Research Article

Yield and N_2 Fixation of Pea (*Pisum Sativum* L.) as Influenced by Mo and Fe Application in Alkaline Calcareous Soil

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Abstract | A field study was conducted to assess the effect of two levels each of Fe (2.5 and 5.0 kg ha^{-1}) and Mo (1.0 and 2.0 kg ha^{-1}) applied alone or in combination on nodulation, nitrogen fixation, fresh pods yield and nutrient uptake of pea [*Pisum sativum* L.] under silt-loam alkaline soil conditions. Treatments were arranged in randomized complete block design (RCBD) with three replications. The required Fe and Mo as $[(NH_4)_6Mo_7O_{24}4H_2O]$ and $FeSO_4.7H_2O$ respectively were applied to soil followed by thorough mixing before sowing. Both Fe and Mo induced significant increases in fresh pods pea yield and nutrient uptake. Their combined effect was more prominent than their alone application. The maximum fresh pods yield of 5458 kg ha^{-1} and biomass yield of 10315 kg ha^{-1} were obtained with the combine application of high levels of Mo and Fe (2 and 5 kg ha^{-1}) and has also enhanced nodules number by 152 %, fresh weight of nodules by 135 %, N concentration in plant by 29 %, N uptake by 50 %, N_2 fixation by 85 % over the control. Application of Mo and Fe at all levels alone or in combinations also improved soil organic matter, mineral N, P and K status that could be associated with more N_2 fixation, enhanced plant and root growths of plants. These results suggested that application of both Mo and Fe is necessary for pea crop enhanced nodulation, N_2 fixation, yield, nutrient uptake and for improved soil fertility.

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Introduction

Pea (*Pisum sativum, L.*) is an important grain legume crop and is counted the third most significant grain legume of the world after soya bean and common beans (Vaughan et al., 2005). It belongs to the legume family and it has the capability to produce its own nitrogen. Pea requires a well drained loose slightly acid soil (pH 6.0-6.5).

The total area under cultivation of pea in Pakistan was 56200 ha producing 36900 tons of dry pods with an average of yield of 657 kg ha^{-1} during the years 2015-2016. Similarly, the total area under pea in Khyber Pakhtunkhwa province was 1200 ha producing 800 tons of dry pods with an average yield of 667 kg ha^{-1} during 2015-2016. (MINFA, 2015-16). The yield of pea per unit area production is low as compared with the production of USA and Australia as 1658 kg ha^{-1} and 1744 kg ha^{-1} respectively (Johana, 2008) and not enough to link the gap between production and consumption. One of the main reasons for lower production is the use of genetically low yielding varieties which are not well adopted to get maximum benefit from the existing resources (Qasim et al., 2001). The pea cultivars usually cultivated in Pakistan, are low in yield and their quality does not compete with the different varieties grown in developed countries

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(Khokhar et al., 1988). Other problem in growing pea include root rot which can be minimized by rotating the planting site and selecting a site with well-drained soil. For obtaining optimum yield of pea, continuous and well organized efforts are needed for the improvement of pea yield.

Pea crop has the ability to obtain much of its N requirement from air with the help of rhizobacteria in the root nodules. The amount of N fixed varies with the type of pea's cultivar, soil type, the presence of indigenous available N in the soil and other environmental conditions. Under ideal conditions, pea crop can fix as much as 50-80 per cent of its total N requirement from air, with the remaining N coming from soil or fertilizer sources. Sun and Liu (2000) reported that pea crop which formed enough nodules can fix approximately 75 kg N ha^{-1}. Another study revealed that pea seed though did not inoculate with rhizobium, even annual nitrogen fixation with regular precipitation varied from 31 to 107 kg N ha^{-1}, and under drought stress condition it was from 4 to 37 kg N/ha (Carranca et al., 1999). Inoculation with *Rhizobium leguminosarium* increased nitrogen fixation in peas to 50 kg N/ha or more. Therefore, in arid and semi arid agricultural regions, pea cultivation significantly reduced the need of chemical fertilizers which is a good indicator towards the sustainable agriculture development and at the same time it protects environment from pollutions hazards caused by inorganic fertilizers.

Biological N_2 fixation is catalysed by the enzyme nitrogenase that contains both Mo and Fe, vital micro elements for the process of biological N_2 fixation (Brkic et al., 2004). Hageman and Burris (1978) reported that the enzyme nitrogenase contained two proteins: Mo-Fe protein and Fe protein. Both components are required for the activity that leads to a simultaneous reduction of N_2 and H consuming up to 28 moles of ATP per mole of N_2 reduced. The legume-rhizobium association requires integrated compounds of organic and inorganic sources which enhances different enzymatic activities and N_2 fixation (Meagher et al., 1991).

In addition, Mo plays significant role in plant enzymes activities as a co-factor that participates in different reactions in plants (Mendel and Hansch, 2002), and enhances assimilation of N by plant. It also stops or control the reduction of inorganic nitrate and assist in fixing nitrogen to ammonia and stimulating nodulation (Meagher et al., 1991; Jongruaysup et al., 1993; Brkics et al., 2004; Westermann, 2005) and hence, Mo is key factor for N-fixation by legumes. Similarly, Fe is also an important micronutrient which plays an essential role in several enzyme systems including plants and animals. The role of Fe in these enzyme systems comprise the catalases, peroxidases and several cytochromes. The cytochromes not only operate the respiratory metabolism of living cell but also correct chemical reactions in plants and animals. Similarly, Fe also plays significant role in photosynthesis, NO^-_2 SO_4^{-2} reduction, N_2 assimilation and other plant biological process. Moreover, Fe plays significant role that is associated with energy transfer, N reduction and fixation, and lignin formation. In addition, iron chlorosis which is caused by mostly Fe deficiency in calcareous soils can be easily recognized on iron-sensitive crops. The iron chlorosis problem can easily be rectified by adding some Fe products into the iron deficient calcareous soils as iron is mostly involved in the production of chlorophyll. Iron deficient plants lack chlorophyll and are chlorotic (Rourke et al., 2007). The chlorotic plants are mainly evident by yellow leaves or almost white, and then brown as leaves die.

The soils of Peshawar region are alkaline calcareous and deficient in N content. As the application of Fe and Mo may enhance the yield and N_2 fixation of pea, therefore it was planned to study the response of pea crop to Fe and Mo to enhance its yield and N_2 fixation in low organic alkaline calcareous soil of Peshawar valley.

Materials and Methods

Experimental description

A field experiment was conducted to study the effect of different levels of molybdenum and iron alone or in combination on the growth, yield and nitrogen fixation of pea (*Pisum sativum* L.) and on soil fertility at the research farm of Agriculture University, Peshawar during 2012-2013. The experiment was comprised of seven treatments including; 1): Control, 2): Fe [$FeSO_4.7H_2O$] low (2.5 kg ha^{-1}), 3): Fe high (5.0 kg ha^{-1}), 4): M_O [$(NH_4)_6Mo_7O_{24}4H_2O$] low (1.0 kg ha^{-1}), 5): M_O high (2.0 kg ha^{-1}), 6): Fe low + M_O low and 7): Fe high + M_O high and arranged in a RCBD with three replications. The field was thoroughly prepared. All Mo and Fe were incorporated in soil just before sowing. The basal dose of 25 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare was applied to all treatment plots of pea and reference wheat crop at the time of sowing.

Each treatment plot was 5 m long and 3 m wide (15 m^2) which accommodated 4 rows of pea 75 cm apart from one another and plant to plant distance was 15-20 cm. Pea variety Climax was used in the experiment. Composite soil sample at 0-30 cm depth was taken prior to fertilizer application for determination of physical and chemical properties and site characterization (Table 1). The required cultural practices were followed during the entire growing period. Data on plant growth and yield such as fresh pod yield (kg ha^{-1}), pea fresh plant biomass (kg ha^{-1}), dry plant biomass (kg ha^{-1}), nodules per plant (nodules $plant^{-1}$), weight of fresh nodules (g $plant^{-1}$), weight of dry nodules (g $plant^{-1}$), N concentration (%), N uptake (kg ha^{-1}), N_2 fixation, Ndfa (%) were recorded. Data on soil organic matter (%), mineral N (mg kg^{-1}), AB-DTPA extractable P (mg kg^{-1}) and K (mg kg^{-1}) were also recorded after crop harvest.

Table 1: *Pre-cultivation physical and chemical properties of the experimental site.*

Parameters	Unit	Value
Soil pH (1:5)	-	8.53
Soil electrical conductivity (1:5)	dSm^{-1}	1.11
Soil organic matter	%	0.94
Total N	%	0.07
Clay	%	5.2
Sand	%	36.4
Silt	%	58.2
Textural class	-	Silt loam
Mineral N	mg kg^{-1}	32.13
AB-DTPA ext. K	mg kg^{-1}	116
AB-DTPA ext. P	mg kg^{-1}	2.45
AB-DTPA ext Fe	mg kg^{-1}	2.85
Reference crop (wheat) analysis		
N (Wheat tissue)	%	0.44
Dry biomass yield wheat	kg ha^{-1}	3318
Nitrogen uptake (Wheat)	kg ha^{-1}	14.60

The results obtained on nodulation, N_2 fixation and yield of pea are presented and discussed.

Pods yield

Two rows in each treatment plot were selected for determination of fresh pod and recorded yield of pea. Four pickings were taken till maturity of pea crop and summed up yield of all the four pickings. The yield so obtained was representing the yield of an area of 7.5 m^2 which was converted into kg ha^{-1}.

Plant biomass

One row (3.75 m^2) in each treatment was harvested for determination of maximum plant biomass. The pea plants along with pods left till maximum growth stage were harvested and weighed. The fresh plants were air dried till constant weight in the laboratory and then weighed to determine total dry matter yield of pea.

Nodulation study

One row in each treatment plot was selected for nodulation study. From each row three pea plants at flowering stage were carefully uprooted. After thoroughly washing, nodules were detached, counted and recorded. Average nodule numbers per plant was calculated. Both fresh and dry weight of nodules were taken and average weight was calculated.

Soil sampling and analysis

Soil samples at 0-30 were collected from each treatment plot at harvest stage for determination of soil organic matter, mineral nitrogen, phosphorous and potassium. The collected samples were then air dehydrated, sieved with 2 mm sieve, labelled and analysed for required investigation. The physical and chemical analysis were determined by different methods as described and suggested by different scientists; Soil texture (Gee and Bauder, 1986), Soil pH (McLean, 1982), EC (Rhoades, 1996), Soil organic matter (Nelson and Sommers, 1996), Mineral N in soil (Mulvaney, 1996), AB-DTPA extractable P (Soltanpour and Schwab, 1977) and AB-DTPA extractable K (Ryan et al., 2000).

Plant sampling and analysis

At full maturity stage of pea plant, an area of 3.75 m^2 was harvested from each treatment plot for the determination of N concentration. Pea biomass was then dehydrated under room temperature. Further, the plant samples were then oven dehydrated at 70 ^{0}C for 48 hrs so that it may dry to the invariant weight. In addition, the plant biomass was cut with a grinder so that it may become fine particle size. The fine particles were then submitted to wet acid digestion according to procedure laid by Walsh and Beaton et al. (1973). At the end, the required total-N concentration in plant biomass was accordingly determined by the procedure of Mulvaney (1996).

Nitrogen fixation

The correct estimation of nitrogen fixation was measured by the N difference method with reference to wheat crop sown as described in Peoples et al. (1989). A non N_2-fixing wheat plants adjacent to pea crop was also grown. The wheat as a reference crop was used to estimate the amount of available soil N taken up by the pea N_2-fixing plant. The nitrogen fixation was then measured and calculated as the difference in uptake of N of the pea fixed and reference wheat plants.

Pea nitrogen fixation = Nitrogen yield by pea – Nitrogen yield by reference wheat plant

While % Ndfa (percent of N derived from air) is the percent ratio between the N fixed and total N uptake by the plant.

Statistical analysis

Data collected were statistically analysed using randomized complete block design (RCBD) by conducting ANOVA tables. In addition, the treatment differences were approximated by LSD-test of significance. Moreover, regression (r values) were also used to investigate the exact relationship between different parameters with each other (Steel et al., 1997).

Results and Discussion

The results of the site under investigation showed that it was alkaline in reaction (pH 8.53), silt loam and mostly non-saline (Electrical conductivity (EC): 1.11 dSm^{-1}). The soil was low in organic fertility containing 0.94 % organic matter, 2.45 mg kg^{-1} soil AB-DTPA extractable P, 116.0 mg kg^{-1}soil AB-DTPA extractable K, 0.07 % total soil N, 32.13 mg kg^{-1} total mineral N and 2.85 mg kg^{-1}AB-DTPA extractable Fe (Table 1). Data were recorded as follows.

Pea plant growth and yield

Number of nodules: Nodules development on plant roots has direct effect on nitrogen fixation and subsequently on plant biomass and grain yield of pea crop. The data on nodulation in pea was recorded at flowering stage (Table 2). The data revealed that the number of nodules per plant varied significantly among treatments. The number of nodules per plant was significantly ($P<0.05$) greater for treatment receiving Fe or Mo alone or in any combination over the control treatment. The maximum nodules of 247 per plant were obtained for treatment receiving combined application of Mo at 2 kg ha^{-1} and Fe at 5 kg ha^{-1}. Differences between the number of nodules obtained with alone application of Mo at 2.0 kg ha^{-1} or with combined application of Mo at 1.0 kg ha^{-1} and Fe at 2.5 kg ha^{-1} were non-ssignificant. The number of nodules obtained with alone application of Fe at both rates (2.5 and 5.0 kg ha^{-1}) were significantly ($P<0.05$) greater than the control but the difference among rates was statistically non-significant. It was observed that ccombined use of Mo at 2 kg ha^{-1} and Fe at 5.0 kg ha^{-1} caused 152 % increase in number of nodules per plant over control. The corresponding increase was 104 % with combined application of Mo and Fe (1.0, 2.5 kg ha^{-1} respectively). Similarly, Mo alone (2 kg ha^{-1}) caused 102 % and iron alone (5 kg ha^{-1}) caused 78 % increase in number of nodules per plant over control. These results indicated that the number of nodules responded significantly to both Mo and Fe applied alone or in combination suggesting the deficiency of both Mo and Fe in the test soil.

Table 2: *Nodulation in pea as influenced by Fe and Mo fertilization.*

Treatment		No of nodules	Wt of fresh nodules	Wt of dry nodules
Fe	Mo			
(kg ha^{-1})		Nodule plant^{-1}	(g plant^{-1})	
0	0	98 d	0.60 c	0.11 b
2.5	0	167 c	0.95 bc	0.20 a
5.0	0	174 c	0.93 bc	0.16 ab
0	1	177 c	1.02 b	0.15 ab
0	2	198 b	1.06 ab	0.17 ab
2.5	1	200 b	1.17 ab	0.16 ab
5.0	2	247 a	1.41 a	0.21 a
LSD<0.05		14.99	0.36	0.07
CV %		4.68	19.93	23.76

Different results mean showed same letter (s) are statistically non-significant at 5 % level of probability.

Fresh weight of nodules: Data regarding fresh weight of nodules per plant of pea as influenced by application of Fe and Mo are presented in Table 2. The data revealed that application of Mo alone or in combination with Fe significantly ($P<0.05$) increased the fresh weight of nodules per plant compared with the control treatment. The maximum fresh weight of nodules (1.41 g plant^{-1}) was obtained for treatment receiving combined application of Mo and Fe (2 and 5 kg ha^{-1} respectively) but this was statistically similar with treatments receiving Mo (2 kg ha^{-1}) alone or Mo and Fe (1.0 and 2.5 kg ha^{-1} respectively). The results further revealed that Mo and Fe (2 and 5 kg ha^{-1} respectively) combined application caused 135 % increase in fresh weight of nodules per plant over control. The corresponding increase was 95 % with combined application of Mo and Fe (1.0 and 2.5 kg ha^{-1}). Similarly, Fe at low level alone caused 58.33 % increase and Mo at high level alone 77 % increase in fresh weight of nodules over control. These results indicated that fresh weight of nodules in pea responded significantly to high level of Mo alone and similarly the high level of Mo and Fe combined treatment.

Dry weight of nodules: The data on dry weight of nodules suggest that differences among treatments were mostly to non-significant level (Table 2). Only one treatment which received Mo and Fe (2 and 5 kg ha^{-1}) exhibited significantly highest nodules weight than the control treatment. There were differences in weight among the remaining treatments but statistically non-significant.

N_2 Fixation in Pea Crop

The N_2 fixation in pea crop was determined by the N difference method. For this purpose, N concentration was determined both in pea plant biomass and non-legumes reference biomass (wheat) at maximum biomass growth stage. Using the N concentration and plant biomass data, total N uptake was determined both in pea and reference plants and subsequently N_2 fixation was calculated by difference. The data obtained on N concentration, N uptake and N_2 fixation in pea crop are presented and discussed in the following section:

Nitrogen concentration in pea plant biomass

The data revealed that N concentration in pea plant biomass increased significantly with all treatments of Fe and Mo applied alone or in combinations (Table 3). The maximum N concentration of 3.09 % was obtained in the treatment receiving both Mo and Fe (2 and 5 kg ha^{-1} respectively) but this was statistically at par with the treatment receiving Mo and Fe (1.0 and 2.5 kg ha^{-1}). The N concentration in other treatments receiving Mo and Fe at any rate was significantly greater than in the control treatment. The minimum N concentration of 2.39 % was recorded in the control treatment.

Nitrogen uptake in pea

The pattern of response of N uptake in pea crop to Mo and Fe fertilization was almost similar to N concentration in pea plant biomass. All the Mo and Fe treatments significantly ($P<0.05$) increased the N uptake in pea crop (Table 3). However, the maximum N uptake in pea was recorded where Mo and Fe were applied together (2 and 5 kg ha^{-1}, respectively). This was closely followed by the treatment receiving Mo and Fe together but at lower rates (1.0 and 2.5 kg ha^{-1} respectively). It was further observed that alone application of Fe or Mo also produced significantly greater N uptake in pea than in the control treatment. However, N uptake response to Mo applied alone was in general greater than the alone application of Fe at all level of treatments. These results suggested that both Fe and Mo applied alone or in combination significantly improved per unit total N uptake in pea.

Table 3: *Nitrogen concentration, uptake and % N derived from air (% Ndfa) in pea as influenced by Fe and Mo fertilization.*

Treatment		Concentration	N uptake	N_2 fixed	Ndfa
Fe	Mo				
(kg ha^{-1})		(%)	(kg ha^{-1})		(%)
0	0	2.39 d	34.99 d	20.39 d	58.17 c
2.5	0	2.98 b	45.02 bc	30.41 bc	71.29 a
5.0	0	2.82 c	43.07 c	28.47 c	72.00 a
0	1	2.99 b	46.68 b	32.08 b	65.17 b
0	2	2.81 c	45.59 b	30.99 b	62.48 bc
2.5	1	3.05 ab	50.51 a	35.91 a	71.08 a
5.0	2	3.09 a	52.30 a	37.70 a	72.10 a
LSD<0.05		0.08	2.45	2.45	4.88
CV %		1.49	3.03	4.46	4.10

Nitrogen fixation

Although N_2 fixed in pea was generally low in all treatments, it was still significantly greater for treatments receiving Mo and Fe or both together compared with control treatment (Table 3). The maximum N fixation (37.70 kg ha^{-1}) was recorded for treatment receiving Mo and Fe (2 and 5 kg ha^{-1}) followed by treatment (35.91 kg ha^{-1}) receiving Mo and Fe (1.0 and 2.5 kg ha^{-1}) and differences between these two treatments were statistically non-significant. It was also observed that alone application of Fe at low level or Mo at low level significantly increased the N_2 fixation in pea compared with the control treatment. However, the increase with alone application of Mo was significantly greater than with alone Fe. The lowest N_2 fixation in pea occurred in the control treatment. The data further revealed that almost 58 % N (% Ndfa) was derived from air and the remaining N derived from soil (Table 3). It was observed that the proportion of N derived from air in pea increased with the application of Mo and Fe and hence dependence on soil N decreased. The maximum N derived from air in pea was 72 % in treatment which had received both at higher level of Mo and Fe (2 and 5 kg ha^{-1} respectively). These results suggested that the dependence of pea on soil N greatly decreased with the application of Mo and Fe.

Fresh plant biomass of pea

Fresh plant biomass of pea increased significantly ($P<0.05$) with application of Mo at both rates (1.0 and 2.0 kg ha^{-1}) and Fe at higher rate (5 kg ha^{-1}) (Table 4). The maximum fresh plant biomass of 10315 kg ha^{-1} was obtained for treatment receiving combined application of Mo and Fe (2 and 5 kg ha^{-1} respectively) followed by 9850 kg ha^{-1} for treatment receiving combined application of Mo and Fe (1.0 and 2.5 kg ha^{-1}). The data showed that the combined application of full doses of Mo and Fe caused 38.19 % increase in fresh biomass of pea over the control treatment. The corresponding increase was 31.96 % with the application of lower rates of Mo and Fe. Alone application of Mo at 1.0 kg ha^{-1} caused 11.87 % increase and at 2.0 kg ha^{-1} caused 26.35 % increase in plant biomass over the control treatment while alone application of Fe at 5.0 kg ha^{-1} caused 7.58 % increase over control. These results suggested that application of Mo at both rates (1.0 and 2 kg ha^{-1}) and Fe at higher rate (5.0 kg ha^{-1}) helped to increase the fresh plant biomass of pea. As mentioned in the earlier section that Fe and Mo have improved nodulation which resulted in greater N_2 fixation and nutrients uptake. Higher nodulation and N_2 fixation could have significantly contributed to the fresh biomass of pea crop. These results indicated that the test soil could be deficient in Fe and Mo as evident from response of pea to Fe and Mo fertilization.

Table 4: *Plant biomass and pods yield of pea as influenced by Fe and Mo Fertilization.*

Treatment		Fresh plant biomass	Dry plant biomass	Pods yield
Fe	Mo			
(kg ha^{-1})		(kg ha^{-1})		
0	0	7464 e	1463 d	3906 d
2.5	0	7835 de	1510 cd	4177 cd
5.0	0	8030 cd	1526 cd	4235 cd
0	1	8350 c	1560 bc	4372 c
0	2	9431 b	1621 ab	4875 b
2.5	1	9850 ab	1656 a	5175 ab
5.0	2	10315 a	1692 a	5458 a
LSD<0.05		485.45	75.54	407.37
CV %		3.12	2.70	4.98

Same letter (s) bearing results means are statistically non-significant at 5 % level of probability.

Dry plant biomass

Like fresh plant biomass, the dry plant biomass of pea also increased significantly ($P<0.05$) with the application of Mo alone and in combination with Fe as compared to the control treatment (Table 4). The maximum dry biomass of 1692 kg ha^{-1} was obtained for treatment receiving combined application of Mo and Fe (2 and 5 kg ha^{-1}) followed by treatment receiving combined application of lower rates of Mo and Fe (1.0 and 2.5 kg ha^{-1} respectively). It was evident that the treatment receiving Mo at high level (2 kg ha^{-1}) alone was statistically at par with both the treatments (T_6 and T_7) in terms of dry matter yield receiving both Mo and Fe (($T_{6:}$ 1 and 2.5 kg ha^{-1}, $T_{7:}$ 2 and 5 kg ha^{-1} respectively) in any combinations. It was determined that combined use of Mo and Fe (2 and 5 kg ha^{-1} respectively) caused 15.65 % increase in dry plant biomass of pea over the control treatment. The corresponding increase was 13.19 % with the application of Mo and Fe at lower rates (1.0 and 2.5 kg ha^{-1}) compared to control. Alone supplementation at lower rate (1.0 kg ha^{-1}) of Mo caused 6.63 % and at higher rate (2.0 kg ha^{-1}) caused 10.80 % raise in dry matter yield over the untreated control treatment. These results showed that application of M_O alone at both levels significantly better than the Fe at both levels or in combination with Fe improved the dry matter yield of pea suggesting the deficiency of micronutrients in the test soil.

Pods yield

The data revealed that application of Mo and Fe alone or in combination significantly ($P<0.05$) increased the pods yield of pea compared with the control treatment (Table 4). The maximum pods yield of 5458 kg ha^{-1} was obtained for treatment receiving combined application of of higher rates of Mo and Fe (2.0 and 5.0 kg ha^{-1}). This was however at par with treatment receiving combined application of Mo and Fe at lower rates (1.0 and 2.5 kg ha^{-1}). It was observed that the combined use of Mo and Fe (2.0 and 5.0 kg ha^{-1}) caused 39.73 % raise in pods yield over control (Figure 1). The corresponding increase was 32.48 % with the combined application of Mo and Fe (1.0 and 2.5 kg ha^{-1}, respectively). The results further showed that alone supplementation of Mo at both rates caused significant increase in pods yield over control and the increase was associated with the rate applied. Alone supplementation of Mo at lower rate (1.0 kg ha^{-1}) caused 11.93 % increase and at higher rate (2.0 kg ha^{-1}) caused 24.81% increase in pods yield over the control treatment. Alone supplementation of Fe at any rate did not cause any significant increase in pods yield compared with the control. These results suggested that pods yield of pea showed significant response both to Mo alone and to combined application of Mo and Fe.

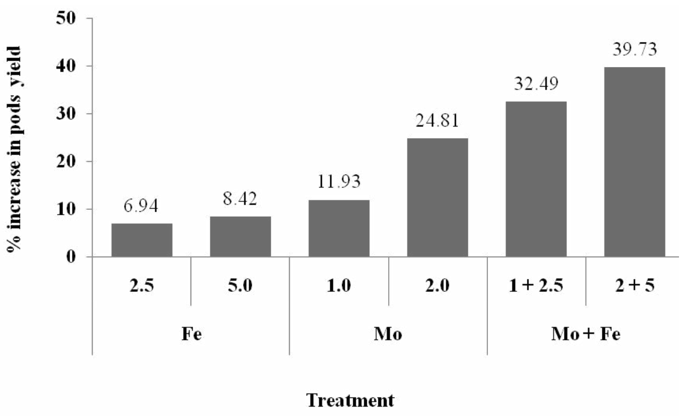

Figure 1: *% increase in pods yield of pea as influenced by Fe and Mo fertilizers application.*

Soil fertility after harvest of pea crop

Soil organic matter, total mineral N, AB-DTPA extractable phosphorous and potassium were determined in each treatment plot after harvest of pea crop. The results obtained are presented and discussed.

Table 5: *Post harvest soil organic matter, total mineral N, AB-DTPA extractable P and K as influenced by Fe and Mo fertilization.*

Fe	Mo	Organic matter	TMN	AB-DTPA-P	AB-DTPA-K
(kg ha^{-1})		(%)	(mg kg^{-1})		
0	0	1.59 c	24.97 e	2.18 d	79.70 b
2.5	0	1.61 bc	31.97 a	3.71 b	84.91 ab
5.0	0	1.73 abc	31.73 ab	2.26 d	90.30 ab
0	1	1.74 ab	26.60 de	4.35 a	85.76 ab
0	2	1.67 bc	29.44 bc	3.05 c	87.88 ab
2.5	1	1.86 a	28.77 cd	3.85 b	94.06 a
5.0	2	1.74 ab	30.23 abc	2.18 d	86.12 ab
LSD<0.05		0.15	2.37	0.45	12.44
CV %		4.92	4.57	8.16	8.04

Soil organic matter

Soil organic matter was generally greater in treatments receiving Mo and Fe alone or in combination than in the control treatment (Table 5). However, significantly higher soil organic matter was obtained in treated plots receiving combined application of Mo and Fe. Although, compared to control, differences between the Mo alone and Fe alone treatments and control were observed, however, they were statistically non-significant. The greater soil organic matter at applied levels of Fe and Me alone or in their combination could be associated with greater biomass production and greater roots developments in same treatments. The correlation analysis between soil organic matter and biomass production confirms the same statement (Figure 6).

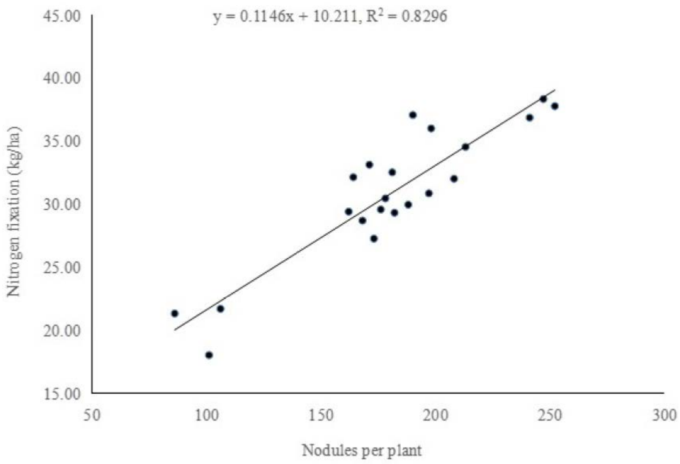

Figure 2: *Relation between N$_2$ fixation and number of nodules per plant plant in pea cultivated on alkaline calcareous soil.*

Total Mineral Nitrogen (TMN)

The result revealed that total mineral N (TMN) in soil was considerably greater in Mo and Fe treated soils as compared with the control treatment (Table 5). The maximum total mineral N of 31.97 mg kg^{-1} soil was obtained in treatment which had received Fe (2.5 kg ha^{-1}) and this was followed closely by treatment receiving Fe at 5.0 kg ha^{-1}. The total mineral nitrogen in soil was also greater in treatments which had received Mo or Fe alone compared with the control treatment. However, the total mineral N was generally greater where Fe was applied alone compared with Mo alone treatment. It was observed that total mineral N values were generally greater for all the treatments including the control treatment. This could possibly be due to the cultivation of a legume crop which fixes atmospheric N and substantial amount of N remain in roots biomass after harvest of the above ground portion.

Soil phosphorous

The data obtained on the AB-DTPA extractable P after harvest of pea crop showed that differences between the fertilized and control treatments were generally inconsistent (Table 5). It looks that P contents were generally low in treatments producing greater plant biomass. Significantly greater P contents were obtained for treatments which had received Mo alone at lower rate (1.0 kg ha^{-1}). However, differences between the control and treatments receiving Mo and Fe (2.0 kg ha^{-1} and 5.0 kg ha^{-1}) were statistically non-significant. The performance of other parameters including plant biomass, grain yield, N uptake and N_2 fixed were all better in the latter treatment. The possible reason could be that a large part of the soil adsorbed P has been taken up by the crop due to increased efficiency of Fe and Mo leaving behind the strongly adsorbed P (2.18 mg kg^{-1}) at soil content nearly at par to its native level (2.45 mg kg^{-1}) (Rahman et al., 2008).

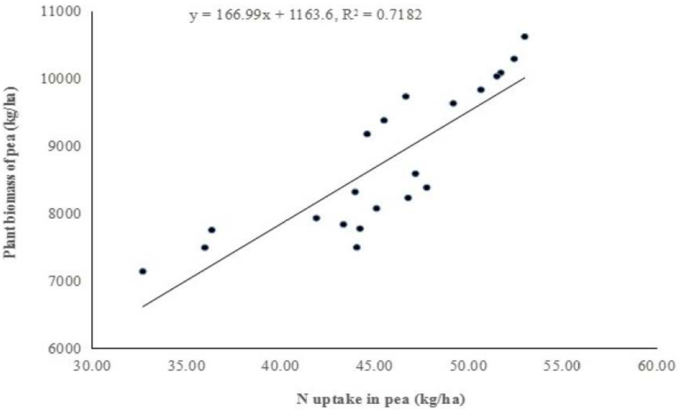

Figure 3: *Relation between N uptake and plant biomass of pea cultivated on alkaline calcareous soil.*

Soil potassium

The data obtained on the AB-DTPA extractable K in soil after harvest of pea crop showed that K contents were generally greater in fertilized than in the control treatment but the differences between the control and most of the fertilized treatments were statistically non-significant (Table 5). The maximum K content of 94.06 mg kg^{-1} soil was obtained in treatment that had received at lower rates of Mo and Fe (1.0 and 2.5 kg ha^{-1})· This treatment was however statistically at par with all other fertilized treatments. The possible reason for these statistically non--significant indifferences could be that the test soil might have contained sufficient K and as a results removal of K in plant biomass had little influence on the residual K content.

Correlation studies

The data revealed that the number of nodules showed significant correlation with fresh and dry plant biomass, pods yield, N concentration, N uptake, N_2 fixation and % Ndfa of pea crop. The number of nodules also showed significant positive correlation with post harvest soil organic matter and mineral N. Other important relationships were between fresh and dry biomass with pods yields, N concentration, N uptake and % N derived from air showing that more the vigour and growth rate higher were the nodulation and N_2 fixation potential of pea. This correlation demonstrated that nodulation played a key role in the plant development, crop yield and N_2 fixation of pea. Greater were the nodulation larger were the yield and N_2 fixation in pea.

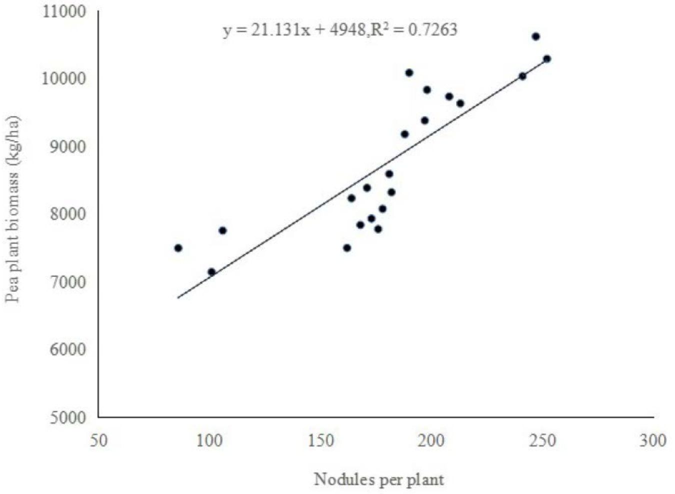

Figure 4: *Relation between number of nodules per plant and biomass of pea cultivated on alkaline calcareous soil.*

Figure 2 exhibited a linear relationship between number of nodules per plant and N_2 fixation in pea with R^2 value of 0.8292. This demonstrates that N_2 fixation is directly dependent on number of of nodules in pea. The N_2 linearly increases with increase in the number of nodules. Similar relationship between number of nodules and plant-N in legumes were observed by Cutcliffe (1986) and Brkic et al. (2004) who reported that as the number of nodules increased the N content in plants also increased and consequently N_2 fixation increased. Moreover, the linear relationship between number of nodules per plant and fresh biomass exhibited in pea (Figure 4) with R^2 value of 0.7263. In addition, Figure 5 exhibited a linear relationship between number of nodules per plant and fresh pods yield in pea with R^2 value of 0.727. This shows that both biomass and fresh pods yield of is also directly dependent on number of of nodules in pea. Similarly, a linear relationship was observed between N uptake and plant biomass of pea (Figure 3) indicating that greater the N uptake larger the plant biomass produced (R^2= 0.718). Significant correlation was observed between plant biomass and soil organic matter (R^2= 0.573 or r= 0.49). Relationship between plant-Fe and N_2 fixation was as well determined by Westermann (2005) and Kaiser et al. (2005) and they determined that Fe played a basic role in the nitrogen fixation in different legumes. The correlation of fresh or dry plant biomass with other soil fertility parameters like total mineral nitrogen, AB-DTPA extractable P and K were non-significant.

The relevance of Mo and Fe to N_2 fixation can be explained as they are integral components of the nitrogenase complex which is responsible for N_2 fixation (Imtiaz et al., 2010; Rashid, 2005; Togay et al., 2015). Therefore, in legumes, Mo and Fe are needed comparatively in greater quantity for enzymatic activities and nodules formation than for host plant growth (Tang et al., 1990) or other non-legume crops. The results have shown that both Mo and Fe application significantly increased number and mass of nodules in pea under field condition suggesting deficiency of these micro nutrients in the test soil. It has been established that Mo deficiency induces N deficiency in most legumes which rely on N_2 fixation particularly in acid soils. Evidences from different research work reported that Mo application as foliar spray on grain legumes crop in the control condition and field increased nodules mass and N_2 fixation which resulted in greater N content and seed yield (Yanni, 1992; Vieira et al., 1998). Further study has shown that Mo increased nodule numbers, nodule weight per plant and nitrogen concentration of nodules (Bhanavase and Patil, 1994). In addition, Singh et al. (2014) revealed that the supplementation of Mo (1 kg ha^{-1}) to pea caused considerable increase in number of nodules from 63.00-97.06 (54.06 %) over control treatment. A number of different legumes grown on Mo deficient soil both in laboratory and field conditions showed more dramatic sign of Mo deficiency (Dilworth and Loneragan, 1991). Shatilov et al. (1978) reported that pea seed treatment with Mo increased number of nodules by 44 % and nodules weight by 31 %. Verma et al. (1988) reported that Mo ranged from 0.5-2 mg/kg seed increased nodulation in chickpea.

Similarly, the Fe which is required in N_2 fixation process of legumes as it is engaged in the chemical process of different enzymes reactions, leghaemoglobin, ferredoxin, hydrogenase and cytochromes (Tang et al., 1992; Scherer et al., 2008). Therefore, it is concluded that Fe chlorosis which affect not only symbiotic N_2 fixation by impairing Rhizobium survival but also affect the establishment of functional nodules or host photosynthesis and energy transfer to the bacteroids (Johnson and Barton, 1993). Similarly, Fe deficiency in peanut has been reported a reduction in enzymatic activity of nitrogenaze in peanut nodules (O` Hara et al., 1988), showing possible direct limitation by iron cholorosis on nodules activity. Another study has shown that Fe chlorosis did not considerably change the shoot growth but badly depressed nodules mass, leghemoglobin content, number of bacteroid and enzymes function, compared with those plants receiving Fe through foliar spray (Tang et al., 1990). O` Hara et al. (1988) has reported that nodules development in lupin and peanut were much sensitised to iron chlorosis as compared with other factors of plant growth such as plant shoot and weights. The application of Mo and Fe increase the plant vigor and nodulation and N_2 fixation. Mo at 2, 4 and 6 g kg^{-1} seed of pea as $NH_4\ Mo_7O_{24}.4H_2O$ and Fe to soil at 5, 10 and 20 kg ha^{-1} as $FeSO_4.7H_2O$ were applied by (Togay et al., 2015). The above dozes were also applied by Kacar and Inal (2008) and Singh et al. (1985) and they reported higher mass of nodules, nodules weight and pods yields at highest Mo and Fe levels in their treatments.

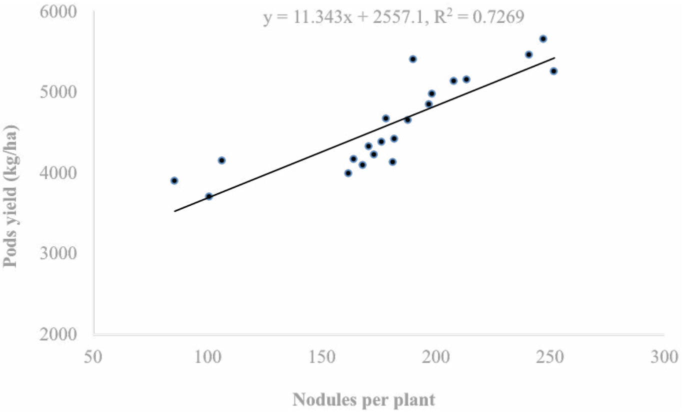

Figure 5: *Relation between number of nodules per plant and fresh pods yield of pea cultivated on alkaline calcareous soil.*

The amount of N_2 fixed varies with the type of pea's cultivar, soil type, the presence of available N in soil and environmental conditions. Under ideal conditions, pea crop can fix as much as 50-80 % of its N requirement from air, with the remaining nitrogen coming from soil or fertilizer sources (Chaudhary and Fujita, 1998; Niehaus and Becker, 1998; Vance et al., 2000). Our study has shown that Mo and Fe (2 and 5 kg ha^{-1}) treated soils enhanced N_2 fixation in pea by 85 % over control (Table 3). The % Ndfa increased from 58 % in the control to 72 % with the application of Fe and Mo. Peoples et al. (2008) reported that Ndfa in pea, chickpea, lentil, cowpea, mungbean, pigeonpea ranged from 8–97 % with an average of 63%. Other studies revealed that Ndfa by various legumes ranged between 40 % and 80 % with an average of

60 % (Van-Kessel and Hartley 2000; Peoples et al., 2008; Salvagiotti et al., 2008). Alves et al. (2003) reported that Brazilian soybean at least derived 70–85 % of crop N from nitrogen fixation process that equal to an amount of 70–250 kg N ha^{-1}. It was reported by Hungria et al. (2005) that Ndfa by inoculated soybean ranged from 69–94% in Brazil. We observed that total N_2 fixed in pea varied from 20.39 kg ha^{-1} in the control to 38 kg ha^{-1} in the treatment receiving combined application of Fe and Mo (5.0 and 2.0 kg ha^{-1}). Brar and Sidhu (1992) reported that N_2 fixation go actively if the crop is healthy and the nutrient supply is adequate. Smil (1999) reported that N_2 fixation in pea ranged from 30-50 kg N ha^{-1} with an average of 40 kg N/ha. Sun and Liu (2000) reported that pea crop with sufficient nodulation fixed an amount of about 75 kg N ha^{-1}. Other study revealed that uninoculated pea fixed from 31 to 107 kg N ha^{-1} with regular precipitation and 4 to 37 kg N ha^{-1} under drought conditions (Carranca et al., 1999). Deibert and Utter (2004) revealed that pea crop derived about 61% of its N from the air with a total amount of 80 kg N ha^{-1} in Australian soils. Further reported that Mo supplementation to the soil at the rate of 4.0 kg ha^{-1} enhanced N content in pods of groundnut by 24.0 % compared with the control (Bhagiya et al., 2005).

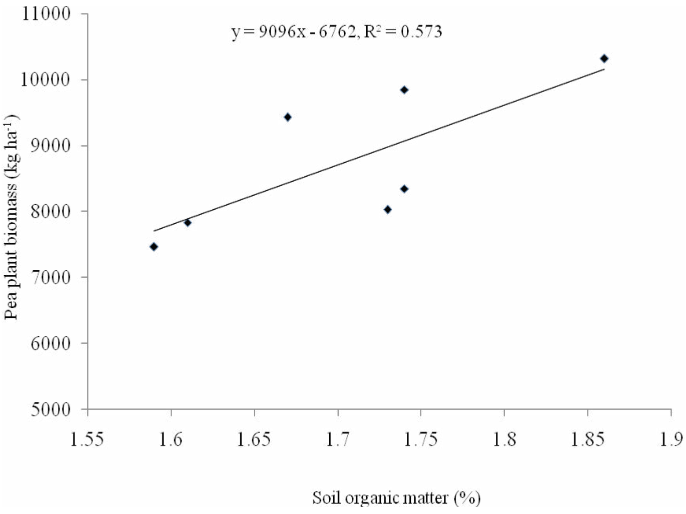

Figure 6: *Relation between soil organic matter and plant biomass of pea cultivated on alkaline calcareous soil.*

This study has shown that Fe and Mo (5 and 2 kg ha^{-1}) treated soil increased fresh biomass of pea by 38 %, dry biomass by 16 % and pods yield by 40 % over control treatment (Table 4). The positive relationship of yield components with Fe and Mo could be related to increasing N_2 fixation, which consequently resulted in increased growth and yield (Maurya et al., 1993; Togay et al., 2008). Chlorosis is a common nutritional disorder caused by Fe deficiency, which has been observed in calcareous soil in different legumes and crops and causes yield losses ranged between 18 and 25 % (Erskine et al., 1993; Rourke et al. 2007). Other researcher evaluated that soil supplementation or foliar spray use of $FeSO_4$ at the rate of between 1 and 25 kg ha^{-1} under diverse environmental conditions enhanced fresh biomass, dry biomass and pods yield of various legumes (Yadav et al., 2002; Balachander et al., 2003; Sahu et al., 2008; Sharma et al., 2010, Singh et al., 2014).

The findings of various studies revealed that Mo at different rates ranged from 2 g to 10 kg ha^{-1} both in soil and foliar application under different environmental conditions enhanced all the growth and yield characters of the pea and other legumes (Deb et al., 2006; Hristozkova et al., 2006; Johansen et al., 2007; Bhuiyan et al., 2008; Togay et al., 2008; Singh et al., 2014). Mo is always deficient in calcareous soils (De Mooy, 1970). Mo application also affects the yield of legumes in those soils where Mo is deficient and unavailable to plants. It has been reported by Richardson et al. (1986) that Mo application to berseem increased green fodder yield and dry matter yield. Similarly, other scientists determined the positive effect of Mo supplementation as it produced significantly greater number of pods per plant (Srivastava and Ahlawat, 1995; Rabbani et al., 2005). Mo application up to 16 mg kg^{-1} enhanced growth, minerals composition, enzyme activity, pods yield of cowpea as well as nutritional and chemical content compared with control plants (Gad et al., 2013).

We also noticed that soil fertility of the alkaline calcareous soil increased substantially where the pea crop was fertilized with both Fe and Mo alone or in combinations. Pea needs both Mo and Fe for its normal growth as Mo at 100 mg kg^{-1} and Fe at 4.5 mg kg^{-1} are critical level needed for legumes crops (Kaiser et al., 2005). Mo application into the soils has increased the contents of K, P and crude protein in legumes. Furthermore, Mo is necessary for translocation and uptake of N in legumes (Togay et al., 2015). It also helps in the absorption of Ca by legumes. Mo also increased the uptake of P and K by the berseem plants. Similarly, Fe deficiency decreases nodule formation, leghemoglobin production and nitrogenase activity, leading to low N concentrations in the shoots in pea (Scherer et al., 2008; Togay et al., 2015). Singh et al. (1995) discovered that iron treated to the soil up to 5 kg ha^{-1} enhanced increased N intake in French beans but P uptake remained unaffected and P deficiency could affect growth and N_2 fixation that have been assessed for determination of nodule development (Almeida et al., 2000; Chaudhary et al., 2008; Tsvetkova and Georgiev, 2003). Yadav et al. (2002) revealed that Fe content and uptake in seed and stover significantly enhanced by Fe supplementation at 6 kg ha^{-1} along with P fertilizer (30 kg P_2O_5 ha^{-1}) but decreased the P content and uptake in mung bean. Hence, it is important to apply the optimum dose of micro nutrients for obtaining the highest results and the nutrients deficiency and excess may not cause the availability of others nutrients.

Our findings suggest that the relative increase in soil organic matter, mineral N, P and K status that could be related to more N_2 fixation, enhanced plant and root growths of pea plants. In fact, leguminous plant play a significant function in the fertility and productivity of soil (Tisdale and Nilson, 1970) and have been considered for green manuring purpose as effective method for improving the soil fertility for succeeding crop (Bhat et al., 2013; Bhattarai et al., 2003; Katyal and Randhawa, 1983). Not only fulfilling its own requirement of N, pea crop is known to leave behind upto 50-60 kg ha^{-1} residual N in soil (Erman et al., 2009). It is only possible due to better uptake and assimilation of available nutrients by the plants during the entire growth period (Kumar et al., 2009; Valenciano et al., 2010). Our results revealed that application of Mo (1.0, 2.0 kg ha^{-1}) and Fe (2.5, 5.0 kg ha^{-1}) along with NPK at 25:60: 60 kg ha^{-1} significantly enhanced the yield attributes of pea as well as the soil fertility status of the alkaline calcareous. Similarly, it has been reported that micro nutrients application to pea crop provides better environment for root growth and nutrient availability in root zone and increase availability of water and nutrients from soil resulting greater translocation of photosynthates in plant and hence improves the soil fertility. Recently, Singh et al. (2014) showed that addition of 60 kg P_2O_5+ 40 kg S+5.0 kg Zn/ha along with Mo (1 kg ha^{-1}), Co (2 kg ha^{-1} and B (0.3 %) were most effective in enhancing the yield of pea and improving the fertility of the soil.

Conclusions and Recommendations

The yield and yield components of pea as well as the number of nodules, nodule weight and its nitrogen fixing ability as indicated by % Ndfa significantly enhanced with application of all levels of Mo and Fe in the given silt-loam calcareous alkaline conditions. The response to Mo, though, was higher than to Fe but their combined supplementation (2.0 and 5.0 kg ha^{-1}) as $(NH_4)_6Mo_7O_{24.}$ H_2O and $FeSO_4.7H_2O$ seemed to be optimal instead of alone application of either nutrient.

The application of 2.0 kg Mo ha^{-1} and 5.0 kg Fe ha^{-1} in the form of $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ and $FeSO_4.7H_2O$ is therefore recommended for increase nodulation, N uptake, N_2 fixation and pods yield of pea and for improved soil fertility in alkaline calcareous soil.

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Author's Contribution

Abdur Rehman: Conducted the research, did experiments and statistical analysis and wrote the manuscript.
Zahir Shah: Planned, designed and supervised the research, did statistical analysis and corrected the manuscript.

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