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Effect of molybdenum levels, bacterium inoculation and chickpea varieties on nodulation under diverse conditions

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Lack of Molybdenum (Mo) in soil affecting nodule formation and its efficiency in legume. To investigate the role of Mo in chickpea for nodulation efficiency along with rhizobium inoculations under irrigated and rainfed conditions. Experiments were conducted at two different locations of Khyber Pakhtunkhwa province of Pakistan (Agronomy Research Farm, The University of Agriculture Peshawar as irrigated and Farmer's field at Karak as rainfed) with the objectives to find out appropriate level of molybdenum and bacterium application method on different chickpea varieties (Chattan and Lawaghar) in order to exploit the nodulation efficiency under agro-climatic condition of Peshawar (irrigated) and Karak (rainfed). The study was carried out in randomized complete block design with three replicates. Analysis of the data showed that application of molybdenum @ 0.3 kg ha^{-1} showed highest number of nodules, active nodules and nodules weight $Pant^{-1}$. Bacteria inoculated with soil have maximum number of nodules, active nodules and fresh weight of nodules as compared to seed treated bacteria. Among chickpea varieties Lawaghar achieved higher number of nodules and active nodules than Chattan variety. While highest nodule weight was observed in Chattan. Chickpea sown in rainfed region of Karak had highest number of nodules, active nodules and nodule fresh weight as compared to irrigated condition of Peshawar. It is concluded that for achievement of higher number of nodules, active nodules $plant^{-1}$ Lawaghar may be sown at Karak.

Keywords: Molybdenum, inoculation methods, chick pea, nodules number, nodules weight

INTRODUCTION

Chickpea (*Cicer arietinum* L.) follows pea and soybean in importance and it contributes about 15% of the world's total pulse production (FAO, 2010). Being rich in protein it has become a key part of human food in the developing world. In Pakistan, it covered an area of 945000 ha producing 312000 tones (GOP, 2016), while in KP chickpea is cultivated on area of 27000 hectares with total production of 12300 tones with average yield of 454 kg ha^{-1}(MNFSR, 2015). Mostly it is cultivated in rainfed conditions in Thal areas of Punjab and Kyber Pakhtunkhwa province. In Sindh and Baluchistan, it is cultivated on left over moisture following removal of previous crop like rice. 80% of the total chickpea production comes from Punjab province where rainfed areas contribute almost 90% of the production.

Molybdenum has a vital function in nitrogen (N) metabolism and protein formation in plants. In bacterial nitrogen fixation it acts as a cofactor for nitrogenase enzymes to catalyze the redox reaction thereby converting elemental N into ammonium (NH +) ions (Mendel and Schwarz, 2011), and nitrate reductase enzymes required for the collection of nitrates from soil. For this reason nitrogen metabolism has a close relation to the ratio of molybdenum in soil, for legume

plants in particular (Mendel and Hansch, 2002). Due to this relationship, Mo-deficient legumes show an unusual proliferation of nodules, which in turn leads to N deficiency (Marschner, 2011).

Molybdenum (Mo) plays an important role in increasing chickpea yield. Growth and yield of chickpea were affected by Mo application significantly. At maturity, plants fertilized with Mo at 4 weeks after emergence produced a greater seed yield, mainly due to an increase in the number of pods per plant. Foliar Mo application was more effective than soil applied Mo. Soil Mo application must be done earlier in the growing season as compared to foliar application. Mo applied at 4 weeks after emergence through foliar fertilization give best response in terms of yield and yield components (Valenciano et al., 2011). Generally, Mo in high concentration has been reported to accelerate the activity of nitrogenase and nitrate reductase, whereas low levels of Mo inhibit the enzyme activities (Imperial et al., 1985), but it depends on a number of factors including type of strains, plant species, environmental factors (Antipov et al., 2005).

The Mo content of soil was considered to be sufficient to meet the requirement of crops in the semiarid areas of Pakistan, but are at the borderline of the requirement is insufficient. It is expected that with time, further depletion of Mo in the soil due to interaction with Cu and other antagonistic elements may take place, which could finally lower Mo levels in being used by the crops. Thus, in the future, addition of standard fertilizers enriched with Mo to the soils may be required (Khan et al., 2011).

Nitrogen fixing microbes need molybdenum during fixation processes as it is a constituent of nitrogenase enzyme. Moreover, molybdenum is needed for nodulation, nitrate reduction, nitrogen fixation and general metabolism in leguminous plants. Molybdenum at 0.85 kg ha^{-1} gave the optimum results for number of pods $plant^{-1}$, seeds pod^{-1}, 100 seed weight and harvest index (%) in groundnuts (Choudhary et al., 2017). Molybdenum improved root and shoot biomass in chickpea plants (Togay et al., 2008). Molybdenum at 16 ppm resulted in maximum growth, nodule number and weight, nitrogenase activity, pod and seed yield as well as nutritional and chemical content of cowpea. However, molybdenum levels more than 16 ppm further decreased the growth on chickpea.

Molybdenum is an essential element it is a constituent of the nitrogenase enzyme, and every bacteria which fixes nitrogen needs molybdenum during the fixation processes. Molybdenum has a positive effect on yield, quality and nodules forming in legume crops. The functions of molybdenum in leguminous plants include nitrate reduction, nodulation, nitrogen fixation and general metabolism (Togay et al., 2008). Molybdenum is required for normal plant growth, reduction supply with molybdenum to the growth medium decreased activities of nitrate reductase and glutamine synthetase involved at initial steps of nitrate assimilation (Hristozkova et al., 2006).

Keeping in view the importance of molybdenum and boron on nodulation efficiency of chickpea, the present study was planned to investigate the effect of molybdenum and boron for improving nodule efficiency of chickpea.

MATERIALS AND METHODS

A field experiment entitled “role of molybdenum and nitrogen fixing bacteria applications methods on yield and quality of chickpea varieties under irrigated and rain-fed conditions” was conducted at Agronomy Research Farm, University of Agriculture Peshawar during winter season, 2017.The experiment was laid out in randomized complete block design (RCBD) keeping three replications with net plot size of 7.2 m^{-2} (6 rows 30cm apart with 4m length). Molybdenum levels were 100, 200 and 300 g ha^{-1} as a foliar spray two weeks after emergence (physiological growth stage V4). The bacterium *Mesorhizobium* strain was applied using two application methods i.e. soil and seed treatment. Chickpea varieties i.e. Chattan 2015 (Desi), Lawghar-2000 (Kabuli) was used during the trial. The experiment will be repeated next year. All other agronomic practices were kept constant for all the experimental units.

Procedure for data collection:

Total numbers of nodules were determined at pod filling stage, five plants were uprooted at proper moisture condition with their roots. Deep in water tub for 10 minutes. All the nodules were counted and averaged it. Number of active nodules at pod filling stage were uproot from each experimental unit carefully and check the colors of nodules. Nodules with pink red coloration was considered active. Nodule weight per plant at full bloom stage, ten plant samples were uprooted carefully through digging, soil was removed carefully then nodules was separated and weighed to get fresh biomass of nodules.

RESULTS AND DISCUSION

Number of nodules plant^{-1} at pod filling

Number of nodules plant^{-1} was significantly affected by different molybdenum levels (M), application methods of nitrogen fixing bacteria, varieties and molybdenum x varieties interaction (Table 1 & 2). While the effect of location and rest of the possible interactions (V x AM, AM x M, M x V x AM, L x M, L x V, L x AM, L x M x V, L x V x AM, L x AM x M, L x M x V x AM) were found non-significant. Application of Mo at 0.3 kg ha^{-1} produced more nodules plant^{-1} (29.17) as compared to 0.2 kg Mo ha^{-1} (28.94) followed by application of 0.1 kg Mo ha^{-1} (25.05) and lowest nodules plant $^{-1}$ (24.49) were obtained from control plots. It was evident that total number of nodules plant^{-1} increased with the application of molybdenum in chickpea crop (Nasar and Shah 2017). The foliar application of molybdenum significantly increased number of nodules of lentil crop. The sole and combine application of molybdenum with iron significantly enhanced nodules number in legumes crops (Brkics et al., 2004). More nodules were obtained by higher application of Mo as compared to control. This would be possibly attributed to more translocation of Mo to the nodules (Campo and Hungria, 2002). More nodules plant^{-1} (27.80) was obtained when nitrogen fixing bacteria was applied directly to the soil followed by seed applied (26.85) and fewer nodules (26.09) were observed in control plots. Chattan-2015 produced lowest nodules plant^{-1} (26.69), while Lawaghar-2000 produced more nodules (27.13) plant^{-1}. The molybdenum treated with pea seeds and molybdenum applied to the soil with rhizobium inoculum increased percent effective nodulations plant (Hidayatullah et al., 2016). The addition of molybdenum under highly alkaline condition increased nodules number with other yield parameters for lentil (Togay et al., 2008).

Number of active nodules plant^{-1} at pod fillin

Active nodules plant^{-1} was significantly affected by various levels of molybdenum (M), application methods inoculation (AM), varieties (V) and interaction (M x V and V x AM), while the effect of location and other possible interactions were found non-significant (Table 1). More number of active nodules (21.17) was obtained by the application of 0.2 kg M ha^{-1} which was statistically at par with 0.3 kg M ha^{-1} (20.94) followed by application of 0.1 kg M (17.05).While minimum active nodules (16.50) were obtained from control plots. The number of nodules increased with increasing application of Mo with both the soil and seed treated (Chandra and Kothari, 2007).

Table 1: Number of nodules, active nodules and weight of nodule as affected by molybdenum levels, application methods of inoculation and different chickpea varieties.

	No. of Nodules			Active nodules			Nodules fresh weight		
Molybdenum levels	Irrigated	Rainfed	**Mean**	Irrigated	Rainfed	Mean	Irrigated	Rainfed	**Mean**
0	23.9	24.7	24.3	16.3	16.7	16.5	1.75	1.64	1.70 c
0.1	24.8	25.3	25.1	16.8	17.3	17.1	1.82	1.75	1.78 b
0.2	28.7	29.2	28.9	20.9	21.4	21.2	2.00	1.91	1.96 a
0.3	28.9	29.4	29.2	20.7	21.9	21.3	2.18	2.12	2.15 a
Means	26.58	27.15	26.88	18.7	19.3	19.03	1.94	1.86	
Application Methods	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean
0	25.9	26.3	26.1	17.9	18.3	18.1	1.89	1.83	1.86 c
Soil	27.3	28.0	27.7	19.6	20.0	19.8	1.97	1.87	1.92 a
Seed	26.6	27.1	26.8	18.6	19.6	19.1	1.95	1.87	1.91 b
Means	26.58	27.15		18.68	19.33		1.94	1.86	
Varieties	Irrigated	Rainfed	**Mean**	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean
Chattan	25.8	27.5	26.6	17.8	19.6	18.7	1.86	2.01	1.94 a
Lawaghar	26.2	28.0	27.1	18.2	20.4	19.3	1.77	1.95	1.86 b
Means	26.0	27.7		18.0	20.0		1.81	1.98	
Conditions	Irrigated	Rainfed		Irrigated	Rainfed	Mean	Irrigated	Rainfed	
Means	26.0	27.7		18.0	20.0		1.81	1.98	

Table 2:Least significant difference test for number of nodules, active nodules and nodules fresh weight as affected by molybdenum levels, application methods of inoculation and different chickpea varieties.

Number of nodules No. Active Nodules Nodules fresh weight

Moly. (M)	LSD	LSD	LSD
Molybdenum	0.5175	0.4959	0.06
Bacteria	0.4482	0.4295	0.05
Varities	0.3659	0.3506	0.04
M x V	***	***	***
V x AM	***	***	***
AM x M	***	***	***
M x V x AM	***	***	***

Foliar application of Mo increase nodules per plant significantly (Togay et al., 2015) and its molecules contribute positively to release the enzymes (nitrogenase) which help for the fixation of nitrogen in legumes. Among application methods nodulation bacteria applied to soil produced more active nodules (19.80) followed by seed applied (18.85) and minimum active nodules (18.10) were obtained from molybdenum control plots. Desi chickpea variety (Chattan-2015) produced lower active nodules (18.69) and kabuli variety (Lawaghar-2000) produced more active nodules (19.14) plant^{-1}. Increasing molybdenum levels from 0 to 0.2 kg ha^{-1} increased active nodules plant^{-1} and further increases in Mo levels (0.3 kg ha^{-1}) increased active nodules for Chattan-2015, while decreased in Lawaghar-2000. Similar results were found by Tantawy et al., (2013) they reported that Fe and Mo significantly increased active nodules number in broad bean. The numbers of active nodules were increased with the application of molybdenum (Bhuiyan et al., 2008).

Nodules fresh weight plant^{-1} was significantly affected by various levels of molybdenum (M), application methods (AM) of nitrogen fixing bacteria, varieties (V) and location. All possible interactions except (M x V) were found non-significant (Table 1& 2). Maximum nodules fresh weight plant^{-1} (2.02 g) was observed in plots applied with 0.3 kg Mo ha^{-1} followed by plots receiving 0.2 kg Mo ha^{-1} plant^{-1} (1.88 g) these results were also found significant. Plots receiving 0.1 kg ha^{-1}Mo produced higher nodules weight plant^{-1} (1.65 g), while lowest nodule weight plant^{-1} (1.60 g) was recorded in control plots. Bhanavase; Patil (1994) reported that molybdenum enhance nodule numbers, nodule weight plant^{-1}. The active nodule activity was increased with increasing Mo levels (Hristozkova et al., 2006). Among application methods of bacteria, soil applied produced maximum nodules fresh weight (1.82 g) as compared to seed treatment (1.80 g). Chattan-2015 produced higher nodule fresh weight (1.94 g) as compared to Lawaghar-2000 (1.63 g). Location showed statistically significant effect on nodules fresh weight plant^{-1} at pod filling stage. Maximum nodule fresh weight (1.89 g) was observed at Peshawar, while Karak had minimum nodule weight (1.68 g). It was observed that molybdenum fertilization can enhance the nitrogen-fixing symbiosis through increased nitrogenase activity rates, due to which larger nodules occurred in soybean and common bean (Adams 1997; Vieira et al., 1998).

CONCLUSION

On the basis of results, it is concluded that Karak has maximum number of nodules, number of active nodules and nodules fresh weight than Peshawar. Among molybdenum levels higher number of nodules, active nodules and nodules fresh weight were achieved with the application of 0.3 Mo kg ha^{-1}. Bacterium application to seed, achieved best results than soil application. Among varieties Karak III showed better performance than Lawaghar variety. It is evident that Karak (rainfed) region is best for the production of chickpea.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Asim Muhammad designed and performed the experiment. Kabir Khan analyzed the data. Sarmad Iqbal reviewed the manuscript and added overall corrections.

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