EFFECT OF ROCK PHOSPHATE AND SUPERPHOSPHATE APPLICATION WITH ORGANIC MANURES ON GROWTH, YIELD AND QUALITY OF POTATO (*Solanum tuberosum* L.) EI-Banna E.N.* and H.Z. Abd EI-Salam**

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ABSTRACT

Two field experiments were carried out at El-Zahraa, Belqas, Dakahlia Governorate, Egypt, during the two summer seasons of 1997 and 1998 on potato cv. Diamant to evaluate the effect of rock phosphate application as a natural source of P in comparison with single super-phosphate (75 kg P_2O_5 for both sources) and four sources of fertilizers, i.e. farmyard manure, livestock manure, poultry manure and mineral fertilization in addition to their interactions on vegetative growth, tubers quality, total tuber yield and the concentrations of N, P and K (%) in both foliage and tubers.

Results indicated that application with rock phosphate increased significantly both fresh and dry weight of foliage plant in the first season and average of tuber weight in both seasons. On the other hand, plant height, number of main stems / plant, total tuber yield ton/fed., tuber dry matter, starch content in tuber, content of N, P and K in foliage and tuber in both seasons were not affected by phosphorus sources (rock or super-phosphate).

Mineral fertilization increased significantly plant height, tuber dry matter, content, N and K content in foliage in both seasons, while application of farmyard manure increased average of tuber weight in both seasons. Results also indicated that applying either farmyard manure or mineral fertilization increased significantly the total tuber yield in both seasons. The interaction between rock phosphate and farmyard manure increased total tuber yield and average tuber weight in both seasons.

Generally, using rock phosphate, or super phosphate together with farmyard manure at the rate of 15 ton/fed. in addition to 180 Kg N + 96 kg P_2O_5 /fed. increased total yield of tubers / fed., but economically application of rock phosphate would save a considerable amount of phosphate fertilizers and consequently would decrease the total costs/fed. as compared with super-phosphate fertilizer. In the meantime, the price of one ton of rock phosphate powder (Abo-Tartour rook 26-28.5% P_2O_5) evaluate between 90-110 L.E., whereas the price of one ton of super-phosphate is about 300 L.E. Moreover, application of rock phosphate together with organic manure may help in decreasing the pollution of environment.

INTRODUCTION

Potato (Solanum tuberosum L.) plant needs more phosphorus for growth and tuber development. This investigation was done to apply a nature source of P (rock Abo-Tartour) to know its effect on growth, yield and some tuber properties, i.e. N, P, K, starch and dry matter contents at harvest time, in addition to estimate N, P and K in foliage at 90 DAP.

Generally, rock phosphate is considered as a poor source of phosphorus for field crop in neutral or alkaline soils, but it is more available when added with organic manures and so in acid soil (Cooke, 1956). The soil microorganisms can play an important role in improving plant growth by releasing phosphorus from rock phosphate (Hauka *et al.*, 1990; Kandeel *et al.*, 1991 and El-Nagar, 1999). Hamail (1992) reported that the mineral fertilization was more effective on vegetative growth, i.e. plant height, number of main stems / plant and fresh and dry weight / plant as well as total tuber yield and tuber dry weight.

Mishra *et al.* (1981) reported that tubers yield, tuber dry matter content, N, P and K contents in the crop (tuber + shoots) were not affected by phosphorus source (rock phosphate or superphosphate), and they found that mixture of rock phosphate and pyrites was more effective than superphosphate on tubers yield, and the results indicated that mixing rock phosphate with pyrites substantially enhanced dissolution of rock phosphate in soil through the actions of H₂SO₄ in oxidation.

Phosphorus sources application increased tuber dry matter content, as well as, total tubers yield, (Mishra *et al.*, 1983; Sud and Negi (1991), Whereas, Hellums *et al.* (1992), did not find significant differences between rock phosphate and single superphosphate on potato tubers yield.

Organic manures such as farmyard manure (FYM), livestock or chicken manure and poultry manure (PM) contribute in plant growth through their effect on physical, chemical and biological properties of the soil.

Sahota *et al.* (1984) reported that application of FYM was indispensable for optimal production and increased the availability of P from the soil. Kaloosh and Koreish (1995) demonstrated that adding checken manure and biofertilizers improved the soil characteristics and soil productivity. Similarly, El-Nagar (1996) mentioned that applying organic manures improved soil structure, areation, retention of moisture and was a good source of essential nutrients and micronutrients as well as its profoundly effect of microflora organisms activities.

Applying organic manures (farmyard manure and poultry manure) increased vegetative growth parameters and yield of tubers as a reflection of increasing of tuber weight (Sujahta and Kirshnappa, 1995 and Ashour and Sarhan, 1998). Sharma and Arora (1987) reported that the application of FYM increased tubers yield as a result of increasing in number of large-sized tubers.

Dass *et al.* (1991) studied the effects of application of rock phosphate and superphosphate in combination with poultry manure and farmyard manure on groundnuts and found that using various sources of P with organic manures were more effective on total yield.

Singh *et al.* (1996) reported that the application of 15 ton FYM / ha + 100 kg P_2O_5 was more effective on tuber yield than using FYM alone, they also added that N, P and K contents in potato plant (shoots + tubers) were also increased with increasing the amounts of FYM and P.

This subject has recently became more important because of the rising costs coupled with growing demands for phosphate fertilizers, particularly in the developing countries.

MATERIALS AND METHODS

This investigation was carried out during the two successive summer seasons of 1997 and 1998 on potato cv. Diamant. Potato tuber seeds were sown on 10th and 14th February 1997 and 1998, respectively at El-Zahraa, near Mansoura, Dakahlia Governorate, Egypt. Some of physical and chemical properties of the experimental soil were as follow in Table (1):

Table 1: Physical and chemical characteristics of the experimental soil.

Physical cha	racteristics	Chemical characteristics								
At depth 0-40 cm										
Sand	33.70 %	PH	7.9							
Silt	23.18 %	Organic matter	2.4 %							
Clay	38.12 %	Total N	0.19 %							
Texture	Clay loam	Available P	42 ppm							
		Available K	360 ppm							

These nutrients of the studied soil are adequate of crops production, according to Hamissa *et al.* (1993). The experiment was in a split plots system of randomized block design with three replications to illustrate the effect of two P_2O_5 sources, i.e. rock phosphate (Abo-Tartour rock 28.13% P_2O_5 , 41.49% CaO, 7.95% F_2O_3 , 2.94% SiO₄, 5.53% SO⁼₄, 0.63% AlO₃ and 0.7% MgO)* and calcium superphosphate (15.5% P_2O_5) with organic and mineral fertilizers in addition to their interaction on growth, tuber quality, yield and NPK concentration in foliage and tubers.

The experimental treatments were as follow:-

- 1- N + K₂O (180 + 96 kg/fed.).
- 2. Farmyard manure.
- 3. Livestock manure.
- 4. Poultry manure.

with rock phosphate and superphosphate (75 kg P₂O₅ /fed.).

Table 2: Organic manure contents and application rates*.

Organic	N% 1997 1998		P%	6	ł	۲%	Rate / fed. (ton)		
Manure			1997	1997 1998		1998	1997	1998	
Farmyard manure	1.20	1.15	0.36	0.40	0.90	0.80	15.00	15.65	
Livestock manure	1.40	1.35	0.25	0.20	1.10	1.20	12.86	13.33	
Poultry manure	2.20	1.90	0.20	0.22	1.15	1.25	8.18	9.47	

* According to Egyptian Fertilizer Development Center (EFDC) analysis, Talkha, DK, Egypt (1997 & 1998).

Organic manures and the two sources of phosphorus were added during soil preparation, while the mineral fertilizers (N + K_2O) were added at three portions during the first half of growing season. The normal cultural practices

were applied according to the Ministry of Agriculture recommendation. The plot area was 11.25 m² (3 ridges each with 5 m long, 0.75 m width and 0.25 m apart).

Plant height (cm), number of main stems / plant and foliage fresh and dry weight were recorded at 90 days after planting (DAP) in a representative samples of 6 plants from each plot. N, P and K contents (%) in foliage at 90 DAP and in tubers at harvest were determined.

Foliage and tubers parts were oven dried at 70°C and then wet digested using sulphoric and perchloric acids mixture according to Chapman and Pratt (1961). Nitrogen was determined by micro-kjeldahl method. Phosphorus was determined colorimetrically as described by Jackson (1967). Potassium was determined by using a flame photometer (Jackson, 1967).

At harvest time after 115 DAP, yield parameters. i.e. tubers yield / fed., average of tuber weight and dry weight of tuber %. Starch content % in tuber were determined according to A.O.A.C. method (1970).

Data were statistically analyzed according to Snedecor and Cochran (1980), and means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Vegetative growth:

Results in Table (3) show that plant height and number of main stems / plant in the two seasons, as well as foliage fresh and dry weight / plant in the second season were not significantly affected by phosphorus sources. Foliage fresh and dry weight / plant in the first season increased significantly by rock phosphate application, this increasing might be due to the contents effect of rock phosphate (CaO, F_2O_3 , SiO₄, SO₄, AlO₃ and MgO), which help in increasing metabolites such as plant growth-promoting substances.

Concerning the effect of organic and mineral fertilization, data indicate that number of main stems / plant, foliage fresh weight / plant and foliage dry weight / plant were not significantly affected by organic manures or mineral fertilization in both seasons, while plant height increased at both seasons by application of mineral fertilization. Similar results were reported by Kandeel *et al.* (1991) and Hamail (1992), who found that using of mineral fertilization was more effective on vegetative growth parameters. The interaction between treatments were insignificant for all vegetative growth parameters in both seasons as shown in the same table (Table 3).

Characters	Plant height (cm)		No. of ste /pla	i main ms ant	Foliage weight (g	e fresh / plant m)	Foliage dry weight / plant (gm)		
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	
Phosphous sources:									
Rock phosphate	45.11 a	43.46 a	3.45 a	3.11 a	260.9 a	293.9 a	35.67 a	35.10 a	
Super phosphate	46.60 a	42.74 a	3.39 a	3.03 a	246.2 b	291.9 a	32.53 b	34.16 a	
Organic & mineral F.:									
N + K [*]	49.97 a	44.74 a	3.83 a	3.17 a	263.1 a	298.0 a	35.93 a	36.96 a	
Farmyard manure	44.64 b	43.83ab	3.50 a	3.11 a	256.2 a	296.0 a	34.19 a	35.53 a	
Livestock manure	44.00 b	1.00 b 42.36bc		.33 a 3.06 a 24		291.5 a	33.69 a	33.50 a	
Poultry manure	42.81 b	42.81 b 41.72 c		3.95 a	245.7 a	286.5 a	32.59 a	32.79 a	
Interactions:									
Rock phosphate with:									
N + K*	46.44 a	45.82 a	3.78 a	3.22 a	269.0 a	302.3 a	37.10 a	37.72 a	
Farmyard manure	45.67 a	44.78 a	3.78 a	3.22 a	263.6 a	300.3 a	35.71 a	37.00 a	
Livestock manure	44.89 a	42.00 a	3.45 a	3.00 a	265.9 a	291.0 a	35.66 a	33.25 a	
Poultry manure	43.44 a	41.22 a	3.78 a	3.00 a	254.2 a	282.0 a	34.20 a	32.36 a	
Super phosphate with:									
N + K (control) *	53.50 a	43.11 a	3.89 a	3.11 a	257.2 a	293.7 a	34.76 a	36.19 a	
Farmyard manure	43.61 a	42.89 a	3.22 a	3.00 a	248.8 a	291.7 a	32.66 a	34.06 a	
Livestock manure	43.11 a	42.72 a	3.22 a	3.11 a	241.8 a	292.0 a	31.72 a	33.75 a	
Poultry manure	42.17 a	42.22 a	3.22 a	3.89 a	237.1 a	291.0 a	30.99 a	33.22 a	

Table 3: Effect of phosphorus sources, organic and mineral fertilization and their interactions on vegetative growth during summer seasons of 1997 and 1998 at 90 DAP^{**}.

(180 kg N+ 96 kg k2O)/fed.

DAP = Day after planting.

In the same column, means followed by the same letter are not significantly different at 5% level by Duncan Multiple Range Test.

Total tuber yield and its components:

Data in Table (4) indicate that average of tuber weight increased significantly by application of rock phosphate in the two seasons, this increasing resulted as a reflection of increasing of plant growth. On the other hand, total tubers yield / fed., tuber dry weight (%) and tuber starch content were not affected by the source of phosphorus, these results agreed with Mishra *et al.* (1981), Sud and Negi (1991) and Hellums *et al.* (1992), who found insignificant differences between rock phosphate and super phosphate on dry matter of tuber and total tubers yield.

With respect of the effect of organic and mineral fertilization on tuber yield and its component, data in Table (4) indicated that both total tubers yield and average tuber weight increased significantly by application of either farmyard manure or mineral fertilization. This was true in the two seasons. This result is in accordance with that obtained by Sahota *et al.* (1984), Sharma and Arora (1987), Sujahta and Kirshnappa (1995) and Ashour and Sarhan (1998). Concerning the positive effect of farmyard manure on total tuber yield and average of tuber weight, this might be due to the role of organic manures in improving soil structure, aeration, retention of moisture, good source of essential nutrients and micronutrients as well as soil productivity (Kaloosh and Koreish, 1995 and El-Nagar, 1996).

Tuber dry matter percentage increased significantly by application of mineral fertilization in the two season as well as farmyard manure in the 2nd

season. These results are in agreement with Kandeel *et al.* (1991) and Hamail (1992), who reported that the mineral fertilization was more effective on tuber dry weight. On the other hand, starch content in tuber was not affected significantly by neither mineral fertilization nor organic manures in both seasons.

Data presented in Table (4) also reveal that mixture of farmyard manure together with rock phosphate and so mineral fertilization at rate of (180 kg N + 75 Kg P_2O_5 + 96 kg K₂O/fed. as a control) gave higher total tubers yield / fed. than the other treatments in the two seasons. Mixture of rock phosphate with farmyard manure was more effective than other treatments in both seasons. This superiorty might be due to that mixing farmyard manure with rock phosphate substantially enhanced dissolution of rock phosphate in the soil through the action of microorganisms which can play an important role in releasing phosphorus and other nutrients from rock phosphate and consequently increased the average of tuber weight and total tubers yield (Hauka *et al.*, 1990 and El-Nagar, 1999). Similar results were reported by Mishra *et al.* (1981); Dass *et al.*, (1991) and Singh *et al.* (1996). Percentage of tuber dry weight and tuber starch content were not affected by the interaction between phosphorus sources and other treatments in both seasons of 1997 and 1998.

Table 4: Effect of phosphorus sources, organic and mineral fertilization
and their interactions on tuber yield / feddan, average tuber
weight, tuber dry weight % and tuber starch content % during
summer seasons of 1997 and 1998 at harvest.

Characters	То	otal	Ave	rage	Tul	ber	Tuber		
	Tubers	s yireld	tuber weight		dry w	eight	starch content		
	(ton/	/fed.)	(g	m)	(%	6)	(%)		
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	
Phosphous sources:									
Rock phosphate	12.88 a	12.71 a	86.25 a	90.28 a	22.38 a	22.61 a	16.29 a	16.35 a	
Super phosphate	12.62 a	12.39 a	83.31 b	84.62 b	22.33 a	22.74 a	16.22 a	16.38 a	
Organic & mineral F.:									
N + K*	13.10 a	12.89 a	83.57 b	87.26 b	23.13 a	23.16 a	16.43 a	16.52 a	
Farmyard manure	13.36 a	12.90 a	88.43 a	91.55 a	22.42ab	23.01 a	16.16 a	16.45 a	
Livestock manure	12.65 b	12.58ab	85.50 b	87.98 b	22.47ab	22.70ab	16.29 a	16.40 a	
Poultry manure	11.90 c	11.83 b	81.62 b	83.01 c	21.42b	21.84 b	16.13 a	16.08 a	
Interactions:									
Rock phosphate with:									
N + K*	12.99 b	12.66ab	84.70 b	87.81 c	23.13 a	22.92 a	16.36 a	16.47 a	
Farmyard manure	13.72 a	13.18 a	90.56 a	95.74 a	22.53 a	23.00 a	16.24 a	16.57 a	
Livestock manure	12.83 b	12.86ab	87.74ab	92.09 b	22.67 a	22.75 a	16.40 a	16.33 a	
Poultry manure	11.99 c	12.14 c	81.98bc	85.46cd	21.20 a	21.77 a	16.14 a	16.03 a	
Super phosphate with:									
N + K (control) *	13.21 a	13.12 a	82.44 b	86.71cd	23.13 a	23.40 a	16.50	16.57 a	
Farmyard manure	13.00 b	12.62ab	86.29ab	87.36cd	22.30 a	23.02 a	16.08 a	16.33 a	
Livestock manure	12.47 b	12.30bc	83.26 b	83.87de	22.27 a	22.65 a	16.19 a	16.47 a	
Poultry manure	11.80 c	11.52 d	81.26bc	80.56 e	21.63 a	21.90 a	16.11 a	16.13 a	

* (180 kg N+ 96 kg k₂O)/fed.

In the same column, means followed by the same letter are not significantly different at 5% level by Duncan Multiple Range Test.

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3. N, P and K concentrations:

Data in Table (5) show that N, P and K in both foliage and tuber were not affected by the source of phosphorus in both seasons. These results are in agreement with those reported by Mishra *et al.* (1981).

The percentage of N and K in foliage were affected significantly by mineral fertilization in both seasons, this result may be due to that the nutrients in mineral fertilizers are directly available and quick release to the plant roots in comparison with organic manures (Cooke, 1972).

Results in Table (5) indicate that the percentage of P in foliage at 90 DAP as well as N, P and K in tuber at harvest were not significantly affected by mineral or organic manures fertilization in both seasons.

The interaction between phosphorus sources and both organic manures and mineral fertilization had no significant effect on the percentage of N, P and K in both foliage at 90 DAP and tubers at harvest in the two seasons of 1997 and 1998.

CONCLUSION

This investigation indicate that application of rock phosphate at the same rate of superphosphate (75 kg P_2O_5 /Fed.) with farmyard manure (at rate of 15-15.65 ton/Fed.) is more effective to obtain the maximum tubers yield. On the other hand, applying rock phosphate will save the amount of superphosphate and will reduce, relatively, the total costs / Fed., Moreover, using both rock phosphate and organic manures together in potato production will reduce the pollution of environment.

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تأثير إضافة صخر الفوسفات والسوبر فوسفات مع الأسمدة العضوية على نمو وجودة محصول البطاطس

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أجريت تجربتان حقليتان في الزهراء – بلقاس – محافظة الدقهلية- مصر - خلال موسمى الزراعة الصيفي ١٩٩٧م ، ١٩٩٨م على نبات البطاطس صنف ديامونت لتقيم تأثير إضافة صخر الفوسفات كمصدر طبيعى للفوسفور مقارنة بالسوبر فوسفات الأحادى وذلك بمعدل (٢٥ كجم فو، أو لكلا المصدرين) وأربعة مصادر سمادية هى السماد البلدى- مخلفات الطيور - سبلة الدواجن- السماد معدنى، بإلاضافة إلى تفاعلهم وذلك على النمو الخضرى- جودة الدرنات – المحصول الكلى للدرنات وتركيز ن ، فو، بو (%) في كل من المجموع الخصرى والدرنات .

أوضحت النتائج أن المعادلة بصخر الفوسفات قد أعطت زيادة معنوية فى كل من الوزن الطازج والوزن الجاف للنبات خلال الموسم الأول وكذلك فى متوسط وزن الدرنة فى كلا الموسمين . ومن ناحية آخرى فإن إرتفاع النبات – عدد السيقان الرئسية / نبات – محصول الدرنات الكلى (طن/ فدان) – المادة الجافة بالدرنة – محتوى الدرنة من النشا – محتوى المجموع الخضرى والدرنة من(ن ، فو، بو) فى كلا الموسمين لن نتأثر بأى من مصدري الفوسفات (صخر الفوسفات أو السوبر فوسفات).

وقد أعطى التسميد المعدني زيادة معنوية في إرتفاع النبات – محتوى المادة الجافة بالدرنة – محتوى (ن، بو) في المجموع الخضرى في كلا الموسمين، بينما أدت إضافة السماد البلدى إلى زيادة متوسط وزن الدرنة في كلا الموسمين. أيضا أوضحت النتائج إلى أن إضافة أى من السماد البلدى أو السماد المعدني قد أعطى زيادة معنوية في محصول الدرنات الكلى في كلا الموسمين مقارنة بالأسمدة الأخرى، كما أوضحت النتائج أن التفاعل بين صخر الفوسفات والسماد البلدى أدى إلى زيادة في محصول الدرنات الكلى ومتوسط وزن الدرنة في كلا الموسمين.

بصفة عامة فإن إستخدام صخر الفوسفات أو السوبر فوسفات مع السماد البلدى بمعدل ١٥ طن/ فدان بإلإضافة إلى ١٨٠ كجم ن + ٩٦كجم بو٢ل قد أدى إلى زيادة المحصول الكلى من الدرنات للفدان ، ومن الناحية الإقتصادية فإن إستخدام صخر الفوسفات سوف يوفر كمية كبيرة من الأسمدة الفوسفاتية وبالتالى سيقلل من التكاليف الكلية للفدان بالمقارنة بسماد السوبر فوسفات حيث أن سعر الطن من صخر الفوسفات المطحون (صخر أبو طرطور ٢٦-٥ر٢٨ % فو٢ أه) يقدر بحوالى ٩٠- ١١٠ جنيه مصرى بينما سعر طن السوبر فوسفات حوالى ٢٠٠ جنيه مصرى، أكثر من ذلك فإن المعاملة بصخر الفوسفات مع قرف أن يساعد فى تقليل تلوث البيئة.

Characters	N%					P	%		K%			
	Foliage at 90 DAp		Tub	Tubers		Foliage at 90 DAp		Tubers		Foliage at 90 DAp		bers
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Phosphous sources:												
Rock phosphate	2.15a	2.07a	1.86a	1.83a	0.19a	0.18a	0.17a	0.17a	3.18a	3.17a	1.23a	1.19a
Super phosphate	2.19a	2.11a	1.87a	1.83a	0.21a	0.20a	0.18a	0.18a	3.20a	3.17a	1.25a	1.21a
Organic & mineral F .:												
N + K*	2.33a	2.17a	1.88a	1.85a	0.22a	0.20a	0.17a	0.18a	3.29a	3.24a	1.28a	1.23a
Farmyard manure	2.13b	2.05b	1.85a	1.81a	0.20a	0.19a	0.18a	0.18a	3.15c	3.14b	1.21a	1.19a
Livestock manure	2.14b	2.04b	1.87a	1.83a	0.20a	0.19a	0.17a	0.17a	3.18b	3.17b	1.22a	1.19a
Poultry manure	2.19a	2.11a	1.87a	1.84a	0.18a	0.18a	0.16a	0.17a	3.14c	3.14c	1.25a	1.19a
Interactions:												
Rock phosphate with:												
N + K [*]	2.18a	2.14a	1.87a	1.86a	0.21a	0.19a	0.17a	0.17a	3.28a	3.20a	1.27a	1.21a
Farmyard manure	2.13a	2.02a	1.84a	1.81a	0.19a	0.18a	0.17a	0.17a	3.13a	3.16a	1.20a	1.17a
Livestock manure	2.14a	2.01a	1.86a	1.82a	0.19a	0.18a	0.17a	0.17a	3.18a	3.18a	1.22a	1.18a
Poultry manure	2.15a	2.10a	1.87a	1.84a	0.18a	0.17a	0.16a	0.16a	3.15a	3.13a	1.24a	1.18a
Super phosphate with:												
N + K (control) *	2.27a	2.20a	1.88a	1.85a	0.23a	0.21a	0.18a	0.19a	3.30a	3.27a	1.29a	1.25a
Farmyard manure	2.12a	2.08a	1.86a	1.81a	0.21a	0.19a	0.19a	0.18a	3.17s	3.16a	1.23a	1.21a
Livestock manure	2.14a	2.06a	1.87a	1.83a	0.21a	0.19a	0.17a	0.18a	3.19a	3.16a	1.22a	1.19a
Poultry manure	2.22a	2.11a	1.87a	1.84a	0.18a	0.19a	0.17a	0.18a	3.13a	3.14a	1.26a	1.19a

Table 5: Effect of phosphorus sources, organic and mineral fertilizations and their interactions on the percentage of N, P and K foliage and tubers (at harvest) during summer seasons during summer seasons of 1997 and 1998.

* (180 kg N + 96 kg K₂O)/Fed.

In the same column, means followed by the same letter are not significantly different at 5% level by Duncan Multiple Range Test.