

EFFECT OF VARIOUS LEVELS OF NITROGEN FERTILIZER ON NODULATION OF PEA CULTIVARS

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Abstract

A field experiment for two consecutive growing years (i.e., 1999-2000 & 2000-2001) was conducted on four pea cultivars viz., Arkel, climax, green feast and olympia in response to different levels of added N fertilizer under the existing climatic conditions of district Mastung, Balochistan. Seven N fertilizer treatments (T_1 to T_7) were applied @ zero (T_1); 60+40 kg P_2O_5 & K_2O ha⁻¹ (T_2) and 25, 50, 75, 100 & 125 kg N ha⁻¹ (T_3 to T_7) plus a constant dose of 60+40 kg P_2O_5 & K_2O ha⁻¹. These N treatments were applied to each sub-plot (except T_1 & T_2) in two halves. The first one was applied at the time of initiation of flowering, while the 2nd one at the time of pod formation. Results showed that most of the mentioned entries of pea cultivars responded significantly and cultivar Arkel took the top position. Results also showed that applied N fertilizer significantly and linearly reduced all mentioned attribute of nodules. However, applied P_2O_5 + k_2O slightly improved it. Numerically a maximum number of nodules (9.23 plant⁻¹), number of effective nodules (6.67 plant⁻¹), number of ineffective nodules (2.65 plant⁻¹), fresh weight of nodules (5.43 g plant⁻¹), dry weight of nodules (1.78 g plant⁻¹), and moisture content of nodules (35.2 g kg⁻¹) were found in applied fertilizer @ 0.0+60+40 kg NPK ha⁻¹. It was further revealed that all mentioned attributes except moisture contents were highly significant ($p < 0.01$) but negatively correlated with fresh pod yield plant⁻¹.

Introduction

Pea (*Pisum sativum* L.) of the family Papilionoideae is an important pulse legume and nutritive cool season vegetable crop. Nitrogen (N) is the key nutrient, limiting crop production under most situations. A major reason for insufficient N supplies being its presence in soil in organic form which must be mineralized before being used by the plants (Azam, 2001). Therefore, legumes including pea have the capacity to harvest free atmospheric nitrogen (N_2) into ammonia (NH_3) with the help of root hairs invaded by specific bacteria (*Rhizobium leguminosaurum* bv. *viciae*) at the expense of C supplied by the host plant. Thereafter, plant can transform it into useable form of plant nitrogen viz., amino acids and proteins. The process of symbiotic N_2 fixation takes place within plant root nodules. Nodules that are fixing N_2 will be pink to red inside (effective) and those which do not so are yellow to green (non-effective) in color (Anon., 2004 b). Effective nodulation caused either by commercial inoculant or by indigenous soil-bacteria, is generally indicated by vigorous growth of the legumes (Vessey, 2002).

In Pakistan soils have either nil or very low viable count of effective rhizobia and low N-supplying capacity because of low organic matter (0.3-1.0%) levels (Ladha *et al.*, 1996) and approximately 1.0% of the total cultivated legume crops are inoculated (Aslam *et al.*, 1997; 2000). Hence, it is generally recommended that producers should always inoculate their pea seeds with a commercially available fresh inoculant of superior quality.

Research revealed that to maximize the N₂ fixation ability of peas, fertilizer application is not recommended, while phosphate (P₂O₅) and potassium (K₂O) are generally recommended @ 30-40 and 30-60 lb/acre, respectively (Anon., 2004 a). Production guide suggest that somewhere between 30-80% of a pea crop's N needs can be met through biological fixation (Bowren *et al.*, 1986; Ali-Khan & Zimmer, 1989). The rest of the N must be provided from the soil pool or from inorganic fertilizer applications. They also concluded that nodule formation became inhibited as soil N levels approached 35 lb/acre and were progressively inhibited as levels exceeded this amount. Small amount of N fertilizer applications has stimulated nodule formation on pea roots in some low N environments (Oghoghorie & Pate, 1971) and with other pulse crops (Kauskik *et al.*, 1995). The soil of the study area is deficient in available nitrogen, need sufficient amount of additional nitrogen fertilizer for non-leguminous and small amount as starter dose for leguminous crops (Anon., 1995). In Pakistan researchers obtained maximum nodulation and their dry weights in inoculated treatments receiving combined N @ 20-50 kg ha⁻¹ (Agha & Mangi, 1983; Ahmedani, 1983; Khan *et al.*, 1986 a,b; Khan, 1988), beyond this N adversely affected nodulation in soybean. However, few other researchers revealed that legume crop fertilized with NPK resulted in decreased nodulation, their number, weight and leghaemoglobin contents as compared with treatment receiving no fertilizer (Hatam, 1977; Jamro *et al.*, 1990). The study of Vessey (2002) has also shown that there is no need to apply N fertilizer to pea when it is inoculated with a good quality inoculant.

The present study was therefore, mainly envisaged to evaluate the beneficial effect of varying levels of nitrogen fertilizer on various attribute of nodules of four cultivars of peas under the existing climatic conditions of district Mastung. The study was also furnished to select suitable dose of nitrogen fertilizer as well as varietal response in maximizing the nodule parameters.

Materials and Methods

The present field experiment was conducted at district Mastung (situated 30 km south of provincial capital, Quetta). The experiment was initiated during winter (rabi) season for two consecutive years i.e., 1999-2000 & 2000-2001. The experimental plots were laid out in a randomized complete block design (RCBD). Four dwarf and early maturing varieties of pea viz., Arkel, climax, green feast and olympia were grown in the experimental plot. The sub-plots were prepared and experiment was arranged in three replicates having plot size of 5 x 15m². Before sowing, a constant dose of phosphorus (P₂O₅) in the form of triple super phosphate (TSP) and potassium (K₂O) in the form of potash @ 60 + 40 kg ha⁻¹ were applied to each sub-plot (except control T₁). The pre-soaked seeds were sown in rows by hand, keeping space of 50 & 25cm inter and intra, respectively. The varying doses of nitrogen fertilizer @ 25, 50, 75, 100 and 125 kg ha⁻¹ in the form of urea were applied to each sub-plot (except T₁ & T₂) in two halves. The 1st one was applied at the time of initiation of flowering, while the 2nd one at the time of pod formation. These nitrogen doses were later on designated as T₃, T₄, T₅, T₆ and T₇, respectively.

Five randomly selected plants from each cultivar and treatment were carefully uprooted at the early pod-filling stage for their nodule studies. Nodules from root cores of pea plants were picked, counted, washed, oven-dried and weighed to record the following data: (1) Total number of nodules plant⁻¹, (2) Total number of effective nodules plant⁻¹, (3) Total number of ineffective nodules plant⁻¹, (4) Fresh weight of nodules plant⁻¹, (5) Dry weight of nodules plant⁻¹ and (6) Moisture content of nodules (g kg⁻¹). More detail about this experiment has already been explained by Achakzai (2006) and Achakzai & Bangulzai (2005).

Data obtained were statistically analyzed, following the procedure described by Steel & Torrie (1980). MSTAT-C computer software package was used and correlation coefficient (r) studies were also made following the procedure described by Fisher & Yates (1953).

Results and Discussion

Data presented in Table 1 showed that number and weight of nodules plant⁻¹ plus moisture contents of pea cultivars were found slightly to highly significant, whereas, fertilizer responses for all mentioned entries were found highly significant (p<0.01). However, interactions between cultivars and fertilizer stood non-significant for all parameters (except number of effective nodule plant⁻¹).

Number of nodules: The total number of nodule significantly differed among pea cultivars, and cultivar Arkel stood at top position (Table 2) whereas, all N levels significantly produced lesser number of nodules (except in T₂) as compared with their respective control treatment (T₁). Therefore, the present findings are in conformity with the results obtained by most of the researchers (Hatam, 1977; Jamro *et al.*, 1985, 1990; Carr, 2000; Vessey, 2002; Anon., 2004 a), but are in contradiction with the results obtained by few others (Agha & Mangi, 1983; Ahmedani, 1983; Khan *et al.*, 1986 a, b; Khan, 1988). Thus it can be safely concluded that nodule formation became inhibited by receiving any dose of applied N fertilizers. However, P+K alone slightly improved it. Therefore, N application for symbiotic N-fixation is not recommended for existing agroclimatic conditions of Mastung.

Effective and ineffective nodules: Data recorded for number of effective and ineffective nodules (plant⁻¹) deciphered that there was no any statistical difference among cultivars (Table 3-4). However, in response to various levels of added N fertilizers, they were significantly reduced whereas applied P+K fertilizers had slightly but non-significantly increased it. The same was also observed by Beg (1982) on the roots of inoculated treatments. In the present studies though no commercial inoculant was used, but the presence of nodules in general and effective ones in particular indicated that soil was having their own native and effective strain of *Rhizobium leguminosaurum*. Keeping grand mean in view, it was also observed that number of effective nodules was 1.45 times greater than those of ineffective nodules. Soil is a big reservoir of bacteria. In addition to host specific, non-host specific strains of *Rhizobium* also exists in soil pool to occupy a proportion of pea root nodules. Therefore, further studies need to be carried to find out the viable count of the appropriate strain of rhizobia in the existing soil. Hence locally adopted indigenous strains could be highly competitive for nodulation and can also prevent the non-host specific strain occupying a significant proportion of pea nodules.

Nodules fresh and dry weight: Data presented in Table 5 & 6 showed that pea cultivars significantly differed and cultivar Arkel produced maximum fresh and dry weight of nodules (i.e., 3.62 & 1.16 g plant⁻¹), while remaining cultivars non-significantly differed at 5% LSD levels. However, by comparing the mean values of applied N fertilizer, they were significantly and linearly decreased as N levels progressively increased. On the other hand applied dose of P+K without N slightly improved it. These are also in line with Hatam (1977) and are in contradiction with the results obtained by Khan (1988).

Table 1. Analysis of variance (ANOVA) for nodulation characteristics of pea (*Pisum sativum* L.) cultivars in response to different levels of applied nitrogen fertilizer.

S. No.	Nodule characteristics	Sum of squares		Mean squares		F-V value of variables		
		Variety (V)	Fertilizer (F)	Variety (V)	Fertilizer (F)	Variety (V) (DF=3)	Fertilizer (F) (DF=6)	V x F (DF=18)
1.	Number of nodule plant ⁻¹ .	9.556	482.116	3.185	80.353	5.5869*	58.5972**	1.3255ns
2.	Number of effective nodule plant ⁻¹ .	3.258	236.338	1.086	39.390	1.9293ns	54.3460**	1.3925**
3.	Number of ineffective nodule plant ⁻¹ .	1.850	27.495	0.617	4.582	1.5992ns	9.0644**	0.7048ns
4.	Fresh weight of nodules plant ⁻¹ , g.	11.683	183.665	3.894	30.611	9.4103**	33.0161**	0.6735ns
5.	Dry weight of nodule plant ⁻¹ , g.	0.743	18.633	0.248	3.106	12.3531**	52.1930**	1.0846ns
6.	Moisture content of nodules, g kg ⁻¹ .	4.481	78.463	1.494	13.077	17.3231**	39.8346**	0.8250ns

* and ** are slightly and highly significant at p<0.05 and p<0.01, respectively. While ns stands for non-significant at both probability levels.

Table 2. Effect of various levels of nitrogen fertilizer on the total number of nodules plant⁻¹ of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	7.99	8.65	9.73	8.29	8.66 a
T ₂	0:60:40	11.56	8.63	8.45	8.28	9.23 a
T ₃	25:60:40	6.93	8.65	6.53	6.45	6.48 b
T ₄	50:60:40	5.73	4.57	6.14	3.74	5.04 c
T ₅	75:60:40	3.59	4.08	3.67	3.01	3.58 d
T ₆	100:60:40	2.75	2.75	3.45	3.36	3.07 d
T ₇	120:60:40	3.30	3.03	3.19	2.99	3.12 d
Means		5.98 a	5.39 bc	5.88 ab	5.16 c	5.60

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 2.51%, LSD 5% = 0.57 for varieties and LSD 5% = 0.96 for fertilizers.

Table 3. Effect of various levels of nitrogen fertilizer on the total number of effective nodules plant⁻¹ of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	5.71	6.20	7.52	5.79	6.30 a
T ₂	0:60:40	7.84	6.08	6.33	6.43	6.67 a
T ₃	25:60:40	5.17	4.85	4.31	4.73	4.76 b
T ₄	50:60:40	4.32	3.06	4.27	2.68	3.58 c
T ₅	75:60:40	2.76	2.73	2.64	2.45	2.64 d
T ₆	100:60:40	2.31	2.06	2.72	3.03	2.53 d
T ₇	120:60:40	2.16	2.55	2.45	2.37	2.38 d
Means		4.32 a	3.93 a	4.32 a	3.93 a	4.12

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 20.64 %, LSD 5% = 0.57 for varieties and LSD 5% = 0.70 for fertilizers.

Table 4. Effect of various levels of nitrogen fertilizer on the total number of ineffective nodules plant⁻¹ of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	2.27	2.45	2.29	2.09	2.27 ab
T ₂	0:60:40	3.72	2.39	2.19	2.19	2.65 a
T ₃	25:60:40	1.77	1.47	1.72	1.72	1.80 bc
T ₄	50:60:40	1.57	1.51	1.27	1.27	1.55 c
T ₅	75:60:40	1.38	1.57	1.02	1.02	1.28 cd
T ₆	100:60:40	0.88	1.07	0.73	0.67	0.83 d
T ₇	120:60:40	1.37	1.83	1.03	1.20	1.35 cd
Means		1.85 a	1.75 a	1.66 a	1.45 a	1.68

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 42.39 %, LSD 5% = 0.47 for varieties and LSD 5% = 0.38 for fertilizers.

Table 5. Effect of various levels of nitrogen fertilizer on the fresh weight of nodules plant⁻¹ (g) of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	4.99	4.67	5.42	4.65	4.93 a
T ₂	0:60:40	7.12	4.84	5.29	4.54	5.43 a
T ₃	25:60:40	3.77	3.17	2.58	3.00	3.13 b
T ₄	50:60:40	3.15	2.29	2.87	1.69	2.50 bc
T ₅	75:60:40	2.19	1.84	1.49	1.90	1.80 cd
T ₆	100:60:40	1.90	1.40	1.54	1.65	1.62 d
T ₇	120:60:40	2.28	1.54	1.30	0.99	1.29 d
Means		3.62 a	2.82 b	2.92 b	2.60 b	2.98

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 32.14 %, LSD 5% = 0.48 for varieties and LSD 5% = 0.79 for fertilizers.

Table 6. Effect of various levels of nitrogen fertilizer on the dry weight of nodules plant⁻¹ (g) of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	1.44	1.67	1.80	1.49	1.60 a
T ₂	0:60:40	2.13	1.70	1.74	1.57	1.78 a
T ₃	25:60:40	1.26	1.07	1.12	1.19	1.16 b
T ₄	50:60:40	1.09	0.75	0.97	0.57	0.84 c
T ₅	75:60:40	0.74	0.64	0.55	0.59	0.63 d
T ₆	100:60:40	0.65	0.49	0.49	0.57	0.65 d
T ₇	120:60:40	0.85	0.60	0.44	0.34	0.55 d
Means		1.16 a	0.90 bc	1.02 b	0.90 bc	1.01

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 24.07 %, LSD 5% = 0.10 for varieties and LSD 5% = 0.20 for fertilizers.

Table 7. Effect of various levels of nitrogen fertilizer on the moisture content (g kg⁻¹) of nodules of four cultivars of pea (*Pisum sativum* L.).

Fertilizer treatments	N:P: K (kg ha ⁻¹)	Arkel	Climax	Green feast	Olympia	Means
T ₁	0:0:0	29.8	29.5	36.2	31.7	31.5 a
T ₂	0:60:40	45.0	31.4	34.7	29.7	35.2 a
T ₃	25:60:40	28.4	21.0	25.0	22.0	24.1 b
T ₄	50:60:40	20.7	15.4	19.0	11.2	16.5 c
T ₅	75:60:40	14.9	9.4	9.4	13.2	11.7 d
T ₆	100:60:40	12.5	9.1	10.5	10.9	10.7 d
T ₇	120:60:40	13.7	9.9	8.7	6.5	9.7 d
Means		23.5 a	17.9 bc	20.5 b	17.8 c	19.9

Means followed by the same letters are not significantly different at 5% LSD levels. CV = 28.85%, LSD 5% = 0.22 for varieties and LSD 5% = 0.47 for fertilizers.

Table 8. Correlation coefficient (r) of nodules with fresh pod yield of pea (*Pisum sativum* L.).

Variables	1	2	3	4	5	6	7
1.	1.000						
2.	0.957 **	1.000					
3.	0.769 **	0.620 **	1.000				
4.	0.917 **	0.872 **	0.759 **	1.000			
5.	0.924 **	0.897 **	0.727 **	0.944 **	1.000		
6.	0.932 **	0.890 **	0.752 **	0.975 **	0.961 **	1.000	
7.	-0.846 **	-0.363 **	-0.608 **	-0.756 **	-0.811 **	0.790 **	1.000

**Symbol indicating that values are highly significantly correlated at 1% level of significance. Variables (1) number of nodules plant⁻¹, (2) number of effective nodules plant⁻¹, (3) number of ineffective nodules plant⁻¹, (4) fresh weight of nodules plant⁻¹ g, (5) oven dried weight of nodules plant⁻¹ g, (6) moisture content of nodules g kg⁻¹ and (7) fresh pod yield plant⁻¹ g.

Nodules moisture content: Data presented in Table 7 showed that pea cultivars significantly differ with each other and cultivar Arkel produced maximum nodule moisture contents (23.5 g kg⁻¹) followed by cultivar green feast (20.5 g kg⁻¹). However, by comparing the mean values of applied N fertilizer, they were significantly but linearly decreased as the N level increases. Prasad *et al.*, (1989) also recorded that application of phosphorous alone positively affected the moisture contents of nodules plant⁻¹. In this parameter, the trend is same as observed in previous parameters of nodulation. Olson *et al.*, (1981) observed that application of N fertilizer in high levels resulted in linear

decrease in fresh and dry weight of nodules as well as nodule moisture contents of cowpea. Faris & Mohamed (1991) reported that cowpea plant produced low nodule moisture contents when it was subjected to even low level of applied N fertilizers. Therefore, all those observation are in support of the present findings.

Correlation: Correlation coefficient values exhibited that all mentioned attributes of nodule were highly significantly and positively correlated with each other. However, they all were (except moisture content of nodules) also highly significantly but negatively associated with fresh pod yield plant⁻¹. The yield of plant is a dependent variable, depends upon all other growth and yield contributing traits. Therefore, it is generally correlated with all other components. Unfortunately researchers have seriously ignored nodule and their attributes in term of correlation. Few studies also showed that reduction in nodule weight and leghaemoglobin content plant⁻¹ was directly correlated with the reduction in nodule number (Tingey & Blum, 1973). Therefore, present findings are also in line with them.

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