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SUBSTITUTION POSSIBILITY OF SOME BIOFERTILIZERS FOR MINERAL PHOSPHORUS FERTILIZER IN PEA CULTIVATION

H. Ibrahim ERKOVAN[1], M. Kerim GULLAP[1], Kamil HALILOGLU[1], Ali KOC[2]*

[1]AtaturkUniversity, Faculty of Agriculture, Department of Field Crops, Erzurum, TURKEY
[2] OsmangaziUniversity, Faculty of Agriculture, Department of Field Crops, Eskisehir TURKEY
[*]Corresponding Author: erkovan@atauni.edu.tr

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ABSTRACT

The interest in the use of biofertilizer as alternative to mineral fertilizer increase continuously due to increasing mineral fertilizer cost and heavy metal accumulation in the soil such as cadmium.The objective of this study was to assess the effects of four biofertilizer (N_2-fixing, P-solubilizing, N_2 fixing-P solubilizing, commercial biofertilizer) with and without mineral phosphorus fertilizer on yield and quality of forage pea(*Pisumsativum spp. arvense* L.). The application of biofertilizeraffected significantly dry matter yield (DM), crude protein (CP), neutral detergent fiber (NDF) and phosphorus contain. The use of mineral fertilizer increased only dry matter yield. The effect of biofertilizer on pea yield and quality varied significantly depending on year. These results indicated that understanding of factors such as biofertilizer, mineral fertilizer and environment will enable us to use biofertilizer as an alternative to mineral fertilizer to optimize productivity and sustainability of pea production.

Keywords: Biofertilizer, mineral fertilizer, phosphorus, yield and quality, pea

INTRODUCTION

Peas are cultivated widely as rotation or second crops for forage and pulse production in semi arid environments. Both seeds and forages of pea are rich in protein and mineral content (Acikgoz et al., 1985). The productivity of peaslike in the other legume crops are restricted by phosphorus deficiency. Thus, producers rely on mineral phosphorus fertilizer to achieve sustainable production. However, prices of chemical fertilizer increase continuously due to increasing energy cost whichrestricted theirutilization economically. On the other hand, phosphorus fertilizers are not environmental friendly input in agriculture due to cadmium content (Al Fayiz et al., 2007). Recently, there has been interest in more environmentally sustainable agricultural practices (Orson 1996). A considerable numbers of bacteria species that are associated with the rhizosphere are able to exert a beneficial effect on plant growth (Rodriguez and Fraga, 1999). These microorganisms secrete different type organic acid (Illmer and Schinner, 1992), thus lowering the pH in the rhizosphere and consequently dissociate the bound form of phosphate (Rodriguez and Fraga, 1999). Phosphorus biofertilizers also help increase nitrogen fixation and availability of some microelements such as Fe, Zn, etc.Generally, only 0.1% of total P in soil is available to plants (Scheffer and Schachtschabel, 1992). The way of increase to P available to plants is enzymatic decomposition or microbial inoculation (Illmer and Schinner, 1992). Hence, bacteria might be partially substitute chemical fertilizer or they can be used.

Many researchershave isolated nutrient solubilising or fixing microorganism in different soils, plant rhizosphere, root or intercellular spaces of plants (Halder et al. 1990; Illmer and Schinner, 1992; Sahin et al. 2004; Cakmakci et al. 2007) and they are described‘plant growth promoting rhizobacteria’ (PGPR)promoting plant growth either increasing nutrient intake or changes enzymatic or hormone synthesis, even some strains had pathogen control by having antibiotic effect (Xie et al. 1996; Glick et al. 1998; Zaccaro et al. 1999; Stirk et al. 2002). There are many successful examples of bacteria applicationabout increasing yield in clover, wheatgrass, perennial ryegrass (Holl et al., 1988), sugar beet (Sahin et al., 2004), barley (Salantur et al., 2005), chickpea (Elkoca et al., 2008), pea (Osman et al., 2010),hungarian vetch (Yolcu et al., 2012).

Plant growth promoting rhizobacteria changes chemical compounds of the applied plants. In general, PGPR application encourage an increase in crude protein content (Peix et al. 2001; Osman et al. 2010; Yolcu et al. 2012), decreases in cellulosic content (Mishra et al. 2010; Yolcu et al. 2012) and an increase in some minerals such as P, Ca, K(Peix et al. 2001; Elkoca et al. 2010; Osman et al. 2010) but decrease some others such as S, Cu, Zn (Yolcu et al., 2012) in dry matter.

In general, there are currently no adequate knowledgeabout the effect of PGPR on the yield and chemical components of forage peas. Objectives of this study were to determine the effects of phosphorus (with and without) and bacteria application on (1) dry matter yield of pea, (2)feeding quality of forage,(3) mineral contents of forage (4) and substitution possibility of biofertilizes for mineral phosphorus fertilizerapplication in pea cultivation in semi arid conditions.

MATERIALS AND METHOD

The field experiment was conducted at the experimental station of Faculty of Agriculture, University of Ataturk, Erzurum ($39^0 51^I$N and $41^0 61^I$E, 1850 m above sea level). The soil of experimental area was loamy with organic matter content of 1.92%, with lime 4.65% and pH of 7.24. Corresponding available P_2O_5 and K_2O contents were 27.3 kg $ha^{-1}$and 120.0 kg ha^{-1}, respectively.In Erzurum, winters are long and extremely cold and summers are cool, short and arid. Long-term annual mean temperature is 5.0^oC, rainfall is 405 mm and relative humidity is 66.5% in the study area.Total annual precipitation and mean annual temperature were 437.8 mm and 5.8 ^0C in 2009 and 475.9 mm and 7.9 ^0C in 2010, respectively in the experiment years. The monthly distribution of precipitation and monthly average temperature were presented in Table 1.

Table 1. Monthly temperature and precipitation values of experimentalyears (2009 and 2010)and long-term average (1990-2010)

	Mean air temperature ^0C			**Total precipitation (mm)**		
	2009	2010	Long-term	2009	2010	Long-term
January	-12.1	-4.3	-10.6	2.3	52.2	16.7
February	-3.1	-1.8	-9.4	18.8	14.8	20.5
March	-0.7	3.1	-2.8	51.1	82.2	35.2
April	4.3	5.6	5.2	42.3	54.2	60.1
May	10.0	10.4	10.4	43.2	63.6	66.7
June	14.7	15.6	14.8	76.2	50.5	41.9
July	17.2	19.5	19.1	29.2	55.5	24.5
August	17.1	20.3	19.3	22.8	9.0	14.8
September	12.4	17.0	13.9	43.7	8.8	20.2
October	8.7	9.2	7.7	51.0	72.2	44.1
November	1.8	1.8	-0.2	41.4	0.0	28.1
December	-1.1	-1.9	-7.2	15.1	12.9	22.8
Total/Mean	5.8	7.9	5.0	437.1	475.9	395.6

The experiment was arranged a randomized complete block design with three replications. Treatment consist of 0 or 50 kg P_2O_5 ha^{-1}, which suggested doses of phosphorus fertilizer in annual legumes cultivation in the region (Serin and Tan 2011). Triple supper phosphate form of the phosphorus fertilizer were used and five different type biofertilizers were (a) control (C), (b) N_2-fixing (NF) (*Bacillus subtilis* OSU-142), (c) P-solubilizing (PS) (*Bacillus megaterium* M3), (d) N_2 fixing-P solubilizing (NF+PS) (*Burkholderia cepacia* GC sup.B) and (e) commercial biofertilizer (CB) (Bio-one) was developed by Texas University which contain *Azotobacter vinelandi* living aerobic condition and *Clostridium pasteurianum* living anaerobic condition.

The biofertilizer were applied sterilized seeds before sowing and phosphorus fertilizer was broadcasted plots surface before sowing and it was incorporated into soil using hand harrow. Forage pea (*P. sativum ssp. arvense* L. cv Taskent) was sown by hand with 100 seeds per m^2 (Uzun and Acikgoz 1998) in May 20^{th} 2009 and May 15^{th} 2010. The plot size was 1.5 m by 5 m = 7.5 m^2, consisting of 5 rows spaced 30 cm apart. Weed control was done by hand hoeing in the beginning of June. The plots were irrigated 3 times in 2009 and 2010 with flooding system when plant colour turns dark green due to lack of moisture in the soil during the growing season.

Harvesting was performed after taking out one row from each side of the plots and a 0.5 m area from beginning or end of each rowin July 29^{th}2009 and August 03^{th} 2010. Dry matter yield was determined in cutting samples at the pod filling stage and samples were oven-dried at the 68 ^0C until reaching a constant weight. After weighting, samples were grounded to pass through a 2 mm sieve and analysed for chemical characteristics.Total N content of the samples was determined by the Kjeldahl method and multiplied by 6.25 to give the crude protein content (Jones 1981). Neutral detergent fiberand ADF content were measured using an ANKOM fiberanalyzer following the procedure described by Anonymous(1995). Mineral content (Ca, P, K andMg) was determined using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) (Mertens 2005).

All data were subjected to analysis of variance usingthe Statview package (SAS Institute1998). Means were separated using Duncan's multiple range tests.

RESULTS

Both phosphorus and biofertilizer application affected dry matter yield significantly but the differences in dry matter yield between years were non-significant(Table 2).Optimum phosphorus doses increased 0.50 t ha^{-1}in dry matter yield. In biofertilizer application, the highest dry matter yield was obtained from NF treatment (6.98 t ha^{-1}).Commercial biofertilizer application also gave statistically similar result to NF.The dry matter yield ofthesetwo treatments (NF+PS and PS) was lower than control (Table 2).Phosphorus fertilizer application increased dry matter yield significantly in the first year,while it was not significant in the second year. This difference was responsible for Y x P interaction (Figure 1a).In the first year, the highest dry matter yields were obtained from both CB and NF treatments but CB had the lowest at the second year.However, NF application had the highest and stable yield increases in dry matter production in both years. The different response of dry matter production to biofertilizer between years conduced Y x BF interactions (Figure 1b).

Table 2. Analysis of variance results with main effects and interactions of biofertilizer and phosphorus fertilizer application on dry matter (DM), crude protein content (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF)

Treatments	**DM (t ha^{-1})**	**CP (%)**	**NDF (%)**	**ADF (%)**
2009	5.71	15.57 B	36.99 B	27.24 B
2010	6.08	18.66 A	46.03 A	34.46 A
Average	**5.89**	**17.11**	**41.51**	**30.85**
P_0	5.60 B	17.40	41.37	31.08
P_{50}	6.18 A	16.83	41.65	30.62
Average	**5.89**	**17.11**	**41.51**	**30.85**
C	5.80 B	17.09 AB	41.58 B	31.88
NF	6.98 A	16.32 B	40.54 B	30.74
PS	5.47 BC	18.56 A	44.49 A	31.34
NF+PS	5.83 C	16.97 AB	40.88 B	31.30
CB	6.38 AB	16.63 B	40.06 B	28.97
Average	**5.89**	**17.11**	**41.51**	**30.85**
Y	ns	**	**	**
P	**	ns	ns	ns
BF	**	**	*	*
Y x P	**	ns	ns	ns
Y x BF	**	**	*	*
P x BF	ns	**	**	**
Yx P x BF	ns	ns	**	ns

ns: non-significant, *: $p<0.05$, **: $p<0.01$.
Means in the same column with different letters are significant.

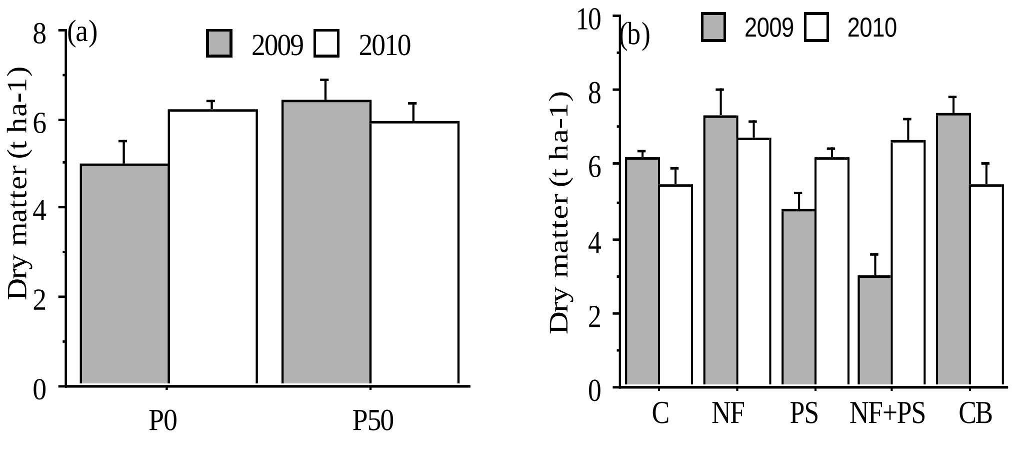

Figure 1. Dry matter yield offorage pea as affected by (a) year x phosphorus, (b) year xbiofertilizer (Bars indicated ± s.e.)

Crude protein content was higher in the second year than in the first year (Table 2). Main effect of phosphorus fertilizer application was insignificant but effect ofbiofertilizer application was significant on the CP (Table 2). Although CP content was higher in all biofertilizer applications in the second year compared to first year results, no significant differences between years was observed in CB applications (Table 2). Thus, Y x BF interaction was significant (Figure 2a). In addition, NF+PS application with phosphorus had an opposite effect. Hence,P x BF interaction was significant (Figure 2b).

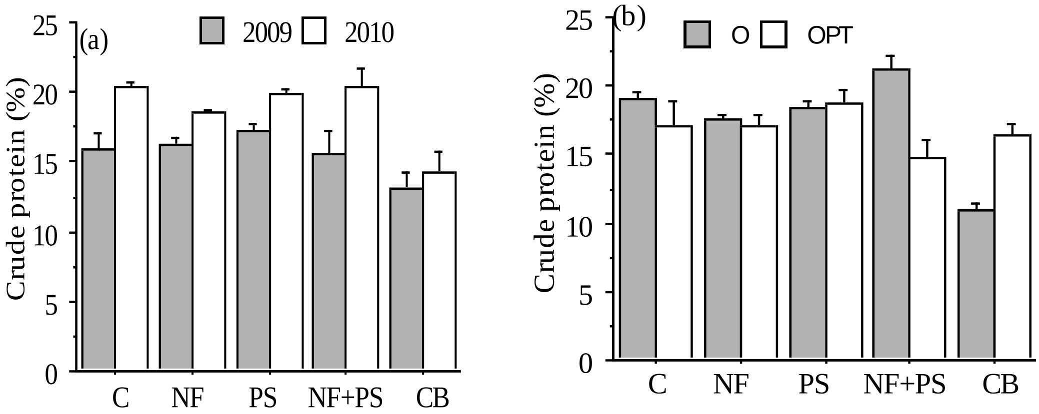

Figure 2. The effect of treatments on crude protein content of forage pea (a) year x biofertilizer,(b) phosphorus x biofertilizer (Bars indicated ± s.e.)

Neutral detergent fiber content was higher in the second year compared to first year results. The main effect of phosphorus fertilizer on NDF content was insignificant. Whereas, the main effect of biofertilizer application was significant(Table 2). Whereas, NDF content harvested PS applied plots was higher than the others. Hence, Y x BF interaction for NDF was significant (Figure 3a).Phosphorus x BF interaction was also significant due to different response of CB to phosphorus fertilizer application (Figure 3b).In addition this interactionsY x P x BF interaction was significant (Figure 3c).

Acid detergent fiber content was higher in the second year than in the first year but main effect of both phosphorus fertilizer and biofertilizer application was insignificant (Table 2).In the first year, biofertilizer applied plots had higher ADF content than alone phosphorus applied plots but in the second year, the highest ADF content was recorded in biofertilizer applied plots. Hence, Y x BF interaction was significant (Figure 4a).Different responses of ADF content to biofertilizer and phosphorus combination caused P x BF interaction (Figure 4b).

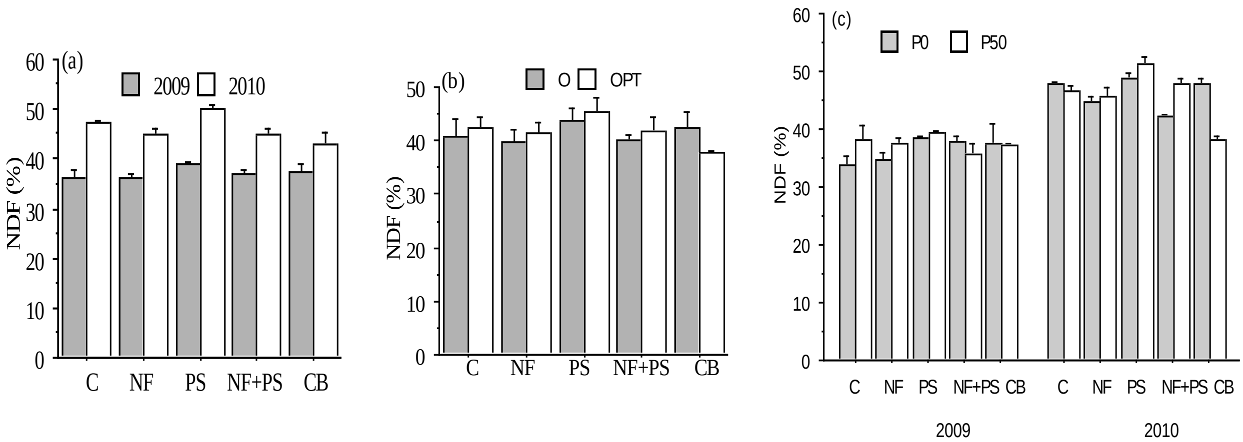

Figure 3.NDF content of forage pea as affected by (a) yearx biofertilizer, (b) phosphorusx biofertilizer, (c) yearx phosphorus xbiofertilizer (Bars indicated ± s.e.)

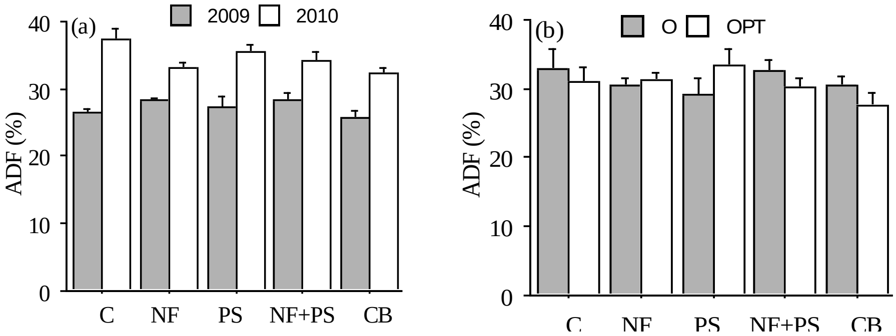

Figure 4. The effect of different fertilizer resources on ADF content of forage pea (a) year x biofertilizer,(b) phosphorus x biofertilizer (Bars indicated ± s.e.)

Year effect was significant on Ca, K and Mg concentrations (Table 3). Calcium content varied from 1.90% to 2.39% and it also varied significantly between the years (p<0.01) (Table 3). Phosphorus content was affected significantly by bacteria application (p<0.05). Commercial biofertilizer and NFapplication causes significant decreases in P content compared to PS application. In the first year, the plant harvested phosphorus fertilizer applied plots had higher P content than untreated plots. But there were no significant differences in the second year. Hence, Y x P and Y x BF interactions were significant for P content (p<0.05)

Table 3. Analysis of variance results with main effects and interactions of biofertilizer and phosphorus fertilizer application on mineral concentration (Ca; Calcium, P; Phosphorus, K; Potassium and Mg;Magnesium)

Treatments	Ca (%)	P (%)	K (%)	Mg (%)
2009	1.80 B	0.29	2.05 B	0.33 B
2010	2.36 A	0.31	3.09 A	0.45 A
Average	**2.13**	**0.30**	**2.57**	**0.39**
P_0	2.19	0.31	2.53	0.38
P_{50}	2.07	0.29	2.61	0.40
Average	**2.13**	**0.30**	**2.57**	**0.39**
C	2.10	0.31 AB	2.25	0.39
NF	1.99	0.28 B	2.45	0.36
PS	2.18	0.32 A	2.70	0.40
NF+PS	2.00	0.31 A	2.67	0.38
CB	2.39	0.27 B	2.79	0.42
Average	**2.13**	**0.30**	**2.57**	**0.39**
Y	**	ns	**	**
P	ns	ns	ns	ns
BF	ns	*	ns	ns
Y x P	ns	*	ns	ns
Y x BF	ns	*	*	ns
P x BF	ns	ns	ns	ns
Yx P x BF	ns	ns	ns	ns

ns: non-significant, *: $p<0.05$, **: $p<0.01$.
Means in the same column with different letters are significant.

(Figure 5a and b).Potassium content was affected significantly among the years (p<0.01). K content was higher in the second year than in the first year. There were no significant effects of CB and phosphorus fertilizer applications on K content in the experiment (Table 3). In the second year, there were no significant effects of CB application on K content, whereas, the hay harvested fromPS, NF and control applied plots had higher K content than the other treatments. As a result of this different response of K content to CB treatment, Y x BF interaction was significant (Figure 6). Neitherbiofertilizer nor phosphorus fertilizer application had significant effect on Mg content but the year effect was significant. The hay harvested in the first year had lower Mg content (0.33 %) than the second year (0.45 %)(Table 3).

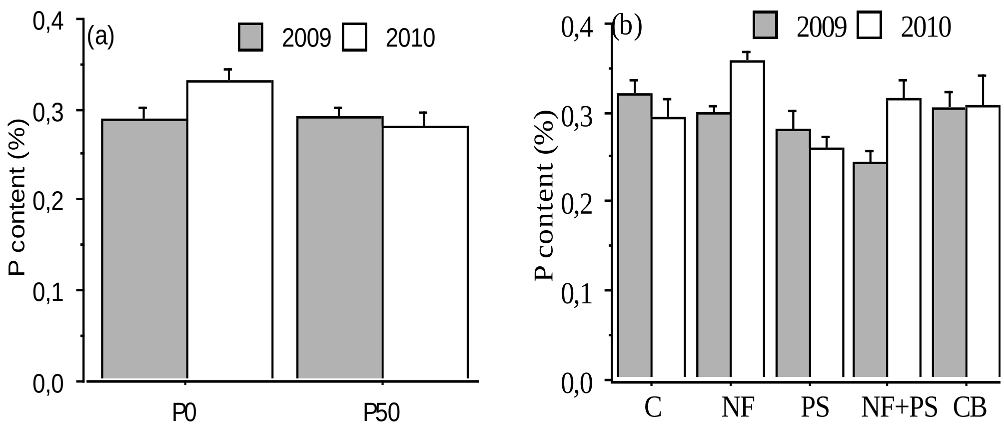

Figure 5.P content of forage pea as affected by (a) yearx phosphorus, (b) year x biofertilizer(Bars indicated ± s.e.)

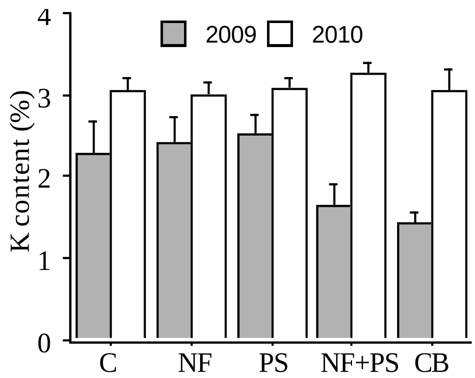

Figure 6. K content of forage pea as affected by (a) year xbiofertilizer(Bars indicated ± s.e.)

DISCUSSION

The data obtained from this study indicated that phosphorus fertilizer and biofertilizer application affected dry matter production of pea crop but there were great differences among biofertilizer treatments. The different response to biofertilizer with respect to dry matter production could be related to microbial content of biofertilizer. Nutrient availability and uptake might be changed as a result of changing rhizosphere microbial activity depending on biofertilizer application. Thus, dry matter production response to treatment must have been changed. Different responses to different microbial inoculant application were reported by the other studies (Elkoca et al. 2008; Osman et al. 2010; Yolcu et al. 2012). Legumes show better response to phosphorus application (Miller and Reetz, 1995; Erkovan et al. 2010).However, while the availability of the nutrient increase in the soil, the response to fertilizer decrease (Read and Ashford, 1968). In the experiment, phosphorus application increased dry matter production significantly in the first year but it was insignificant in the second year. These differences might be originated from microbial content of the experimental soil and the adding biofertilizer cause significantly changes in microbial content ofrhizosphere and as a result of this changes fluctative effect of biofertilizer on dry matter production was recorded due to changing nutrient uptake (Cakmakci et al., 2007). These biofertilizer could attribute to plant growth and yield by providing biologically fixed nitrogen, solubilized immobilized phosphorus and produced phytohormones (Hewedy 1999).

Chemical content of pea crops were affected significantly by years. Higher crude protein content was recorded second year in the experiment.The weather was warmer in the second year than in the first year. Since warmer weather restricted photosynthetic period, carbohydrate accumulation occurs in vegetative tissue as a result of this effect, therefore crude protein content could be higher in the first year. Because initially protein skeleton is constituted in the cell thereafter carbohydrate accumulation occurs (Osman et al., 2010).While sole phosphorus application had no significant effect on CP content, the combine effect biofertilizer and phosphorus fertilizer application varied depending on application combinations. For example, CB plus phosphorus fertilizer applications resulted in significant increases in CP content compared to sole application of CB.Phosphorus fertilizer or PS application causes significant changes in chemical content and it generally increases crude protein and mineral content (Peix et al., 2001). Because biofertilizer supports phytohormones production, which stimulate nutrients absorption as well as photosynthesis process, as a result of this protein content increases (Cakmakci et al., 2007). But phosphorus fertilizer in the experiment did not affect crude protein content. However, PS bacteria causes significantly increase in crude protein content in hay.Crude protein content is generally increasedby existence of phosphorus which plant can intake easily but environmental factors responsible for very wide changes in crude protein content are not fully understood (Reichert and McKenzie, 1982).The protein content of pea appears to be highly variable. Reichert and MacKenzie (1982) found that protein content varied between 14.5 to 28.5% in genetically identical pea plants grown under same conditions such as year and field.

Since the second year was warmer than the first year, the NDF and ADF contentwere higher in the second year in the experiment. P-solubilizing causes increases in NDF and ADF content. These increases might berelated to increases in cellulosic component synthesis in the plant depending on phosphorus supply (Avila et al., 2011). Since warm weather hastened plant growth in the second year, carbohydrate accumulation decreased in the plant, hence, except phosphorus, investigated minerals content were increased in the second year.Warm weather causes generally an increase in cellulosic content (Osman et al., 2010).Commercial biofertilizer application with phosphorus fertilizer conduced a significant decrease in NDF content compared to alone application, whereas the other biofertilizer applications did not show interactive effect with phosphorus treatment. Acid detergent fiber content was higher in NF and PS applied plots fertilized with phosphorus than unfertilized with phosphorus. Especially, PS application with phosphorus fertilizer caused evidently increases in ADF content compared to alone application of it.As plant growth advanced, mineral acquisition decrease and carbohydrate content increase as long as plant growth.These results indicate that there is an antagonistic effect between biofertilizer and mineral fertilizer depending on microorganism species based on NDF and ADF. Similar results reported also by Mehrvarz and Chaichi (2008).

Earlier ceasing of plant growth always causes higher mineral content compared to normal growing plants (Cakmakci et al., 2007). The decrease in P content of phosphorus fertilizer applied plots might be related to acquisition rate of applied fertilizer phosphorus. Under unfavourable conditions phosphorus fixation increase quickly in the soil. However, PS application causes an increase in P content.These results implied that when phosphorus availability increased in the soil, the plants acquired more P, and consequently, P content inplant tissue increased. Similar supportive results were also reported by other researches(Peix et al. 2001; Cakmakci et al. 2007; Dasci et al. 2010). Our findings implied that efficiency of phosphorus fertilizer is strongly related to soil condition that affects availability of phosphorus fertilizer because PS application have positive effect for phosphorus acquisition for plant due to increase availability of phosphorus.

In conclusions, inorganic phosphorus application had slightly positive effect on hay production in forage pea under Erzurum ecological condition (a high altitude semi arid environment). The effect of mineral phosphorus fertilization closely related to availability rather than the applied amount because there was not any increase in P content of plant under mineral phosphorus application, whereas, it was significant increases under PS application. NF and CB application causes an increase in dry matter. Hence, in order to achieve higher hay production in pea cultivation NF inoculation can be suggested. But in the microbial fertilizer except for NF, there is need of more improvement study with respect to increase stability of their effect underdifferent environment because the responses of microbial fertilizers changed depending on years. In these studies, understanding of interaction between microbial fertilizer and soil microbial content will enable us to use microbial fertilizer as an alternative to mineral fertilizer. Because biofertilizer x environment interaction isvery common phenomenon in the microbial fertilizer studies. These results indicated that biofertilizer could be partially but not completely substitute chemical fertilizer in pea cultivation.

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