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Effect of *Bacillus subtilis* Ch-13, Nitrogen and Phosphorus on Yield, Protein and Gluten Content of Wheat (*Triticum aestivum* L.)

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Abstract: The effects of *Bacillus subtilis* Ch-13 (BS_0 *Bacillus subtilis* Ch-13 not applied and BS_1 *Bacillus subtilis* Ch-13 applied) and chemical fertilizer CF_0 (0 kg N da^{-1}, 0 kg P da^{-1}), CF_{25} (5 kg N da^{-1}, 0.7 kg P da^{-1}), CF_{50} (10 kg N da^{-1}, 1.4 kg P da^{-1}), CF_{75} (15 kg N da^{-1}, 2.1 kg P da^{-1}), CF_{100} (20 kg N da^{-1}, 2.8 kg P da^{-1}) on yield, protein and gluten content of wheat (*Triticum aestivum* L. cv. Pehlivan), grown under natural climatic conditions were investigated.

It is determined that yield, wet gluten and protein content of seed were increased by *Bacillus subtilis* Ch-13 application with increasing chemical fertilizer applications. Those effects of *Bacillus subtilis* Ch-13, nitrogen and phosphorus were found significantly important ($P<0.01$). However, the positive effect of *Bacillus subtilis* Ch-13 on yield decreased with increasing doses of chemical fertilizer. The highest yield (466.2 kg da^{-1}) was obtained from $CF_{100}BS_1$ application. The maximum gluten and protein content have been determined in $CF_{100}BS_0$ (33.5 %) and $CF_{100}BS_0$ (10.6 %) applications respectively.

Key Words: Wheat (*Triticum aestivum* L.), *Bacillus subtilis* Ch-13, chemical fertilizer, wet gluten, protein.

Bacillus subtilis Ch-13, Azot ve Fosfor Uygulamalarının Buğdayda (*Triticum aestivum* L.) Verim, Protein ve Gluten Miktarına Etkisinin Belirlenmesi

Özet: Bu çalışmada *Bacillus subtilis* Ch-13'ün (BS_0 *Bacillus subtilis* Ch-13 uygulanmayan ve BS_1 *Bacillus subtilis* Ch-13 uygulanan) artan dozlarda azot ve fosfor uygulamalarıyla CF_0 (0 kg N da^{-1}, 0 kg P da^{-1}), CF_{25} (5 kg N da^{-1}, 0.7 kg P da^{-1}), CF_{50} (10 kg N da^{-1}, 1.4 kg P da^{-1}), CF_{75} (15 kg N da^{-1}, 2.1 kg P da^{-1}), CF_{100} (20 kg N da^{-1}, 2.8 kg P da^{-1}) doğal iklim koşullarında kuruda yetiştirilen buğday bitkisinde (*Triticum aestivum* L. cv. Pehlivan) verim, protein ve yaş gluten içeriğine olan etkisi araştırılmıştır.

Deneme sonunda artan dozlarda azotlu ve fosforlu gübreleme ve mikrobiyolojik gübre uygulamasının buğday bitkisinde verim ile tanenin yaş gluten ve protein içeriğini istatistiksel olarak önemli düzeyde arttırdığı belirlenmiştir (P<0.01). Ancak mikrobiyolojik gübrenin olumlu etkisi artan dozlarda azot ve fosfor uygulamalarıyla azalmıştır. Denemede en yüksek verim (466.2 kg da^{-1}) $CF_{100}B_1$ uygulamasından, en yüksek yaş gluten ve protein içeriği ise sırasıyla $CF_{100}B_0$ (% 33.5) ve $CF_{100}B_0$ (% 10.6) dozlarından elde edilmiştir.

Anahtar Kelimeler: Buğday (*Triticum aestivum* L.), *Bacillus subtilis* Ch-13, kimyasal gübre, yaş gluten, protein.

Introduction

For optimum plant growth, nutrients must be available in sufficient and balanced quantities. Soils contain natural reserves of plant nutrients, but these reserves are largely in unavailable forms for plants, and only a minor amount is released each year through biological activity or chemical processes. This release is too slow to compensate for the removal of nutrients by agricultural production and to meet crop requirements.

Among the materials used in agriculture, fertilizer is the most widely used. Recently, use of chemical fertilizers is increasing. Because of unconscious and excessive usage of chemical fertilizers, they tend to cause groundwater pollution and cause changes the pH levels of the soil.

Therefore, new alternative systems have been developed for agricultural production to reduce or completely eliminate the negative effects of excessive use of synthetic fertilizers and chemicals in agricultural production on human health and ecological balance.

Quality of agricultural products is influenced by many factors such as maturity, climate, soil, specie and agricultural applications. These factors have directly effect on quality of the soil (Moscatello et al., 1996). Application of organic fertilizers have positive effects as plant germination, root growth, soil suitability for processing and increasing the water holding capacity by improve the physical properties of the soil. Organic fertilizers increase available amount of nutrients by affecting the chemical properties of soil. Also ensures the continuity of productivity by positive impact on soil flora and fauna (İlbaş, 2009).

Microbiological fertilizer usage is one of the new approach in many countries in order to increase the productivity of plants. It has been found that, several bacteria species provide an increase in biomass of plants.-Also these are effective in increasing dry matter, antioxidant enzymes, carotenoids, chlorophyll and soluble protein in various agricultural, industrial and forest plants by improving availability forms of nitrogen (N), phosphorus (P) and potassium (K) in soil (İlbaş, 2009). Microbiological fertilizers have several benefits in agricultural production such as it replaces chemical N and P, stimulates plant growth through increased root formation, activates the soil biologically by stimulating nitrogen fixation and symbiotic plant-microorganism interaction, restores natural soil fertility through the production of organic material and N, K, and P will be produced naturally by the microorganisms, so few soil amendments will not need to be added.

Bread is the most important and basic food in humans diet. While bread has been produced from meals and flours milled from most cereal grains, the type of bread accepted by the customer in the Western World is normally prepared from wheat flour. It is generally agreed that bread marking of flour depends on the quality and quantity of the flour proteins.

The flour proteins milled from common wheat process the unique and distinctive property of forming gluten when wetted and mixed with water. Wheat gluten imparts to doughs made physical properties that differ from those of doughs made from other cereal grains. It is gluten formation, rather than any distinctive nutritive property, that gives wheat its prominence in the diet (Pomeranz and Shellenberger, 1971).

In this study, the effect of *Bacillus subtilis* Ch-13 (BS) used in combination with nitrogen and phosphorus on yield, protein and gluten content of wheat is evaluated.

Materials and Methods

The experiment carried out in Uludag University Agricultural Application and Research Center at 2012-2013 and experiment parcels were randomized as 10 x 3 m size. The crops were sown in 50 seed m^2 density with seeder at 2012 November. In experiment, wheat (*Triticum aestivum* L. cv Pehlivan) was used as plant material. The experiment conducted in natural climatic conditions and without irrigation. Meteorological data related to experimental region is presented in Table 1.

Table 1. Meteorological data of the experimental region (Anonim, 2015)

	Long-term mean; 1954-2013		**2012**		**2013**	
Months	**Temperature (C°)**	**Rainfall (kg/m²)**	**Temperature (C°)**	**Rainfall (kg/m²)**	**Temperature (C°)**	**Rainfall (kg/m²)**
January	6.3	86.3	3.0	121.2	7.2	105.6
February	6.2	72.3	3.4	123.5	9.3	95.5
March	8.4	69.8	7.2	89.6	10.9	85.6
April	12.9	64.0	15.2	100.0	13.8	51.8
May	17.6	43.3	17.8	80.6	20.2	26.2
June	22.2	32.6	24.3	3.6	22.6	62.2
July	24.6	16.6	26.7	7.0	24.8	21.5
August	24.2	15.8	25.2	1.8	26.0	1.6
September	20.1	37.8	21.9	16.6	20.4	18.3
October	15.3	68.1	18.6	34.6	13.0	140.1
November	10.7	78.9	6.9	53.3	11.7	68.6
December	7.4	106.0	7.5	178.5	4.8	50.0
Total	175.9	691.5	177.7	810.3	184.7	727
Average	14.7	57.6	14.8	67.5	15.4	60.6

As microbiological fertilizer *Bacillus subtilis* Ch-13 (BS) (Extrasol™) was used in this study. BS sprayed on to seeds 1-2 hours before sowing as 1L/tone seed, its mixed by hand and dried in shade. Chemical and physical properties of microbiological fertilizer and experiment soil are shown in Table 2 and Table 3.

Table 2. Chemical and physical properties of microbiological fertilizer.*

Preparation parameters	Data of analysis			In house standard	Results
	Sample No	(CFU)	Average		
Colony forming units (CFU)of *Bacillus subtilis.* Ch-13 in cm^3	1	1.18x10^8	1.03x10^8	>1.00x10^8	Complies in house standards
	2	0.89x10^8			
Contamination With other microorganism (%)	1	4.3	5.75	<6.0	Complies in house standards
	2	7.2			
pH	1	7.37	7.69	7.00 - 7.50	Increased comparing with house standards
	2	8.02			
Appearance (color.state.smell)	Liquid with dark brown color and special smell		Liquid with dark brown color and special smell	Liquid with color from milk-yellow to dark brown and special smell	Complies in house standards
Volume of 1 sample	1	1000 mL	1000 mL		Complies with label description
Shelf life (storage period)	Stable during 24 months when stored in temperature from +5 to +20°C		Stable during 24 months in temperature from +5 to +20°C		Valid according conditions of storage

* Certificated by Bisolbi-Inter Ltd. Product Name: EXTRASOL -microbiological preparation. Active ingredient: Bacillus subtilis Ch-13. Date of manufacture: 21.04.2011. Date of analysis – 24.05.2011

Soil samples were taken from 0-20 cm depth from the experiment area dried in shade and sieved with 2 mm thin sieve (Kacar, 2009).

Five doses of chemical fertilizer (CF_0, CF_{25}, CF_{50}, CF_{75} or CF_{100}) and two doses of BS (BS_0: *Bacillus subtilis* Ch-13 not applied and BS_1: *Bacillus subtilis* Ch-13 applied) was threated in experiment. Fertilizer application doses are shown in Table 4.

Table 3. Some physical and chemical properties of experiment soil.

Properties	Interpretation		Methods
Soil Texture	Clay		Bouyoucos, 1951
Clay	57 %		
Silt	29 %		
Sand	14 %		
pH	7.6	Slightly alkaline	Richards, 1954
Electrical Conductivity (EC 1:2,5)	281 µS cm^{-1}	Saltless	Richards, 1954
Lime	14.8 g kg^{-1}	Low	Richards, 1954
Organic Material	1.55 %	Very low	Nelson and Sommers, 1982
Total Nitrogen (N)	0.44 g kg^{-1}	Very low	Bremner, 1965
Available Phosphorus (P)	16.72 mg kg^{-1}	Sufficient	Olsen et al., 1954
Exchangeable cations			
Potassium (K)	106 mg kg^{-1}	Low	Pratt, 1965
Calcium (Ca)	4250 mg kg^{-1}	Excess	Pratt, 1965
Sodium (Na)	35.65 mg kg^{-1}	Sufficient	Pratt, 1965
Magnesium (Mg)	987.6 mg kg^{-1}	Sufficient	Kalra ,1998
Available microelements (mg kg^{-1})			Lindsay and Norvell, 1978
Iron (Fe)	6.76	High	
Copper (Cu)	1.48	Sufficent	
Zinc (Zn)	3.12	Excess	
Manganese (Mn)	92.88	Excess	

Table 4. Nitrogen and phosphorus application doses.

	CF_0	CF_{25}	CF_{50}	CF_{75}	CF_{100}
N, kg da^{-1}	0	5	10	15	20
P, kg da^{-1}	0	0.7	1.4	2.1	2.8

Before seeding, 20-20-0 compose fertilizer was applied and, ammonium nitrate (NH_4NO_3; 33 % N) was used at two growth stages in vegetation period of wheat (beginning of tillering and stem elongation) described by Zedox et al (1974).

After plants were harvested (first week of 2013 July), the samples dried at 70 °C, dry weights were determined and plant samples were wet digested by using HNO_3 +H_2O_2 mixture. Nitrogen was determined by the Kjeldahl method (Bremmer, 1965) and protein contents of samples were calculated as factor 5.8 x N % in dry matter according to Jones (1941) and Watt and Merrill (1963). Wet gluten content of wheat seeds was determined by ICC method106 (IACC, 1986).

The experiment was conducted according to randomized block design with three replications. Analysis of variance (ANOVA) of data obtained from this research were analyzed by JMP 9.0.2. and the differences were compared by Least Significant Difference Test (LSD) at alpha 0.05 and 0.001.

Results and Discussion

The effect of BS on yield of wheat is presented in Table 5. According to data, BS applications increased the yield by 10.29 %, 20.84 %, 11.38, % 9.61 % and 0.56 % respectively. Highest yield is obtained from $CF_{100}BS_1$ (maximum dose chemical fertilizer + BS) application and the highest increase on yield is obtained CF_{25} applications by 20.84 % ($p>0.001$).

BS application has led to an increase on yield in all doses of chemical fertilizer. Panhwar et al. (2011) reported that BS strains (PSB9 and PSB16) increased yield of aerobic rice thanks to positive effect of these BS strains on producing organic acids from soil and plant roots. Many researchers reported similar results of BS effect on yield (Young et al., 2003; 2004 and Yao et al., 2007). Meanwhile chemical fertilizer dose increase, positive effect of BS on yield is decreased.

BS transforms the nutrients to bio-available form. In case of the nutrient concentration in soil is sufficient, BS could not work effective, but in nutrient deficiency situations the symbiosis between plant and bacteria is effective. Effect of BS on yield in different chemical fertilizer doses are shown in Table 5.

Table 5. Effect of BS on yield with different chemical fertilizer applications

CF Doses		**Yield (kg da^{-1})**		**Average**
	% Difference	**BS_0**	**BS_1**	
CF_0	10.29	133.73 f	147.50 f	140.61 E
CF_{25}	20.84	266.16 e	321.63 d	273.90 D
CF_{50}	11.38	416.20 b	463.60 a	439.90 B
CF_{75}	9.61	422.56 b	463.20 a	442.88 B
CF_{100}	0.56	463.60 a	466.20 a	462.06 A
Average		340.45 B	372.43 A	
CF Doses			***	
BS			***	
CF Doses x BS			***	

*** $p\leq0.001$ F: 526.2768

Significant effects of chemical fertilizer and BS applications on protein content are shown in Table 6. Fertilization and BS applications increased protein content of wheat grain for all levels (except CF_{100}). Maximum protein content (10.58 %) was determined in $CF_{100}BS_0$ ($P\leq0.001$).

Table 6. Effect of microbiological fertilizer on protein content of wheat in different chemical fertilizer doses

CF Doses	Protein (%)			Average
	% Difference	BS_0	BS_1	
CF_0	9.05	8.17 f	8.91 e	8.54 D
CF_{25}	9.74	8.93 e	9.80 bc	9.39 C
CF_{50}	4.27	9.36 d	9.76 c	9.56 B
CF_{75}	4.00	9.75 c	10.14 b	9.94 A
CF_{100}	-7.80	10.58 a	9.75 c	10.16 A
Average		9.35 B	9.68 A	
CF Doses			***	
BS			***	
CF Doses x BS			***	

*** p≤0.001 F: 31.7039

Protein increases due to N fertilizer have been associated with changes in the distribution patterns of high molecular weight (HMW) and low molecular weight (LMW) proteins (Doekes and Wennekes, 1982; Lásztity et al., 1984; Wieser and Seilmeier, 1998).

BS effect on wet gluten content of wheat is shown in Table 7. The peak level of wet gluten amount (33.5%) reached at $CF_{100}BS_0$ application. Except the highest dose of chemical fertilizer, the increase of wet gluten content by the application of BS is found significantly important (p>0.001).

BS increased wet gluten content by 3.21 % (CF_0), 8.66 % (CF_{25}), 17.06 % (CF_{50}) and 11.74 % (CF_{75}) respectively. Maximum increase of wet gluten content was determined at $CF_{50}BS_1$ application (Table 7). Several authors have demonstrate that there is a significant relation between chemical fertilizers (N-P fertilizers) and wet gluten content (Borkowska et al., 1999 and Wang et al., 2002). Especially increasing levels of N-fertilization leads to increase on protein components, processing quality and wet gluten content of wheat grain (Parades-Lopes et al., 1985; Peltonen and Virtanen, 1994; Achremowicz et al., 1995 and Chengfu el al., 2004).

Under nitrogen (N) limiting soil conditions, application of N fertilizer is known to increase protein content and alter flour functionality in bread wheat. Fewer studies are available on the effects of N fertilizer on quality traits in durum wheat, particularly regarding the effects on extra-strong gluten types. Protein content and gluten strength are two of the major quality criteria used to predict pasta quality of durum wheat cultivars Matsuo et al (1972).

Conclusion

Prices of chemical fertilizers in the world are increasing every day and this situation causes economic difficulties for agricultural production. Even if, chemical fertilizers are not used as excessive amounts in Turkey, the reduction in use of chemical fertilizer provides economic benefits to the farmers; furthermore nitrate leaching might be limited on the agricultural lands. According to the result obtained in this research, it is suggested that chemical fertilizer application doses can be reduced to about 25 % with *Bacillus subtilis* Ch-13 applications in wheat production.

Table 7. Effect of microbiological fertilizer on wet gluten in different chemical fertilizer doses

CF Doses	Gluten (%)			Average
	% Difference	BS_0	BS_1	
CF_0	3.21	24.90 f	25.70 e	25.43 D
CF_{25}	8.66	27.70 ef	30.10 c	27.90 C
CF_{50}	17.06	25.20 ef	29.50 c	27.35 C
CF_{75}	11.74	28.10 d	31.40 b	29.75 B
CF_{100}	-1.50	33.50 a	33.00 a	33.25 A
Average		27.48 B	29.99 A	
CF Doses			***	
BS			***	
CF Doses x BS			***	

*** p≤0.001 F: 84.1928

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