNS/BAFPS 35:2005

```
1   Initialize image acquisition device
2   Set Image Acquisition device
3   Access image acquisition
4   Get image snapshot
5   Save snapshot
6
7   Load image on the workspace
8   Convert grayscale image to binary image
9
10  Filter binary image
11  Remove objects inside the image which pixel less than 20
12  Fill holes enclosed the boundary
13
14  Locate the image's center of gravity
15  Get the minor axis length of the object, in pixel
16  Convert pixel value to centimeter
17
18  Locate the major axis length of the object
19  Get the major axis length in pixel
20  Convert pixel value to centimeter
21
22  Compute the egg volume
23  Compute the egg mass/weight
24  Classify the egg's size through the calculated egg mass/weight
25  If mass/weight is greater than or equal to 70, print "Jumbo",
26      Else if the mass/weight is greater than or equal to 65, print "Extra Large"
27      Else if the mass/weight is greater than or equal to 60, print "Large"
28      Else if the mass/weight is greater than or equal to 55, print "Medium"
29      Else if the mass/weight is greater than or equal to 50, print "Small"
30      Else if the mass/weight is greater than or equal to 45, print "Pullet"
31      Else, print "Peewee"
```

Figure 5. Algorithm for Chicken Egg Weight's Estimation

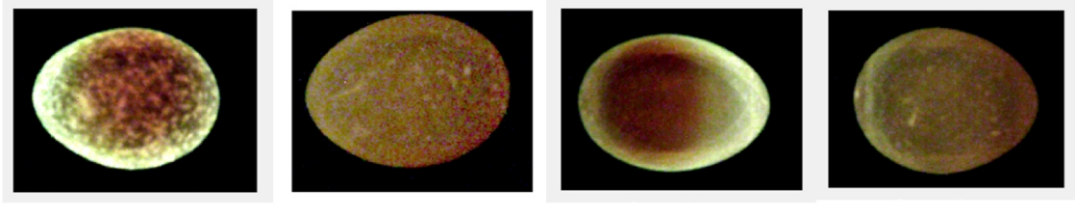

Figure 6. Captured Image of Different Egg Positions

The reason for having these different positions is to evaluate the efficiency and accuracy of the system in estimating chicken egg weight and size classification even when the positions of the eggs are at different perspectives.

Using percent error formula on Eq. 3, the researcher was able to check the developed system's accuracy. It compares the system's obtained value to the measured value. The manual measurement (weight) in Eq. 3 is the measured value of the egg's weight using a weighing scale, while computerized measurement (weight) is the value of the egg's weight generated from the developed system.

$$\% \text{ Error} = \frac{\text{manual measurement (weight) - computerized measurement (weight)}}{\text{manual measurement (weight)}} \times 100\% \quad \text{(Eq. 3)}$$

Results and Discussion

Figure 7 shows a sample output of the egg weight estimation system. This user interface would be prompted on the screen for visual inspection to see the egg's properties during inspection and classification. It displayed the images used in the image processing and analysis, like the captured image, binary image, and the measured image. It also displayed the calculated egg weight and egg classification. For demonstration purposes, the egg inspector clicked the Inspect Egg button on the interface after the egg was positioned on the egg holder. In not less than 5 seconds, the result was then displayed on the screen.

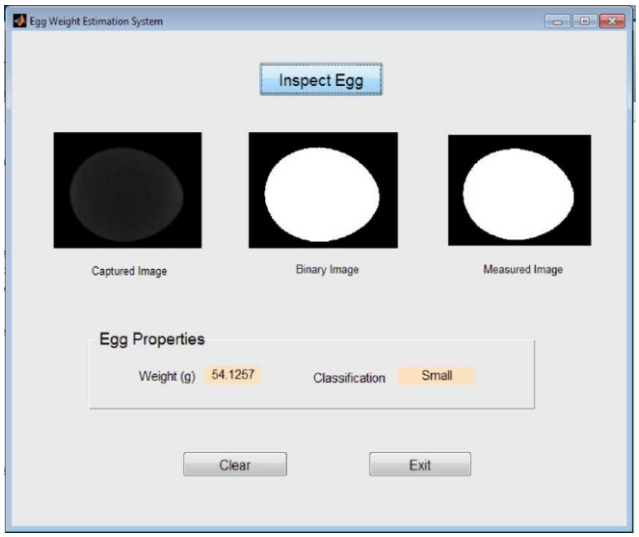

Figure 7. Sample Egg Weight Estimation System Result Output

Comparison between Manual and Computerized Method of Measurement Result

With the obtained egg grading and inspection data from both manual and computerized method, percent error was used to determine the accuracy of the system. The eggs were positioned in four different ways as follows:

a. Egg positioned parallel to the webcam

In this position (Position 1), the egg was laid on the egg holder parallel to the camera's orientation. Table 2 shows that the percent error of the egg samples ranged from 0.45% - 5.97%, with an average percent error of 3.69%. A percentage error very close to zero means very close to the targeted value. Therefore, this position of the egg samples where the eggs were laid parallel to the camera was a good position to get the egg weight using this system.

Table 2. Egg Weight Estimation Value and its Percent Error (Position 1)

Egg Sample	Weight Estimation (g)		
	Computerized Method	Manual Method	% Error
1	72.87±0.416	74.20	1.80
2	80.36±0.072	80.00	0.45
3	62.39±0.243	64.40	3.12
4	49.55±0.275	52.20	5.07
5	60.35±0.258	62.40	3.29
6	60.81±0.449	64.40	5.58
7	57.17±0.133	60.80	5.97
8	63.35±0.583	65.00	2.54
9	54.09±0.473	56.40	4.10
10	53.85±2.918	56.00	3.83
11	50.38±0.321	53.40	5.65
12	51.26±0.159	53.70	4.54
13	53.26±0.257	55.50	4.04
14	51.48±0.344	53.00	2.87
15	50.67±0.104	52.00	2.56

b. Egg positioned held inclined from its 1st position

In this second position (Position 2), the egg was held inclined or rotated 10-15° from its original position (Position 1). As shown in Table 3, the percent error of the egg samples ranged from 0.03% - 5.22%, with an average percent error of 3.05%. It had a percentage error closer to zero which means that this position could still estimate the egg's weight.

Table 3. Egg Weight Estimation Value and its Percent Error (Position 2)

Egg Sample	Weight Estimation (g)		
	Computerized Method	Manual Method	% Error
1	72.45±0.459	74.20	2.36
2	81.00±0.305	80.00	1.25
3	62.28±0.303	64.40	3.29
4	49.64±0.202	52.20	4.90
5	60.26±0.214	62.40	3.42
6	61.47±0.264	64.40	4.56
7	57.63±0.34	60.80	5.22
8	63.29±0.723	65.00	2.64
9	54.46±0.055	56.40	3.44
10	55.98±0.051	56.00	0.03
11	52.17±0.276	53.40	2.30
12	51.60±0.204	53.70	3.91
13	53.52±0.241	55.50	3.57
14	51.56±0.354	53.00	2.73
15	50.85±0.271	52.00	2.21

c. Egg positioned parallel to the webcam (180° from Position 1)

The egg samples in this position (Position 3) were rotated up to 180° from its first position (Position 1). As shown in Table 4, the percent error of the egg samples ranged from 0.77% - 6.33%, with an average percent error of 3.49%. It also had a percentage error closer to zero which means that this position could still estimate the egg's weight.

Table 4. Egg Weight Estimation Value and its Percent Error (Position 3)

Egg Sample	Weight Estimation (g)		
	Computerized Method	Manual Method	% Error
1	72.84±0.174	74.20	1.83
2	80.71±0.337	80.00	0.88
3	62.30±0.023	64.40	3.26
4	49.50±0.163	52.20	5.18
5	59.93±0.381	62.40	3.96
6	61.64±0.304	64.40	4.28
7	56.95±0.111	60.80	6.33
8	63.60±0.388	65.00	2.15
9	53.86±0.341	56.40	4.50
10	55.57±0.191	56.00	0.77
11	50.46±0.129	53.40	5.50
12	51.47±0.066	53.70	4.15
13	53.06±0.243	55.50	4.40
14	51.91±0.066	53.00	2.05
15	50.39±0.191	52.00	3.10

d. Egg positioned held inclined from its previous position

For this Position 4, the egg was held at 10-15° inclination point from its original position (Position 3). As shown in Table 5 that the percent error of the egg samples ranged from 0.01% - 6.14%, with an average percent error of 3.18%. It had a percentage error still closer to zero which means that this position could still estimate the egg's weight.

Table 5. Egg Weight Estimation Value and its Percent Error (Position 4)

Egg Sample	Weight Estimation (g)		
	Computerized Method	Manual Method	% Error
1	73.32±0.167	74.20	1.19
2	79.99±0.487	80.00	0.01
3	62.30±0.239	64.40	3.26
4	49.75±0.225	52.20	4.69
5	59.50±0.430	62.40	4.65
6	62.03±0.184	64.40	3.69
7	57.07±0.081	60.80	6.14
8	63.54±0.302	65.00	2.24
9	54.24±0.189	56.40	3.84
10	55.85±0.179	56.00	0.26
11	51.47±1.279	53.40	3.61
12	51.75±0.098	53.70	3.63
13	52.86±0.058	55.50	4.76
14	51.90±0.066	53.00	2.08
15	50.12±0.006	52.00	3.62

The estimated egg weight could still be determined mathematically using the obtained measurement value of the egg's minor and major axis length from the image. The results on the previous tables (Tables 2-5) show that the average percentage error ranged only from 3.05% to 3.69%. Comparing the manual measurement and the computerized egg weight estimation system pointed to 3.69% error or 96.31% accuracy. Therefore, this system is capable of estimating the egg's weight through getting the exact egg's dimensions from the captured image by an inexpensive webcam with the aid of a program.

Egg Weight Estimation System over Manual Processing Cost Effectiveness

Small to medium-scale or even large-scale poultry farms determine an egg's weight using either an egg weighing scale or a balance weighing scale. With this presented system, there is no need to acquire an expensive high-resolution camera and a dedicated egg weighing scale at the same time. This system uses an inexpensive webcam in estimating egg's weight. This helped minimized expenses regarding acquiring additional devices and materials.

The more laborers an industry has, the bigger the money to spend for the laborers' wages and additional benefits. With this system embedded in an egg-sorting machine or conveyor, poultry farm owners do not need to hire redundant laborers just to inspect and weigh the eggs individually. For example, instead of hiring two laborers (assigned for inspection and weighing), farm owners may hire only one laborer to do both task's just to supervise and oversee the system. It is already the task of the system to determine the egg's weight for further classification.

As regards to productivity, it is expected that this device could be more productive than manual laborers who operate only during working hours. This system could operate 24/7 if it is integrated with a conveyor within a grading machine.

Conclusion

This automated chicken egg estimation system was tested using 15 fresh chicken egg samples in different sizes. The result showed that the manual measurement and the computerized egg weight estimation system had up to 3.69% error or 96.31% accuracy.

Results in Table 2 to 5 show that image measurement must be carefully measured to obtain the precise measurement value. Using computer vision, the determination of the egg mass is dependent on a measurement taken from the image. Once the computed measurement value is far from the exact value, it affects the determination of the eggs' mass. Indeed, a low-cost webcam could be used as a replacement for weighing regarding determining the egg's mass with the aid of the right image processing and analysis algorithm.

With the 96.31% accuracy, this study could greatly be an alternative way in the chicken egg weight estimation method especially in large-scale poultry farms in the Philippines to minimize human interaction during the process as well as to save time and energy.

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Research Article

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Facilitation of olive harvest by microbial indole acetic acid and an enzyme mixture

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Abstract

In this study, the effect of indole acetic acid (IAA) and/or a commercial enzyme mixture on the olive harvest was analyzed. IAA was produced by *Gibberella fujikuroi*. IAA (1500 and 3000 ppm) and/or the commercial enzyme preparation (1%) was mixed and applied to the olive trees before (3 weeks and 1 week) the harvest. The changes in protein, pectin and cellulose contents of fruits were determined monthly. While the cellulose and protein contents showed a decrease in 2 periods of 3 months (July-September and October-December), pectin contents increased in the same period. IAA and/or enzyme applications did not lead to any significant changes in the cellulose and protein contents of the fruit (p>0,001). However, the pectin amount in the trees where the enzyme application was performed before the harvest, showed a tendency to fall. The most convenient application with regard to the fruit and leaf fall was 3000 ppm IAA+1% enzyme application 3 weeks before harvest. While the fruit fall amount increased more when compared to the control and other groups with this application, the leaf fall decreased significantly.

Keywords: *Gibberella fujikuroi*, Pectinolytic Enzymes, Protein, Pectin

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Introduction

The harvest is one of the most significant processes of the olive cultivation because the choosing of the form and time of the harvest affects the quantity and quality and manufacturing cost of the annual yield, and the yield of the next years.

The olive harvest both constitutes more than 40% of the manufacturing cost (Castillo-Ruiz et al., 2015) and affects particularly the physiology of flowering of a tree along with the yield and quality. The harvest can be done with mechanical and traditional methods. However, in Turkey, the utilization of the harvest machines used in the countries such as Italy and Spain, where the modern cultivation is made, is rather difficult. This is because of the fact that the condition of the some gardens and trees is not suitable for the use of these machines (Guler and Cesur, 2011).

The traditional harvesting methods are hand-picking, whisking, shaking, dropping with a rod. Especially as a result of the harvest done with rods, both the fruit and the annual shoots carrying the buds that will yield next year's products are damaged. Therefore, in the harvest done with the rod, a contamination from the cancerous areas to the healthy ones occurs with the branch and offshoot breaking. This situation both reduces the quality of the fruit and increases the severity of the periodicity (Bulbul, 2008). From the point of the yield quality, the best form of the harvest is hand-picking (Bulbul, 2008; Morales-Sillero and Garcia, 2015).

The joint usage of the hormones for the plant development and some enzymes (cellulolytic and pectinolytic) can be an alternative in order to prevent the damage in the harvest, either with the machine or traditional methods, and the loss of leaf.

The hormones can be used for the purpose of thinning the blossoms and fruit, rooting the slips, germination, fruit setting and parthenocarpy, affecting the dormancy mechanism, gender formation, blossoming, increasing the fruit quality, diminishing the pre-harvest falls, slowing down the aging process, preservation, fighting the diseases and weed (Barut, 1995). Auxins rank among the first-discovered plant hormones. Auxin is a general term used for the compounds having the characteristic of initiating the growth of the shoot cells. The most significant representative of this class is indole-3-acetic acid (IAA). The IAA is responsible for the regulation of each stage of the plant development (Palavan-Unsal, 1993). It was determined that not only the plants (Palavan-Unsal, 1993) but also fungi (Cakmakci, 1981; Ergun, 1997; Unyayar, 2000), lichen, algae (Ergun, 1997) and bacteria (Cakmakci, 1981; Martinez-Toledo et al., 1988; Davies, 2010) form IAA.

In this study, in order to create an alternative to the existing harvesting practices, the IAA hormone produced naturally by *Gibberella fujikuroi* was extracted and its facilitative effects on the olive harvest were observed by using it with a commercial enzyme preparation.

Materials and Methods

Materials

In this study, *Gibberella fujikuroi* was used in the production of IAA. This fungus was obtained from Hacettepe University, Faculty of Science, Department of Biology Prof.

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The experiment was carried out in the olive garden belonging to Murat Paşa Foundation in Antalya. Five olive trees of Gemlik cultivar were chosen for the experiment. A commercial enzyme preparation that contains a small amount of cellulase and hemicellulase enzymes as well as pectintranseliminase, polygalacturonase, and pectinesterase enzymes (Pectinex Ultra SP-L, Novo Nordisk Ferment Ltd., Switzerland) was used for facilitation of harvest.

Methods

IAA Production

The enriched synthetic Czapek-Dox broth was used for the IAA production (Mahadevan & Sridhar, 1982). The medium was inoculated (1%) with the suspension prepared from *Giberella fujikuroi* spores. Then the culture was incubated for 8 days at 30 ^{0}C and 150 rpm (Yalcinkaya, 1993). During the incubation, the IAA levels were determined in the samples taken on the 2nd, 5th, and 8th days (Cakmakci, 1981). Concentrated HCl (0.05 mL) was added to the 5 mL sample for the IAA extraction. After the samples had been thoroughly mixed, they were extracted two times with 5 mL ether. Then ether was evaporated under the nitrogen gas. The residue was dissolved in water and the IAA level was spectrophotometrically determined. The IAA extract (3 mL) was added into 1.5 mL 0.05 M $FeCl_3.6H_2O$ + 35% perchloric acid mixture. It was waited for 20 min until the characteristic red color to form. Afterwards, the absorbances were measured at 525 nm on a spectrophotometer (UV-VIS 1601 Shimadzu) against to the blind. IAA amount was calculated by comparison to the standard curve.

The IAA and/or enzyme application to the olive trees

The applications performed with the IAA produced by *Gibberella fujikuroi* and/or commercial enzyme preparation are shown in Table 1.

Table 1. The amount of IAA and/or enzyme

Trees	*3 weeks before the harvest*	*1 week before the harvest*
1	1500 ppm IAA	1500 ppm IAA
2	1500 ppm IAA + 1% enzyme	1500 ppm IAA + 1% enzyme
3	3000 ppm IAA	3000 ppm IAA
4	3000 ppm IAA + 1% enzyme	3000 ppm IAA + 1% enzyme
5	Control	Control

IAA and enzyme mixture were applied to the trees by a back pulverizer. The experiment was properly conducted according to the randomized parcel design. The picking of the olive samples began in July, at the fruit set stage, and it was sustained at intervals of one month until the harvest stage. The sample picking process ended on 30th December. The olive samples were maintained at -20ºC in 250-gram portions until they were analyzed.

The analyses of protein, pectin, and cellulose contents

Olive seeds were removed and the flesh was homogenized before the analysis. The protein, pectin, and raw cellulose amounts were determined in the samples. The protein content was determined by the Kjeldahl method (AOAC, 1990). The crude cellulose analysis was made according to ASTM (1980) with slight modifications and the pectin ratio according to Cemeroglu (1976).

Statistical Analyses

The analysis results of the olive samples were subjected to the variance analysis in accordance with the randomized parcel design. The SAS package program was used in the statistical evaluations and the results were analyzed by using the univariate analysis (ANOVA) test (SAS Institute Inc., 2011).

Results and Discussion

Gibberella fujikuroi produced the maximum IAA with 5-day incubation. It was observed that the amount of IAA increased gradually within the period of 5-day incubation and on the 5th day, the maximum production was reached. Following the 5th day, the production of IAA declined rapidly and was determined to be close to the level of the initial values. The production of IAA reached 38850 ppm (unpublished data) in consequence of triplicate performed. In the other studies, it was observed that the production rates of various fungi were lower and their incubation periods were longer (Palavan-Unsal, 1993; Yalcinkaya, 1993; Khan et al., 2012; Jaroszuk-Ściseł et al., 2014). The increasing of the production level and the shortness of the incubation period are significant in terms of reducing the production cost.

Cellulose, protein, and pectin contents of the olive fruit samples

The cellulose, pectin, and protein amount of the olive within the period from the fruit set to the harvest (July-December) is shown in Table 2.

It is possible to analyze the changes which occurred during the olive ripening in two periods. A rapid change occurred in the cellulose amount in the July-September period. Fewer changes were observed in the October-December period. In various studies, in which the changes in the polysaccharide compounds on the cell wall were analyzed, it was notified that the cellulose amount of the cell wall of the olive fruit decreases at the unripe, green, turning to green and turning to purple stages (Vierhuis et al., 2000; Mafra et al., 2001).

The differences in the protein amount in the ripening period compared to the beginning were also considered to be statistically significant ($p<0.001$). It was determined that the changes in the protein contents, as the ones in the cellulose amount, could also be analyzed in 2 periods. While the decline in the protein amount in the July-September period was considered to be statistically insignificant ($p<0.001$), the differences between this period and the October-December period were considered to be significant ($p<0.001$). Especially, the protein contents of the samples of October showed a certain decline compared to the samples of September. Similar findings were encountered in the studies where the domestic and foreign olive cultivars were analyzed. Lazovic & Miranovic (1999) examined the protein content and amino acid composition of the olive fruit and determined that the protein content in the fruit was 1.50%-2.61%. They used Picholine, Itriana, and Zutica cultivars and notified that the average protein content in the September-November period declined at the rates of 12% in Picholine type, 23% in Itriana and 38% in Zutica. The highest protein content was detected in the shoots, then in the leaf and the lowest in the fruit. In the study conducted by Ozay & Borcaklı (1995), it was found that the protein content of Gemlik olive changed between 1.76-1.95 g/100 g after it had been processed as the table olive. The pectin amount showed a regular increase. This result resembles the findings of Vierhuis et al. (2000) which notify that the pectin amount

Table 2. Changes in the cellulose, pectin, and protein amount in the ripening period

	Months					
	July	August	September	October	November	December
Cellulose (%)	7.34±0.125a*	6.92±0.300b	6.18±0.076c	5.98±0.031cd	5.74±0.014d	5.66±0.029d
Protein (%)	1.97±0.113a	1.88±0.118a	1.82±0.091a	1.43±0.046b	1.37±0.016b	1.30±0.016b
Pectin (%)	1.70±0.043c	1.79±0.051cb	1.89±0.043b	2.13±0.078a	2.29±0.094a	2.26±0,024a

*Means in the same row with different superscript letters are significantly different (P<0.001).

As a result of the studies conducted to determine the effects of the IAA and enzyme applications on the cellulose, protein, and pectin contents, it was concluded that the applications performed 3 weeks and 1 week before the harvest did not cause any changes in the cellulose and protein amount (p>0.001). However, the pectin amount showed the tendency to decline in the trees on which the enzyme applications are performed (p<0.001). However it was determined that the IAA application did not significantly affect the pectin content (p>0.001).

Harvest Results

The trees, on which the enzyme and/or hormone application were made 1 or 3 weeks before the harvest, were harvested on the same day. Harvesting was performed with a rod. The main branches of the trees, on which the application was performed, were hit once in a way that their external skin was not damaged and the olives that fell onto the tarp that was stretched under the tree were picked. In the control trees, this practice, because of not being effective in the fruit fall, was realized with the traditional method by hitting the areas where the fruit was present with the stick. The amount of the leaf and fruit that fell due to natural causes and practices are shown in Table 3.

Table 3. The amount of fruit and leaf that fell with the effect of the application

Trees	Fruit and leaf	1st Application (4th December)			2nd Application (18th December)		
		Before Harvest	Harvest	Total	Before Harvest	Harvest	Total
1*	Fruit (g)	1980	750	2730	1000	400	1400
	Leaf (pcs)	1966	99	2065	59	103	162
2	Fruit (g)	2211	1100	3311	1000	1250	2250
	Leaf (pcs)	650	203	853	76	160	236
3	Fruit (g)	2040	900	2940	750	570	1320
	Leaf (pcs)	1382	113	1495	48	79	127
4	Fruit (g)	4550	600	5150	850	600	1450
	Leaf (pcs)	741	153	894	232	253	485
5	Fruit (g)	850	3500	4350	200	4350	4550
	Leaf (pcs)	908	1250	2158	524	1250	1774

*1; 1500 ppm IAA, 2; 1500 ppm+%1 enzyme, 3; 3000 ppm IAA, 4; 3000 ppm+%1 enzyme, 5; Control

As can be seen in Table 3, as a result of hormone and/or enzyme, a significant amount of the fruit fell before the harvest procedure. The amounts that fell before the harvest were rather close to each other in the groups on which 1500 ppm IAA and/or enzyme was applied 3 weeks before the harvest. While the fruit amount that falls due to natural causes before the application shows close values with each other, the differences occurring after the application can be clearly observed. The amount of the fruit that fell from the trees, on which 1500 ppm IAA and/or enzyme was applied, during the harvest showed rather close values with each other. In consequence of the comparison of the same experimental groups in terms of the application periods, it was determined that the applications performed 3 weeks before the harvest were more efficient.

In the groups in which the IAA concentration was increased to 3000 ppm, it was observed that a significant amount of the fruit fell before the harvest. It was observed that the use of the enzyme in addition to 3000 ppm IAA expedites the fruit fall. Since a significant amount of the fruit fell before the harvest, the fruit amount obtained after the harvest was less that the one before the harvest. Upon considering the total amount of the fruit, it can be said that 3000 ppm IAA+1% enzyme with 5150 g yield provided the best result. However, the similar effect could not be provided with the applications performed 1 week before the harvest. Therefore, in order for the harvest to be expedited, 3000 ppm IAA+1% enzyme application, 3 weeks before the harvest can be recommended. With the studies conducted before, it was aimed to expedite the harvest by using various chemicals. In the study conducted by Cavusoglu (1973), the Ethrel® (2-chloroethylphosphonic acid) was used in various concentrations in order to expedite the harvest. The Ethrel® caused the offshoot break and the high level of leaf loss. In another study, Ethrel® was considered to be ineffective in pre-harvest fall and insufficient in fruit fall during the harvest (Cavusoglu, 1977). The chemical applications could not find a common usage area because they leave residues as well as cause leaf loss.

According to the results, it was determined that an effective harvest performed with the rod, a total of 4550 g fruit was obtained and 1250 leaves fell during the harvest as a result of hitting the tree multiple times with the rod. Along with 1432 leaves that fall due to natural causes before the harvest, the total loss is 2652 leaves. However, with the effect of 3000 ppm IAA+1% enzyme application, a total of 894 leaves fell.

The most leaf fall was caused by 1500 ppm IAA application performed 3 weeks before the harvest. Although the data obtained in 1500 ppm IAA application did not show any significant differences compared to the control, upon taking all the applications into account, the decline of the leaf fall compared to the control, along with expediting the fruit harvest, is significant for the efficiency of the application. From the data obtained, it can be said that while the IAA+enzyme application creates a synergistic action in fruit fall, it can offer an advantage in terms of declining the leaf fall. The harvest method practiced in the control group causes both the aggravation of the periodicity and diseases, as well as the high level of leaf loss, because of the damages it creates in the branches and the cells. However, upon considering the leaf loss occurring during the harvest, it was observed that the most leaf fall occurs in the tree on which 3000 ppm IAA+1% enzyme was applied 1 week before the harvest and on the other hand, the leaf loss was more in the control tree. Percentage ratios of the leaf loss caused by the applications were calculated by accepting the leaf number of the branches on which the application was made to be 5000 pieces (Table 4).

The leaf loss at the rate of 10-57.6% was determined with the effect of the application performed 3 weeks before the harvest. In the control group, the loss was determined as 57.9%. The loss in the applications performed 1 week before the harvest was much less compared to the control tree (96.15%). The total loss caused by 3000 ppm IAA+1% enzyme application 3 weeks before the harvest, recommended in this study, was limited to 2%.

The different concentrations (2000, 4000 and 8000 ppm) of the Ethrel® were applied to the Manzanialla olive 1 month before the harvest. These trees were harvested with a mechanical shaker. One of the 2 control trees was harvested by hand and the other was harvested with a pneumatic shaker. The amount of the fruit that fell as a result of the harvest was found to be 68.7% at 2000 ppm; 95.1% at 4000 ppm; 99.1% at 8000 ppm, and 100% in the control. In consequence of this application, it was determined that the Ethrel® causes a lot of leaf fall. The leaf fall at the rate of 0.2% at 2000 ppm; 18% at 4000 ppm; 22% at 8000 ppm, and 32% in the control was notified. The highest pre-harvest fall was determined to be 59.93% at 8000 ppm. This rate is 7.23% in the control tree (Ozguven et al., 1998). There are some resemblances between the two studies in terms of the leaf fall in the control. However, the loss caused by the IAA and enzyme applications is much less. In another study, it is notified that usage of glycerol and NaH_2PO_4 increased the leaf loss from 9% to 18% in addition to increasing the fruit fall from 50% to 80% (Martin, 1994).

The pectolytic enzyme preparation used in this study will affect the quality and amount of the oil in addition to the positive effects of the fruit and leaf fall because the technical enzyme preparations are used for this purpose in the olive oil production. More products of much higher quality are obtained by means of changing the structure of pectic polysaccharides on the cell wall of the olive fruit with the pectolytic enzyme. It was determined that with the effect of the enzyme, the methyl esterification decreased, the molecular weight profile changed, the galactan chains that are bound with the (1-4) bond broke (Vierhuis et al., 2003). The pectolytic and cellulolytic enzymes are the enzymes bound to the cell wall of the olive fruit and of which amount increases with the ethylene production during the ripening. This enzyme group, formed with seven glycosidases and Cx-cellulose, tears down the cell wall and ensures that the fruit softens and ripens (Fernandez-Bolanos et al., 1995). Depending on the increase of the ethylene production, the enzyme synthesis or activation in especially the black olives is promoted (Fernández-Bolaños et al., 1997; Morales-Sillero and García, 2015). Therefore, the studies aimed at using the chemicals promoting the ethylene production were conducted in order to expedite the harvest (Cavusoglu, 1973; 1977; Ben and Wodner, 1993; Martin, 1994; Ozguven et al., 1998). It will be possible to benefit mutually from the pre-harvest application with the determination of the effects of the enzyme applications, performed in order to expedite the harvest, on the yield and quality of the olive oil. Thereby, an advantage in terms of the cost can also be created.

Conclusion

The olive harvest is an efficient and important factor on the quality and amount of the yield. The procedures carried out during the harvest are significant in developing these features. The findings of this study, conducted in order to diminish the yield loss in Turkey, which is an important olive

Table 4. Amount of the leaves that fell as a result of the applications

Trees	Applications*	Leaves that fell before the harvest (%)	Leaves that fell by the wind, etc. before the harvest (%)	Leaves that fell before the harvest with the effect of the application (%)	Leaves that fell during the harvest (%)	Leaves that fell as a result of the application (%)
1	1	56.78	51.56	5.22	43.21	48.43
	2	32.20	21.18	11.01	67.79	78.81
2	1	95.20	43.97	51.23	6.39	57.62
	2	36.41	30.86	5.55	0	5.55
3	1	91.00	89.90	1.68	8.41	10.09
	2	73.31	16.07	57.23	26.68	83.92
4	1	93.00	90.80	2.20	7.80	10.00
	2	47.83	10.30	37.52	52.16	89.69
5	1	42.07	42.07	-	57.92	57.92
	2	3.84	3.84	-	96.15	96.15

*1; 3 weeks before harvest, 2; 1 week before harvest

producer, and meet the product quality that the world markets demand, have shown that the IAA and enzyme applications can give the successful outcome. It is thought that the hormones and enzymes used in the experiment do not pose any danger to the health as they are already present naturally in the plant tissues. With the studies to be conducted from now on, the effect of the IAA and enzyme application on the periodicity, amount, and quality of the olive oil will be determined.

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Research Article

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Effects of deficit irrigation on the potato tuber development and quality

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Abstract

This study was conducted to determine the effects of deficit irrigation on tuber growth and quality of potato (*Solanum tuberosum* L.). Certified seeds of potato variety "*Agria*" were used as study material. Irrigation treatments was consisted of one irrigation interval (5 days) and five different levels (I_{100}, I_{85}, I_{70}, I_{55}, I_{40}) of soil water deficit measured before irrigations. First irrigation was applied by drip irrigation up to field capacity the soil water content in 0-60 cm depth in all treatments. Subsequent irrigations were applied according to the treatments.

The irrigation water and evapotranspiration (*ET*) values of treatments ranged from 243.0 to 311.9.4 mm and from 337.1 to 385.9 mm in the first year, respectively, and from 166.7 to 223.2 mm and from 204.0 to 255.7 mm in the second year, respectively. Yields varied from 30.85 to 47.13 t/ha in the first year and from 28.77 to 44.45 t/ha in the second year. The yields were decreased based on water deficit levels. The highest yields were obtained from I_{100}treatment.

The results have indicated that water restriction had a significant effect on yield, single tuber weight, percentage of marketable tuber, plant length, mean tuber length, mean tuber diameter and percentage of tuber peeling. The results were showing that the I_{100} treatment in especially was of the most importance for the highest percentage marketable tuber and tuber yield obtained per unit water applied. Therefore, the I_{100} treatment can be recommended for potato cultivation under similar climatic and soil conditions.

Keywords: Potato, drip irrigation, deficit irrigation

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Introduction

Potato cultivated in many countries of the world ranks as the fourth-most-important food crop, after wheat, corn and rice in terms of the amount produced. It is an essential food in the human diet with regard to carbohydrates, proteins, minerals and vitamins within which it includes. As it is usually consumed as fresh by boiling or frying, it is marketed after processing in various forms like canned food, frozen finger potato, chips, mashed, granules and powder in the industry in developed countries. It is also utilized in the production of livestock feed, starch, flour and alcohol as a byproduct (Onaran et al., 2000). Potato grown for early or off-season production plays a crucial role in the economy of several areas in the Mediterranean countries (Cantore et al., 2014).

The average potato production in the world a yearly 323 million tonnes and its production area is 20 million hectares and average yield per hectare is 17 tonnes. The five largest potato producers are China (33.9%, 87.3 million tons), India (11.4%, 41.5 million tons), the Russian Federation (8.1%, 29.5 million tons), Ukraine (6.4%, 23.3 million tons), and finally the United States (5.7%, 21.0 million tons) (FAOSTAT, 2014). Turkey ranks 13th with 4.8 million tons in potato production (Anon., 2014a).

Potato grows more densely in especially Afyon, Niğde and Nevşehir (Turkey) regions where temperate climate is dominant. These regions where potato production generally makes during the summer could be among the world's most fertile potato areas with their suitability of the soil structure (Vural et al., 2000).

The essential objective of irrigation is not only to enhance productivity in agricultural production, but also to boost the production, thereby maximizing net income by exploiting the water in an efficient manner in the long-term and without having negative effects on the environment and thus water resources. It is essential to convey and distribute the water in a way to minimize loss of water from its source and to control its amount in the soil regularly (Korukcu et al., 2007). Unconscious and inefficient use of water in the agriculture will lead to waste of scarce water resources and polluted the groundwater over time, thereby become drought in agricultural lands.

Potato is a crop highly sensitive to soil water deficits. To optimize yields, the total available soil water should not be depleted by more than 30 to 50%, and the soil should be maintained at a relatively high moisture content. Irrigation at 40% of field capacity (*Fc*) is adequate for seed grade tubers, while "processing/table" crops benefit from irrigation at 65% *Fc* (Doorenbos and Kassam, 1979; Van Loon, 1981).

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Potato plants are more productive and produce higher quality tubers when watered precisely using soil water tension than if they are under- or over irrigated (Ati et al., 2012).

The study was conducted to determine the effects of water deficits on the tuber growth and quality and the water consumption of potato grown under field conditions in Afyon where it is the largest potato cultivation area of Turkey.

Materials and Methods

Research area and climate

This study was carried out in a farmer's field in 246,5 m^2 situated in the Ihsaniye district of Afyonkarahisar Province in the Aegean region of Turkey. The experimental area is located between +39° 7'14.01" N latitude and +30° 23' 15.54" E longitude. The Ihsaniye has a characteristic of a plateau by all appearances (Anon., 2011).

Afyonkarahisar prevails a steppe climate with cold snowy winters and with hot and dry summers and with rain in the springs and autumns. The hottest and the coldest monthly average temperature was 22.3°C and 0.3°C, respectively. The total average rainfall was 416 mm (Anon., 2011). The average rainfall and water resources are limited in the potato growing season between April and August months.

Soil structure

Soil texture of experiment area is sandy-loamy (SL). The soil bulk density in depth of 0-60 cm varies between 1.08 and 1.28 gr/cm^3, water content at field capacity ranges from 20.49 to 17.68% by weight and wilting point varies between 11.36 and 9.87%. The available water holding capacity of soil is 59.57 mm 60 cm^{-1}.

Sowing and fertilizing

The experiment was carried out as a randomized complete block design with three replications. The experiment consisted of 15 plots. The potatoes were planted at a depth of 15 cm through sowing machine 39 potato seeds (13 seeds per row) in 3 rows with 5 m long, 35 cm intra row and 70 cm between rows in each experimental plot. *Agria* potato variety was used in the treatment. Per hectare were applied 150 kg of DAP (*Diammonium Phosphate)* fertilizer and 200 kg of AS (*Ammonium Sulfate*) fertilizer, considering soil analysis laboratory report. In both years, DAP fertilizer was applied before planting, and one half of AS fertilizer and its other half were implemented during first hoeing and first irrigation, respectively.

Irrigation water

Irrigation water was supplied from the main pipe near to experimental field. The analysis results indicated that water used in the experiment is in C_2S_1 class (sodium risk is low; EC is medium) and it can be used for irrigation (USSL, 1954).

Plots were irrigated by drip irrigation system, that it was composed of main pipeline, side pipelines (manifold) and laterals. Lateral pipes, which had inline drippers at 33 cm intervals, were 16 mm in diameter. Discharge of dripper was 4 L h^{-1} to 2 kPa pressure. The amount of irrigation water was checked by a volumetric meter.

The treatments were formed with five different levels of soil water deficit before irrigation, as explained below.

1- I_{100}: Irrigation up to field capacity of available soil water deficit before irrigation (K_1: 1.00)

2- I_{85}: Irrigation up to 85% of available soil water deficit before irrigation (K_2: 0.85)

3- I_{70}: Irrigation up to 70% of available soil water deficit before irrigation (K_3: 0.70)

4- I_{55}: Irrigation up to 55% of available soil water deficit before irrigation (K_4: 0.55)

5- I_{40}: Irrigation up to 40% of available soil water deficit before irrigation (K_5: 0.40)

Before the regular irrigations, all the treatments were irrigated until field capacity. Then, the subsequent irrigations were applied according to the prescribed program with 5-day intervals. The amount of irrigation water applied in the treatments was determined using Equation 1 and 2 (Ertek and Kara, 2013).

$$Ir = Wsd \; x \; Kn \; x \; A \qquad (1)$$

$$Wsd = (Fc - WA) \qquad (2)$$

Where, *Ir*– the irrigation water (liter); *Wsd*– soil water deficit before irrigation (mm); *Kn*– the deficit rate of water applied to treatments; *Fc*– field capacity (mm); *WA*– available soil water before irrigation (mm) and *A*– *p*lot area (m^2)

Water consumption by plants in all treatments was calculated using Equation 3 on the basis of the water budget. The soil water content was measured by gravimetric method during sowing, before each irrigation and in the last harvest (Allen et al., 1998).

$$ET = Ir + P + Cr - DP - RO \pm DSW \qquad (3)$$

Where *ET*– plant water consumption (mm), *Ir*–irrigation water (mm), *P*– the precipitation (mm), *Cr*– the capillary rise (mm), *Dp*– the deep percolation losses (mm), *RO*– the runoff losses (mm), and *DSW*– the moisture stored in the soil profile (mm).

DP and *RO* values were neglected because irrigation was applied to field capacity by drip system. The groundwater problem in the experimental area was not available. Therefore, *Cr* was ignored (Kanber et al., 1993; Ertek et al., 2006). The effective rooting depth of the potato plant is about 60 cm and approximately 85% of the root length is concentrated in the upper 0.3-0.4 m of the soil (Efetha, 2011; Cantore et al., 2004). For this reason, the water consumptions (*ET*) were calculated for the soil layers at the depths of 0-30 cm and 30-60 cm.

Regression analysis was performed to determine the relationship between the yield obtained from treatments and irrigation water and plant water consumption. Furthermore, the water use-yield relationship was determined using the Stewart Model (Doorenbos and Kassam, 1979) (Eq. 4).

$$(1 - \frac{Y_a}{Y_m}) = Ky(1 - \frac{ET_a}{ET_m}) \qquad (4)$$

Where Y_a– the real yield (kg ha^{-1}), Y_m– the maximum yield (kg ha^{-1}), ET_m– the maximum plant water consumption (mm), *Ky*– the yield-response factor for ET_a.

Water use efficiency, also expressed as rate of water use and used in comparing the irrigation methods or in evaluating the irrigation programs, was determined using the following Eq. 5 and 6 that were given by Tanner and Sinclair (1983) (Ertek et al. 2006).

$$WUE = \frac{Ey}{ET} \qquad (5)$$

$$IWUE = \frac{Ey}{I} \qquad (6)$$

Where *IWUE*: the irrigation water use efficiency (t ha^{-1} mm^{-1}), *WUE*: the water use efficiency (t $ha^{-1}mm^{-1}$), and *Ey*: the economical root yield (t ha^{-1}).

Plant observations and measurements

The edge rows of plots were not harvested to avoid the edge effect. The harvest was done in the middle row. Plant observations and measurements were performed on labelled plants in the middle row.

Statistical Analysis

The data obtained from the study were analyzed using Minitab®16.2.4.packaged software.

Results and Discussion

Irrigation water and plant water consumption

First irrigation was applied up to field capacity the soil water content in 0-60 cm depth in all treatments. Then, the regular irrigation was started to on June 26, 2013 and July 11, 2014. The irrigations were ended on August 11, 2013 and August 25, 2014. All the plots were irrigated for 10 times at intervals of 5 days throughout the irrigation periods in both years.

The lowest and the highest values of irrigation water in 2013 and 2014 years were observed in I_{40} (243.0 mm, 166.7 mm) and I_{100}(311.9 mm, 223.2 mm) treatments, respectively. In both years, the highest water consumption was calculated as 385.9 mm and 255.7 mm in the I_{100} treatment, respectively, and the lowest values were determined as 337.1 mm and 204.0 mm in the I_{40} treatment, respectively. The *ET* values increased with increasing irrigation levels.

When analyzed the figures after and before irrigation, it can be seen that the soil water contents before irrigation didn't drop below the wilting point (WP) in all treatments during both years of the experiment (Figures 1 and 2). Furthermore, the soil water contents after irrigation was field capacity in I_{100} treatment.

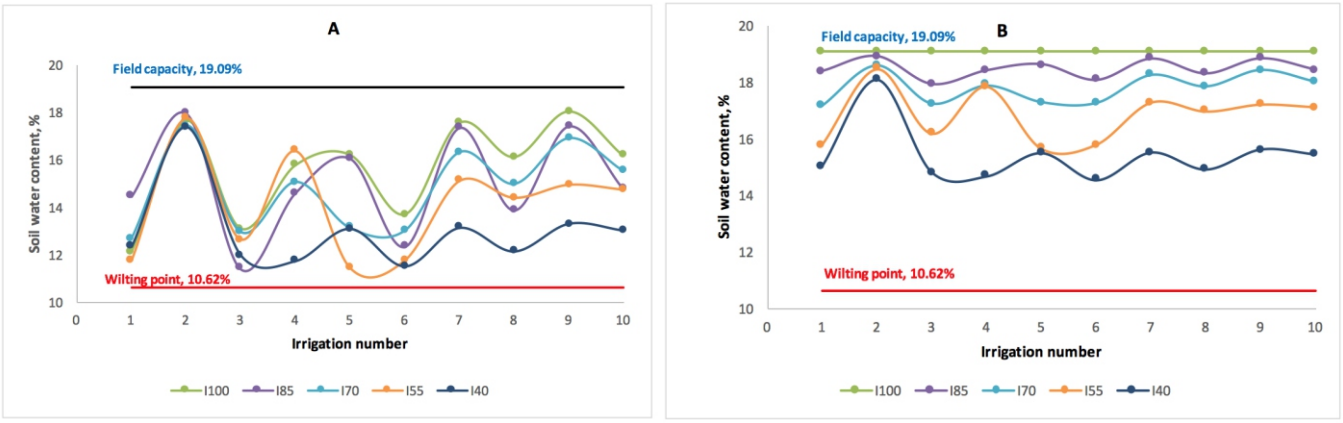

Figure 1. The soil water contents before (**A**) and after (**B**) irrigations during the growth season (2013)

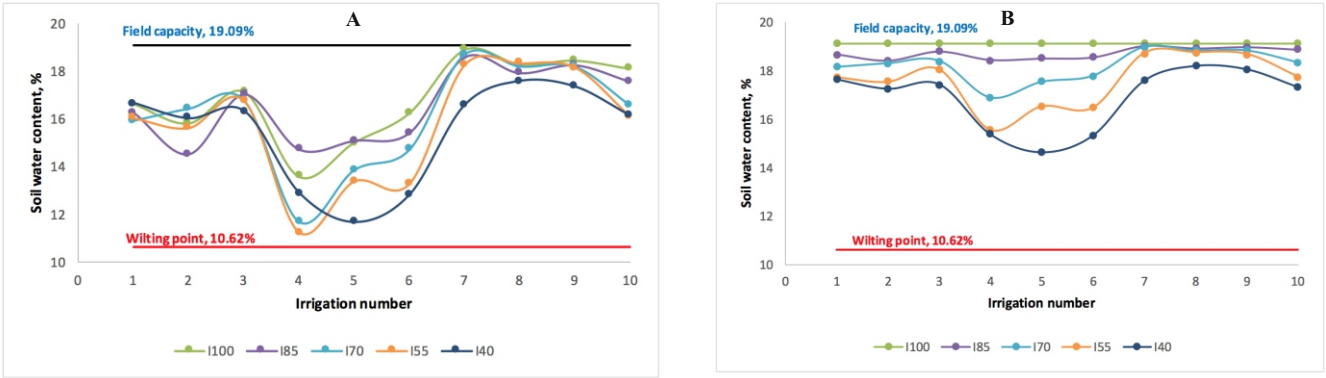

Figure 2. The soil water contents before (**A**) and (**B**) after irrigations during the growth season (2014)

In 2013, the highest and the lowest values of soil water content before irrigation reached at the 9th irrigation of I_{100} treatment by 18.05% and the 5th irrigation of I_{55} treatment by 11.45%, respectively. In 2014, the highest and the lowest values of soil water content before irrigation reached at the 7th irrigation of I_{100} treatment by 18.89% and the 4th irrigation of I_{55} treatment by 11.23%, respectively.

Some differences between years have been observed when analyzed the figures related to soil water contents before irrigations in the years 2013 and 2014. The soil water content in the second year of the study was higher than first year, as a result of local rainfalls, especially during August. Moreover, in both years of the trial, the soil water content before irrigation in the periods between 3rd and 7th irrigations were higher than the other irrigation periods. The reason is that this interval was corresponded to a period at which maximum plant growth and development occurred.

Similarly, the amount of irrigation water required for potato in the Baghdad–Iraq was determined as 300–338 mm by Ati et al. (2012). When compared with the research results of Ayas and Korukcu (2010), it is clear that there was a similarity with the values obtained in the first year of our study, and the values in the second year were lower. The differences can be attributed to the variations that have occurred in climatic conditions during the irrigation period.

Yield components

In both years of study, the yield, tuber starch ratio, tuber dry-matter content, marketable tuber ratio, tuber length, tuber diameter, tuber weight and number of tubers per crop were found to be the highest at I_{100} treatment.

The yield, marketable tuber ratio, tuber length, tuber diameter, tuber weight and number of tubers per crop were seen to be the lowest at I_{40} treatment (Table 1). The quality and yield parameters in both years were considerable similar to each other.

According to Tukey test results by means of two years, number of tubers per plant were statistically significant at the 1% level and divided into three different groups. Results obtained from this study were significantly similar to previous studies (Yilmaz et al., 1996; Tugay et al., 1997, Yilmaz and Tugay, 1999; Kiziloglu et al., 2006; Aksic, 2014).

It has been found that there was a statistically at 1% significance level between treatments in terms of tuber yield per hectare, tuber weight, tuber length, tuber diameter, marketable tuber ratio, tuber peeling ratio, tuber dry-matter content, tuber starch ratio, while was not statistically significant difference between 2013 and 2014 years. Moreover, the significant effect of irrigation regime on tuber yield was mainly due to the average tuber weight, tuber diameter and tuber length, because the differences in the number of tubers per plant were not significant.

Furthermore, graphical analysis of the relationship between tuber yield and water consumption of treatments has shown to be a statistically significant correlation in both years ($P<0.01$) (R^2: 0.98, 2013 and 0.94, 2014). The tuber yields per hectare from this study were higher than that from Yilmaz et al. (1996) and Tugay et al. (1997). It can be said that this might result from potato variety used and variation in the amount of precipitation between years. In addition, it is seen that tuber weights were higher than those from Yilmaz (1995), Yilmaz et al. (1996) and Yilmaz (1999), and similar to the values from Yilmaz et al. (2003) and Didin (1999).

In a study conducted by Kashyap and Panda (2003) in order to investigate the water-yield relationships in potato, it irrigates when water available in the soil consumes at the rate of 10, 30, 45, 60 and 75%. The study results showed that when falling at 60% and 75% levels of available water in soil, irrigation has caused a significant decline in the tuber yield. Furthermore, in a study conducted by Fakhari et al. (2013) were determined to be the higher potato tuber yields at higher irrigation levels. Mokh et al. (2015) stated that full irrigation regime resulted in the highest tuber yield under all nitrogen levels and there were significant reductions in total yield when applying smaller amounts of irrigation water.

It has been shown that the largest average tuber diameter from this study was greater than those from Ubeyitoğulları (2005) and smaller than those Didin (1999) and Ayas (2007). This can be due to differences between plant varieties used and annual rainfall. The tuber lengths from this study were similar to those from Didin (1999), Ubeyitoğulları (2005) and Ayas (2007).

The percentage of marketable tubers were indicated to be similar to those of a study carried out by Ayas (2007). However, the percentage of marketable tubers were higher when compared with the researches by Yilmaz et al. (1996). This might be attributable to the potato variety used and differences in amount of precipitation.

The tuber peeling ratios in the study were similar to research of Ubeyitoğulları (2005) and Didin (1999), and the average tuber dry-matter content was similar to Ubeyitoğulları (2005), Morales et al. (1992) and Didin (1999) and, however, higher than those worked out by Ertan (1980), Harada et al. (1985), Kara (1995), Yilmaz and Tugay (1999). This can be attributed to the potato varieties and to effective rainfall, especially during tuber development period.

It is clear that the average tuber starch ratio was similar to reported by Didin (1999), but higher than those reported by Ubeyitoğulları (2005), Ertan (1980), Harada et al. (1985) and Kara (1995). In a similar manner, this could also attribute to the potato variety cultivated and to effective rainfall, especially during tuber development period. The previous studies pointed out that the potato varieties and amount of rainfall were effectively on quality and yield of potato. Furthermore, Lynch and Tai (1989) stated that the differential tolerance to moisture stress among potato genotypes may be associated with differences in sensitivity during the ontogeny of yield development.

Generally, the *ky* values in the higher water applied treatments are higher than others. This situation shows that the unit water deficit in the higher water applied treatments will be caused higher yield decrease than other treatments. Yield response factors (*ky*) of treatments were 2.49 in the first year, 2.08 in the second year and 2.43 when both years were evaluated together. This indicates that yield might decrease 2.49 per one unit of water deficiency in the first year, 2.08 in the second year and 2.43 when both years were taking into consideration together (Figure 3).

Table 1. Variance analysis results related to some yield and quality components in the years 2013 and 2014

Treatments	Number of tuber per plant	Tuber weight (g)	Tuber diameter (mm)	Tuber length (mm)	Marketable tuber ratio (%)	Tuber peel ratio (%)	Tuber dry-matter ratio (%)	Tuber starch ratio (%)	Yield (t/ha)
2013									
I_{100}	11.9a	102.0a	64.4a	81.9a	95.0a	4.1bc	22.6ab	20.5a	44.45a
I_{85}	11.0a	94.8b	59.4ab	77.2a	86.7ab	3.6c	22.1ab	17.4a	43.89a
I_{70}	11.2a	87.3c	53.1bc	66.5b	80.0abc	3.6c	22.5ab	16.9a	36.70ab
I_{55}	11.8a	77.9d	53.9bc	63.0b	69.5bc	5.4ab	24.1a	18.2a	32.73b
I_{40}	10.8a	72.1d	45.8c	59.3b	61.7c	6.0a	20.7b	17.8a	28.77b
2014									
I_{100}	12.0a	104.2a	59.7a	72.7a	91.7a	3.6c	25.2b	14.7a	47.13a
I_{85}	11.8a	102.3a	57.2ab	73.8a	88.3ab	4.0bc	27.1a	16.8a	42.71ab
I_{70}	10.8a	95.4ab	56.0ab	71.1ab	83.3ab	4.6bc	28.2a	16.3a	40.02ab
I_{55}	11.0a	76.8ab	52.2bc	67.7ab	74.5bc	5.2ab	26.4ab	15.4a	32.23ab
I_{40}	10.7a	66.9b	49.1c	64.7b	63.3c	6.5a	27.8a	15.0a	30.85b

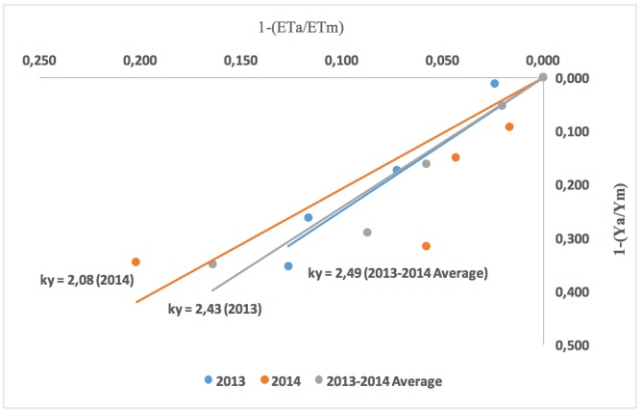

Figure 3. Relationship between deficit in seasonal water consumption and proportional reduction in yield.

Doorenbos and Kassam (1979) reported that effects on different plant species of the equal *ET* deficit during the entire growing season were different, and the seasonal *ET* deficit (*ky*<1) for crops such as peanut, grape, cotton and soybean caused less yield loss than the same *ET* restriction (*ky*>1) in crops like potato and pepper.

In both years, the highest values were obtained for the highest water consumption treatment. This is showing that potato is sensitive to water stress. The fact that yield-response factor (*ky*) values by years are very larger than 1 (one) also corroborates these results.

In both years, the highest and the lowest *IWUE* values were determined in I_{100} and I_{40} treatments, as 0.183 and 0.092 t ha^{-1}mm in first year and 0.283 and 0.138 t ha^{-1}mm in the second year, respectively. In the second years *IWUE* values were the higher than one in the first year (Table 2).

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In the first year, the highest and the lowest *WUE* values were determined in I_{85} and I_{40} treatments as 0.117 t ha^{-1}mm and 0.085 t ha^{-1}mm, respectively. During the second year, the highest and the lowest *WUE* were determined as 0.184 t ha^{-1} mm in I_{100} and 0.151 t ha^{-1}mm in I_{55} treatment, respectively. Due to the reduction in the yield per unit of water, the first year *WUE* and *IWUE* were lower than the value of the second year. The different effect of drought on *WUE* observed in different studies can be attributed to the level of water stress encountered by the crop (Cantore et al., 2014). Similarly, *WUE* values were determined as 0.081-0.098 t ha^{-1}mm for the highest and the lowest evapotranspiration treatments by Aksic et al. (2014). Furthermore, Wang et al. (2006) found that *WUE* was 0.103-0.132 t ha^{-1}mm in the conditions of North China Plain. It was closer to the values in our study.

The water use efficiencies and *ky* values in this study are indicated that the yield and quality of potato will be significantly affected by deficit irrigations. Early studies have shown that water is the most important limiting factor in potato production and it is possible to increase production levels by well-scheduled irrigation programs throughout the growing season (Shock et al., 1998; Shock et al., 2003; Yuan et al., 2003; Onder et al., 2005; Erdem, et al., 2006).

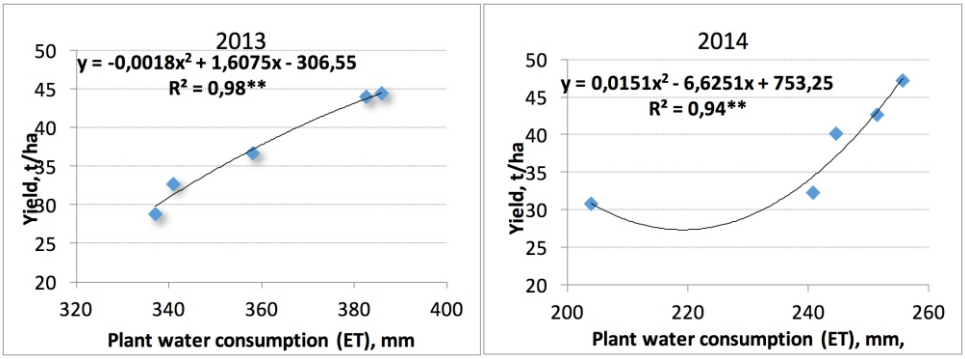

Figure 4. Relationship between water consumption and yield in 2013 and 2014

Table 2. Water use efficiencies

Treatments	2013		2014	
	IWUE (t /ha/ mm)	*WUE* (t/ha/mm)	*IWUE* (t /ha/ mm)	*WUE* (t/ha/mm)
I_{100}	0.183	0.115*	0.283	0.184*
I_{85}	0.167	0.117*	0.220	0.170
I_{70}	0.132	0.103	0.190	0.164
I_{55}	0.107	0.096	0.149	0.134
I_{40}	0.092	0.085	0.138	0.151

Conclusion

Results have indicated that the water stress were statistically significant effected on quality and yield components of potato such as yield per hectare, single tuber weight, percentage of marketable tuber, tuber length, tuber diameter, percentage of tuber peeling.

It can be said that potato yield and quality will occur considerable losses for soil water deficit in 70, 55 and 40%. The I_{85} and I_{100} treatment is more appropriate to prevent the loss of potato yield and quality. The results were showing that the I_{100} treatment in especially was of the most importance for the highest percentage marketable tuber and tuber yield obtained per unit water applied. Therefore, the I_{100} treatment can be recommended for potato cultivation under similar climatic and soil conditions.

As a conclusion, considering to the results of our and previous studies, it is clear that deficit irrigation is not suitable in the potato cultivation, because the profits from the reduced water applications cannot compensate for the income loss from the yield reduced.

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Research Article

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Physicochemical properties and morphological observations of selected local rice varieties in northern Afghanistan

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Abstract

In this study, milled rice (*Oryza sativa* L.) samples of local varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi) were procured from Kunduz province, Afghanistan and check varieties (Koshihikari and Super Basmati) from Japan. We conducted the research in the Laboratory of Crop Science of Ibaraki University, Japan during February in 2018, to clarify the physicochemical and morphological traits on different local rice varieties. The results demonstrated that local rice varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi) from Afghanistan including Super Basmati (Check) with long and slender grains, associated with significantly higher grain amylose and protein contents of 22.9 and 8.1%, respectively, which created in declined taste points. While, Koshihikari with short and medium grain types demonstrated the lowest grain amylose and protein contents of 17.7 and 5.5%, respectively, which amplified grain taste point. The micrographs observations revealed that there were no obvious alterations in the endosperm of translucent grains across varieties. In contrast, the endosperm of chalky grains in local rice varieties were differed compared to check varieties (Super Basmati and Koshihikari), irregularly developed starch granules together with single spherical shape and dent-portion on their surfaces with numerous airgaps were observed due to high temperature. Such irregular arrangement leads in lower grain weight and quality.

Keywords: Rice, Physicochemical properties, Grain morphology, Scanning electron microscope

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Introduction

Rice (*Oryza sativa* L.) is the world's single most significant food crop and the primary source of calories for around 50% of the human population (Wei et al., 2007). Around 90% of rice is produced and being consumed in Asia (Gealy et al., 2003). It is the second vital staple crop after wheat in Afghanistan (Hassanzoy et al., 2017; Sarhadi et al., 2009; FAO, 2017). Kunduz, Baghlan and, Takhar are the top three rice producing provinces in the country, which jointly considered to be the grain basket of Afghanistan, holding strategic significant for food security at the national level (Thomas and Ramzi, 2010). The rice yield has been increased in Afghanistan from 3.22 t/ha in 2008 to 3.50 t/ha in 2010 reported by ICARDA (2011). Unlike the rice cultivation area has been decreasing due to climate change (FAO, 2017). Although grain quality is a composite of physical and chemical specifications needed for a particular exertion by a specific customer class (Zhou et al., 2015). In particular, grain quality is one of the greatest significant traits, as it applies a great influence on the rice price in the market (Hosoya, 2013). Though preferences for some of the quality characteristics differ over countries and regions (Calingacion et al., 2014), the priority for some of the specifications is widely shared.

The chalky rice grain appears when the rice grain has a white part within the rice grain, and it is a consequential quality trait that decides rice price (Hosoya, 2013; Xi et al., 2016). The micrographs observation of chalky endosperm, compound starch granules were irregularly arranged with numerous airspaces, which could have resulted in poor grain weight and quality. While in the translucent endosperm, starch granules (polyhedral shape) were regularly filled (Xi et al. 2016; Shi et al., 2017). There is no information available on the physicochemical and morphological traits of selected local rice varieties in Afghanistan. To attain the enhancement elongate in local rice varieties in Afghanistan, it is decisive to deliver more information. Therefore, the study was conducted to determine some physicochemical properties and as well as morphological analysis on selected local rice varieties by using scanning electron microscope.

Materials and Methods

Milled rice samples

The rice samples of local varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi) in the form of milled rice were procured from Kunduz province and these varieties are grown enormously in the north of Afghanistan, additionally, to the upward-mentioned local rice varieties, Super Basmati (*indica*) and Koshihikari (*japonica*) varieties were procured from the Laboratory of Crop Science, the College of Agriculture, Ibaraki University, Japan.

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Physicochemical properties of milled rice

We measured the grain length and width of 15 milled rice grains to the nearest mm using a mini vernier caliper (Niigata, SK-M100, Japan) and followed the method of IRRI (2002). The milled rice for grain amylose, protein, and the taste point (as a reference) assays were measured by using a taste analyzer (RCTA11A; Satake Co. Ltd., Japan).

Preparation for scanning electron microscopy of cross-sections of milled rice

For the observations of endosperm of chalky and translucent grains (Figure 1), followed the method determined by Zakaria et al. (2002). Briefly, the milled rice grains for each of the six varieties were selected and rapidly were submerged into slush nitrogen (solid and liquid, -210 °C). After vacuum freeze-dried with a freeze vacuum dryer (-60 °C, 10^{-3} Pa, LFD-100NDPS1; Nihon Techno Service). Then grains carefully divided into halves by using a razor blade. The separated halves were attached on specimens, and the specimen's surface was coated with platinum (JUC-5000; JEOL, Japan), and then were observed by using scanning electron microscope (JSM6360A; JEOL, Japan). All the above-mentioned data were analyzed in the Laboratory of Crop Science, the College of Agriculture, Ibaraki University Ami-machi, Japan during February 2018.

Statistical Analysis

Data obtained for the physicochemical properties were analyzed by variance analysis (ANOVA) using Statistical Package for the Social Sciences (SPSS) software 13.0.

Results and Discussion

Physicochemical properties of milled rice

The physical parameters (grain weight, length, width, and type) results were summarized in Table 1. The grain weight ranged from 21.0 to 16.8 mg in all varieties. The maximum grain weight was recorded for Sarda Barah (21.0 mg) followed by Koshihikari, Garma Barah, Surkha Zurahti and Shah Lawangi, respectively, while the significant minimum grain weight was recorded for Super Basmati (16.8 mg) across all varieties (Table 1). There were variations in terms of grain length across all varieties. Thus, significant lower grain length was recorded in Koshihikari (4.8 mm). While the longer grain length was recorded in Super Basmati followed by Sarda Barah and Surkha Zurahti ranged from 7.4, 7.3, and 7.1 mm, respectively. In our previous study, there were differences in grain width and grain length in different varieties (Noori et al., 2017). In addition, grain width in Koshihikari (2.8 mm) significantly higher across all varieties, while lower grain width was recorded for Super Basmati (1.9 mm) followed by Surkha Zurahti, Sarda Barah, Garma Barah, and Shah Lawangi ranged from 1.9, 2.1, 2.1, 2.0, and 2.0 mm, respectively. Besides, grain size and shape are among the first rice quality indicator that breeders consider when progressing new varieties for release and marketable production (Rani et al., 2006). Grains were mainly long and slender, followed by medium-sized and shaped types (Juliano & Villareal, 1993). Furthermore, in the current study, according to the grain size and shape results, Koshihikari was classified as short and medium grain types. Whereas, the remain local rice varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi), including Super Basmati were as long and slender grains, respectively (Table 1). In Afghanistan, generally, consumers prefer rice (*indica*) with long, slender, and translucent grains, which has intermediate or high amylose and protein contents that cook into delicious food (Qabuli palaw).

Although average amylose content differed from 8% to 16% between places and from 5% to 22% among varieties (Jing et al., 2010), variations in amylose content is mostly described by variety (68%) and less by the environment (25%). Tsukaguchi et al. (2016) found high significant differences in grain protein content among different genotypes and the protein content was grew by applying nitrogen as topdressing. In the current study, the grain amylose content, protein content, and the taste point differed among rice varieties. Super Basmati was associated with significantly higher grain amylose (22.9%) and protein (8.0%) contents across all varieties, which created in declined taste point (60) and followed by local rice varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi), from Afghanistan (Table 2). While, Koshihikari demonstrated the lowest grain amylose and protein contents of 17.7 and 5.5%, respectively, which amplified grain taste point (69.3). Juliano & Villareal. (1993) found that, Koshihikari had lower amylose content and gave the softest cooked rice among other varieties.

Scanning electron microscope of cross-sections of milled rice

To observe the contrast between the endosperm of translucent and chalky grains, cross-sections of the central area of the milled rice grains were observed under a scanning electron microscope (Figure 1). The micrographs observation showed that there were no apparent variations in the endosperm structures of all varieties in translucent grains. Besides, regular starch granules with polyhedral shape were build-up without airgaps (Figure 2, T1-T3, Figure 3, T4-T6). In particular, in the translucent endosperms of all varieties, demonstrating that the grain developing process were more advanced compared to chalky grains. This result agrees with the previous findings of Liu et al. (2017) and Shi et al. (2017).

In contrast, there were obvious variations in the endosperm of chalky grains across all varieties. Briefly, irregular starch granules (round shape and small size), were loosely packed. Numerous dent-portions on the surface, airgaps and cell walls were observed among starch granules in the chalky endosperm of Sarda Barah (Figure 2, C1). Multiples single starch granules with round shape and small sizes had poorly developed with numerous dent-portions and airgaps on their surfaces and among starch granules were appeared in the chalky endosperm of Garma Barah (Figure 2, C2). The endosperm of chalky grain, which caused by high temperature during ripening period, the small starch granules with round shape were abnormally filled, and numerous airgaps were also appeared among amyloplasts (Ishimaru et al., 2009; Chen et al., 2016; Liu et al., 2017; Shi et al., 2017). In the chalky endosperm structure of Surkha Zurahti, abnormal starch granules (round shape and small size) were irregularly arranged with numerous airgaps among them (Figure 2, C3). Whereas, the endosperm structure of chalky grain in Shah Lawangi variety was quite differed compared to other varieties (Figure 2, C4). Furthermore, irregularly developed starch granules together with the single round shape and dent-portion on the surface and multiple sizes of starch granules with numerous airgaps were observed. It might be due to high temperature condition at ripening period. In Afghanistan, variation in temperature during the day may range from freezing at dawn to nearly

40 °C at noon (Saidajan, 2012).

Hosoya. (2013) found that, poor starch granules build-up in the endosperm of chalky rice implies that chalky rice grain is influenced by a shortage in assimilation products because of high temperature condition. These authors described that generally, assimilation products produced by photosynthesis in the leaf transfer to the grain via the transport system. Although high temperature rises respiration rate, which afterward declines the amount of assimilation products for individual grain (Vong and Murata, 1977).

Interestingly, the micrographs observation displayed that a few alterations of starch accumulation in the chalky grains were observed in the endosperms of Super Basmati and Koshihikari (Figure 2, C5-C6). Additionally, high density of large amyloplasts with round shape and airgaps among them were packed. Consequently, the micrographs result demonstrated the imperfect development of starch granules in the endosperm of chalky grains, which facilitates in lower grain weight and grain quality. Our results coincided by findings of Shi et al. (2017) and Chen et al. (2016) who displayed that the poor arrangement of starch granules, which influenced by high temperature condition, implies the reduction of lower grain weight and quality.

Conclusion

In conclusion, it is the first time that the physicochemical properties and morphological traits were explored in different local rice varieties from Afghanistan. Our results exhibit that Super Basmati including local rice varieties (Sarda Barah, Garma Barah, Surkha Zurahti and Shah Lawangi) from Afghanistan with long and slender grains, companioned with significantly higher grain amylose and protein contents, which coincided in decreased taste points. While, Koshihikari with short and medium grain types showed the lowest grain amylose and protein contents of 17.7 and 5.5%, respectively, which amplified grain taste point. The scanning electron micrographs displayed that, there were no obvious variations in the endosperms of translucent grains across all varieties. Furthermore, starch granules with polyhedral shape were regularly build-up without airgaps. Particularly, in translucent endosperms of all varieties, displaying that the grain filling process were more advanced compared to chalky grains. In contrast, the endosperm of chalky grains in local rice varieties were varied, irregularly developed starch granules together with the single round shape and dent-portion on their surfaces with numerous airgaps were observed due to high temperature. Such loose arrangement amplifies in lower grain weight and quality. But, there were no apparent alterations in the chalky endosperms of Koshihikari and Super Basmati.

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Table 1. Physical parameters of selected (milled grain) rice varieties.

	Varieties	Grain weight (mg)	Grain length (mm)	Grain width (mm)	Size	Shape
1	Sarda Barah (*indica*)	21.0 a	7.3 a	2.1 bc	Long	Slender
2	Garma Barah (*indica*)	19.5 ab	7.1 ab	2.0 c	Long	Slender
3	Surkha Zurahti (*indica*)	19.4 ab	7.1 a	2.1 b	Long	Slender
4	Shah Lawangi (*indica*)	17.6 bc	6.7 b	2.0 c	Long	Slender
5	Super Basmati (*indica*)	16.8 c	7.4 a	1.9 d	Long	Slender
6	Koshihikari (*japonica*)	20.0 ab	4.8 c	2.8 a	Short	Medium

Same letters in two columns under the same title reveal no significant difference at the 5% probability level.

Table 2. Comparison of selected rice varieties on milled grain amylose content, protein content and taste point.

	Varieties	Type	Varietal origin	Amylose (%)	Protein (%)	Taste point (as reference)
1	Sarda Barah	Local	Afghanistan	20.5 b	8.4 a	63.0 d
2	Garma Barah	Local	Afghanistan	20.0 b	8.2 ab	63.0 d
3	Surkha Zurahti	Local	Afghanistan	19.9 b	8.0 b	66.0 c
4	Shah Lawangi	Local	Afghanistan	18.9 c	7.9 b	67.4 b
5	Super Basmati	Improved	Pakistan	22.9 a	8.1 ab	60.0 e
6	Koshihikari	Improved	Japan	17.7 d	5.5 c	69.3 a

Same letters in two columns under the same title reveal no significant difference at the 5% probability level.

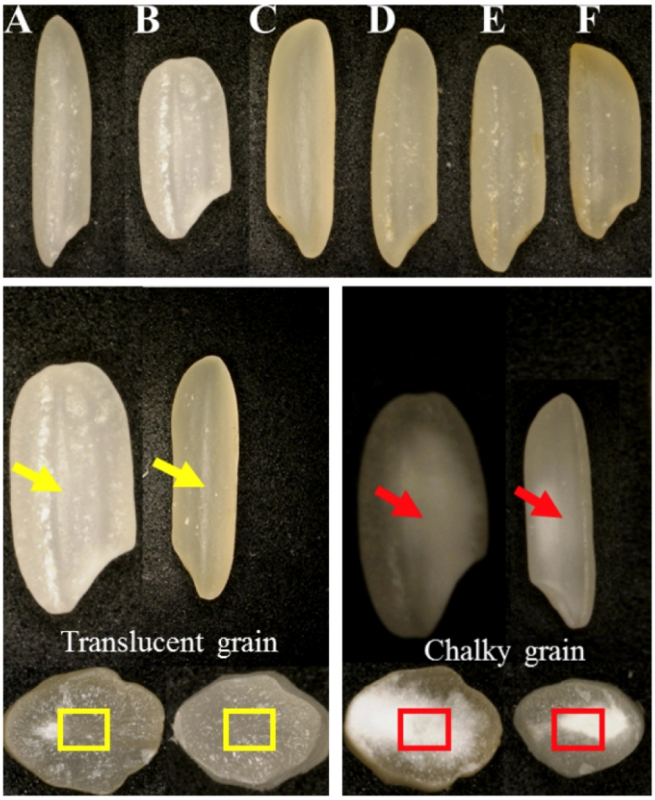

Figure 1. Comparison of grain shape and appearance of translucent rice grains (T) and chalky rice grains (C) of Super Basmati (A), Koshihikari (B), Sarda Barah (C), Garma Barah (D), Surkha Zurahti (E), and Shah Lawangi (F). Photographs of milled rice were taken by a microscopy (Keyence-VH500, Japan). Yellow and red rectangles on the central portions of cross-sections of milled rice grains reveal the chalky and translucent portions for scanning electron microscopic observations.

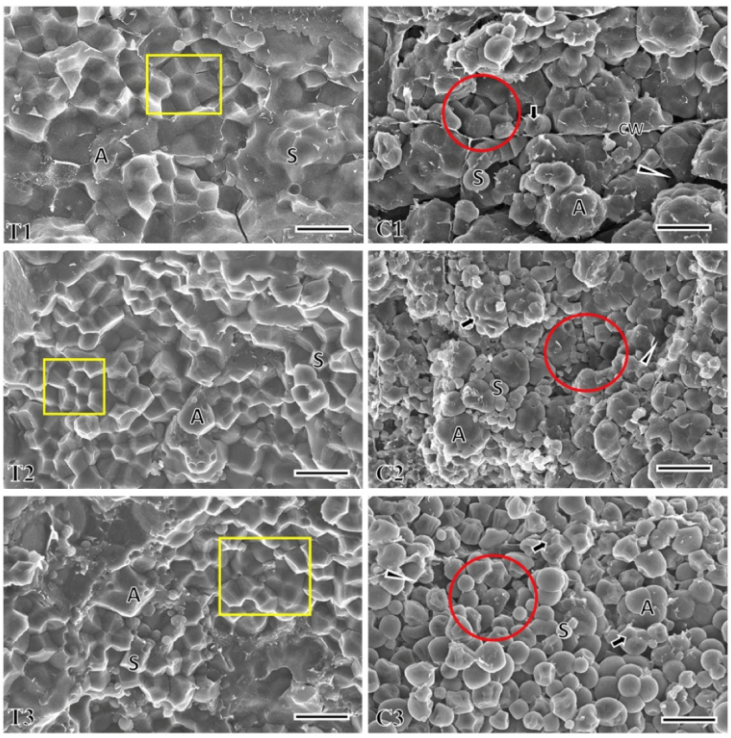

Figure 2. Scanning electron micrographs of the cross-sections of the central portion of milled rice grains of Sarda Barah (1), Garma Barah (2), and Surkha Zurahti (3). T: translucent grains, C: chalky grains. A: amyloplasts, CW: cell wall, S: starch granules, arrows: dent portion, arrowheads: airgap and Scale bars: 10-µm. Yellow rectangles reveal the polyhedral shape of starch granules grouping into amyloplasts without airgaps. Red circles reveal loosely developed starch granules together with the single round shape and multiple size and shape of starch granules with numerous airgaps.

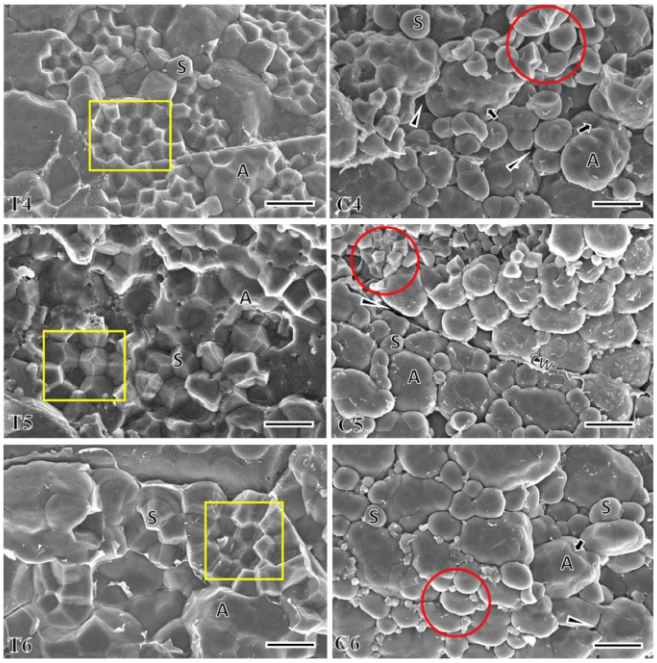

Figure 3. Scanning electron micrographs of the cross-sections of the central portion of milled rice grains of Shah Lawangi (4), Super Basmati (5), and Koshihikari (6). T: translucent grains, C: chalky grains. A: amyloplasts, CW: cell wall, S: starch granules, arrows: dent portion, arrowheads: airgap and Scale bars: 10-µm. Yellow rectangles reveal the polyhedral shape of starch granules grouping into amyloplasts without airgaps. Red circles reveal loosely developed starch granules together with the single round shape and multiple size and shape of starch granules with numerous airgaps.

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Research Article

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Determination of the effect of some pesticides on honey bees

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Abstract

Although the bee deaths that started in 2006 have passed for a long time, no solution has been found and even bee deaths have started to increase again in recent years. The end of winter and spring months are periods when bee deaths are seen intensely. When these periods are examined, it can be seen that many factors (disease-harmfulness, hunger, cold, etc.) cause bee deaths. One of these factors is the pesticides used in springtime in the wintering region. In this study, the effects of pesticides, which are commonly used against factors damaging agricultural crops grown in regions where bee deaths is high, on the body motor movements of the bees are investigated. The most commonly used product used for agricultural combat in pesticides used in our study and the label dose (recommended dose) used for this product was fed twice with the label dose and half by oral gavage, after 1, 4 and 24 hours, the bees were checked and some of the body parts (antenna, leg, abdomen and mouth parts) were rated according to motor movements. As a result of the study, pesticides affecting body motor movements of bees are listed as Chlorpyrifos-Ethyl, Imidacloprid, Deltamethrin, Thiacloprid, Acetamiprid, Abamectin and Tau-fluvalinate active substances from high to low. Spirodiclofen, Glyphosate Potassium Salt, and Penconazole active substance chemicals arranged in the same group with control and did not changed their body motor movements.

Keywords: Pesticide, Apis mellifera, Honey bee, Bee mortality, Colony looses

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Introduction

Bees continue to exist under pressure in today's world with many factors. The decrease in the amount and variety of flora, the effects of agro-chemicals, the beekeepers' unnecessary applications, the new parasites peculiar to bees, climate change will further widen the size of this problem in the future (Goulson et al., 2015; Mitchell et al., 2017; Williamson and Wright, 2013). In recent years, the decline in the population of honey bees and other pollinizers has also been a source of concern for food safety (Abbo et al., 2016).

Colony losses are found in Turkey (Karahan and Karaca, 2016) and Europe and America (vanEngelsdorp et al., 2011). Severe colony loss in honey bees, called Colony Collapse Disorder (CCD), is widespread across Europe and North America (Stokstad, 2007). Pesticides used against agricultural pests are at the forefront, as are many of the losses in bee populations (Goulson et al., 2015; Krupke et al., 2012; Paradis et al., 2013). In addition, bee deaths constitute environmental risks (Tosi et al., 2017).

Honey bees are important not only because they produce honey and honey products, but also because they offer pollinating services (Yalçın, and Turgut, 2016). In addition to pollinating services (Klatt et al., 2013), it also makes a significant contribution to human nutrition with products such as honey, pollen, wax, propolis and bee milk (Ellis et al., 2015; Gray and Peterson, 2017).

Pesticides are also the most important risk factors for honey (Connolly 2013; Baron et al., 2017) and other insect pollinators (Glenny et al., 2017; Tihelka et al., 2017; Garibaldi et al., 2013), as well as beneficial organisms such as parasitoid bees in the wild (Aydoğdu and Kanev, 2017). For example, only about 5% of the neonychotinoid active ingredient used is consumed by plants (Sur and Stork 2003) and a large part is dispersed in the environment (Goulson, 2014).

Information on how honey bees are affected by pesticides is based on very old ones. It is stated that the Paris Green used against the apple in the USA caused a considerable amount of bee death in 1870 and Carbaryl application as powder in the cotton fields in 1967 destroyed 15% (70,000 bee colonies) of total colony in California (Yıldırım, 2012).

Honey has a different structure than other insects in feeding the baby, and the care of the baby is made by worker bees (Winston, 1991; He et al., 2016). In accordance with the technique, unincorporated chemicals transport nectar and pollen workers through plants and cause collective larvae and adult bee deaths through contact and feeding (Bonmatin et al., 2015; Zhu et al., 2017; Efsa, 2013; Özbek, 2010; Yıldırım, 2012)

It has been determined that the bees in contact with the pesticide become more susceptible to parasites (Goulson et al., 2015) and the life span of the immune system weakens (Woyciechowski and Moroń, 2009). Pesticides affect the queen's bee mating flight negatively and shorten the life span

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of sperm as a result of insufficient sperm intake of sperm (Williams et al., 2015). In the case of workers; this creates a change in the performance of movement, incoherence, orientation and gathering (Desneux et al., 2007; Gill et al., 2012; Schneider et al., 2012; Williamson and Wright, 2013). The beekeeping products in the colonies which are indirectly exposed to beekeeping products are threatening human health (Böhme, et al., 2017).

Materials and Methods

The main material of this work is the Anatolian bee species (*Apis mellifera anatoliaaca*) which is the most common bee in our country (Sirali, 2017) and pesticides used in regions where bee deaths were intensively observed. The usage and quantities of these pesticides widely used in agricultural production areas are given in Table 1 (Karahan and Karaca, 2016).

Table 1 lists the licensed products and doses used for the pesticides used intensively in the area where the bees died. These pesticides are used intensively, regardless of the license group in regions where agricultural production. It is also thought that the target is better struggled by using over the label dose against the harmful factors. These chemicals are used extensively when the bees are spread (food collection) or during the flowering period.

In the study, the most commonly used product of agricultural use of pesticides is fed the label dose (recommended dose) twice the label dose and half of the label by oral (in 2M sugar syrup) and some of the body parts of the bees (legs, abdomen and mouth parts) were evaluated by looking at the motor movements. After 1, 4 and 24 hours after feeding, the bees were checked and scored. In this point; "1" for each organ, if all the organs are healthy and two "2" points if all antennas, mouthpieces, legs and abdomen are moving slowly and irregularly (Duell, 2012). If all of the organs of the bees followed according to this scoring system were working, " 8 ", if not, they got " 0 ".

In order to obtaine the bees used in the study, 2M of sugar water was placed in a petri dish in front of the hives 10 meters. Worker bees that came to the petri dish were caught randomly through small plastic boxes and brought to the laboratory. Afterwards, these bees were kept frozen for about 3-4 minutes in the freezer of the refrigerator (Hranitz et al., 2010). The immobilized bees were removed from the boxes and attached to the previously prepared injector reservoirs between the head and thorax. After the bees have been soaked, they have been chosen to react with their ankles by touching their antennae with the aid of an ear garbage impregnated with water and syrup (sugary syrup), and the responding healthy bees have been selected for use in the study (Abramson et al., 2004). All of these selected bees were fed with 2 moles of prepared syrup until saturated and kept at normal room temperature for 24 hours (Duell, 2012). At the end of this training, a second selection was made to compensate for mistakes caused by practice, leaving the healthy ones in groups of 5.

The solution containing pesticide in 2M sugar syrup was fed to the bees with 10 µl of micropipette in each experimental group. The control group was not given pesticide, but only 2M sugar syrup was given.

Five trials were established for each pesticide. In each trial 5 bees were used per dose. A total of 1000 bees for 10 different pesticides in Table 1 were used in this study.

In this study, it was determined how body motor movements of bees exposed to pesticides change.

As a result of this process it was revealed that reactions of bees given different dose. In statistical analysis, one-way variance analysis was used with the help of SPSS (ver.17) program and benefitted from Tukey test ($P<0.05$).

Table 1. Type and amount of pesticides used in the study

	Pesticides* (Active Ingredient)	Registered Crops	Product Type	Rate (Dose)	Formulation Type
1	Chlorpyrifos-Ethyl	Corn	Insecticide	180 ml/100 L water	EC (Emulsion concentrate)
2	Deltamethrin	Corn / Apple	Insecticide	50 ml/100 L water	EC (Emulsion concentrate)
3	Imidacloprid	Apple	Insecticide	20 ml/100 L water	SC (Suspension concentrate)
4	Thiacloprid	Apple	Insecticide	40 ml/100 L water	OD (Oil based suspension concentrate)
5	Acetamiprid	Apple	Insecticide	20 g/100 L water	SP (Water soluble powder)
6	Abamectin	Citrus	Acaricide	25 ml/100 L water	EC (Emulsion concentrate)
7	Tau-Fluvalinate	Apple	Insecticide	30 ml/100 L water	EW (Emulsion, Oil in water)
8	Penconazole	Grape	Fungicide	25 ml/100 L water	EC (Emulsion concentrate)
9	Spirodiclofen	Citrus	Acaricide	20 ml/100 L water	SC (Suspension concentrate)
10	Glyphosate Potassium Salt	Citrus	Herbicide	600 ml/100 L water	SL (Water soluble concentrate)

* Pesticides used in areas where bee deaths occur

Results and Discussion

The doses were consumed to bees as stated in the material method section and the scores and statistical groups were given in Table 2. According to the body motor movements, Table 3 shows the percentage of decrease in the body motor movements of the bees, which are given the label dose depending on the control group. These scores obtained by bees according to their body movements indicate the health status of bees. When bees are released, the bees whose body motion score below 5 cannot fly but the bees who receive more than 5 points can fly. The bees, whose score is above 6, fly comfortably, and the bees, which score between 5 and 6, have difficulty flying.

The average body movement scores obtained after 1, 4 and 24 hours after consumption of the pesticides used in the study are given in Table 2. Among the pesticides used, Chlorpyrifos-Ethyl is the pesticide that affects the body movements of bees the most. The control bees were included in 3 groups and statistically different group in the follow-up period. Among the pesticides used, Imidacloprid, Deltamethrin, Thiacloprid all of the bees were included in the different statistic group according to the control group. Acetamiprid and Abamectin active pesticides did not affect the bees at the end of 1 and 4 hours but at the end of 24 hours they were statistically in different groups with control bees affecting the body movement scores of the bees. Spirodiclofen, Glyphosate Potassium Salt, Penconazole active substance pesticides and control group were similar to each other and statistically, they were in the same group after 1, 4 and 24 hours. After the recommended dose of Chlorpyrifos-Ethyl was administered, 96.00% of the bees died at the end of the first hour and all at the end of 4 hours (Table 3).

After applying the label dose of pesticide used in Table 3, the average score of bees was calculated and the percentage of decrease in body movements was given depending on the control group. Table 3 shows that there was no change in the body movements of the control group bees in the experiment with Chlorpyrifos-ethyl at the end of 1 hour, while 96.00% decrease in the body movements of the bees fed with Chlorpyrifos-ethyl label dose was observed. After 4 and 24 hours after the application of Chlorpyrifos-ethyl, the body movements of bees have stopped completely (100%) that is, bees have died. The decrease in body motor movements after the application of other pesticides was given in Table 3.

Table 2. Average points given to body motor movements of pesticide applied bees (Average ± SE)

Pesticides	Doses	1 Hour	4 Hour	24 Hour
Chlorpyrifos-Ethyl	Control (0 ml/100 L)	8.00 ± 0.00 a	7.88 ± 0.08 a	7.28 ± 0.18 a*
	3. Dose (90 ml/100 L)	0.76 ± 0.11 b	0.00 ± 0.00 b	0.00 ± 0.00 b
	2. Dose (180 ml/100 L)**	0.32 ± 0.04 c	0.00 ± 0.00 b	0.00 ± 0.00 b
	1. Dose (360 ml/100 L)	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Imidacloprid	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.48 ± 0.12 a
	3. Dose (10 ml/100 L)	3.68 ± 0.33 b	0.20 ± 0.08 b	0.56 ± 0.24 b
	2. Dose (20 ml/100 L)	1.92 ± 0.41 c	0.08 ± 0.10 c	0.00 ± 0.00 c
	1. Dose (40 ml/100 L)	1.16 ± 0.26 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Deltamethrin	Control (0 ml/100 L)	7.96 ± 0.04 a	7.92 ± 0.08 a	7.12 ± 0.12 a
	3. Dose (25 ml/100 L)	3.84 ± 0.20 b	1.72 ± 0.21 b	1.12 ± 0.16 b
	2. Dose (50 ml/100 L)	2.44 ± 0.22 c	0.64 ± 0.19 c	0.12 ± 0.08 c
	1. Dose (100 ml/100 L)	1.40 ± 0.14 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Thiacloprid	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.48 ± 0.10 a
	3. Dose (20 ml/100 L)	4.96 ± 0.24 b	1.20 ± 0.12 b	1.16 ± 0.14 b
	2. Dose (40 ml/100 L)	3.20 ± 0.20 c	0.36 ± 0.04 c	0.28 ± 0.08 c
	1. Dose (80 ml/100 L)	2.16 ± 0.22 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Acetamiprid	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.12 ± 0.23 a
	3. Dose (10 g/100 L)	7.36 ± 0.07 a	7.04 ± 0.11 b	4.48 ± 0.34 b
	2. Dose (20 g/100 L)	6.68 ± 0.24 b	6.28 ± 0.28 c	2.92 ± 0.31 c
	1. Dose (40 g/100 L)	5.04 ± 0.20 c	4.52 ± 0.18 d	1.76 ± 0.14 d
Abamectin	Control (0 ml/100 L)	8.00 ± 0.00 a	7.96 ± 0.04 a	7.48 ± 0.08 a
	3. Dose (12.5 ml/100 L)	7.88 ± 0.08 a	7.24 ± 0.20 a	7.36 ± 0.27 a
	2. Dose (25 ml/100 L)	6.96 ± 0.16 b	5.08 ± 0.23 b	5.32 ± 0.19 b
	1. Dose (50 ml/100 L)	4.56 ± 0.20 c	2.56 ± 0.31 c	1.92 ± 0.16 c
Tau-fluvalinate	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.52 ± 0.10 a
	3. Dose (15 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.36 ± 0.26 a
	2. Dose (30 ml/100 L)	8.00 ± 0.00 a	7.88 ± 0.08 a	7.44 ± 0.07 a
	1. Dose (60 ml/100 L)	8.00 ± 0.00 a	7.48 ± 0.08 b	6.56 ± 0.13 b
Penconazole	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.64 ± 0.17 a
	3. Dose (12.5 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.40 ± 0.27 a
	2. Dose (25 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.48 ± 0.17 a
	1. Dose (50 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.48 ± 0.16 a
Spirodiclofen	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.48 ± 0.17 a
	3. Dose (10 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.20 ± 0.26 a
	2. Dose (20 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.32 ± 0.21 a
	1. Dose (40 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.44 ± 0.19 a
Glyphosate Potassium Salt	Control (0 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.56 ± 0.16 a
	3. Dose (300 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.24 ± 0.24 a
	2. Dose (600 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.44 ± 0.29 a
	1. Dose (1200 ml/100 L)	8.00 ± 0.00 a	8.00 ± 0.00 a	7.60 ± 0.07 a

* Within same columns, the values (±standard error) means sharing a letter are not significantly different from each other (Tukey's at P<0.05).
** 2. Dose is label dose of all pesticides used (recommended dose).

Table 3. Decrease rate of body movements of label-administered bees (%)

	Decrease in body motor movements (%)			
	Doses	**1 Hour**	**4 Hour**	**24 Hour**
1	Chlorpyrifos-Ethyl	96.00	100.00	100.00
2	Imidacloprid	76.00	99.00	100.00
3	Deltamethrin	69.50	92.00	98.50
4	Thiacloprid	60.00	95.50	96.50
5	Acetamiprid	16.50	21.50	63.50
6	Abamectin	13.00	36.50	33.50
7	Tau-fluvalinate	0.00	1.50	7.00
8	Spirodiclofen	0.00	0.00	8.50
9	Glyphosate Potassium Salt	0.00	0.00	7.00
10	Penconazole	0.00	0.00	6.50

Conclusion

As seen in the findings of the work, some pesticides have restricted the body motor movements of bees or caused the death of bees. Some of the pesticides used did not affect the body movements of the bees. These pesticides may not have affected the body function of the bees, but may have affected some sense organs (such as direction, vision, hearing, smell, taste). This can end their lives because the bees lose their properties such as smell, taste, direction or sight, cannot find their husk, or mix their husk. The most important result of this study is that some pesticides do not directly kill the bees and they are transported to the hives by worker bees and they pass on products like honey, pollen. These pesticide-depleted foods are consumed by human beings as well as consumed by other bees in the hive, creating a major problem for human health.

The bees exposed to different pesticide mixtures in the field conditions are lost on the field and between the field and the colony (Zhu et al., 2017). Therefore, as the strength of the colon weakens, the movements of the bees are diminished and their duties are delayed, the abdomen swells, the wings and legs become paralyzed and die (Yıldırım, 2012). The study showed similarity with the studies of ' Williamson et al. 2014; Oliver et al.,2015; Karahan et al., 2015; Zhu et al., 2017; Bovi et al., 2018 ', and it was observed that some pesticides used were found to be extremely harmful in honey bees and cause paralysis and deaths afterwards.

In order to reduce pesticide damages and protect bees, the spraying should not be done during the flowering periods, and if the pesticide has to be expelled, it should be applied in the evening hours when there are no bees. In addition, the beekeeping site should be located far away from areas where intensive spraying is made, if it cannot be transported during the application, it should be kept closed and covered in order to protect it from pesticides (Yıldırım, 2012).

As a result, the awareness of the harm that all pesticides have on the bees and the environment has not yet been understood. The substances used to make benefits can damage the environment and cause the bees to die in climate changes. New works should be done to protect the lives of the bees and the surrounding area. Protection of bees and the environment is more important and necessary for people than anything else.

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Research Article

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Performance and variation in phenotypic characters of maize genotypes in Mid-Western Region of Nepal

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Abstract

An experiment was conducted to evaluate the morphological characters of maize genotypes at Horticultural Research Station, Dailekh in two consecutive years (2016 and 2017). Altogether, seven genotypes consisting of BGBY POP, BLSPRSO7, Across 9942/9944, Across 9331/RE, TLBRSO7 along with check varieties (Deuti and Local) were experimented. The trial was laid out in Randomized Complete Block Design with three replicates. Data on morphological parameters and yield attributing traits were recorded. The results over the years of experiment revealed significant differences for days to maturity, plant height, ear height, number of kernel per row and grain yield. Furthermore, highest grain yield was recorded in Across 9944/9942 (7.24 tha^{-1}) followed by BLSPRSO7 (6.73 tha^{-1}). Similarly, number of kernel per row was maximum in Across 9942/9944 (37.6) followed by Deuti (37.1). The significant variation in ear height was observed which ranged from 125.4 cm (Across 9944/9942) to 146 cm (local variety). It showed local variety to be early maturing (114 Days After Sowing); however, Across 9942/9944 was late maturing (119 DAS). Correlation analysis revealed that ear population, plant population and plant height were yield determinative and simultaneous selection for these traits might bring an improvement in grain yield. This study revealed that Across9931/RE can be a potential genotype for yield and for further maize breeding program.

Keywords: Correlation, Genotypes, Maize, Phenotype, Yield

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Introduction

Maize (*Zea mays* L.) is the world's widely grown cereal and primary staple food in many developing countries (Shrestha, 2015). In Nepal, it is the second most important staple food crop both in terms of area and production after rice. It is grown in 891,583 hectare of land with total production of 2,231,517 mt and yield of 2.5 t/ha^{-1} (ABPSD, 2016). It shares about 7% to Agricultural Gross Domestic Product (MOAD, 2015). It has higher yield potential than any other cereals and thus is popularly known as the 'queen of cereal'. It is a traditional crop cultivated as food, feed and fodder on sloping uplands in the hills. Maize is grown under rain-fed condition during the summer (April-August) as a sole crop, relayed with millet later in the season (Paudyal et al., 2001). About 80% of the rainfall occurs in Nepal during June to September. However, the mid-western and western regions are relatively dry in comparison to other regions, which affects on the yield of crops in those dry areas. Thus, development of new crop varieties to such areas is essential.

Maize is one of the five cereal crops meeting the food requirement, which contributes approximately 22.6% of the total requirement. It contributes to food security in the hills, while in the accessible areas it is gradually becoming a commercial commodity due to increasing demand of nutrients in poultry and animal feed (Pathik, 2002). In Nepal, improved seeds are produced in 841596 ha whereas local are produced in 49987 ha (ABPSD, 2016). The second most important agricultural land area is the mid-hills where 15% of land is cultivated (Paudyal et.al., 2001). In mid-western region, maize is produced in 8394 ha with production of 12030 ton and yield of 1.4 t/ha-1 whereas in Dailekh it is produced in 20150 ha with production of 35292 ton and yield of 1.7 t/ha-1. The overall demand for maize has been estimated to grow up by 6-8% per annum for the next two decades because of the increased demand for food in the hills as population increases and for livestock feed in accessible areas in the Terai and inner-Terai as the demand for milk, meat, and meat products is increasing (Pathik, 2002).

Several production factors are responsible for the yield reduction of maize such as; declining soil fertility, low adoption of high yielding varieties, meager availability of the essential inputs and partly due to the management practices of the crop (Kaini, 2004). Further, many farmers of Dailekh district are dependent on their local variety of maize which is less yielding , susceptible to disease, pest and intolerance to changing climatic condition. The performance of maize crop isn't satisfactory in mid-hills due to unavailability of improved varieties. Thus, the selection of maize genotypes is an importance for propagation of desirable traits and development of new varieties. The need of new variety for farmers is realized for ensuring food security by developing new varieties appealing characteristics like higher yield, disease resistant, drought tolerant or regionally adapted to different environments and growing conditions. Hence, to address these issues, replace

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the local variety with new varieties, and strengthen the farmers with superior maize cultivars an experiment was conducted at HRS, Dailekh during 2016 and 2017 and the performance of promising maize genotypes was studied under Mid-Western condition of Nepal.

Materials and Methods

To study the performance of different genotypes of maize in mid-hills region of Nepal, an experiment was conducted at research blocks of Horticultural Research Station, Dailekh (28°85'N, 81°72'E, 1300 m asl) during summer season in two consecutive years: 2016 and 2017. Five genotypes of maize (BGBYPOP, BLSPRSO7, ACROSS 9942/9944, ACROSS 9331/RE and TLBRSO7) procured from NMRRP, Rampur including one released variety Deuti, and a local variety (Standard Check) were used in the experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) with seven genotypes as treatments and was replicated thrice. Each plot consisted 4 rows of 3 m long with spacing of 75cm x 25cm and the plot size was maintained at 3m x 3m. The recommended dose of fertilizers and manure were applied at the rate of 120:60:40 kg NPK/ha and 6 t/ha-1 FYM respectively. 50% (60 kg per ha urea) of the recommended dose of Nitrogen and full dose of Phosphorous and Potassium were applied during the land preparation and remaining 50% dose of Nitrogen, in the form of urea, was applied in two split doses: 25% during the knee height stage and 25% during the silking stage.

The parameters were taken with frequent field supervision. Days to tasseling and days to silking were recorded with regular field observation after 60 days of sowing, at which emergence of 50% of tassel followed by silking started to be observed in the early genotypes. Similarly, the days to maturity was noted with the observation of matured cob color and dryness. And the remaining parameters were taken during the time of harvest.

Five sample plants of each plots based on the genotypes were taken for measurement of plant height, ear height, number of kernels row per year, and number of kernels per row and eventually averaged. Plant height was measured from the ground level (soil surface) to the base of tassel at the time of harvest. Similarly, the measure of ear height was taken from the soil surface to the base of the ear at the harvesting time. Further, the maize and ear population was counted at the standing crops stage during the time of harvesting the maize. Cobs were harvested, husks were removed and five sample cobs of each genotype were weighted and averaged and the final grain yield was calculated.

Similarly, the correlation coefficients among different traits were carried out using the formula given by Steel and Torrie (1980) by using SPSS program.

$$\text{Correlation coefficient } (r) = \frac{\sum XY - (\sum X)(\sum Y)/N}{[\{\sum X^2 - (\sum X)^2\}\{(\sum Y^2) - (\sum Y)^2/N\}]^{1/2}}$$

Where,

r= correlation coefficient

$\sum XY$ = Sum of product of two variables X and Y

$\sum X^2$ =Sum of squares of variables X

$\sum Y^2$ = Sum of squares of variables Y

N = Number of pairs of observations X and Y

Results and Discussion

The analysis of variance revealed significant differences for days to maturity (DM), plant height (PH), ear height (EH), number of kernel per row (NKPR) and grain yield among the tested genotypes whereas, days to tasseling (DT), days to silking (DS), plant population (PP), ear population (EP), ear length (EL), number of kernel row per ear (NKRE) and five hundred kernel weight (FKW) showed non-significant differences (Table 1, 2 and 3).

The effect of genotypes was non-significant for days to tasseling and days to silking over the years of experiment. However, both the parameters were varied significantly between genotypes in the year of 2017. Despite of significant effect of genotypes in days to maturity during 2017, the overall data revealed the variation with non-significant differences in maturing days. The local variety was found to be early maturing (114 DAS), which was statistically similar with Across 9331/RE (115 DAS), BLSPRSO7 (115 DAS) and TLBRSO7 (116 DAS). Contrastingly, Across 9942/9944 was observed to be late maturing (119 DAS) among the tested genotypes.

Statistical analysis for plant height and ear height revealed significant difference among tested genotypes over the years (Table 2). Local variety was significantly tallest (256.7 cm) with maximum ear height (146 cm). However, shortest plant height (225.5 cm) and ear height (125.4 cm) was recorded in TLBRSO7 and Across 9942/9944 respectively. Genotypes Deuti (252.8 cm) and BLSPRSO7 (241.3 cm) were statistically at par with local variety of maize in terms of plant height. Similarly, the maximum ear height in local variety was considerably followed by Deuti (135.2 cm) and TLBRSO7 (132.0 cm), whereas, the minimum ear height was measured in Across 9942/9944 (125.4 cm).

Plant population and ear population varied insignificantly among the genotypes. However, highly significant difference was observed among the tested genotypes for ear population during 2017, where the plots of TLBRSO7 were counted with maximum number of ears per plot (45) among the tested genotypes.

The number of kernel row per ear revealed non-significant differences among tested genotypes over the experimental years (Table 3). However, highly significant difference was recorded during year of 2017 among the tested genotypes and BGBY POP was collectively recorded to have maximum number of kernel row per ear (15.3). Further, the number of kernel per row varied significantly. Maximum number of kernel per row (37.6) was counted in Across 9942/9944, which was insignificantly followed by other tested genotypes except BGBY POP and BLSPRSO7. BLSPRSO7 was noted with the minimum number of kernel per row (32.7).

The observation in grain yield varied significantly among the tested genotypes over the year of experimentation. Across 9944/9942 has recorded to have maximum grain yield of 7.2 tha^{-1}, which is statistically at par with BLSPRSO7 (6.7 t/ha-1), Local (6.1 t/ha-1), Deuti (6.1 t/ha-1) and BGBYPOP (5.8 t/ha-1). Despite of local check having the highest plant population per plot, Across 9942/9944 was recorded to have the maximum grain yield due to the maximum plant population per plot and highest number of kernel per row, which is in partial conformity with the finding of Abuzar et al. (2011), in which the higher grain yield was found from the plot with higher number of ears per plant and number of grains row per ear. Similarly, Emam (2001) verified that kernels per ear and kernel row per ear is the most important yield adjusting components in response to plant population density in maize. The genotype Across 9331/RE, however, produced minimum grain yield (4.1 t/ha-1).

Correlation coefficient among the phenotypic variables is given in Table 4. Days to tasseling was strongly, positively correlated with days to silking which is similar to the observations of Noor et al. (2010). Plant height had significantly positive association with ear height (0.59**), and plant population per plot (0.57**). The similar result is in conformity with Jhakar et al. (2017). Further, the plant height is observed to be positively and significantly associated with the grain yield (0.31*). This positive and statistically significant relationship between plant height and grain yield found in our research is also supported by Sadek et al. (2006); Halidu et al. (2015); Jhakar et al. (2017), under different experimental conditions. Further, Silva et al. (2016) is in partial agreement with our findings as they found negative correlation between the plant height and grain yield. However, the negative correlation existed between ear length and grain yield in our research is supported by their results which recorded negative phenotypic relation between those traits. Also, the positive and significant correlation between plant population and grain yield is corroborated by Emam (2001), who described that the grain yield increased at the higher population densities of maize plant.

Moreover, highly significant and positive correlation existed between ear population and plant population (0.57**). Days to maturity had highly negative significant correlation with plant height (-0.45**). The plant population demonstrated the significant and positive correlation with plant height and ear height. Many research showed that increasing crop density increases PH and EH (Sharifi et al., 2009; Shafi et al., 2012; Silva et al., 2014; Mandic et al., 2016).

Ear length was found to have the negatively significant correlation with plant height (-0.40**). The observation of Noor et al. (2010), however contradicts this finding as they noted positive and significant correlation between ear length and plant height. Ear length exhibited positive and highly significant correlation with days to maturity (0.73**). NKPR showed positive and highly significant correlation with days to maturity (0.42**) and ear length (0.60*). This result is in partial conformity with Dar et al. (2015) as they observed the negative correlation of no. of kernels per row (NKPR) with days to maturity and highly positive correlation between NKPR and ear length.

Conclusion

The variation in phenotypic characters is observed among the maize genotypes. The genotype Across 9944/9942 was found to be superior in terms of grain yield producing 18.7% more grain yield than the standard check along with the highest ear population. Plant height, plant population, and ear Population are highly and significantly associated with yield and are needed to be considered for selection. Thus, we could recommend to the farmers of this area (mid-hills) to select the genotype Across 9944/9942 in order to improve overall performance and yield of the crop.

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Table 1. Performance of different genotypes of maize in terms of growth parameters during 2016/17 in research block of HRS, Dailekh

Maize Genotypes	Days to Tasseling			Days to Silking			Days to Maturity		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
BGBY POP	68	68	68	70	71	71	122	114	118
BLSPRS07	62	67	65	64	70	67	119	112	115
Across 9942/9944	67	69	68	69	72	71	120	118	119
Across 9331/RE	60	68	64	64	70	67	117	112	115
TLBRSO7	65	68	67	67	70	69	118	114	116
Deuti (Check)	67	72	70	69	74	72	120	115	118
Local (Check)	69	62	66	71	64	68	119	108	114
Grand Mean (GM)	66	68	67	68	70	69	120	113	116
LSD $_{(0.05)}$	NS	3.8*	NS	NS	3.6*	NS	NS	4.7*	3.5*
CV (%)	7.5	3.1	6.5	7.4	2.9	6.1	2.5	2.3	2.6

Note: NS and * indicate non-significant and significant at $P<0.05$ respectively.

Table 2. Phenotypic characters of maize genotypes tested at HRS, Dailekh during 2016 and 2017

Maize Genotypes	Plant Height (cm)			Ear Height (cm)			Plant Population/Plot			Ear population/Plot		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
BGBY POP	219.3	243.1	231.2	125.0	128.5	126.8	30	36	33	35	36	36
BLSPRS07	233.0	249.5	241.3	128.7	127.9	128.3	31	35	33	34	43	38
Across 9942/9944	230.0	248.1	239.1	121.7	129.2	125.4	28	37	33	32	44	38
Across 9331/RE	229.0	225.3	227.2	137.3	122.9	130.1	29	29	29	30	28	29
TLBRSO7	217.3	233.6	225.5	125.3	138.6	132.0	26	31	28	29	45	37
Deuti (Check)	242.7	262.9	252.8	134.5	135.9	135.2	33	36	35	32	37	35
Local (Check)	244.0	269.4	256.7	141.0	151.1	146.0	34	37	36	31	36	33
GM	230.8	247.4	239.1	130.5	133.4	132.0	30	34	32	32	38	35
LSD $_{(0.05)}$	NS	NS	16.28*	10.8*	13.93*	9.44*	NS	NS	NS	NS	5.5**	NS
CV (%)	4.8	6.8	5.8	4.7	5.9	6.1	11.6	18.9	15.3	17.5	8.1	15.1

Note: NS, * and ** indicate non-significant, significant and highly significant at $P<0.05$ and. $P<0.01$ respectively.

Table 3. Performance of different genotypes of maize in terms of yield and yield attributing during 2016/17 in research block of HRS, Dailekh.

Maize Genotypes	No. of kernel row/ear			No. of kernel per row			FKW (g)			Yield (t/ha-1)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
BGBY POP	14.8	15.9	15.3	36.1	31.6	33.9	223.3	235.0	229.2	6.0	5.7	5.8
BLSPRS07	13.3	15.1	14.2	33.2	32.2	32.7	226.7	240.0	233.3	5.3	8.2	6.7
Across 9942/9944	13.3	14.8	14.0	39.3	35.9	37.6	216.7	223.3	220.0	8.2	6.6	7.2
Across 9331/RE	14.5	13.1	13.8	37.7	34.7	36.2	213.3	243.3	228.3	4.9	3.4	4.1
TLBRSO7	13.9	13.7	13.8	36.6	35.2	35.9	213.3	260.0	236.7	4.4	6.5	5.4
Deuti	13.1	13.9	13.5	39.3	34.9	37.1	220.0	220.0	220.0	6.1	6.1	6.1
Local (Check)	13.7	13.5	13.6	37.7	32.6	35.2	243.3	253.3	248.3	6.1	6.0	6.1
Grand Mean	13.8	14.3	14.1	37.1	33.9	35.5	222.4	239.3	230.8	5.8	6.1	5.9
$LSD_{(0.05)}$	NS	1.2**	NS	3.5*	NS	2.9*	NS	NS	NS	2.1*	2.3*	1.7*
CV (%)	10	4.5	8.1	5.3	9.1	7	15.2	18	15.5	20.1	21.6	24

Note: FKW, NS, * and ** indicate Field Kernel Weight, Non-Significant, significant and highly significant at $P<0.05$ and. $P<0.01$ respectively.

Table 4. Correlation coefficient among different yield and yield attributing traits of seven genotypes of maize experimented at HRS, Dailekh, Nepal during 2016/17.

	DT	DS	PH	EH	PP plot^{-1}	EP plot^{-1}	DM	EL	NKRE	NKPR	FKW	Yield
DT	1	0.97**	0.07	-0.13	0.10	0.09	0.14	-0.15	0.20	0.05	-0.08	0.14
DS		1	0.08	-0.12	0.04	0.04	0.15	-0.16	0.17	0.08	-0.04	0.13
PH			1	0.59**	0.57**	0.35*	-0.45**	-0.40**	0.01	-0.27	0.24	0.31*
EH				1	0.36*	0.18	-0.33*	-0.12	0.02	-0.13	0.13	0.07
PP plot^{-1}					1	0.57**	-0.27	-0.23	0.17	-0.15	0.10	0.33*
EP plot^{-1}						1	-0.12	-0.34*	0.25	-0.18	0.16	0.60**
DM							1	0.73**	-0.07	0.42**	-0.25	0.06
EL								1	-0.16	0.60**	-0.27	-0.01
NKRE									1	-0.23	-0.16	0.11
NKPR										1	-0.19	0.05
FKW											1	0.23
Yield												1

Note: PP plot^{-1}= Plant Population per Plot, EP plot^{-1}= Ear Population per Plot. * and ** indicate significant at $P<0.05$ and. $P<0.01$ respectively.

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Review Article

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Different formulations in gluten-free bread production: A review

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Abstract

Studies in recent years have shown that the market demand for gluten-free products is considerably increasing to fulfill celiac patients' needs. Celiac disease is a food allergenic disease in humans induced by gluten in wheat, barley, rye, kamut, spelt and hybrids like triticale. For individuals with this disease, the one and only cure is to keep away from gluten-containing foods for perpetuity. Because of this reason, production and development of gluten-free bakery products, particularly bread because it is a basic food consumed daily in the world, have become popular and have been improved by the addition of different cereals, flours and starches, dough treatment or changing processing conditions and the method of baking. It needs to improve gluten-free bakery products' quality because the absence of gluten is a big problem for the quality of dough and bread. For example, a bread made from gluten free flour has lower volume, weaker texture and aroma than the traditionally ones . However the increase of gluten-free market, there are still some problems such as their high prices, limited variety, and availability and low nutritional quality. This review focuses on the finding suitable alternatives for gluten free bread to improve their baking and sensory quality and nutritional properties.

Keywords: Celiac, hydrocolloids, gluten-free bread, bread quality

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Introduction

Celiac disease is a lifelong intolerance to gluten disease, caused by the intake of gluten-containing cereals such as wheat, rye, barley, oat, kamut, spelt and their products. (Korus et al. 2009; Padalino et al. 2016; Wang et al. 2017). Celiac disease can appear in the early childhood and people with the Celiac disease have some symptoms such as chronic diarrhea, failure to thrive, fatigue and weight loss when consumed gluten-containing foods (Korus et al. 2009; Demirkesen et al. 2010; Foschia et al. 2016). Nowadays, the only and effective treatment for people with Celiac disease is strict keep to a gluten-free (GF) diet, which means a permanent withdrawal of all types of bread and food prepared with wheat flours and similar proteins including kamut, spelt, triticale, barley and rye from daily food (Mariotti et al. 2013; Lamacchia et al. 2014; Foschia et al. 2016).

Gluten, the protein present in wheat which contains glutenin and gliadin fractions, is a major protein component which is responsible for water absorption capacity, cohesivity, viscosity elasticity and gas holding ability of bread dough and producing high quality baked goods with desired volume and texture (Gallagher et al. 2004; Demirkesen et al. 2010; Janawali et al. 2016; Tsatsaragkou et al. 2016). The production of bread using gluten-free flours is a major problem for bakers and researchers. Because, it can't be produced desirable bread which has some quality properties such as taste, texture, specific volume, flavour, colour and nutritional value without gluten (Matos and Rosell 2014; Tsatsaragkou et al. 2016).

Nowadays, numerous studies have been investigated on GF to eleminate these problems which means manufacturing GF breads with similar quality properties to wheat breads such as the use of rice flour (Demirkesen et al. 2010; Torbica et al. 2010; Hager and Arendt 2013; Mohammadi et al. 2014; Nicolae et al. 2016), corn flour (Sanchez et al. 2002), soybean flour (Sciarini et al. 2012), patato flour (Liu et al. 2018), buckwheat flour; (Torbica et al. 2010; Hager and Arendt 2013; Mariotti et al. 2013; Buresova et al. 2016), chestnut flour (Demirkesen et al. 2013; Moreira et al. 2013a;b;), corn starch (Lazaridou et al. 2007; Korus et al. 2009; Aguilar et al. 2015), quinoa white flour (Elgeti et al. 2014), hydrocolloids (Lazaridou et al. 2007; Demirkesen et al. 2010; Hager and Arendt 2013; Mariotti et al. 2013; Mohammadi et al. 2014; Nicolae et al. 2016; Mir SA et al. 2016; Ferrero 2017; Liu et al. 2018;), emulsifiers (Demirkesen et al. 2010; Houben et al. 2012; Sciarini et al. 2012), enzymes (Gujral and Rosell 2004a,b; Moore et al. 2006; Renzetti et al. 2008; Buresova et al. 2016), water-soluble dietary fibres (Tsatsaragkou et al. 2016; Capriles et al. 2016), dairy ingredients (Buresova et al. 2016) as alternatives to gluten, to development of properties of GF bakery products.

Effect of some different flours and starches on the quality of GF breads and batters

Cereal flours such as rice, corn, millet species, sorghum, finger millet and foxtail millet are widely used for GF bakery products due to they don't contain gluten forming proteins (Houben et al. 2012; Foschia et al. 2016; Padalino et al. 2016). These flours are used for enhancing the texture of several bakery products such as tarhana, cookies, bread, pasta, tagliatelle, cake and spaghetti (Jnawali et al. 2016).

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In addition general applications used to improve GF pasta and bread such as adding different GF flours, hydrocolloids, proteins, and enzymes was investigated by Padalino et al. (2016). These researchers also explained that dough heating and cooling practices are more important than starch gelatinization and starch retrogradation for GF products technology. Foschia et al. (2016) reported that rice flour 59.3%, maize flour 40.7%, buckwheat flour 22.2%, whole grain maize flour 18.5%, tapioca flour 11.1%, potato flour 7.4%, millet flour 7.4% and quinoa flour 3.7% were used in commercial GF bread formulations. Among these flours, rice flour is especially interesting because of its hypoallergenic properties, bland taste, white color, digestibility and easy availability (Sanchez et al. 2002; Torbica et al. 2010; Foschia et al. 2016).The use of rice flour, corn, and cassava starch to replace wheat flour in the production of free-gluten white bread has been produced (Lopez et al. 2004). Evaluating the physical parameters (crumb appearance, specific volume, and moisture) and the sensorial parameters (flavor, appearance, crumb texture, crust color and satisfaction) they reported that rice flour bread presented the best parameters, followed by corn starch bread and cassava starch bread. They also optimized a mixture of flours that composed of 45% rice flour, 35% corn starch and 20% cassava starch.

Similar results were obtained from GF bread containing some different flours such as rice, husked buckwheat or unhusked buckwheat flour by Torbica et al. (2010). They have explained that the rheological properties of GF bread formulations containing mixtures of these flours by using Mixolab. They have also investigated textural and sensory properties of GF bread formulations. They found that GF products with unhusked buckwheat flour had highest water absorption values, lowest stability, and weakest protein network structure in consisted of husked buckwheat flour.

Addition of starch in GF products is one of the used methods as a replacement for gluten. Starch and its derivates (chemically modified, resistant starches, maltodextrins etc.) are very important for bread making because of their ability to gelatinize, positively affects on bread volume and crumb softness (Naqash et al. 2017). In a study by Sanchez et al. (2002), rice flour, corn starch, and cassava starch were used in GF breadmaking to statistically establish optimal amounts of each ingredient by using a central composite design. According to the obtained results, the optimal GF bread can be prepared from 74.2% corn starch, 17.2% rice flour, and 8.6% cassava starch. In the same study, it was also found that addition of soy flour improved crumb-structure quality of bread. In another study, Korus et al. (2009) showed that the addition of corn resistant starch preparations gave GF bread with less hard crumb than bread without resistant starch addition.

Impact of dietary fibre and pseudocereals on the quality of GF breads and batters

Dietary fibres have a significant role in improving GF bread quality because of it increases the nutritional value of bread and also uses for improving rheological, texture characteristics of dough and sensory properties of final baked products (Gomez et al. 2003; Tsatsaragkou et al. 2016). Saturni et al. (2010) reported that their use in GF diet can help to increase fibre intake in celiac disease patients. Moreover, some researchers have investigated the addition of dietary fibres in GF bakery products formulations. For example, Talens et al. (2017) applied two different orange fibres; one obtained by hot air coupled with microwave drying of orange peels and the other commercially available to effect on texture and sensory properties of GF muffins. With the application of hot air coupled with microwave drying of orange peels, total dietary fibre, water retention capacity, viscosity and viscoelastic properties were higher and resulted in a new alternative for citrus by-products valorisation and transformation into a fibre ingredient suitable for GF baking. In a previous study, some different cereal fibres based on wheat, maize, oat and barley were used to improve the quality, sensory and nutritional properties of GF dough and bread by Sabanis et al. (2009). Results showed that among the dietary fibres maize and oat fibres had significantly affect the loaf volume and crumb softness of GF bread. In the same study, researchers have also found that the addition of wheat fibre resulted in decreased bread volume and a much firmer crumb texture than the control due to the high water binding capacity of this fibre. Similar results were explained in a review by Tsatsaragkou et al. (2016) and they indicated that the each category of dietary fibres such as flours/seeds, isolated fibres/commercial formulations (insoluble and soluble fibres), fruit/vegetable fibres and products alternative flours etc. can be positively affected the final quality of GF product due to their ability to increase bread volume, improve water and gas holding capacity of dough.

In addition, the pseudocereals, which are considered as protein supplementation on GF products, such as amaranth, yellowpea, chickpea and lentil flour, psyllium flour, teff flour (fermented), quinoa flour, dehulled buckwheat flour, and puffed buckwheat flour are often used for GF bakery products because of increasing batter volume, elasticity and shelf life; improving essential amino acids, dietary fiber, fatty acids, mineral and ash content, and baking properties of GF bakery products (Alvarez-Jubete et al. 2010; Houben et al. 2012; Elgeti et al. 2014; Lamacchia et al. 2014; Alencar et al. 2015; Naqash et al. 2017). Because many GF bakery products are made using GF flours or starch and in this way, they do not contain the same levels of B-vitamins, iron and fibre as their gluten-containing counterparts (Alvarez-Jubete et al. 2010). Among these pseudocereals, buckwheat flour was investigated due to it has high nutritional value and health benefits for humans by Mariotti et al. (2013). They have been also used hydroxypropylmethylcellulose (HPMC) on the breadmaking properties of commercial GF bread mixtures and found that the combination of both 0.5% HPMC and 40% dehulled buckwheat flour increased in bread height and specific volume and also decreased significantly crumb hardness of GF bread.

Elgeti et al. (2014) indicated that the addition of quinoa white flour gave GF bread with significantly higher specific volume and homogeneous and finely distributed gas bubbles crumb compared to the typical GF control recipe based on rice and corn flour. Similarly, Alverez-Jubete et al. (2010) pointed out that the quinoa, amaranth and buckwheat flours have been extensively used in formulations of GF products due to their high nutritional properties such as high protein, fiber and mineral content and health-promoting effects. In a study by Alencar et al. (2015) evaluated the temporal profile and instrumental analysis of different GF bread's formulations containing amaranth and quinoa flours and sweeteners. The researchers found that the addition of pseudocereals and sweeteners was shown to be similar

effects on sensory and physicochemical properties of GF bread, compared to starch-based formulations which consisting of potato, cassava and sour tapioca starches.

Application of hydrocolloids and emulsifiers in GF bread

Hydrocolloids, also known as water-soluble gums, are one of the food additives with the intention of improving dough handling properties and resulting on positive effects of crumb structure, taste, acceptability and staling of GF breads due to their ability to increase water retention capacity, rheology, viscosity and texture of dough (Anton and Artfield 2008; Padalino et al. 2016; SAMir et al. 2016; Wang et al. 2017). The GF bread quality is influenced by the presence of hydrocolloids which increase dough rheology and quality of final bread (Houben et al. 2012; Nicalae et al. 2016; Liu et al. 2018).

Among hydrocolloids, cellulose derivates such as carboxymethylcellulose (CMC) and HPMC; guar gum and xanthan gum are extensively used in recipes of GF bakery products (Wang et al. 2017). For example, Sciarini et al. (2010) showed that hydrocolloids such as carrageenan, alginate, xanthan gum, CMC and gelatine increased batter consistencies of GF bread made from rice, corn and soy flours and 158% water. Similarly, Liu et al. (2018) reported that hydrocolloids such as HPMC, CMC, xanthan gum and apple pectin improved the mixing and thermal behaviour of GF potato dough. In particular, they also suggested that the addition of hydrocolloids in the GF potato steamed bread was improved the specific volume, hardness and porosity of the crumb.

GF dough structure is highly affected by the addition of hydrocolloids, such as CMC. Lazaridou et al. (2007) used different hydrocolloids into GF bread made from rice flour, corn starch and sodium caseinate and studied their effect on dough rheology and bread quality. Among the hydrocolloids, they found that CMC and pectin seemed to be the best hydrocolloid improvers of GF bread, at 1% for CMC and 2% for pectin, resulted in bread with significantly increased volumes and high values of crumb porosity and elasticity and also the addition of these hydrocolloids did not alter the firmness of the crumb, and the supplemented bread had high acceptability ratings by consumer panel. Likewise, Buresova et al. (2016) examined that the effect of calcium and sodium caseinate was compared to the effect of xanthan gum and CMC on the behaviour of rice-buckwheat dough and bread quality. At the end of the study, they found that the incorporation of calcium and sodium caseinate could be used as an alternative supplement positively effected of the both rheological properties of rice-buckwheat dough and bread quality.

Hager and Arendt (2013) studied the effects of different gums such as HPMC and xanthan gum and their combination on GF model systems consisting of rice, maize, teff and buckwheat flours using response surface methodology. They showed that with the addition of HPMC and xanthan gum at very low levels contributed to improve bread properties, but might also deteriorate loaf quality. Moreover, Sabanis and Tzia (2011) suggested that 1% and 1.5 % addition of HPMC promoted to increase loaf volume and color than control GF bread and also bread containing 1.5% HPMC was preferred by a trained panel for sensory evaluation. These positive impacts of HPMC on GF bread quality can be explained by because of its moisture absorption ability, gas binding capacity (Houben et al. 2012). In another study, McCarthy et al. (2005) optimized formulation for GF bread based on rice flour containing different levels of HPMC (0.5-2.5% flour/starch base) and the water levels (70-95% flour/starch base) using response surface methodology. They found that the optimized formulation was at the level of 2.2% HPMC and 79% water. They also determined that the increasing water addition considerably effected on bread quality properties and HPMC and water showed significant interactions in their effect on crumb grain structure.

Mohammadi et al. (2014) determined the effects of xanthan gum and CMC on the development of GF flatbread. Evaluating the moisture, firmness, elasticity, crumb and crust color, sensory evaluation, porosity appearance, dough, bread yield and weight loss, they reported that the xanthan gum showed the best bread quality properties as compared to all the samples.

The effects of hydrocolloid addition on rheological properties and breadmaking performance of rice-buckwheat batter at different water levels have been reported (Peressini et al. 2011). In their study, xanthan gum and propylene glycol alginate were added to rice-buckwheat blend (60:40) at levels of 0.5-1.5%. The researchers showed that propylene glycol alginate provided higher quality bread regarding specific volume, crumb mechanical properties and crumb structure than xanthan gum and also it gave promising results for the production of high quality to rice-buckwheat bread.

Another hydrocolloid like Sodium Carboxymethylcellulose (NaCMC) has been tested for rheological properties of GF dough (Nicolae et al. 2016). They found that the addition of 1% NaCMC was the most appropriate dose for a good quality GF product comparable as structure and volume with a standard wheat bread. Contrary to common opinion, Sciarini et al. (2012) explained that additives used in GF bread like emulsifiers, enzymes and hydrocolloids did not improve final bread technological quality and they also showed that the presence of additives is not essential for GF bread production.

Bourekoua et al. (2018) investigated the effect of agar-agar, gum arabic, locust bean gum, tapioca starch and corn starch and their combinations on the quality of GF bread. GF bread was made from rice semolina supplemented with field bean semolina and thermal properties of additives and GF bread were evaluated using differential scanning calorimeter (DSC). According to results, they reported that gum arabic was found to be the best additive for producing optimum GF rice-based bread (with 1.5% of gum arabic and 71.5 g/100 g of water).

Morreale et al. (2018) studied with HPMC to understand the role of hydrocolloids viscosity and hydration in developing GF bread. They confirmed that the role of the HPMC in effecting the viscoelastic behaviour of the GF batter and influencing the rheology characteristics of bread by the inclusion of a 2.2% of HPMC 15000 cP with hydration level to 110%. And finally they obtained desirable GF bread regarding crumb hardness, cohesiveness and resilience.

Additives such as emulsifiers have also been used in GF bread for improving bread structure and staling (Houben et al. 2012; Selomulyo and Zhou 2007). DATEM is anionic oil-in-water emulsifiers that are used for improving dough and bread quality by improving mixing tolerance, gas retention and resistance of the dough to collapse (Selomulyo and Zhou 2007).

Demirkesen et al. (2010) conducted studies to show the effect of different gums and emulsifiers on GF bread made from rice flour. In their study, they found that emulsifiers in addition to gums were necessary to obtain the desired physical properties in dough formulations. In another study by the same researchers, they evaluated that the GF bread formulated with different chestnut/rice flour ratio with/without gum blend and emulsifier DATEM using rheological, baking and sensory measurements. They found that the bread containing chestnut/rice ratio 30/70 with an addition of the blends of xanthan-guar and emulsifier had the best quality parameters.

Onyango et al. (2009) studied that the effect of cellulose-derivatives and emulsifiers on creep-recovery and crumb properties of GF bread made from sorghum and gelatinised cassava starch. At the end of their study, they found that emulsifiers strengthened the doughs and decreased crumb firmness and staling rate when compared the control. These effects were most pronounced at 2.4% w/w fwb concentration. Similarly, the effects of some emulsifiers such as lecithin, DATEM, distilled monoglycerides or sodium stearoyl lactylate were studied by Nunes et al. (2009). It was found from their study that emulsifiers have a positive effect on the GF bread. For example, they suggested that the bread containing with distilled monoglycerides at high levels the specific volume reached a maximum improvement as well as reducing significantly the staling rate of the crumb.

Conclusions

The primary focus of this review is to discuss the current approaches used to develop the rheological and baking properties of GF bread. Because people suffering from celiac disease consists in a lifelong GF diet. With this aim different kinds of additives such as different flours, starch, dietary fibre, pseudocereals, hydrocolloids, enzymes, diary ingredients, and emulsifiers have been commonly used for as alternatives to gluten and to improve the properties of GF bakery products. They have been used for diverse purposes like to make GF bread with good bread quality, sensory and nutritional properties available to consumers with celiac disease patients and also to increase the variety of these products. The obtained results have been showed that more research is needed in this area, in particular improving nutritional quality and increasing variety of GF products.

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