RESPONSE OF PEAS TO MOLYBDENUM APPLICATION WITH AND WITHOUT RHIZOBIUM INOCULUM UNDER ALKALINE CALCAREOUS SOILS

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ABSTRACT. *Molybdenum (Mo) deficiency affecting nodule formation in legumes is common in acid soils and rare in alkaline calcareous soils. But due to intensive cropping and lack of Mo in fertilization programme, its deficiency may also occur in calcareous soils. So, the study was aimed to investigate the role of Mo in pea's growth and yield performance along with rhizobium inoculums under calcareous soils. A field study was conducted on sandy clay loam soil to determine the response of peas to Mo, applied as foliar spray, through soil and seed treatment with and without rhizobium. The experiment was designed in RCBD with factorial arrangement and replicated four times. The two treatments, Mo treated seeds of peas (T5) and Mo applied through soil (T6) with rhizobium inoculum increased percent effective nodulations plant^{-1} (94.70) which enhanced N, P, and K (5.23, 0.80 and 3.58 %) and Cu, Fe, Mn and Zn concentration (20.86, 259.92, 284.37 and 115.37 mg kg^{-1}) in leaf resulting in improved root and shoot ratio (0.31), pod length (9.13 cm) and pod yield (6.6 t ha^{-1}). Without rhizobium inoculum, only the Mo application also increased all investigated variants over control but lower as compared to Mo with rhizobium strains. However, foliar application of Mo did not show significant results. It was concluded that the best way of Mo application was to treat the seeds with Mo with rhizobium inoculum which affected all growth parameters under study and its importance in calcareous soil for leguminous crops cannot be ignored.*

Key words: pea; molybdenum; rhizobium; nutrients; growth; nodules; yield

INTRODUCTION

Molybdenum is a transition element, which can exist in several oxidation states ranging from zero to VI, where VI is the most common form found in most agricultural soils [1]. Similar to most metals required for plant growth, Mo has been utilized by specific plant enzymes to participate in reduction and oxidative reactions [2]. Intensive cropping and lack of organic matter in alkaline soils have led to micronutrient deficiencies of Mo including Zn, Fe, B and lower microbial activities resulting in low yields, particularly that of leguminous crops. Rhizobium bacteria play an important role in symbiotic nitrogen fixation by producing effective nodulation which improves growth and yield of legumes when used as seed inoculant [3]. In grain legumes, rhizobium inoculants (biological nitrogen fixation technology) can be an alternative of costly chemical nitrogen fertilizer for legume production [4]. The genus Rhizobium and Bradyrhizobium can fix approximately 175x10^{6} tons of nitrogen yearly [5] which is higher than the total global chemical nitrogen fertilizer production of 30x10^{6} tons [6]. In higher plants the most important enzymes are nitrate reductase and glutamine synthetase, which are responsible for nitrogen metabolism [7]. Their activities are accompanied with the mineral nutrition and Mo metabolism [8]. The process of biological nitrogen fixation is catalysed by nitrogenase enzyme which works properly in the presence of Mo. This nitrogenise enzyme contains two proteins i.e. Mo-Fe protein and Fe protein and nitrogen fixation will not take place without these proteins [9]. Molybdenum requirements of crops that develop symbiosis with rhizobium [10] are higher and Mo itself is not biologically active but is rather predominantly found to be an integral part of an organic pterin complex called the Mo co-factor (Moco). The Moco binds to Mo-requiring enzymes (molybdoenzymes) found in most biological systems including plants, animals and prokaryotes [1]. Studies show that application of Mo on soybean and common beans increase nitrogen fixation and produce larger nodules through nitrogenase activity [11: 12] and legume yields can be improved by applying Mo in foliar form which increases nitrogen fixation and nodulation [13]. The application of B and Mo on chickpea significantly influenced yield and yield components like plant height, number of nodules plant^{-1}, and 100-seed weight and contributed 32-53% higher yield over control [14]. Keeping in view the importance of Mo and microorganisms like rhizobium in leguminous crops, a field study was conducted to investigate the effect of Mo application with and without rhizobium on growth and yield of peas.

MATERIAL AND METHODS

Field experiment

A field experiment was conducted during Rabi at Agriculture Research Institute Sariab Quetta (Pakistan) on response of peas to Mo application with and without rhizobium inoculum under alkaline calcareous soils. The experiment was laid in a randomized complete block design (RCBD) with factorial arrangement having six treatments replicated four times. Molybdenum in the form of NH_4-molybdate was applied as a foliar spray, through soil and seed treated with and without rhizobium inoculation. The treatments included: 0 Mo (T1), three rates of Mo as foliar spray (0.07% Mo (T2), 0.06% Mo (T3), 0.05% Mo (T4), seed treatment (T5) as a 1/8 ounce acre^{-1} and soil application (T6) as 1 lb acre^{-1}. All the cultural practices were followed as per agronomic procedure. The seeds of peas (Olympia variety) were sown @ 60 kg ha^{-1}. Recommended dose of N, P and K fertilizer was applied @ 40-80-40 kg ha^{-1} in the form of urea, single supper phosphate and potassium sulphate respectively. Seeds of peas were treated by priming in Mo solution (1/8th ounce acre^{-1}) for two hours, dried in shed and 1 pound acre^{-1} of Mo was applied directly to the soil. For foliar application, 0.05, 0.06 and 0.07 % Mo solution was sprayed twice as per treatment. First one was applied, when the plants were between 12 and 13 cm height and the second one before blooming. The rhizobium inoculum was purchased from the bacteriology section of Ayub Agriculture Research Institute Faisalabad Pakistan and seeds were inoculated before sowing following the standard inoculating method (reference). Tube-well water was used for irrigating the peas with 10 days interval time. At maturity, the root and shoot ratio, length of pod, total number of nodules plant^{-1}, number of effective nodules plant^{-1} and pod yield was recorded. The data regarding root and shoot ratio, length of pod, pod yield and some macro and micro nutrient contents (N, P, K, Cu, Fe, Mn and Zn) in leaf were subjected to analysis of variance and least significance difference at 0.05 alpha level in order to study the effect of treatments on peas nodulation and yield. All the statistical analysis was computed using MSTAT-C software [15].

Analytical methods

Composite soil samples at 0-15 cm depth were collected before the sowing of peas and analysed for some physical and chemical properties, macro and micronutrients. Soil pH and EC were determined in 1:5 soil-water suspension, organic matter by potassium dichromate oxidation [16: 17], soil texture by Bouyoucos method [18] and total N by Kjeldhal's method [19]. The soils were extracted for P, K, Cu, Fe, Mn and Zn using ammonium bicarbonate dietylene triamine penta acidic acid (ABDTPA) method of [20]. The P in the extract was analysed by ascorbic acid color development method using spectrophotometer (Jenway 6100), K using flame photometer (Jenway PFP7) and Cu, Fe, Mn and Zn were determined directly by atomic absorption spectrophotometer (Perkin Elmer 3100). Boron in soils was extracted by hot-water method and was measured spectrophotometrically using azomethine-H method of [21]. Where as Mo was extracted from the soil with a solution of ammonium oxalate-oxalic acid in 1:10 soil solution ratio and the intensity of the orange coloured Mo-thiocyanate complex was measured using spectrophotometer following the method described by [22,23]. Plant samples from recent fully developed leaflets at first blooming were collected, decontaminated and washed with distilled water, dried in oven at 80^{o}C, ground to a 20 mesh size and stored at 4 ^{o}C for analysis [24]. The plant samples were digested by wet oxidation method using hot H_2SO_4 and repeated additions of 30% H_2O_2 [25]. The digests were analyzed for P by vandomolybdo phosphoric acid yellow color method [26], K by emission spectroscopy and Cu, Fe, Mn and Zn using atomic absorption spectrophotometer. Total N was determined by Kjeldahl's method by digesting the contents in H_2SO_4 followed by distillation [27].

RESULTS

The pre soil analysis of the experimental site revealed that the soil texture was sandy clay loam and had pH (8.30), EC$_e$ (1.24 dSm^{-1-}), organic matter (1.13%), total nitrogen (0.061%) , AB-DTPA extractable P, K (1.23, 245.70 %), Cu, Fe, Mn, and Zn (0.45, 4.23, 0.94 and 0.86 mg kg^{-1}) and ammonium oxalate-oxalic acid extractable Mo (0.37 mg kg^{-1}). The statistical analysis of the agronomic data showed that root and shoot ratio, length of pod (cm), total number of nodules plant^{-1}, percent effective nodules plant^{-1} and pod yield (t ha^{-1}) with and without rhizobium were significantly influenced by the Mo application (Table 1). All six treatments had higher length of pod (cm), total number of nodules plant^{-1}, percent effective nodules plant^{-1} and pod yield (t ha^{-1}) except root and shoot ratio in rhizobium inoculated plots as compared to the ones which were not treated with rhizobium. Among rhizobium applied plots, T5 (seed treated with Mo) had the maximum root and shoot ratio (0.305), pod length (9.13 cm), total number of nodules plant^{-1} (54.75), percent effective nodules plant^{-1} (94.70) and pea yield (6.60 t ha^{-1}) followed by T6 (soil applied with Mo) and minimum in T1 (0 Mo), whereas the foliar applied Mo treatments (T2, T3 and T4) were statistically at par with each other in root and shoot ratio, pod length, percent effective nodules plant and yield. In case of plots without rhizobium inoculation, all the growth parameters were at maximum in T5 (seed treated with Mo) and minimum in T1 (0 Mo) but were lower than rhizobium inoculated plots. However, root and shoot ratio and percent effective nodules were significant among all treatments except foliar applied Mo treatments (T2, T3 and T4). Whereas, pod length, total number of nodules plant^{-1} and yield were statistically similar among all treatments but were different from the foliar applied treatments. The data regarding percent yield increase over control due to Mo application with and without rhizobium inoculant has shown in fig. 1. The error bar scale indicated that seed treated with Mo (T5) with and without rhizobium inoculant non significantly shown high percent yield (56.82 and 53.61) increase of pea over control followed by soil applied Mo (T6), T2, T3 and T4 (foliar applied Mo) which were statistically differed from each other in percent yield increase over control. However, the foliar applied Mo without rhizobium inoculant also increased percent yield of pea over control, but were lower as compared to one which were inoculated with rhizobium. Same yield pattern of peas was observed when Mo was applied without rhizobium but yield were lower than those which received rhizobium. Nitrogen concentration in pea leaves ranged from 2.07 to 5.11 % with a mean value of 3.92%, P from 0.08 to 0.74% with mean value of 0.46%, K from 1.20 to 3.69 % with a mean value of 2.43%, Cu from 3.78 to 26.38 mg kg^{-1} with a mean value of 16.88 mg kg^{-1}, Fe from 30.24 to 290.77 mg kg^{-1} with a mean value of 205.75 mg kg^{-1}, Mn from 20.37 to 325.66 with a mean value of 231.24 mg kg^{-1} and Zn from 19.43 to 120.75 mg kg^{-1} with a mean values of 85.96 mg kg^{-1}. The data in Table 2 showed that the leaf nutrient concentration of pea plant was significantly affected by Mo application with and without rhizobium inoculant. In rhizobium inoculated plot, all the Mo levels showed significantly higher N, P, K, Cu, Fe, Mn and Zn contents of pea plant as compared to control (0 Mo). The maximum N, P and K contents (5.23, 0.80,and 3.58%) and Cu, Fe, Mn and Zn concentration (21.02, 259.92, 284.37 and 115.00 mg kg^{-1}) were noted in T5 (seed treated with Mo) which were statistically at par with T6 and were significant over control (0Mo) and with the remaining treatments in case of N, P, Fe and Zn. Whereas, the N, P, Cu, Fe, Mn and Zn concentration in T2,T3 and T4 (foliar applied Mo) were

Table 1. Effect of Molybdenum application with and without rhizobium inoculum on growth and yield of peas under alkaline calcareous soil.

Without Rhizobium inoculum	**Yield (t ha^{-1})**	2.425 e	3.530 de	3.933 cde	3.790 cde	5.228 abc	4.538 bcd	**3.907 B**
	%effective nodules plant^{-1}	11.250 e	81.612 bc	78.130 bc	72.447 c	87.880 ab	85.803 abc	**69.520 B**
	Total no. of nodules plant^{-1}	6.000 h	19.500 ef	17.000 f	14.250 fg	36.000 c	29.500 cd	**20.375 B**
	Pod length (cm)	5.215 f	7.390 de	7.223 e	7.070 e	7.845 bcde	7.460 cde	**7.034 B**
	Root & shoot ratio	0.138 d	0.200 cd	0.133 d	0.163 cd	0.338 a	0.215 bc	**0.198 A**
With Rhizobium inoculum	**Yield (t ha^{-1})**	2.850 e	5.725 ab	4.975 bcd	4.775 bcd	6.600 a	5.740 ab	**5.111 A**
	% effective nodules plant^{-1}	30.080 d	84.583 abc	83.045 abc	81.370 abc	94.707 a	91.023 ab	**77.468 A**
	Total number of nodules plant^{-1}	8.500 gh	31.750 cd	28.500 d	24.500 de	54.750 a	47.000 b	**32.500 A**
	Pod length (cm)	5.803 f	8.068 bcd	8.203 bc	8.040 bcd	9.125 a	8.518 ab	**7.940 A**
	Root & shoot ratio	#0.136 c	0.194 b	0.135 c	0.163bc	0.305 a	0.208 b	**0.183 A**
Treatments		Control (T_1)	0.07% Mo spray (T_2)	0.06% Mo spray (T_3)	0.05% Mo spray (T_4)	1/8-ounce acre^{-1} as a seed treatment (T_5)	1 lb acre^{-1} as a soil application (T_6)	**Mean**

Mean carrying the same letters is statistically alik

Table 2. Effect of molybdenum application with and without rhizobium on leaf nutrient concentration of peas underalkaline calcareous soil.

Treatment	**With Rhizobium**			**Without Rhizobium**		
	Macronutrients (%)					
	N	**P**	**K**	**N**	**P**	**K**
Control (T_1)	3.430 de	0.203 e	1.745 f	2.800 e	0.145 e	1.475 f
0.07% Mo spray (T_2)	4.603 abc	0.548 bc	3.358 ab	3.885 bcd	0.525 bc	2.523 de
0.06% Mo spray (T_3)	4.870 ab	0.613 b	2.565 cde	3.853 bcde	0.453 cd	2.345 e
0.05% Mo spray (T_4)	4.855 ab	0.603 b	2.338 e	3.750 cde	0.410 d	2.235 e
1/8 ounce acre^{-1} as a seed treatment (T_5)	5.233 a	0.800 a	3.588 a	4.658 abc	0.620 b	3.018 bc
1 lb acre^{-1} as a soil application (T_6)	5.040 a	0.785 a	3.508 a	4.568 abc	0.580 b	2.948 bcd
Mean	**4.672 A**	**0.592 A**	**2.8500 A**	**3.920 B**	**0.4554 B**	**2.424 B**

Micronutrients (mg kg^{-1})								
	Zn	**Fe**	**Cu**	**Mn**	**Zn**	**Fe**	**Cu**	**Mn**
Control (T1)	21.550 d	38.340 d	4.840 d	28.350 d	17.240 d	31.050 d	4.113 d	22.540 d
0.07% Mo spray (T2)	21.550 d	38.340 d	4.840 d	273.470 a	70.930 c	181.440c	16.213c	217.410 c
0.06% Mo spray (T3)	88.670 b	224.000 b	19.770 ab	272.030 a	70.310 c	176.160c	16.060c	216.260 c
0.05% Mo spray (T4)	87.880 b	217.480 b	19.663ab	271.410 a	70.000 c	176.040 c	15.640c	215.770 c
1/8 ounce acre-1 as a seed treatment (T5)	87.480 b	217.340 b	19.260 ab	284.370 a	92.300 b	210.540 b	17.648bc	226.070bc
1 lb acre-1 as a soil application (T6)	115.370a	259.920 a	21.015 a	257.820ab	91.860 b	224.700 b	17.520 bc	204.970 c
Mean	114.830a	277.410 a	20.863 a	**231.240 A**	**68.771 B**	**166.65 0 B**	**14.532 B**	**183.840 B**

Mean carrying the same letters is statistically alike

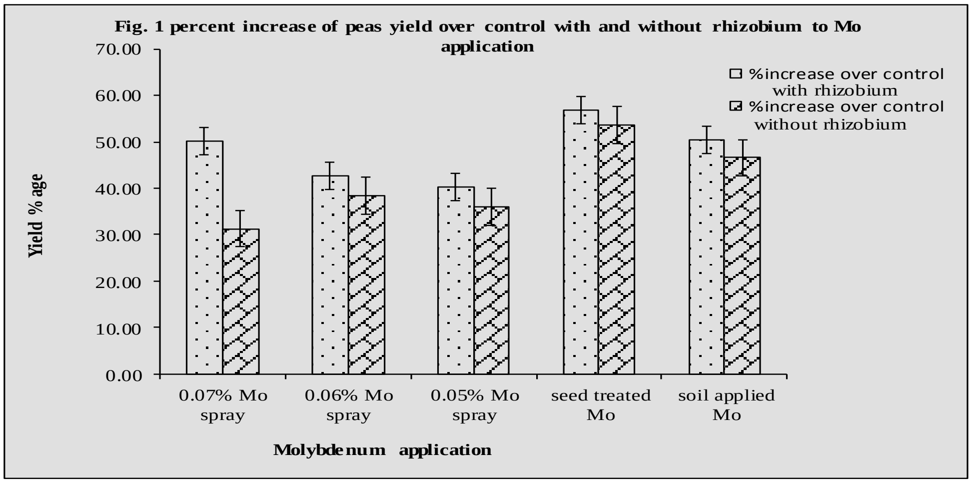

Fig. 1 percent increase of peas yield over control with and without rhizobium to Mo application

statistically at par with each other, but the K concentration in T2 was different from T3 andT4. However, the Cu and Mn concentration in leaf of pea plant were same at all Mo levels. In case of without rhizobium inoculated plot, the Mo application also significantly enhanced nutrient contents in pea plant over control, but lower when compared to plot receiving Mo with rhizobium (Table 2). It was noted that N contents were statistically same in all Mo levels and P concentration was non-significant in T5 (seed treated with Mo), T6 (soil applied Mo) and T2 (foliar applied Mo) but were significant over control and T3 and T4 (foliar applied Mo). Similar statistical trend was also observed in K contents of pea plant. In case of micronutrients, the leaf concentration of Cu and Mn were statistically at par with each other on all Mo levels. However, Fe and Zn concentration in T5 (seed treated with Mo) and T6 (soil applied Mo) were same with each other but were statistically significant over control (0 Mo) and T2, T3 andT4 (foliar applied Mo). The highest concentration of Cu, Mn and Zn (17.65, 226.07 and 92.30 mg kg^{-1}) pea plant was recorded in T5 (seed treated with Mo) and Fe (224.70 mg kg^{-1}) was in T6 (soil applied Mo). The minimum concentration of Cu, Fe, Mn and Zn (4.12, 31.05, 22.54 and 17.24 mg kg^{-1}) in leaf was observed in T1 (0 Mo). The lowest concentration was recorded in treatment where no Mo was applied except rhizobium inoculum. Nonetheless, foliar application of Mo non-significantly increased leaf macro (NPK) and micro nutrients (Zn, Fe, Cu and Mn) concentration over control but less than seed treated and soil applied Mo.

DISCUSSION

According to Table 1 that root and shoot ratio, length of pod (cm), total number of nodules plant^{-1}, percent effective nodules plant^{-1} and pod yield (t ha^{-1}) of peas with and without rhizobium were significantly influenced by the Mo application. The effective nodules were resulted in meeting the nitrogen requirement of peas through symbiosis which improved all the growth variants under study. These results were found in agreement with the findings of [28,29] who observed that rhizobium compatible strains inoculated seed along with Mo application showed positive effects on yield and quality parameters in soybean, pea and alfalfa crops. Similar studies conducted by [30] showed that the highest plant height in chickpea was where Mo was applied as seed treatment @ 6 g kg^{-1}seed. Percent yield increase over control due to Mo application with and without rhizobium inoculant has shown in Fig. 1. The high pods yield of peas might be due to more nutrient uptake that was examined in the leaf nutrients concentration and high root shoot ratio of pea. Because the yield in control (without Mo) was lower that also indicated low leaf nutrient concentration. These results were in line with findings of [11] and [12] who observed that Mo fertilization can enhance the nitrogen-fixing symbiosis through increased nitrogenase activity rates and larger nodules in soybean and common bean. [31] also reported that in Mo-deficient chickpea, the flowers produced were less in number, smaller in size and many of them did not mature, leading to lower seed yield. The findings of this research are in conformity with the results of [32] who observed the effect of Mo and phosphorus on growth and yield variants of lentil under highly alkaline condition and reported that plant height, number of branches, number of pods plant^{-1}, number of seed plant^{-1}, 1000 seed weight, grain yield, harvest index, biological yield, number of nodules plant^{-1}, root dry weight, and shoot dry weight, and protein contents of lentils were improved significantly. The increase in leaf macro (NPK) and micronutrients (Zn, Fe, Cu and Mn) concentration had increased all the above mentioned growth and yield parameters of peas. The maximum root and shoot ratio, pod length, number of effective nodules per plant, and yield (t ha^{-1}) was observed in treatment five and six which was the result of high uptake of N, P, K, Zn, Fe, Cu and Mn concentration in these treatments. Low uptake of these nutrients has shown minimum root and shoot ratio, pod length, number of effective nodules per plant, and yield (t ha^{-1}) as indicated in control and in the treatments receiving Mo in foliar form. Plant growth was affected by the Mo application; at maturity plants fertilized with Mo had greater total dry matter production, because the Mo foliar application caused an increase in plant growth. Most of the time the best way to apply Mo is as a seed treatment. Seed application has distinct advantages over both soil and foliar treatments. Seed treatment results in an even Mo application. Seed treatment also requires lower Mo rates than those used for soil application that 1/8 ounce per acre of Mo applied as a seed treatment will increase pea yields the same amount as pound per acre of Mo applied directly to the soil.

CONCLUSION

The seeds of peas treated with Mo and soil applied Mo with rhizobium inoculum increased number of effective nodulations which enhanced the macro (N, P and K) and micro (Zn, Fe, Cu and Mn) nutrient concentrations in leaf resulting in improved root and shoot ratio, pod length, and pod yield (t ha^{-1}). Without rhizobium inoculum, where only Mo was applied, there was increase in all variants over control but lower as compared to Mo with rhizobium strains. However, foliar application of Mo did not show significant results. It was concluded that the best way of Mo application was seed treatment which affected all growth parameters under study and its importance in calcareous soils for leguminous crops cannot be ignored.

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