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Yield and Rhizosphere - microflora of Cowpea in Response to Magnesium Fertilization in Lateritic Soils of Kerala

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ABSTRACT

Background: Magnesium deficiency has become a major nutritional disorder in lateritic soils of Kerala. Appropriate magnesium fertilization is the best strategy to combat deficiency issues. Apart from correcting nutritional deficiency, magnesium fertilization has an influence on the growth of beneficial microbes such as nitrogen fixing bacterias and arbuscular mycorrhizal fungi. The experiment aimed to investigate the effect of magnesium fertilization on crop yield and population rhizosphere micoflora of cowpea in lateritic soils of Kerala.

Methods: A pot culture experiment was conducted with a gradient of magnesium additions ranging from 5 mg kg^{-1} to 80 mg kg^{-1} of soil along with recommended dose of fertilizers. Population of rhizobium, free living nitrogen fixing bacteria, spore count of arbuscular mycorrhizal fungi and per cent root colonization of arbuscular mycorrhizal fungi were studied during flowering. The available magnesium and magnesium uptake were also worked out during harvest. Yield and yield contributing characteristics of cowpea were measured during harvest stage.

Result: Magnesium addition produced significant variations in population of rhizobium and free- living nitrogen fixing bacteria whereas spore count of AMF and per cent root colonization of AMF did not vary according to the added doses of magnesium. A higher population of rhizobium, free living nitrogen fixers, root nodules, magnesium uptake, plant height and yield were obtained in the treatment where magnesium was applied @ 10 mg kg^{-1} soil.

Key words: Arbuscular mycorrhizal fungi and yield, Cowpea, Free living nitrogen fixing bacteria, Magnesium carbonate, Rhizobium.

INTRODUCTION

Soils of Kerala had developed from acid igneous rocks under humid tropical climate and heavy rainfall situations (Sureshkumar *et al*., 2018). Lateritic soils occupy more than 50 per cent of the total geographical area of Kerala (Krishnan *et al*., 1996). Due to poor cation retention capacity of lateritic soils, magnesium deficiency has become a major nutritional disorder in these soils. Mitigation of magnesium deficiency by fertilization practices improves nutrient availability and has an influence on growth of rhizosphere microflora.

Magnesium is an essential element for growth of beneficial microbes in soil. Jones and Huber (2007) reported an increase in the reproduction of soil bacteria with the application of magnesium carbonate. Calcium and magnesium are essential elements for efficient nitrogen fixation by rhizobia and magnesium deficiency results in reduced nitrogen fixation (Dechen *et al*., 2015). Sufficient magnesium supply considerably increased nodule number, size, weight, mass, nodulation index and nitrogenase activity in nodules of soybean plant validating that magnesium supply plays crucial role in nodule formation and development (Khaitov, 2018).

Cowpea (*Vigna unguiculata*) is an annual herbaceous legume belonging to fabaceae family, cultivated as main crop and as an intercrop in Kerala. It constitutes 13.88% (5803.05ha) of total area under vegetables. The area, production and productivity of cowpea in Thrissur district are 406.88 ha, 1924 tonnes and 4730 kg per ha during 2018-19 (GOK, 2020). The roots of leguminous plants have higher cation exchange capacity than graminaceous plants. They requires higher proportion of basic cations in their nutrition. Along with increasing yield of cowpea, magnesium management will improve soil fertility through nitrogen fixation by promoting the growth of symbiotic and non-symbiotic nitrogen fixing bacteria in the rhizosphere region. The low retention of magnesium in lateritic soil limits cowpea cultivation in these areas. Hence the present experiment was carried out to study modifications in the rhizosphere microflora and yield of cowpea under graded doses of magnesium fertilization.

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MATERIALS AND METHODS

The experiment was carried out at Radiotracer Laboratory, College of Horticulture, Kerala Agricultural University during January/february- April/may, 2019 to investigate the effect of magnesium nutrition on rhizosphere micro-flora, growth and yield of cowpea. Bush cowpea variety Bhagyalakshmi

was used in the study. Top soil (0-15 cm depth) representing lateritic origin was collected, air dried, ground with wooden mortar and pestle, sieved through 2 mm sieve and characterized for available nutrient content and initial soil microflora. The texture of experimental soil was sandy clay with a pH of 4.70 which belongs to very strongly acidic category. The soil was medium in organic carbon (1.32%), available nitrogen (476.67 kg ha^{-1}) and potassium (240.18 kg ha^{-1}) and high in phosphorus (98.04 kg ha^{-1}). The secondary and micronutrients except magnesium (64.53 mg kg^{-1}) and boron (0.22 mg kg^{-1}) were sufficient.

The treatments consisted of 9 levels of magnesium *viz.*, magnesium @ 5 mg kg^{-1} of soil (T_2), magnesium @ 10 mg kg^{-1} of soil (T_3), magnesium @ 15 mg kg^{-1} of soil (T_4), magnesium @ 20 mg kg^{-1} of soil (T_5), magnesium @ 30 mg kg^{-1} of soil (T_6), magnesium @ 40 mg kg^{-1} of soil (T_7), magnesium @ 50 mg kg^{-1} of soil (T_8), magnesium @ 60 mg kg^{-1} of soil (T_9) and magnesium @ 80 mg kg^{-1} of soil (T_{10}) and absolute control (T_1). The recommended dose of fertilizers as per the package of practices of KAU (KAU, 2016) includes the application of 20 t ha^{-1} of organic manure, 250 kg ha^{-1} calcium carbonate and 20:30:10 kg ha^{-1} of N, P_2O_5 and K_2O which was applied in all treatments except absolute control. The experiment was laid out in completely randomized design (CRD) with four replications in earthen pots of 5 kg capacity and five pots were maintained in each replication.

The quantity of fertilizers were calculated for 5 kg soil. Organic manure in the form of vermi-compost was added after one week of application of calcium carbonate (AR grade). Magnesium required for each treatment was supplied through magnesium carbonate (AR grade) two weeks after organic manure application. Seeds were treated with rhizobium culture and kept for half an hour in shade then sown manually by putting 2-3 seeds per pot and one healthy plant retained one week after emergence. Complete dose of phosphorus, potassium and half split of nitrogen was applied after thinning of plant population and second dose of nitrogen was supplied 15 days later. Foliar application of boron (0.05%) was done twice to combat boron deficiency. Irrigation with de-ionized water, weed control and plant protection measures were adopted uniformly in each pot.

Rhizosphere soil samples were collected during flowering by uprooting 5 plants from each replication and keeping the soil around root system intact. After removing the bits of plant roots and other debris, the soil, strongly adhered to the roots was immediately used for enumeration of symbiotic nitrogen fixing bacteria and free living nitrogen fixing bacteria. Population was counted on agar plates containing appropriate media following serial dilution technique and pour plate method (Pramer and Schimdt, 1965). Yeast extract mannitol agar medium (Vincent, 1970) and Jenson's medium (Smita and Goyal, 2017) were used for rhizobium and free living nitrogen fixing bacteria respectively.

Spore count of AMF was determined in the rhizosphere soil of plants using wet sieving and decanting method (Gerdmann and Nicolson, 1963). The soil water suspension after removing heavier particles was passed through a series of different size sieves (250 µm, 106 µm, 75 µm, 45 µm, 37 µm) arranged in descending order of their mesh size. Seivates were collected from each sieve separately in beakers. Supernatant from each beaker was separately filtered through Whatman No.1 filter paper and spores were examined under stereo zoom microscope (LABOMED).

The roots collected from cowpea plants were used for analyzing percentage root colonization of AMF. The roots used to measure AMF colonization were cut into approximately 1 cm length and were cleared in 10% (w/ v) KOH at 90°C in a water bath for 60 minutes or 121°C for 10 minutes to remove host cytoplasm and nuclei. After removing KOH acidify with 1 per cent HCl for 10 minutes to neutralize the extreme KOH. Then root bits were stained with 0.05% (w/v) Trypan blue in lactophenol and heated gently for 10 minutes. The excess stain was removed by lactophenol and 30 root segments of each sample were examined under microscope for percentage of root length colonized by Arbuscular Mycorrhizal Fungi (Philips and Haymen, 1970).

$$\text{Per cent root colonization} = \frac{\text{No. of root segments colonized}}{\text{Total no. of root segments examined}} \times 100$$

Biometric parameters such as plant height, number of pods per plant, yield per plant, root nodules per plant were observed at harvest. Available magnesium content of soil and uptake of magnesium was determined at harvest. All the data collected were subjected to analysis of variance in CRD using OPSTAT software package (Sheoran *et al.*, 1998). Duncan's multiple range test was employed to test the significance of difference between means of treatments at 5% level of significance.

RESULTS AND DISCUSSION

Effect of magnesium doses on rhizosphere microflora

The population of symbiotic nitrogen fixing bacteria *viz;* Rhizobium and Bradyrhizobium during flowering of cowpea varied significantly as per grades of magnesium added (Table 1). The highest population of rhizobia was recorded in treatment supplied with 10 mg kg^{-1} magnesium (Fig 1). The effect of further addition of magnesium showed no significant increase. Dechen *et al.* (2015) also reported that calcium and magnesium are essential elements for efficient nitrogen fixation by rhizobia and Mg deficiency results in reduced nitrogen fixation. As per Kiss *et al.* (2004) magnesium has important role in metabolism of rhizobium bacteria and nodule development because nitrogen fixing bacteria requires ATP that must exist as a magnesium complex. Another possible mechanism for improved rizhosphere colonization of symbiotic nitrogen fixing bacteria may be magnesium induced increase in carbon flow to roots.

However, a linear response of nitrogen fixers to magnesium availability could not be recorded in this study. The free living nitrogen fixing bacteria and number of root nodules (Table 1) showed a similar pattern to that of Rhizobium. Among various treatments significantly higher free living nitrogen fixing bacteria (Fig 2) and number of root nodules was recorded in plants treated with 10 mg kg^{-1} of magnesium during flowering stage. According to Peng *et al*. (2018), nodule growth under nitrogen limited conditions was enhanced by external Mg supply due to higher partitioning of photosynthates to roots as nitrogen fixation require large amount of energy. The medium to high status of available N in the experimental soil can be attributed to the lack of linear response to graded dose of magnesium.

Spore count of AMF (Fig 3) in the rhizosphere soil during flowering of cowpea (Table 1) could not exhibit a significant variation with varying levels of magnesium supplied. However, per cent root colonization with AMF (Fig 4) was higher in treatments with higher availability of magnesium when compared to absolute control (Table 1). Similar reports were given by Gryndler *et al.* (1992), where they observed pronounced positive effect of magnesium on per cent root colonization of maize and substitution of magnesium by potassium or calcium significantly reduced infection.

Effect of magnesium doses on available magnesium and biometric parameters

The variations in available Mg content in soil corresponded to the gradation in magnesium through added sources with the highest content in treatment supplied with 80 mg $kg^{-1}$of magnesium (Table 2). Similar increase in available magnesium with magnesium fertilization was observed by Fageria (1991). The highest uptake of magnesium was recorded in treatments supplied with 20 and 60 mg $kg^{-1}$of magnesium (Table 2). Yield and related biometric attributes were significantly influenced by the varying levels of magnesium added (Table 2). Significantly higher plant height was obtained in treatment supplied with 10 mg kg^{-1} of magnesium with a mean value of 61.65 cm followed by plants treated with 30 and 15 mg kg^{-1} of magnesium. The treatments differed significantly with respect to number of pods per plant. Significantly higher number of pods per plant was obtained in plants received 50 mg kg^{-1} of magnesium and was at par with that of 5, 10, 15, 20 and 80 mg kg^{-1} of magnesium.

Table 1: Effect of treatments on microbial populationduring flowering.

	Treatments	Rhizobium (cfu/g of soil)	Total N-fixing bacteria (cfu/g of soil)	No of root nodules	Arbuscular mycorrhizal fungi	
					Spores /g soil	Root colonization (%)
T_1	Absolute control	2.00^{e}	43.50^{f}	20.5^{g}	23.45	75^{c}
T_2	RDF + Magnesium @ 5 mg kg^{-1} of soil	28.50^{b}	115.00^{b}	32.0^{cde}	24.15	80^{bc}
T_3	RDF + Magnesium @ 10 mg kg^{-1} of soil	41.50^{a}	149.00^{a}	50.0^{a}	24.65	90^{a}
T_4	RDF + Magnesium @ 15 mg kg^{-1} of soil	4.50^{e}	47.00^{ef}	41.0^{b}	24.30	85^{ab}
T_5	RDF + Magnesium @ 20 mg kg^{-1} of soil	20.50^{c}	84.50^{c}	36.5^{bc}	22.77	90^{a}
T_6	RDF + Magnesium @ 30 mg kg^{-1} of soil	16.50^{d}	88.50^{c}	26.0^{efg}	22.98	90^{a}
T_7	RDF + Magnesium @ 40 mg kg^{-1} of soil	20.50^{c}	50.50^{ef}	27.5^{ef}	23.18	90^{a}
T_8	RDF + Magnesium @ 50 mg kg^{-1} of soil	18.00^{cd}	59.50^{de}	35.0^{bcd}	23.99	85^{ab}
T_9	RDF + Magnesium @ 60 mg kg^{-1} of soil	25.50^{b}	68.50^{d}	27.5^{ef}	23.3	85^{ab}
T_{10}	RDF + Magnesium @ 80 mg kg^{-1} of soil	28.00^{b}	102.00^{b}	25.0^{fg}	25.33	90^{a}

Treatment means with common superscript do not differ significantly.

Table 2: Effect of magnesium doses on available magnesium, magnesium uptake, yield and biometric parameters of cowpea.

	Treatments	Available Mg (mg kg^{-1})	Mg uptake (mg per plant dry weight)	Plant height	Pods per plant (cm)	Length of pods (cm)	Yield (g $plant^{-1}$)
T_1	Absolute control	63.10^{g}	26.67^{j}	37.90^{f}	8.25^{f}	9.60^{g}	33.04^{e}
T_2	RDF + Magnesium @ 5 mg kg^{-1} of soil	70.52^{f}	58.88^{de}	52.80^{c}	19.5^{abc}	17.70^{a}	70.39^{c}
T_3	RDF + Magnesium @ 10 mg kg^{-1} of soil	79.49^{b}	66.62^{a}	61.65^{a}	19.50^{abc}	15.80^{bc}	79.33^{a}
T_4	RDF + Magnesium @ 15 mg kg^{-1} of soil	93.10^{d}	55.59^{ef}	56.95^{b}	18.75^{abc}	15.95^{bc}	73.33^{bc}
T_5	RDF + Magnesium @ 20 mg kg^{-1} of soil	82.55^{e}	63.40^{abc}	49.65^{d}	20.25^{ab}	15.05^{cd}	76.57^{ab}
T_6	RDF + Magnesium @ 30 mg kg^{-1} of soil	89.42^{d}	48.34^{g}	58.95^{b}	17.25^{bc}	17.00^{ab}	75.28^{abc}
T_7	RDF + Magnesium @ 40 mg kg^{-1} of soil	100.80^{c}	52.45^{f}	48.40^{d}	18.00^{bc}	15.50^{c}	73.30^{bc}
T_8	RDF + Magnesium @ 50 mg kg^{-1} of soil	110.40^{b}	61.25^{cd}	49.80^{d}	21.75^{a}	16.35^{abc}	76.12^{abc}
T_9	RDF + Magnesium @ 60 mg kg^{-1} of soil	126.82^{a}	64.94^{ab}	48.25^{d}	16.50^{cd}	17.70^{a}	74.64^{abc}
T_{10}	RDF + Magnesium @ 80 mg kg^{-1} of soil	130.95^{a}	62.07^{bcd}	49.55^{d}	18.75^{abc}	13.95^{de}	74.19^{abc}

Treatment means with common superscript do not differ significantly.

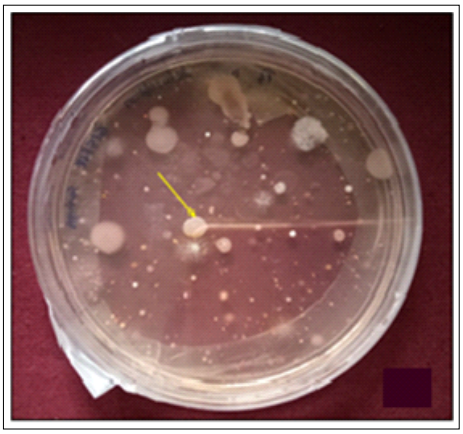

Fig 1: *Rhizobium.*
T_3 - Magnesium @ 10 mg kg^{-1}.

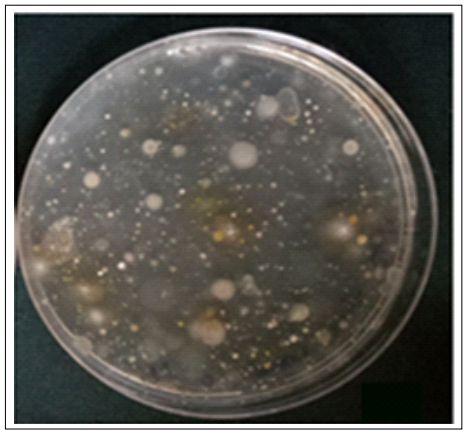

Fig 2: Nitrogen fixing bacteria.
T_3 - Magnesium @ 10 mg kg^{-1}.

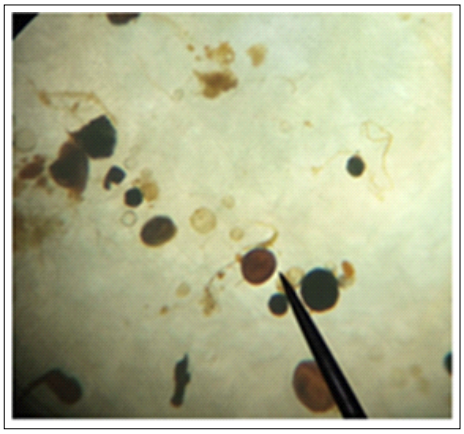

Fig 3: Spore count of AMF (10X).
T_3 - Magnesium @ 10 mg kg^{-1}.

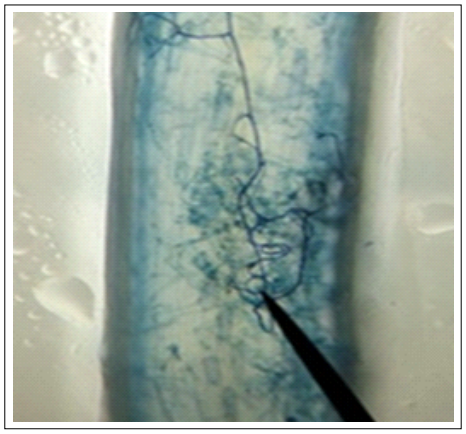

Fig 4: Per cent root colonization of AMF (45X).
T_3 - Magnesium @ 10 mg kg^{-1}.

Significantly long pods were observed in plants supplied with 5, 30 and 60 mg kg^{-1} of magnesium. The treatments differed significantly with respect to the yield per plant. Plants treated with 10 mg kg^{-1} of magnesium recorded significantly higher yield but was at par with that of 50, 20, 30, 60 and 80 mg kg^{-1} magnesium received plants. The absolute control treatment recorded the lowest yield. The lack of growth response to higher dose of magnesium addition indicated that moderate level of magnesium *i.e.* 10 mg kg^{-1} would be sufficient to meet the magnesium requirements of cowpea. Kasinath (2014) Critical level of magnesium for maximum yield response in tomato was 75 mg kg. Supplying 10 mg kg of magnesium through magnesium carbonate raise the available magnesium status to 75 mg kg and contributes to maximum yield response. This can be attributed to optimum calcium-magnesium ratio in the treatment.

CONCLUSION

The results suggest that moderate magnesium fertilization improved plant growth and altered plant nutrient concentration via enhancement of mutualistic interactions between cowpea and rhizoshere microflora. A higher response of rizhosphere microflora and yield of cowpea were obtained in treatment supplied with 10 mg kg^{-1} of magnesium.

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