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THE EFFECT OF NITROGEN AND PHOSPHORUS ON THE YIELD AND EXTRACTABILITY OF PROTEIN FROM TRIFOLIUM ALEXANDRIUM

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Abstract. A study was conducted to determine the effect of nitrogen and phosphorus fertilizers on the yield of dry matter and extractability of protein from *Trifolium alexandrium*. Application of nitrogen, phosphorus and nitrogen–phosphorus mixture increased the yield from 118.00 to 124.20, 127.80 and 132.0 ton/ha, respectively. Extractability of protein increased from 1977 to 2269 kg/ha after the application of fertilizers containing nitrogen and phosphorous.

Trifolium alexandrium (Egyptian clover) is an important forage crop of Pakistan. It is cultivated over a large irrigated area during 'kharif' (September–May). The crop occupies approximately 75% of the irrigated area used for forage production and efforts are being made to increase its production with the use of fertilizers.

Tomer[1] reported an increase in the yield of Egyptian clover when nitrogen and phosphorus were applied to the soil. Uehling[2] recommended nitrogen and phosphorus mixture for best yield of Egyptian and Persian clovers. Effects of agronomic factors on the yield of extractable leaf protein from various leaves was reported by Arkcoll and Festenstein.[3] Filimonov *et al.*[4] reported an increase in the level of crude protein when fertilizer was applied to meadow grasses.

The present studies were conducted to find individual and combined effect of fertilizers containing nitrogen and phosphorus on the yield of dry matter and extractability of protein from Egyptian clover.

Materials and Methods

Crop Cultivation. Seeds of Egyptian clover and fertilizers (urea and single superphosphate) were purchased from the local market. Experimental field was divided into 16 plots of 19×5 m measurement for the present study. Four plots each were selected for the application of urea, urea+single superphosphate, single superphosphate and control by randomization. Thus there were four replicates for each treatment.

Crop was sown according to the method of Khan and Khan.[5] Single superphosphate was applied at the time of ploughing at the rate of 56.6 kg P_2O_5/ha, whereas urea (56.6 kg N/ha) was applied immediately after seeding.

First cut was taken after two months of sowing. The regrowths were harvested after an interval of 1 month. A total of 6 cuts were made.

Experimental

An IBP Pulper and the Belt Press[6] were used for the extraction of juice. Proteinous nitrogen (PN) was precipitated by adding an equal volume of 20% solution of trichloroacetic acid (TCA) to the juice and centrifuging at 3,000 rev/min. The supernatent was analysed for non-prteinous nitrogen (NPN) and the precipitate for PN. Protein was also coagulated by heating the juice and then separated by passing the coagulum though a long-cloth bags. The volume of leaf-juice filtrate was measured and the protein concentrate (LPC) was dried at 70–80°C. Proximate analysis of the pulp, residue, LPC and the filtrate was carried out.

Dry matter was estimated by heating the samples at 100±5°C for 20 hr. Nitrogen was estimated by a microkjeldahl method using $CuSO_4$–K_2SO_4–SeO_2 (1:9:0.02) mixture.[7] Fat, fibre and ash were determined by AOAC methods.[8] Sugar present in the filtrate was determined by Fujita and Iwatake method.[9]

Result and Discussion

Effect of Fertilizers on the Yield and Composition of Trifolium alexandrium (*Egyptian Clover*). Egyptian clover responded to the application of fertilizers (Table 1). The yield increased from 118.0 to 124.20 and 127.80 ton/ha when urea and superphosphate were applied at the rate of 56.6 kg N/ha and 56.6 kg P_2O_5/ha respectivley (Fig. 1). Nitrogen and phosphorus fertilizers when applied in conjunction with each other further increased the yield of the crop to

TABLE 1. EFFECT OF FERTILIZERS ON THE YIELD AND COMPOSITION OF *Trifolium alexandrium*.

Treatment	Control						Urea*						Single superphsosphate†						Urea+single superphosphate**					
Cut No.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Crop yield (ton/ha)	22·5	22·5	13·5	15·0	27·0	17·5	22·5	22·5	16·8	16·3	26·1	20·0	22·5	25·0	16·0	18·8	30·5	15·0	25·0	22·5	16·0	16·3	28·5	23·8
Dry matter (%)	10·1	8·4	9·4	10·0	10·3	16·4	10·0	8·4	9·0	10·0	9·8	14·3	10·4	8·7	10·0	10·5	10·1	16·6	10·4	8·6	10·0	10·4	10·0	16·5
Total nitrogen (%)	3·9	3·8	4·7	4·7	3·8	3·1	4·0	4·0	4·8	4·8	4·0	3·1	3·9	3·9	4·7	4·8	3·8	2·5	4·5	4·2	5·0	5·0	4·0	2·6
Fat (%)	9·0	11·3	5·2	5·5	8·7	4·9	11·6	14·1	11·5	5·8	10·3	6·0	9·0	11·1	9·2	5·4	10·0	7·1	10·0	12·0	10·4	5·6	11·7	7·5
Fibre (%)	24·6	25·6	16·7	15·4	29·7	30·0	21·2	23·7	29·5	16·2	23·6	25·1	28·5	30·7	16·0	26·7	28·0	33·0	28·0	26·8	19·8	17·2	28·1	30·5
Ash (%)	12·0	13·7	13·1	13·5	15·8	12·1	13·5	13·5	12·0	13·5	16·2	12·3	16·1	16·3	14·8	14·0	17·4	11·2	15·3	15·5	12·3	13·5	15·9	12·4

* Supplying 56·6 kg N/ha, † Supplying 56·6 kg P_2O_5/ha, ** Supplying 56.6 kgN+56.6 kg P_2O_5/ha.

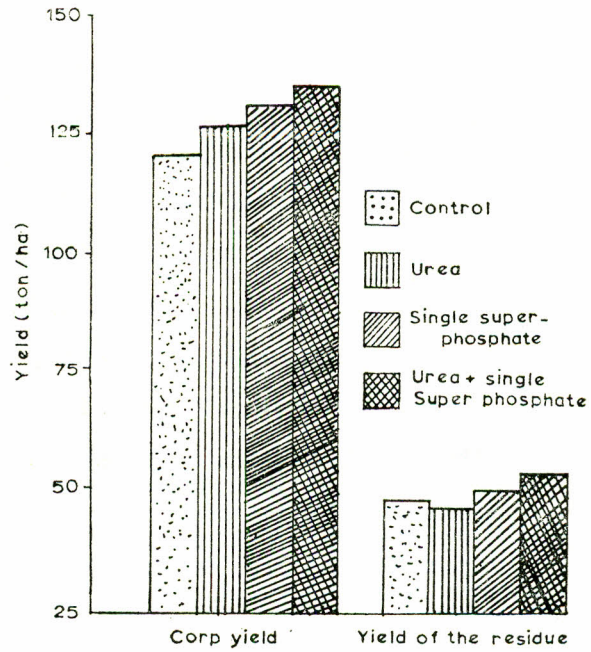

Fig. 1. Effect of fertilizers on the yield of crop and the riesidue (total of 6 cuts).

132 ton/ha. The improvement in the yield of the crop with the application of phosphorus seems to be due to the fact that Egyptian clover is a leguminous plant which have a better response to phosphorus fertilizers than nitrogen fertilizers.[1] Uehling[2] observed a higher yield of Egyptian and Persian clover with the application of nitrogen and phosphorus mixture. Phosphorus is also reported to have a stimulatory effect on the absorption of nitrogen by the roots of Egyptian clover from the soil.[5]

Treatment with nitrogen and phosphorus also affected the composition of the plant (Tabl 1). It is evident from the table that crude protein level of plant increased with the application of nitrogen and nitrogen–phosphorus mixture. Higher protein level with the application of fertilizers has been reported by many workers.[3,4] Arkcoll and Festenstein[3] observed an increase in the protein level of wheat leaves with nitrogen application. A similar increase in the protein content of meadow grasses has been reported by Filimonov *et al.*[4]

Nitrogen, phosphorus or nitrogen-phosphorus mixture when applied also affected the fat, fibre and ash-contents of the plant. The increase in fat contents ranged from 5.8 to 14.1% over the 6 cuts, when nitrogen was applied. In case of phosphorus or a combination of phosphorus and nitrogen the increase in fat contents ranged from 5.4 to 11.1% and from 5.6 to 12.0% respectively. A decrease in fibre content of the first two and 4–6th cuts accompanied by an increase in the third cut was noticed when nitrogen was applied (Table 1). Phosphorus applied alone or in conjunction with nitrogen increased the fibrous and ash-contents of the plant.

TABLE 2. EFFECT OF TREATMENTS ON THE EXTRACTABILITY AND COMPOSITION OF JUICE.

Treatment	Control						Urea*						Single superphosphate†						Urea+single superphosphate**					
Cut No.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Vol. of juice k. litre/ha	12·8	14·4	7·5	8·2	16·4	7·2	13·0	14·5	8·9	8·9	15·9	9·6	12·3	15·8	8·6	10·0	18·6	5·5	14·4	14·4	8·9	9·1	17·5	8·5
Dry matter (%)	6·7	4·9	5·7	6·0	6·0	7·1	6·3	5·1	5·8	6·0	5·9	6·9	6·0	5·1	5·9	6·4	5·4	6·8	6·1	5·2	6·2	6·4	5·4	8·0
Total nitrogen (%)	5·4	6·0	6·2	6·5	6·0	5·0	6·2	6·2	6·6	6·9	6·3	5·5	6·0	6·1	6·5	6·8	6·1	5·0	6·4	6·3	7·0	7·0	6·3	5·0
PN (%)	4·6	5·2	5·6	5·6	5·0	3·8	5·5	5·4	5·9	6·1	5·3	4·3	5·2	5·3	5·8	5·9	5·3	3·4	5·6	5·5	6·3	6·2	5·3	3·5
NPN (%)	0·9	0·8	0·6	0·9	1·0	1·2	0·7	0·8	0·7	0·8	1·0	1·2	0·8	0·9	0·7	1·0	0·8	1·6	0·8	0·9	0·7	0·8	1·0	1·5

*Supplying 56·6 kg N/ha, † Supplying 56·6 kg P_2O_5/ha, **Supplying 56·6 kgN+56·6 kg P_2O_5/ha.

TABLE 3. EFFECT OF FERTILIZERS ON THE YIELD AND COMPOSITION OF PROTEIN COAGULATED (LPC) FROM THE JUICE.

Treatment	Control						Urea*						Single superphosphate†						Urea+single superphosphate**					
Cut No.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
LPC yield (kg/ha)	441·0	371·0	234·4	273·0	437·0	221·0	441·0	387·0	295·0	298·5	438·0	262·4	431·0	431·0	269·0	361·0	495·0	119·0	527·8	378·0	300·0	318·5	492·0	253·0
Dry matter (%)	95·5	95·5	93·8	95·7	95·8	93·4	96·0	95·0	95·4	98·5	93·5	95·5	95·5	95·9	96·2	94·5	92·7	92·8	96·0	95·9	95·0	95·1	93·5	95·6
Total nitrogen (%)	8·8	8·2	10·4	9·2	9·1	8·5	9·9	8·6	10·1	9·7	9·1	9·0	9·0	8·5	9·9	9·2	8·8	8·0	10·8	8·6	10·9	9·7	9·3	8·2
Fat (%)	14·6	15·0	19·0	15·8	17·2	10·1	14·0	17·1	16·9	17·2	15·5	10·0	12·0	14·1	16·5	17·0	17·3	10·2	12·0	14·4	18·9	17·1	16·5	10·0
Fibre (%)	0·8	0·8	1·1	1·0	1·1	1·8	1·0	1·0	1·0	1·0	1·2	1·0	1·5	1·2	1·2	1·3	1·0	1·0	1·1	0·9	1·5	1·2	1·2	1·9
Ash (%)	11·2	5·8	7·2	8·2	10·8	10·4	10·4	5·0	5·7	8·0	10·5	9·7	9·8	5·7	7·1	6·2	9·6	8·5	11·2	8·7	7·4	6·2	10·1	8·9

*Supplying 56·6 kg N/ha, †Supplying 56.6 kg P_2O_5/ha, **Supplying 56·6 kgN+56.6 kg P_2O_5/ha.

TABLE 4. EFFECT OF FERTILIZERS ON THE YIELD AND COMPOSITION OF RESIDUE.

Treatment	Control						Urea*						Single super phosphate†						Urea† single superphosphate**					
Cut No.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Residue yield (ton/ha)	9·2	8·9	5·6	6·2	10·1	8·8	9·0	6·8	6·7	6·3	9·8	8·2	9·0	8·1	7·1	8·0	11·4	7·7	10·0	7·3	7·0	6·9	10·7	12·1
Dry matter(%)	17·0	15·5	15·0	15·6	18·0	25·3	16·0	15·6	15·0	16·8	17·2	24·3	17·5	15·2	15·0	16·0	17·0	27·0	17·0	15·5	15·5	15·0	17·7	25·6
Total nitrogen (%)	2·5	2·8	3·9	3·6	2·5	2·8	2·5	2·7	3·0	3·9	2·4	2·3	2·8	4·0	4·0	3·5	2·8	3·0	2·6	2·6	3·4	4·0	2·8	2·9
Fat (%)	10·0	11·9	10·0	8·5	10·4	5·2	10·0	12·4	10·8	8·6	10·4	5·1	9·9	13·1	10·5	8·7	9·4	5·0	10·0	12·1	10·1	8·5	10·1	5·7
Fibre (%)	25·6	26·3	24·2	25·0	31·2	33·0	23·7	21·8	22·7	24·0	30·8	31·0	30·4	30·2	26·2	30·3	36·0	40·4	26·8	25·5	23·2	26·0	35·2	37·0
Ash (%)	11·3	8·9	9·6	10·5	14·8	8·3	12·4	9·1	10·1	10·9	15·0	10·0	13·0	11·2	10·7	11·6	15·7	7·9	12·5	10·3	11·7	11·1	15·0	9·3

*Supplying 56·6 kg N/ha, †Supplying 56·6 kg P_2O_5/ha, **Supplying 56·6 kgN+56·6 kg P_2O_5/ha.

TABLE 5. EFFECT OF FERTILIZERS ON THE COMPOSITION OF LEAF JUICE FILTRATE.

Treatment	Control						Urea*						Single superphosphate†						Urea† single superphosphate**					
Cut No.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Vol. of filtrate (k. litre/ha)	10·0	12·2	6·0	6·5	14·1	5·9	10·0	12·8	7·1	6·1	13·9	7·0	10·0	14·0	6·9	8·0	16·3	4·3	11·4	12·6	7·1	6·9	15·2	7·4
Dry matter(%)	3·4	3·0	3·3	3·4	3·5	5·3	3·5	3·2	3·3	3·4	3·1	5·0	3·0	3·5	3·5	3·8	3·4	3·4	3·8	3·0	3·6	3·2	3·3	5·8
Total nitrogen (%)	2·5	3·1	2·6	3·0	2·7	3·0	2·3	3·0	2·5	3·0	3·1	3·5	3·0	3·2	3·7	3·5	3·5	3·5	2·5	3·2	3·3	3·1	3·3	5·6
Ash (%)	23·0	27·4	27·0	27·5	28·0	28·7	21·1	25·2	26·1	27·0	27·8	28·1	23·4	25·9	26·7	27·7	28·7	29·0	23·8	26·2	26·5	27·5	27·8	28·4
Sugar(g/100 ml)	0·5	1·0	1·0	1·1	1·5	1·9	0·5	0·8	1·0	1·2	1·3	1·4	1·0	1·1	1·3	1·4	1·7	2·2	0·6	0·8	1·2	1·3	1·5	2·0

*Supplying 56·6 kg N/ha, †Supplying 56·6 kg P_2O_5/ha, **Supplying 56·6 kgN+56·6 kg P_2O_5/ha.

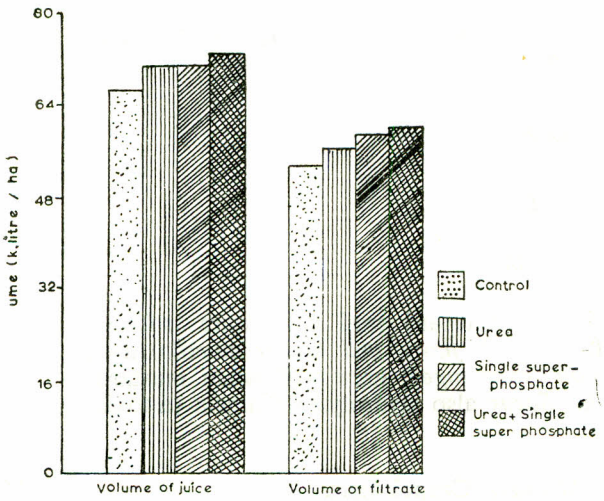

Fig. 2. Effect of fertilizers on the extractability of juice and the filtrate.

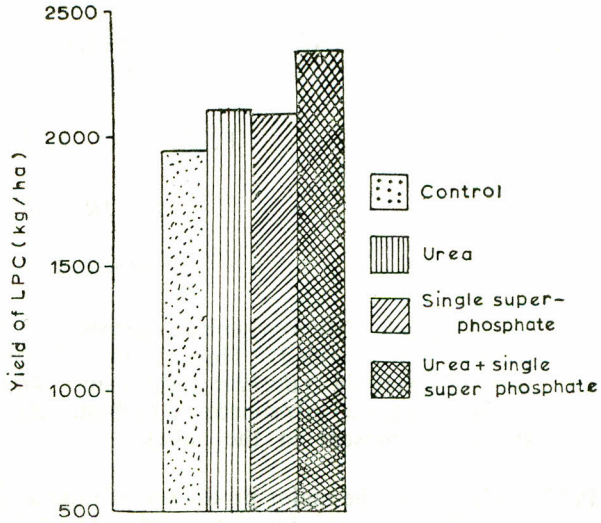

Fig. 3. Effcet of fertilizers on the yield of leaf protein corcentrat.

Effect of Treatments on the Extractability and Composition of Juice. The extractability and composition of juice was affected by the treatment of the crop with nitrogen and phosphorus (Table 2, Fig. 2). Nitrogen treatment resulted in a higher extractability of juice and nitrogenous matter as compared to phosphorus treatment. This seems to be due to the tender texture of the plant as a result of succulent and luxurious growth caused by nitrogen supply.[3] Phosphorus alone or when applied with nitrogen resulted in an increase in the NPN and a decrease in PN contents of juice (Table 2). This decrease in PN of the juice seems to be due to the increase in fibrous contents of the plant (Table 1) which retained the chloroplasts.[6] This view is further supported by the fact that residues from phosphorus-treated plants contained higher percentage of nitrogen than the residue of nitrogen–treated plants (Table 4).

Effect of Fertilizers on the Yield and Composition of the Protein Coagulated from the Juice and the Residue. The yield and composition of the protein coagulated from the juice is presented in Table 3. Treatment of Egyptian clover with nitrogen or nitrogen–phosphorus mixture increased the yield of LPC from 1977 to 2121.9 and 2269 kg/ha respectively (Fig. 3). LPC was found to contain 8–11% nitrogen, 14–19% fat, 5–10% ash and a fairly low amount of fibre (1–2%).

Table 4 shows the yield and composition of the residue left after extraction of the juice. The residue yield in the case of phosphorus, and nitrogen–phosphorus mixture treated-plants was higher than that of nitrogen-treated plants (Table 4, Fig. 1). The residues contained 2.3–4% nitrogen, 5.0–12.4% fat and 7.9–15% ash and can be used in animal feed as such or with some supplementation. The protein contents of phosphorus or nitrogen and phosphorus treated residues were invariably more than nitrogen treated residue. A decrease in the extraction of chloroplastic protein with an increase in the fibre-contents of the plants has been reported by Davys and Pirie.[6] Application of nitrogen+phosphorus or phosphorus alone resulted in an increase in the fibre-contents of the plant (Table 1) and this seems to be the reason for retention of more protein in the residue.

Effect of Fertilizers of Leaf Juice Filtrate. Leaf juice filtrate left after the coagulation of protein contained 2.5–5% nitrogen, 1–2 g/100 ml reducing sugars and fairly large amount of ash (23–29%, Table 5). Filtrates obtained after phosphorus or nitrogen–phosphorus mixture treatment contained higher amount of nitrogen, ash and sugar than those obtained after nitrogen treatment. These filtrates can be used for the propagation of yeast. Propagantion of yeast on leaf juice filtrate was reported by Butt *et al.*[10]

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