Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5705

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (4):** 1666-1670 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Influence of Carrier and Liquid Rhizobial Biofertilizer on Nodulation, Nitrogenase Activity and Yield in Mungbean Crop by Different Method of Applications

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Received: 19.07.2017 | Revised: 28.07.2017 | Accepted: 2.08.2017

ABSTRACT

Present pot culture studies undertaken to study the influence of Rhizobial Biofertilizer with 12 treatments following Complete Randomized Block Design (CRD) on nodulation, nitrogenase activity by Acetylene Reduction Assay and yield. Application of liquid based biofertilizers i.e T_9 - Seed treatment with LBF at the time of sowing + soil application at 40 DAS affected the nodule formation, growth, yield of mungbean and microbial population in the soil. Application of biofertilizers twice through seed treatment and soil application at 40 DAS appeared to influence positively the Nitrogenase activity, growth, yield of the plants. This is due to the availability of viable Rhizobia supplied through biofertilizers in the soil.

Key words: *Rhizobia, Biofertilizers, Acetylene Reduction Assay.*

INTRODUCTION

Leguminous crops are one of the most important component of the sustainable agriculture which play vital role in the economy and sustainability of the environment. With rapidly increasing population of the world, demand for legumes is also increasing. Among the legumes, mung bean is one of the most important crop, well adopted to the sub humid and semi-arid zones. It requires the temperature round about 30°C to grow. Clay loam to sandy loam texture of soil is suitable for its growth (Ahmad and Jan, 2002). It is a rich source of vegetable protein about 25% of the total seed weight, can be used as food or fodder crop. It also contains is of flavonoids that have antioxidant activities which prevent from many diseases Mungbean (*Vigna radiata*) is an important pulse crop of India. It is highly nutritious and easily digestible. Greengram is the third most important pulse crop of India after chickpea and pigeonpea. It is well suited for all rainfed areas with annual rainfall of 600-1000 mm. India alone accounts for 65 % of the world acreage and 54 % of the world production.

Cite this article: Bhavya K., Reddy R. S., Triveni S., influence of carrier and liquid rhizobial biofertilizer on nodulation, nitrogenase activity and yield in mungbean crop by different method of applications, *Int. J. Pure App. Biosci.* **5(4):** 1666-1670 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5705

Considering the increased cost of chemical fertilizers and their ill effects on soil, there is a need to use organic and biofertilizers to minimize the cost on inorganic fertilizers and bring down the cost of production. Pulse crops have unique properties of nodulation through rhizobium bacteria. These bacteria, through biological nitrogen fixation, meet about 80% to 90% of total N requirements of legumes and also increases the yield in mungbean.

MATERIALS AND METHODS

Collection of biofertilizers.

Different types of carrier and liquid based biofertilizers were collected from following different firms and stored at 4°C in refrigerator

Equipment and apparatus used

Samples were weighed using a single pan electric balance, gas chromatograph (Agilent 7820 A, India) fitted with Porapak R column and Flame ionization detector (FID). A pot culture experiment was carried out at College of Agriculture, Rajendranagar, Hyd. Soil was red soil and neutral in reaction (pH 7.8). It was medium in available nitrogen. The pot culture experiment was conducted by following Complete Randomized Block Treatments consisted of Control : 100% RDF, T_1 : 75% RDF + 25%CBF (Seed treatment) at the time of sowing, T_2 : 75% RDF + 25%CBF (Soil application) before sowing, T_3 : 75% RDF +25% CBF- Liquid culture (soil application) at the time of sowing, T_4 : 75% RDF + 25% LBF-Liquid culture (Soil application) before sowing, T_5 : 75% RDF + 25%LBF (Seed treatment) at the time of sowing, T_6 : 75% RDF+ 25%LBF (Soil application) before sowing, T_7 : 75% RDF + 25%CBF (Seed treatment at the time of sowing) + soil application at 40DAS, T_8 : 75% RDF+25%CBF (Soil application before sowing) + soil application at 40 DAS, T_9 : 75% RDF+25% LBF (Seed treatment at the time of sowing) + soil application at 40 DAS,T_{10} : 75% RDF+25%LBF (Soil application before sowing) + soil application at 40 DAS,T_{11} : 75% RDF +25% CBF-Liquid culture(soil application at the time of sowing soil application at 40DAS)T_{12} : 75% RDF +25%LBF- Liquid culture (Soil application before sowing) + soil application at 40 DAS.

Seed treatment with Biofertilizers

The seeds were soaked for 10 minutes and drained off the water. Jaggery solution was prepared by dissolving 120 g guar in one litre water and was boiled for ½ h and cooled to room temperature. Carrier based Biofertilizers of *Rhizobium* and PSB @ 250 g each per 10 kg seed were transferred to the cooled jaggery solution to make a slurry. The soaked seeds were thoroughly mixed with cultures slurry so as to obtain a uniform coating of the cultures on the seeds. The seeds thus inoculated were spread on a clean gunny bag in shade and dried. These dried seeds were used for sowing. Seed treatment with liquid based biofertilizers and liquid culture of rhizobium @ 10 ml for 10 kg seed and PSB @ 300 – 500 ml per acre.

Soil application

Mixed 3 to 5 kg biofertilizer with 50kg finely powdered FYM and broadcasted in experimental pot.

Thinning - Thinning was carried out to achieve five plants per pot.

Nitrogenase activity by Acetylene Reduction Assay (ARA)

The nitrogen fixing capacity of the plants were evaluated by using acetylene reduction assay (ARA) following the standard procedure. Legume plants were removed from the soil without disturbing the root nodules. The root along with nodules was placed in a 100 ml conical flask. The flask was sealed with rubber septum (serum cap). Ten percent (v / v) of the inert gas was removed from the flask with an air tight syringe. 10 ml of acetylene was injected into the flask and incubated for 24 h at room temperature. 1 ml gas mixture was removed from the flask with an airtight syringe. After incubation, 1 ml of gas sample was withdrawn and injected into the gas chromatograph (Agilent 7820 A, India) fitted with Porapak R column and Flame ionization detector (FID). The column

temperature was maintained at 60^{oC}. Nitrogen gas was used as carrier gas at the flow rate of 30 ml min^{-1}). The acetylene and ethylene peaks were observed and ethylene peak height was measured. The acetylene reduction activity of the isolates was calculated using the formula:

$$\frac{\text{Sample peak length of ethylene (mm) x Attenuation x Volume of gas phase of flask x 0.0006}}{\text{Incubation time (h) x Volume of gas sample injected into gas chromatograph (ml)}}$$

The acetylene reduction assay of the sample was expressed as n moles of ethylene formed mg of protein h^{-1}. At the end of experimental period the cell protein content of the cultures were determined following the method described by Lowery et al.

Number of Nodules per plant

Number of root nodules were counted carefully after uprooting munbean plants from the field, followed by dipping them in water to remove soil clods without losing the nodules and counted by detaching the nodules from the root.

Yield attributes - All the plants from one m^2 were harvested at maturity to record data on yield attributes i.e. number of seeds pod^{-1}, weight of 100 seeds.

RESULT AND DISCUSSION

Number of root nodules per plant

At maximum vegetative stage, maximum root nodules (14.0) was observed in response to T_9 - Seed treatment with LBF at the time of sowing + soil application at 40 DAS was on par with T_{10} - LBF as soil application before sowing + soil application at 40 DAS (13.0), T_5 - LBF Seed treatment at the time of sowing (13.0)) and T_6 - LBF Soil application before sowing (12.0). The significantly lowest nodule number (7.0) was recorded by treatment T_3 – CBF liquid culture as Soil application before sowing. Compared to control (6.0) in all other treatments root length was significantly higher.

Nitrogen fixation ability of mungbean

Acetylene Reduction Assay (ARA) was carried out to examine the nitrogen fixing efficiency of the plant roots under pot culture conditions .For nitrogen fixation efficiency all the treatments have showed positive results. Among all treatments T_9 - Seed treatment with LBF at the time of sowing + soil application at 40 DAS showed highest nitrogen fixing ability (230.12 µmoles C_2H_4) followed by T_{10} LBF as Soil application before sowing + soil application at 40 DAS (211.13 µmoles C_2H_4). Significantly lowest nitrogen fixing ability was recorded with T_3 – CBF liquid culture as soil application before sowing (70.42). Compared to control (54.65 µmoles C_2H_4) in all other treatments root length was significantly higher. These results are in the line with the findings Morad *et al*. studied that seed inoculation with proper *Rhizobium* strain together with minor amounts of phosphorous at early growth stage stimulated root nodulation and increased biological nitrogen fixation eventually improving yield components.

YIELD ATTRIBUTING CHARACTERS

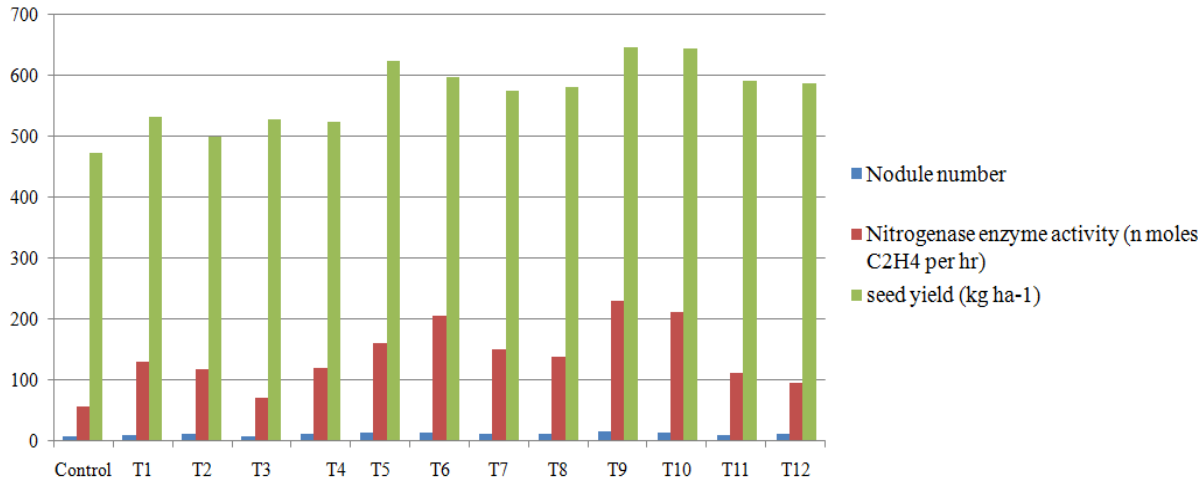
Seed yield per hectare (kg ha^{-1})

Seed yield per hectare at harvesting stage was significantly influenced by application of carrier and liquid based biofertilizers.Among all the treatments, significantly highest seed yield calculated per hectare (645.85kg ha^{-1}) was recorded in T_9 - seed treatment with LBF at the time of sowing + soil application at 40 DAS and was on par with T_{10} – LBF as Soil application before sowing + soil application at 40 DAS(643.84 kg ha^{-1}) followed by treatment T_5 – seed treatment with LBF at the time of sowing (625.20 kg ha^{-1}) .The significantly lowest seed yield per plant was observed in T_2 - CBF soil application before sowing(500.00 kg ha^{-1}). Compared to control (471.81 kg ha^{-1}) in all other treatments seed yield was significantly higher.

Treatment T_9 (75% RDF + 25% LBF Seed treatment at the time of sowing + soil application at 40 DAS) recorded higher yield attributing characters and yield. This might be due to seed treatment of liquid based biofertilizer at the time of sowing and soil

application at 40 DAS increases no of pods, no of seeds per pod, inturn seed yield. The highest yield and yield attributing characters could be attributed because of enhanced supply of N and P, production of several phytohormones and mobilization of reserve food material to developing seed which act as sink for carbohydrate and nitrogenous compounds present in plant. Nearly equal yield was observed in T_{10} - LBF Soil application before sowing + soil application at 40 DAS. Biswas and Bhowmick reported that seed yield was high using liquid inoculants which is followed by carrier inoculants in black gram. Similar results were observed by Bhattacharya and Kumar, Brahmaprakash *et al*., in soyabean , Gupta in chickpea.

Treatments	Nodule number	Nitrogenase enzyme activity (n moles C_2H_4 per hr)	seed yield (kg ha^{-1})
Control	6	54.65	471.81
T1	9	129.08	533.18
T2	10	116.23	500.00
T3	7	70.42	528.15
T4	10	119.56	523.12
T5	13	158.90	625.20
T6	12	205.60	598.57
T7	10	149.26	575.43
T8	11	138.02	581.46
T9	14	230.12	645.85
T10	13	211.13	643.84
T11	8	110.52	592.53
T12	10	94.82	586.49
SEm	1.05	1.192	1.99
CD(P=0.05)	3.093	0.983	5.01



Influence of application of carrier and liquid based biofertilizers on Nodule number, Nitrogenase activity, seed yield of mungbean.

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