Eco. Env. & Cons. 26 (2) : 2020; pp. (793-797)
Copyright@ EM International
ISSN 0971–765X

A study on potential *of Rhizobium spp as* bio fertilizers in sustainable agriculture

Chetan D.M.*[1] and Rao C.V.[2]

Department of Biotechnology Engineering, N.M.A.M. Institute of Technology (Visvesvaraya Technological University, Belagavi) Nitte, 574 110, Udupi District, Karnataka, India

(Received 22 November, 2019; accepted 6 January, 2020)

ABSTRACT

In this project *Rhizobium* bacteria was isolated from the root nodules of *"Mimosa pudica"* and used as a microbial culture for the production of biofertilizers. The biofertilizers were prepared using the *Rhizobium* with two different carriers charcoal and seed cake powder of *"Pongamia pinnata"*. To study the effect of biofertilizers, soil treatment was carried out and plants were cultivated in it. At regular intervals certain parameters of plants such as height, number of flowers, and number of buds were tabulated. Other parameters such as soil pH, nitrogen and phosphorous content in the soil were tested before the addition of biofertilizer and after the completion of experiment. Results of each carrier and without carrier has been compared with different parameters and plants.

Key words : *Biofertilizers, Mimosa pudica, Rhizobium spp and Pongamia pinnata*

Introduction

Plants have been known to be highly useful to humans all over the world, not only for food, but for ornamental and medicinal purposes as well. To meet the demands of the growing population for all such needs, the agriculture, horticulture, and medical sectors play a major role.Vast advancements in the field of agricultural biology have led to the reinforcement of new techniques to improve production of plants in order to meet with the demands of the population. Through the decades of improvement and advances in technology, there have been many methods utilised by the agricultural industry to do the same and showed the most significant effect on yield. Utilisation of green house needed for higher yield (Anabel Gonzalez and Diaz, 2019). Some of the methods are as listed below: Hybridization, crop rotation, better irrigation, hybrid seeds, tissue culture, fertilisers, organic pesticides and many more. Fertilisers are natural or artificial substances which enhance the growth and productiveness of the plant. For good growth and yield fertilizers are essential (Kecskes *et al.,* 2016). Fertilizers provide essential nutrients to the crops during their growth, which is generally lacking in the soil or deficient and help in increasing the yield. Fertilisers ensure the most effective use of both land and water. Bruno Rodriguez *et al.* (2017) worked on *Bacillus licheniformis* where they used combinations and applied several treatment before field application and analyse tihe influence of biological and chemical characteristics.

Chemical fertiliser contains combined synthetic forms of the primary macro-nutrients as well as variety of micro-nutrients to the plants to enhance their growth and yield. Adekunle Raimi *et al.* 2017). The extract from the flowers may be utilized to ease women's menstrual problems and to improve blood circulation. Herbal concoctions utilizing elements

from the plant are utilized to treat arthritis, coughs and boils. Many beauty products such as creams and oils utilize extracts from the plant especially the flowers and Phosphate-solubilizing bacteria (PSB) play a crucial role in soil Bruno Rodríguez and Morgado (2019).

Collection of sample: *Mimosa pudica* root nodules were collected and used for the isolation of *Rhizobium*.

Isolation of bacteria: Sterilisation of Root nodules: Roots were thoroughly washed with the tap water followed by several washings with distilled water, then with 95% ethanol for 10 seconds. Finally, washed once again 3 times with distilled water. Treated with 0.1N mercuric chloride for 30 seconds.

Procedure: Serial dilutions were prepared using saline. Set of test tubes were numbered from 1 to 9 each, which contained 9 mL of saline solution each and was subjected to sterilization. Root nodules were crushed with 20 mL of sterile saline. 1 mL of root nodule suspension was measured separately and added to the test tube number 1 and mixed well. This is the first dilution (10^{-1}). Then spread plate method was performed by using a sterile glass rod to spread the diluted sample evenly on the YMA media plates respectively. The plates were incubated for 24-48 hrs at 37 ^{o}C.

Cultivation of vegetable and flowering plants: Locally available soil was taken for the cultivation of plants and for soil analysis. Plants were cultivated in the green house. (Jelen *et al.*, 2016). Vegetable plants: *Abelmoschus esculentus* (okra) was selected for the cultivation. *Abelmoschus esculentus* seeds were sown in green house in soil treated with cow dung to get their plantlets. Plantlets of *Abelmoschus esculentus* were transferred to plastic bags after 15 days. Transferred plants were treated with the bio-fertilizers prepared. 4 sets of plants were maintained for the observation, and to study the effect of bio-fertilizers. 4 sets contained triplets Set 1: Soil treated with only cow dung (control). Set 2 : Soil treated with Rhizobium broth culture with charcoal. Set 3: Soil treated with Rhizobium broth culture with seed cake. Set 4: Soil treated with only Rhizobium broth culture. Shahzad *et al*.(2012).

Flowering plants: *Rosa chinensis* and *Catharanthus roseus* were selected for the cultivation. Plants were bought from the nursery and cultivated in green house. Haney *et al.* (2015). 4 sets of plants were maintained for the observation, and to study the effect of bio-fertilizers. 4 sets contained triplets. Both Rosa chinensis and Catharanthus roseus containing 4 sets with triplets. (Gomare *et al.,* 2013).

Results

Shoot Parameter Analysis

Rose Length

In control, linear increase in length was shown till week 2, there was a break in growth rate and then linear increase till week 4. In Biofertilizer containing seedcake, increase in length was shown in week 1, growth rate remained constant for 2 weeks and there was increase in week 4.In biofertilizer containing charcoal, and broth linear increase in length was shown throughout the growth (Adekunle Raimi *et al.,* 2017).

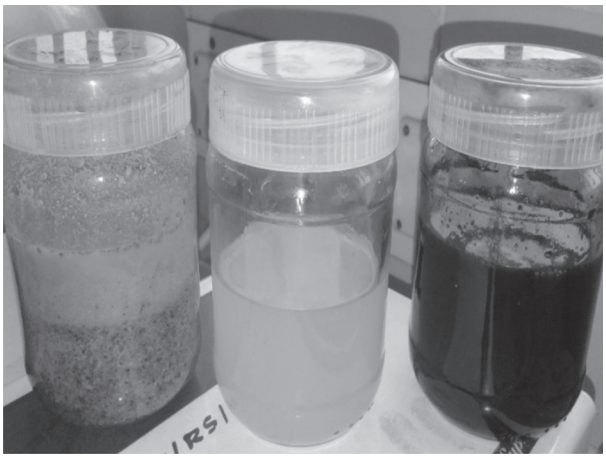

Fig. 1. Bio-fertilizer prepared with seed cake broth, rhizobium broth and charcoal (left to right).

Fig. 2. Control set of vinca, rose and okra

Vinca length

Biofertiliser (Fig.1) prepared in seedcake broth, *Rhizobium* broth and charcoal broth. From the flower count and plant weight, it is observed that *Vinca rosea* plant growth is enhanced by the use of biofertilizer with charcoal as carrier followed by broth. Control set of vinca, rose and okra Shoot length measurement of *Rosa* recorded (Fig. 2, 3). Rose and okra showed best growth results under control conditions due to the type of plant that it is. In the greenhouse trial, bacterial inoculations increased shoot and root weights of rice plants significantly Kecskes *et al.,* (2016). Different plants have different nutrient requirements and this in turn plays a major role in the effect of biofertilizer on the plant. Shahzad *et al.* (2012) in their investigation on root nodules of alfalfa, 25 variants showed positive results for the isolation of *Rhizobium spp* and subsequent increase in growth yield (Fig. 4, 5). Rose plant shows linear growth with biofertiliser containing charcoal as carrier but with seed cake as carrier it shows more flower count and with broth biomass is increased (Fig. 6) Despite its lesser effect than in control conditions, it shows that the biofertiliser is capable to affect the intended plant. From the flower count and plant weight, it is observed that *Vinca rosea* plant growth is enhanced by the use of

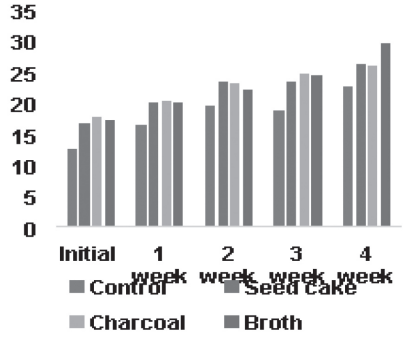

Fig. 3. Shoot length measurement of *Rosa chinensis*.

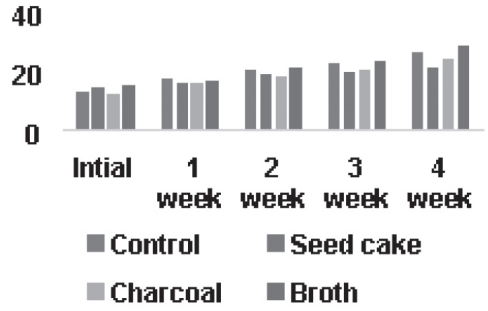

Fig. 4. Shoot length measurement of Vincarosea

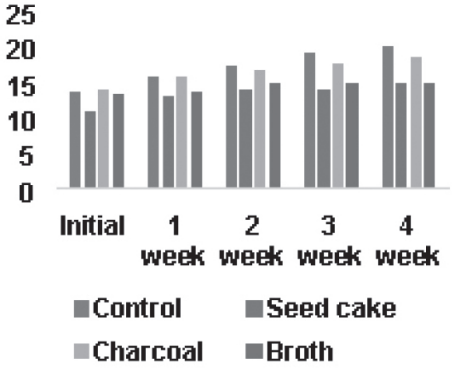

Fig. 5. Shoot length measurement of Okra

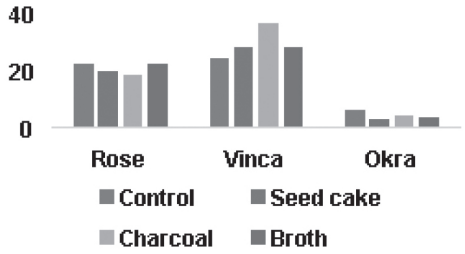

Fig. 6. Weight measurement of Rose, Vinca

biofertilizer with charcoal as carrier followed by broth. Rose and okra showed best growth results under control conditions due to the type of plant that it is (Fig.7). Different plants have different nutrient requirements and this in turn plays a major role in the effect of bio fertilizer on the plant. Rose plant shows linear growth with biofertiliser containing charcoal as carrier but with seed cake as carrier it shows more flower count and with broth biomass is increased. Despite its lesser effect than in control conditions, it shows that the biofertiliser is capable to affect the intended plant. We also see that there is high root length values for plants treated with bio fertilizer. *Rosa chinensis* may have shown the high root length in case of control, but charcoal is identi-

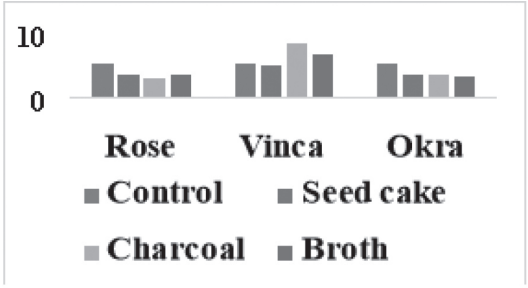

Fig. 7. Shoot branch count of Rose, Vinca and Okra plants.

fied as the condition under which roots have shown highest length for all the 3 plants. Root length also gives us an idea as to how much nutrients the plant has utilized for growth (Fig. 8, 9). Number of root and shoot branches was observed in order to give us an idea as to how much of the soil nutrients is utilized by the plant for maturation. We see high number of root branches for *Vinca rosea* and Okra under the influence of charcoal and in case of *Rosa chinensis* under the influence of seed cake. *Vinca rosea* showed

Fig. 8. Vinca rosea Flowers

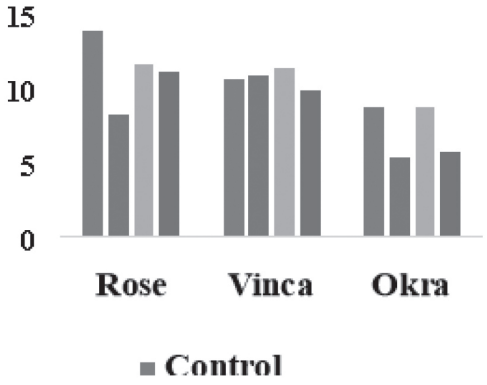

Fig. 9. Root length measurement of Rose, Vinca and Okra plants.

highest number of root branches under the influence of charcoal, while the rest had better results in control condition (Fig. 8, 9, 10). Similar positive results for the utilisation of biological fertilizers by Gomare *et al.* (2017); Rajendra (1998); Mishra (2012); Carrapico (2000); Kumar (2010); Panwar (2000); Rajasekaran, and Sundaramoorthy (2010) recorded.

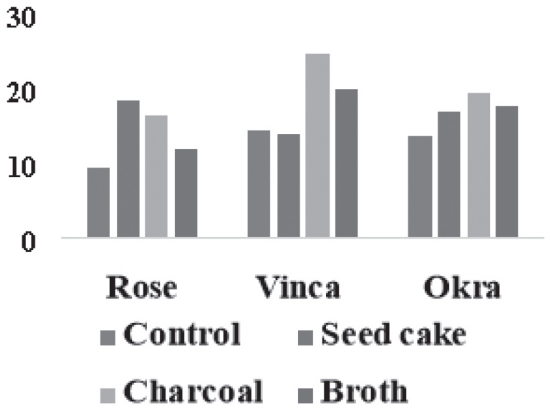

Fig. 10. Root branch count of Rose, Vinca and Okra plants.

Conclusion

Vinca rosea showed highest number of root branches under the influence of charcoal, while the rest had better results in control condition further yield can be improved by standardising procedure

References

Adekunle Raimi, 2017. Cogent Food & Agriculture Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa, 3(1) : 1-27.

Anabel Gonzalez-Díaz, Marcia E. Ojeda-Morales, 2019. *Journal of Plant Nutrition*. 42(19) : 2560-2576.

Bruno Rodríguez-Morgado, Pablo Caballero, Patricia Paneque, Isidoro Gomez, Juan Parrado and Manuel Tejada, 2017. 40, 2019 - Issue 3 28 Oct Pages 399-406

Carrapico, F., Teixeira, G. and Diniz, M.A. 2000. Azolla as a biofertiliser in Africa: A Challenge for the future. *Revesta Cincies Agraries.* 23 : 120-138.

Gomare, M. Mese and Y. Shetkar, 2013. Isolation of Rhizobium and coat effective production of Biofertilizer. *J. l. Sci.* 2(2) : 49-53.

Gomare, 2017. Haney, Cara H.; Ausubel, Frederick M. 2015. Plant microbiome blueprints. *Science.* 349 (6250) : 788-789.

Jelen, Benjamin, I., 2016. Giovannelli, Donato; Falkowski, Paul, G. 2016. The Role of Microbial Electron Transfer in the Coevolution of the Biosphere and Geosphere. *Annual Review of Microbiology*. 70 (1): 45–62

Kecskes, A. T. M. A., Choudhury, A. V., Casteriano, R., Deaker, R. J., Roughley, L. Lewin, Ford, R. and Kennedy, I. R. 2016. Effects of bacterial inoculant biofertilizers on growth, yield. *Journal of Plant Nutrition.* 39(3) : 377-388.

Kumar, V., Chandra, A. and Singh, G. 2010. Efficacy of fly-ash based bio-fertilizers vs perfected chemical fertilizers in wheat (*Triticum aestivum*). *Int. J. Sci. Technol.* 2 : 31-35.

Mishra, D.J., Singh Rajvir, Mishra, U.K., Shahi Sudhir Kumar. 2012. Role of Bio-Fertilizer in Organic Agriculture: A Review. *J. Recent Sciencies.* (2): 39-41.

Rajendra, P., Singh, S. and Sharma, S. N. 1998. Interrelationship of fertilizers use and other agricultural inputs for higher crop yields. *Fertilizers News.* 43: 3540.

Panwar, J.D.S. and Singh, O. 2000. Response of *Azospirillum* and *Bacillus* on growth and yield of wheat under field conditions. *Indian J. Plant Physiol.* 5 : 108-110.

Rajasekaran, S. and Sundaramoorthy, P. 2010. Response of organic manures and biofertilizer on germination, growth and yield of selected leguminous corps. *J. Ecotoxicol. Environ. Monit.* 20 : 161-168.

Shahzad, M., Shafee, F. Abbas, S. Babar, M. M. Tariq and Z. Ahmad. 2012. Isolation and Biochemical Characterisation of *Rhizobium meliloti* from root of Alfalfa (*Medico sativa*). J 22(2): 522-524.