

RESEARCH ARTICLE

EFFECT OF IRON, MOLYBDENUM AND *RHIZOBIUM* ON BIOLOGICAL PROPERTIES OF SOIL IN SUMMER GROUNDNUT (*ARACHIS HYPOGAEA* L.)Divya Gaur^{1*}, N.J. Jadav², Aishwarya Maheta³ and Shruti Gaur⁴^{1,2,3}Department of soil science and agricultural chemistry, AAU, Anand, Gujarat⁴Department of soil science and agricultural chemistry, JAU, Junagadh, GujaratEmail: divyagaur777@gmail.com

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Abstract: The study was carried out to determine the effect of iron, molybdenum and *Rhizobium* on biological properties of soil in summer groundnut. The field experiment comprised of eighteen treatment combinations with three levels of iron 0, 5 and 10 kg Fe ha⁻¹, three levels of molybdenum 0, 1 and 2 kg Mo ha⁻¹ and two levels of *Rhizobium*: without *Rhizobium* and with *Rhizobium* inoculation were studied with GG-34 variety of groundnut in randomized block design in factorial concept with three replications. Among all the treatments application of 10 kg Fe ha⁻¹, 1.0 kg Mo ha⁻¹ and seed inoculation with *Rhizobium* proved superior over other treatments.

Keywords: Iron, Molybdenum, *Rhizobium*, Groundnut

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop belonging to family *Fabaceae* (or *Leguminosae*). Groundnut is the king of oilseed crops and vegetable oil economy of country depends very much on it. It is mostly grown for seeds and oil production in the world. India ranks second in groundnut production in world. In India, during 2020-21 groundnut crop cultivated in about 6 million hectares with the total production of 10.24 mt and productivity of 1703 kg ha⁻¹. Iron is one of the most deficient nutrients in Indian soils. It is a structural component of cytochrome, hematin and leghaemoglobin. It is also important in activation of several enzymes including: fumaric hydrogenase, catalase, dehydrogenase, oxidase and peroxidase. It helps in absorption of other plant nutrients. Iron is also associated with chloroplast and protein synthesis. Molybdenum is an essential plant nutrient found in soil. It is also known as ultra-micronutrient as this is required in very less amount. As its fewer amounts are required, the deficiency and sufficiency range are narrow. Molybdenum requirement of legumes are mostly higher than grasses. Molybdenum is more accessible in anion form (MoO₄²⁻) to plants in alkaline soils as it becomes more soluble at higher pH. While in acidic soils its availability decreases due to anion adsorption. The inoculation of *Rhizobium* for legumes has been used worldwide. Seed inoculation with an efficient *Rhizobium* strain is the cheapest and most important input in leguminous crop production. Inoculation of

legume crops with proper strain of *Rhizobium* can fulfill up to 90% of their nitrogen requirements (Anandham *et al.*, 2007).

MATERIALS AND METHODS

Two years field experiment was conducted at Agronomy Farm, BACA, Anand Agricultural University, Anand, Gujarat during summer seasons of 2021 and 2022 in randomized block design in factorial concept with three replications. The experimental field was well drained and sandy loam in texture. Geographically, Anand is situated at 22° 35' N latitude, 72° 55' E longitude with an elevation of 45.1 m above the mean sea level. The climate of this region is typically semi-arid and sub-tropical as Anand is almost 70 km away from Arabian Sea Coast. Maximum temperature ranged between 15.4°C to 39.5°C and minimum temperature ranged between 5.3°C to 26.3°C during the period of experimentation in 2021. While maximum temperature ranged between 24.5°C to 41.7°C and minimum temperature ranged between 10.4°C to 27.9°C during the period of experimentation in 2022. The eighteen treatment combinations involving three factors, each of Fe and Mo at 3 levels and *Rhizobium* at 2 levels were taken, in which Fe at 0, 5 and 10 kg ha⁻¹, molybdenum at 0, 1 and 2 kg ha⁻¹ and two levels of *Rhizobium*; without *Rhizobium* and with *Rhizobium* inoculation were taken. Iron and molybdenum were applied in soil before sowing through FeSO₄ and Ammonium molybdate (NH₄)₆Mo₇O₂₄·4H₂O, respectively. The *Rhizobium*

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biofertilizer was inoculated through seed treatment (5 ml kg⁻¹ seed). Recommended dose of fertilizer for summer groundnut was 25:50:0 kg of N, P₂O₅ and K₂O/ha, respectively. The whole dose of fertilizer was applied at the time of sowing.

Total microbial count: Total microbial count was estimated using serial plate dilution technique. Soil samples were collected from each plot before sowing and at harvest. One gram of fresh soil sample with 10 ml sterilized water, transferred to a 250 ml flask and vigorously shaken for 1 hour. From this, 1 ml of suspension transferred aseptically to 9 ml sterilized water. Similarly, dilutions were made up to 10⁻¹¹. Now 0.1 ml suspension from each tube spread to nutrient agar plates. These plates were incubated for 24-48 hours and colony forming units (CFU g⁻¹) were counted using colony counter.

Microbial Biomass Carbon (MBC): Microbial biomass carbon was determined by chloroform fumigation method suggested by Voroney *et al.* (1993). 10 g moist soil sample was fumigated with ethanol free chloroform for 24 hours in vacuum fumigator. Following fumigant removal, soil was extracted with 0.5 M K₂SO₄. After half an hour shaking filtrate was placed in water bath with 0.1 N NaOH in another small tube. This CO₂ trapped NaOH was titrated by 0.1 N HCl.

Urease activity: Urease activity was determined following the method suggested by Tabatabai and Bremner (1972). Five gram of soil was incubated with 9 ml THAM buffer, 0.2 ml toluene and 1 ml of 0.2% of urea solution at 37°C for 2 hours. Then 50 ml KCl-AgSO₄ solution was added, and shaking was done for 30 minutes. Soil suspension was filtered. Taking 20 ml aliquot from filtrate, NH₄-N was determined by steam distillation method.

Statistical Analysis: The data on different aspects of groundnut crop under investigation were subjected to statistical analysis as per the procedure described by researchers at the Computer Center, Department of

Agricultural Statistics, Anand Agricultural University, Anand. The values of calculated “F” was worked out and compared with the value of table “F” at 5 % level of significance. The value of SEM, critical difference (CD) and co-efficient of variation (CV %) were also calculated.

RESULTS AND DISCUSSION

Total microbial count: A perusal of data presented in Table 1 revealed that total microbial count increased with increasing level of iron during both the individual years as well as in pooled. Highest microbial count *i.e.*, 3.34 × 10⁷, 3.42 × 10⁷ and 3.38 × 10⁷ CFU g⁻¹ recorded with Fe application @ 10 kg ha⁻¹. Molybdenum application at 1 kg ha⁻¹ significantly increased microbial count 3.37 × 10⁷ CFU g⁻¹ in pooled. Application of Mo increased microbial population and these important effects of Mo on soil conditions were due to the role of Mo-enzymes in nitrogen metabolism. Similar results were also reported by Sun *et al.* (2013). Highest total microbial count 6.33 × 10⁷ CFU g⁻¹ in pooled recorded with seed inoculation with *Rhizobium*. Higher microbial population might be due to biofertilizer inoculation. Similar results were also observed by Gupta *et al.* (2020).

Rhizobium count: *Rhizobium* count increased with increasing level of iron during both the individual years as well as in pooled. Highest microbial count 3.24 × 10⁵ CFU g⁻¹ in pooled recorded with Fe application @ 10 kg ha⁻¹. Molybdenum application @ 1 kg ha⁻¹ significantly increased *Rhizobium* count 3.28 × 10⁵ CFU g⁻¹ in pooled. Similar findings were also reported by Biswas *et al.* (2020). *Rhizobium* count increased significantly with seed inoculation with *Rhizobium*. Highest *Rhizobium* count 5.49 × 10⁵ CFU g⁻¹ was observed in pooled. Increase in CFU at the end of experiment was observed that suggests increase in population of nitrogen fixers. Similar results were also reported by Biswas *et al.* (2020).

Table 1. Effect of iron, molybdenum and *Rhizobium* on total microbial count, *Rhizobium* count, microbial biomass and urease activity

Treatments	Total microbial count (CFU g ⁻¹ soil)			<i>Rhizobium</i> count (CFU g ⁻¹ soil)			Microbial biomass carbon (µg g ⁻¹ soil)			Urease activity (µg urea g ⁻¹ hr ⁻¹)		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Iron (kg ha⁻¹):												
Fe ₀	2.98 × 10 ⁷	3.14 × 10 ⁷	3.06 × 10 ⁷	2.52 × 10 ⁵	2.85 × 10 ⁵	2.69 × 10 ⁵	200.30	206.48	203.39	171.62	173.06	172.34
Fe ₅	3.08 × 10 ⁷	3.21 × 10 ⁷	3.15 × 10 ⁷	2.88 × 10 ⁵	3.15 × 10 ⁵	3.01 × 10 ⁵	208.32	209.70	209.01	174.82	177.21	176.01
Fe ₁₀	3.34 × 10 ⁷	3.42 × 10 ⁷	3.38 × 10 ⁷	3.11 × 10 ⁵	3.37 × 10 ⁵	3.24 × 10 ⁵	217.12	223.97	220.54	179.23	180.79	180.01
SEm ±	0.10	0.07	0.06	0.10	0.10	0.07	4.46	4.38	3.12	2.74	3.07	2.40
CD(P=0.05)	0.28	0.23	0.17	0.38	0.29	0.19	12.81	12.58	8.21	NS	NS	NS
Molybdenum (kg ha⁻¹):												
Mo ₀	3.02 × 10 ⁷	3.11 × 10 ⁷	3.07 × 10 ⁷	2.70 × 10 ⁵	2.83 × 10 ⁵	2.76 × 10 ⁵	199.01	203.43	201.22	170.27	172.76	171.52
Mo ₁	3.34 × 10 ⁷	3.40 × 10 ⁷	3.37 × 10 ⁷	3.08 × 10 ⁵	3.47 × 10 ⁵	3.28 × 10 ⁵	215.58	221.69	218.64	178.48	182.36	180.92
Mo ₂	3.03 × 10 ⁷	3.25 × 10 ⁷	3.14 × 10 ⁷	2.73 × 10 ⁵	3.07 × 10 ⁵	2.90 × 10 ⁵	211.15	215.02	213.08	174.92	175.94	175.43

	10 ⁷	10 ⁷	10 ⁷	10 ⁵	10 ⁵	10 ⁵						
SEm ±	0.10	0.07	0.06	0.13	0.10	0.07	4.46	4.38	3.12	2.74	3.07	2.40
CD(P=0.05)	0.28	0.23	0.17	0.38	0.29	0.19	12.81	12.58	8.21	NS	NS	NS
Rhizobium :												
R ₀	5.80 × 10 ⁵	6.20 × 10 ⁵	5.99 × 10 ⁵	4.45 × 10 ⁴	4.96 × 10 ⁴	4.71 × 10 ⁴	174.45	180.60	177.53	172.82	174.88	173.85
R ₁	6.20 × 10 ⁷	6.45 × 10 ⁷	6.33 × 10 ⁷	5.23 × 10 ⁵	5.75 × 10 ⁵	5.49 × 10 ⁵	242.71	246.16	244.43	176.30	179.83	178.06
SEm ±	0.08	0.06	0.05	0.11	0.08	0.07	3.64	3.57	2.55	2.24	2.51	1.92
CD(P=0.05)	0.23	0.19	0.15	0.31	0.23	0.21	10.46	10.27	7.20	NS	NS	NS
Significant Interaction	-	-	-	Fe × R, Mo × R	Fe × R, Mo × R	Fe × R, Mo × R	-	-	-	-	-	-
CV (%)	13.28	10.35	11.85	14.20	13.75	14.06	9.09	8.70	8.88	6.67	7.34	7.02

Interaction effect of Fe × R

Data given in Table 2 revealed that combination of Fe and *Rhizobium* on *Rhizobium* count found to be significant in both the individual years as well as in

pooled. Significantly higher *Rhizobium* count were recorded under seed inoculation with *Rhizobium* along with application of Fe @ 10 kg ha⁻¹.

Table 2. Interaction effect of iron and *Rhizobium* on *Rhizobium* count after harvest

Treatments	Rhizobium count(CFU g ⁻¹ soil)					
	2021		2022		Pooled	
	R ₀	R ₁	R ₀	R ₁	R ₀	R ₁
Fe ₀	3.80×10 ⁴	4.67×10 ⁵	4.34×10 ⁴	5.27×10 ⁵	4.07×10 ⁴	4.97×10 ⁵
Fe ₁	4.73×10 ⁴	5.29×10 ⁵	4.79×10 ⁴	5.82×10 ⁵	4.76×10 ⁴	5.56×10 ⁵
Fe ₂	4.82×10 ⁴	5.74×10 ⁵	5.76×10 ⁴	6.16×10 ⁵	5.29×10 ⁴	5.95×10 ⁵
SEm ±	0.14		0.14		0.09	
CD	0.39		0.41		0.28	

Interaction effect of Mo × R

A perusal of data given in Table 3 explicit that combination of Mo and *Rhizobium* on *Rhizobium* count found to be significant in both the individual

years as well as in pooled. Significantly higher *Rhizobium* count were recorded under seed inoculation with *Rhizobium* along with application of Mo @ 1 kg ha⁻¹

Table 3. Interaction effect of molybdenum and *Rhizobium* on *Rhizobium* count after harvest

Treatments	Rhizobium count (CFU g ⁻¹ soil)					
	2021		2022		Pooled	
	R ₀	R ₁	R ₀	R ₁	R ₀	R ₁
Mo ₀	3.89×10 ⁴	5.01×10 ⁵	4.29×10 ⁴	5.23×10 ⁵	4.09×10 ⁴	5.12×10 ⁵
Mo ₁	4.58×10 ⁴	5.71×10 ⁵	5.74×10 ⁴	6.37×10 ⁵	5.16×10 ⁴	6.04×10 ⁵
Mo ₂	4.87×10 ⁴	4.98×10 ⁵	4.86×10 ⁴	5.64×10 ⁵	4.86×10 ⁴	5.31×10 ⁵
SEm ±	0.14		0.14		0.09	
CD	0.39		0.41		0.28	

Microbial biomass carbon: Significantly higher microbial biomass carbon in soil at harvest *i.e.*, 217.12, 223.97 and 220.54 µg g⁻¹ soil observed with application of Fe @ 10 kg ha⁻¹ compared to control in both the individual years as well as in pooled, but these results were at par with 5 kg Fe ha⁻¹. Application of 1 kg Mo ha⁻¹ obtained highest microbial biomass carbon in soil at harvest *i.e.*, 215.58, 221.69 and 218.64 µg g⁻¹ soil in 2021, 2022 and pooled, respectively. The corresponding values of microbial biomass carbon in soil at harvest were

242.71, 246.16 and 244.43 µg g⁻¹ soil in 2021, 2022 and pooled, respectively with *Rhizobium* inoculation. It might be due to more crop biomass owing to *Rhizobium* inoculation that resulted in more organic residues and exudates in soil resulting higher microbial biomass carbon. Similar results were observed by Zhang *et al.* (2010) and Bhattacharya and Chandra (2013).

Urease activity: Urease activity in soil did not differ significantly with different level of iron, molybdenum and biofertilizer application during

both the individual years as well as in pooled. Application of 1 kg Mo ha⁻¹ obtained highest urease activity in soil *i.e.*, 178.48, 182.36 and 180.92 µg urea g⁻¹ hr⁻¹ in 2021, 2022 and pooled, respectively. The results suggested that excess amount of trace element present in soil can disturb biochemical activities in soil. Similar findings of inhibition of urease enzyme with higher amount of Mo were also reported by Zaborowska *et al.* (2016).

CONCLUSION

Based on obtained results of this study it can be concluded that application of 20 kg ha⁻¹ Fe, 1 kg ha⁻¹ Mo and seed treatment with *Rhizobium* culture in groundnut led to improve biological properties of soil.

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