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Comparison of Conventional and Non-Conventional Carriers for Bacterial Survival and Plant Growth

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Abstract – **Bio-fertilizers havegained immense interest for their use in agriculture to break the stagnancy of major crop yields that is exerting extra pressure on bio-fertilizers to perform better. To enhance the efficiency of bio-fertilizer, carrier materials play vital role for their consistent performance, longer shelf life and effectiveness under field conditions. The present study was conducted to evaluate the role of carrier materialsfor survival of plant growth promoting rhizobacteria (PGPR) and their efficiency to improve the growth and yield of mungbean. Pre-isolated and wellcharacterized strain of PGPR was loaded onto different carrier materials. Result showed that efficacy of different carrier materials was highly variable to improve the performance of PGPR inoculation. Inoculation of PGPR with different carrier materials had positive impact on all the growth, physiological and yield parameters of mungbean compared to sole inoculation andwith control (only NPK was applied).It also narrates the positive impact of carriers mounted inoculation on survival of bacteria. Among the carriers, biochar and compost based inocula improved the yield of mungbean by 14% as compared with the other carriers based inoculations. Comparison among carriers revealed that biochar and compost can serve ascarrier material for the production of efficient bio-fertilizer.**

Keywords – **Biochar, Carrier, Compost, Mungbean, PGPR.**

I. Introduction

Bio-fertilizers are usually the carrier based formulationscontaining beneficial microbes that enhance the growth and yield of plants when applied in soil or coated on seed [1]. Carrier material plays very important role in microbial survival. In general, shortly after the application of bio-fertilizers in soil, microbial population starts declining. It is because of heterogeneous and unpredictable environment nature of soils. After applying bio-fertilizers, these beneficial bacteria have to compete with indigenous micro-flora and protozoans for energy, carbon and nutrients [2]. On contrary, carrier material supports the inoculated bacteria by providing them conducive conditions for their survival, increases the effectiveness and allows easy handling and long storage of the bio-fertilizers [3]. Selection of carrier material is very import so it should be selected keeping in view the specific characteristics. These characteristicsincludeease in their availability, sticky nature, high organic contents, availability in bulk quantity, ease in processing, should not contain toxic substances and high water holding capacity [4].A wide array of conventional and non-conventional materials can be used as carriers but the need of hour is to find out the most appropriate carrier that fulfills all the requirements for formulation of efficient bio-fertilizer.

Some of the conventionally used carriers are humic acid, compost, peat and vermicompost. Product produced by the organic matter degradation under controlled conditions of temperature, pressure and oxygen is compost. It contains both easily degradable and stable portion of carbon [5]. So it provides carbon for microbial assimilation as well as stable carbon that can hold nutrients and does not allow them to leach down [6]. It is a well-known source of nutrients for plants and its application to soil also promotes the microbial activity [7]. The activity of microbes and soil fertility are closely interconnected because of their ability to enhance the availability of nutrients like carbon, nitrogen, phosphorus and sulphur [8]. Other commonly used carrier materials for the formulation of bio-fertilizers are peat, vermicompost and humic acid [9]. All of these have high organic content and enhance the overall growth of plants by improving nutrient uptake. These carriers also improve the physical and chemical properties of soil [10]. However, limited work is available about the use of biochar as a carrier for bio-inoculants. Biochar is a rich source of carbon, produced by the pyrolysis of organic matter under oxygen deficient conditions [11]. Its properties like large surface area and vary high amount of carbon are very much beneficial for soils. It enhances nutrient uptake, promotes microbial activities by providing suitable micro-environment, improvesthe physical properties of soil and most importantly provides the stable organic carbon content in soil.

So keeping in view the recent scenario of bio-fertilizers application, present study was conducted to compare the various conventional and non-conventional carrier materials and to select the most suitable one that can enhance the effectiveness and shelf life of bio-fertilizers.

II. Materials and Method

Fresh inoculum was prepared for pre-isolated and well characterized PGPR *Bacillus thuringiensis* strain S5 (KT750960). Strain was inoculated in sterilized broth and incubated at 28 ± 1°C and 100 rpm for 72 hours in shaking incubator. Required population of 10^7 mL^{-1} of bacterium was maintained by using densitometer (Den-1, Densitometer, McFarland, UK) to be used as inoculum for different carrier materials.

Four different materials i.e. peat, biochar, humic acid and compost were used as carriers for bacterial inoculation of mungbean along with sole inoculation and control treatment where only recommended NPK fertilizers were applied. The carriers were collected and processed by grinding, sieving and sterilization before the application. These carriers were analyzed for the physical and chemical characteristics (Table, I).

For inoculation, mungbean (AZRI-06) was used and seeds were coated by slurry method. Seed coating was done by mixing 10 mL sugar solution, 10 mL bacterial culture and 50 g carrier material for coating of 100 g of mungbean seed. Inoculated seeds were kept overnight for drying before sowing in pots, filled with 10 kg of soil. Pots were filled with sandy clay loam soil having EC_e 2.2 dS m^{-1}, pH 7.6 and saturation percentage 31.5 %. Chemical fertilizers were applied according to the recommended dose for NPKto all the treatments. Experiment was conducted in completely randomized design (CRD) and each treatment was replicated six times. Data regarding photosynthesis and transpiration (µ mol^{-2} S^{-1}) was recorded by using Infrared Gas Analyzer (IRGA) at physiological maturity. For nodulation data, half of the replications were harvested at flowering stage, Number of nodules per plant, nodule fresh weight and nodule dry weight was recorded. Pods were harvested when their color changed from green to brown to dark brown. Yield and agronomic attributes of the plants were recorded at harvesting. For chemical analysis, Nitrogen was measured by Kjeldhal method, phosphorus by Olsen P method and potassiumcontent in root, shoot and grain was measured by flame photometer (Jenway PFP-7). Data was analyzed by using software "Statistix 8.1".

III. Results

This study was conducted to compare the effectiveness of different conventional and non-conventional carriers to enhance the shelf life of bio-fertilizer, its survival in rhizosphere and effect on growth and yield of mungbean. Among these carriers compost, peat and humic acid are conventionally used while biochar is still in the initial phase of evaluation as a carrier in bio-fertilizer. Results of our study showed that carrier based formulations performed better as compared to the sole inoculation (carrier was not used) and control (only chemical fertilizer was applied). Loading of PGPR onto various carriers clearly demonstratedthe improvement in bacterial survival. Data regarding bacterial survival is mentioned in the Table-II. Bacterial survival was checked after 30, 60 and 90 days of inoculation. Maximum survival was in inoculated peat followed by the inoculated biochar. Among the agronomic attributes, root length was enhanced by 28% with the use of inoculated compost as compared to the sole inoculation of PGPR. Peat based inoculation increased the shoots length upto 31% followed by compost based inoculation (18%), biochar based inoculation (13%) and humic acid based inoculation (6%) over sole inoculation of PGPR (Table-III). Maximum increase in root dry weight was observe due to 29% with biochar based inoculation, 24% with compost based inoculation, 17% with peat based inoculation and 7% with humic acid based inoculation over sole inoculation of PGPR, respectively. Maximum increase in shoot dry weight was recorded upto 43% by humic acid based inoculation compared with sole inoculation of PGPR in the table-I. While in case of number of nodules, maximum increase was up to 38% by using compost as carrier material. Hundred grain weightwas significantly improved by27% with peat based inoculation, 20% with biochar based inoculation, 13% with compost based inoculation and 2% with humic acid based inoculation as compared to sole inoculation of PGPR (no carrier).

The response of various carrier based inoculation was also highly variable for improving the physiology of mungbean. Transpiration rate and photosynthetic rate were significantly improved in response to inoculated carrier as compared to sole inoculation of PGPR. Maximum transpiration was observed by compost based inoculation which was almost doubleas compared to the control (where only NPK fertilizers were applied). Similarly, photosynthetic rate was improved upto 27% with compost and biochar base inoculations that were statistically at par with each other (Table V).

Plant chemical analysis showed that NPK contents were significantly increased in response to various carrier based inoculations. Biochar based inoculation enhanced the N contents in shoot by 21% over sole inoculation of PGPR, followed by compost base inoculation (18%) and then peat based inoculation (9%) and humic acid based inoculation (6%). Similarly, maximum P contents in shoot were up to 18% by compost based inoculation as compared to sole inoculation of PGPR. Maximum increase in potassium contents of shoots was up to 6% by biochar and compost based inoculations as compared to sole inoculation of PGPR (Table IV).

Nitrogen contents in grain were improved up to 12%by compost based inoculation, 10% by biochar based inoculation, 6% by peat based inoculation and up to 0.04% by humic acid based inoculation over the control. Similarly, P contents in grain were significantly enhanced upto 26, 18, 13 and 7% by biochar, compost, peat and humic acid based inoculations, respectively, as compared to control. Potassium contents of grain were alsoincreased up to 17%, by compost and biochar based inoculations, as compared to control which were maximum (Table IV).

Certain activities like P mineralization and solubilization were increased in the rhizosphere by the application of carrier based inoculations. Maximum P mineralization was observed in case of compost based inoculation which enhanced solubilization by 53% followed by biochar based inoculation which was up to 47% as compared to sole inoculation of PGPR (Table VI).

IV. Discussion

Results of this study demonstrated that loading of PGPR onto various carriers enhanced the growth and yield of mungbean. All the carriers enhanced the bacterial survival, improvedthe effectiveness of bio-fertilizer and increased the shelf life [12]. Among all the carrier materials, peat was better in bacterial survival efficiency. It might be due to the more availability of labile carbon and nitrogen to the microbes [13]. Another reason might be that the pH of peatwas more favorable for bacterial survival. Biochar was next to peat in enhancing the bacterial survival efficiency. It might be due to the easily degradable carbon portion that is required by bacteria for the body mass production. It also provides the micro-pores to bacteria to protect against the predation by the protozoa. Ultimate purpose of formulations is to provide suitable micro-environment to inhibit the rapid decline of our required bacteria so that we can secure the sufficient number of bacteria in the rhizosphere of crops to get better growth and yield of crops.

Carrier based inoculation has significantly enhanced the growth, physiological characteristics of the plants, microbial activities and crop yield. Among the inoculated carriers biochar and compost performed better as compared to the other carriers. This might be due to more surface area of the biochar that provides the seat for bacterial survival in harsh ambient conditions. As the bacterial survival improved, it resulted in increased solubilization of P. Increased uptake of P might have resulted in enhanced root growth that ultimately enhanced the uptake of all the nutrients [14]. This all results in better root weight, shoot weight, root length, shoot length and number of nodules [15]. It also provides the proper moisture, carbon and nitrogen availability to the microbes [16]. Another reason can be the fact that inoculated carriers help PGPR to increase their population and activity in the rhizosphere and ultimately has positive influence on plant growth attributes [17].

Increase in yield (grain and hundred weights) by loading PGPR onto various carriers is evident from our experiment. Inoculated carriers (Peat, biochar and compost) had shown better results in enhancing the yield of mungbean. This increase in yield might be due to better root growth and enhanced nutrient uptake, high nutrient retaining capacity (biochar) and providing large surface area to the PGPR for their survival and functioning [18]. This improved architecture of root system and microbial activity also resulted in enhanced accumulation of NPK content in shoot and grain [19]. The immediate effects might be due to the higher availability of nutrients [20].

From this study it is concluded that carrier based inoculation improved bacterial survival, growth, yield of mungbean and microbial activities in soil as compared to the sole inoculation and over the control (chemical fertilizer only). Among the inoculated carriers biochar and compost performed better as compared to the other carriers. Hence an effective bio-fertilizer can be prepared by using these as carriers of PGPR.

References

[1] O. Karaguzel, I. Baktir, S. Cakmakci and V. Ortacesme, "Growth and flowering responses of *Lupinusvarius* L. to paclobutrazol", *Horticultural Science*, vol. 39, 2006, pp.1659-1663.

[2] S. Dobbelaere, J. Vanderleyden and Y. Okon, "Plant growth promoting effects of diazotrophs in the rhizosphere". *Critical Review in Plant Sciences,* vol. 22, 2003, pp.107-149.

[3] H. Muraleedharan, S. Seshadri and K. Parumal, "Carrier material", 1st Ed. 2010, pp.12-13.

[4] M.H. Abd-Allaand S.A. Omar, "Survival ofrhizobia/ bradyrhizobia and a rock-phosphate solubilizing fungus *Aspergillusniger* on various carriers from some agro-industrial waste and their effects on nodulation and growth of faba bean and soybean", *Journal Plant Nutrition*vol.24, 2001, pp. 261-272.

[5] Tuomela, M., M. Vikman, A. Hatakka and M. Itavaara (2000), "Biodegradation of lignin in a compost environment",*A Review. Bio resource Technology*, Vol. 72, 2000, pp. 169-183.

[6] S.M. Abit, C.H. Bolster, P. Cai and S.L. Walker, "Influence of feedstock and pyrolysis temperature of biochar amendments on transport of *Escherichia coli* in saturated and unsaturated Soil", *Environmental Science and Technology,* vol.46, 2012, pp. 8097-8105.

[7] A. Daza, C. Santamaroa, D.N.R. Navarro, M. Camacho, R. Orive and F. Temprano, "Perlite as a carrier for bacterial inoculants", *Soil Biology and Biochemistry*, vol. 32, 2000, pp. 567-572.

[8] A. Hartmann, A., M. Rothballer and M. Schmid (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil.* vol. 312, 2008, pp. 7-14.

[9] P. Sivasakthivelan, and P. Saranraj, "Azospirillum and its Formulations: A Review", *International Journal of Microbiogy Research,* vol. 4, 2013, pp. 275-287.

[10] S. Nardi, D. Pizzeghello, A. Muscolo and A. Vianello, "Physiological effects of humic substances on higher plants", *Soil Biology and Biochemistry*. vol. 34, 2002, pp. 1527-1536.

[11] J.W. Gaskin, C. Steiner, K. Harris, K.C. Das and B. Bibens, "Effect of low temperature pyrolysis conditions on biochar for agricultural use", *American Society of Agricultural and Biological Engineers,* vol. 51, 2008, pp. 2061-2069.

[12] N. Manivannan and D. Thilagavathy, "Use of Vermicompost as carrier material for microbial inoculants for enhanced crop production", *Journal of Pure and Applied. Microbiology*, vol.3, 2009, pp. 255-260.

[13] L. Hale, M. Luth, R. Kenney and D. Crowley, "Evaluation of pinewood biochar as a carrier of bacterial strain *Enterobacter cloacae* UW5 for soil inoculation", *Applied Soil Ecology*, vol. 84, 2014, pp. 192-199.

[14] B.D. Roy, B. Deb and G.D. Sharma, "Evaluation of carrier based inoculant of *Azotobacterchroococcum* strain SDSA-I12/2 in improving growth and yield of summer rice IR-36", *Biofrontiers*, vol. 1, 2010, pp. 36-40.

[15] M.K., Hussain, V. Strezov, K.Y. Chan and P.F. Nelson, "Agronomic properties of wastewater sludge, biochar and bioavailability of metals in production of cherry tomato (*Lycopersiconesculentum*)", *Chemosphere*, vol. 78, 2010, pp. 1167-1171

[16] A. Rizwan, M. Arshad, A. Khaliq and Z.A. Zahir, "Effectiveness of organic/bio fertilizer supplemented with chemical fertilizer for improving soil water retention aggregate stability, growth and nutrient uptake in maize (*Zea mays*)", *Journal of Sustainable Agri*culture, vol. 31, 2008, pp. 57-77.

[17] A. Perez-Piqueres, V. Edel-Hermann, C. Alabouvette and C. Steinberg, "Response of soil microbial communities to compost amendments", *Soil Biology and Biochemistry*, vol. 38, 2006, pp. 460-470.

[18] S. Steinbeiss, G. Gleixner and M. Antonietti, "Effect of biochar amendment on soil carbon balance and soil microbial activity", *Soil Biology and Biochemistry*, vol. 41, 2009, pp. 1301-1310.

[19] H. Asai, B.K. Samson, H.M. Stephan,K. Homma, Y. Kiyono and T. Horie, "Biochar amendment techniques for upland rice production in Northern Laos, soil physical properties, leaf SPAD and grain yield", *Field Crops Research*, vol.111, 2009,pp. 81-84.

[20] S.M. Haefele, Y. Konboon, W. Wongboon, S. Amarante, A.A. Maarifat, E.M. Pfeiffer and C. Knoblauch, "Effects and fate of biochar from rice residues in rice-based systems", *Field Crops Research*, vol.121, 2011, pp. 430-440.

Table I. Physical and chemical characteristics of carriers

Properties	Carrier materials			
	Biochar	Humic acid	Compost	Peat
WHC (%)	62.9±4.5	64.2±2.16	58.4±4.45	51.5±4.16
EC (dS m^{-1})	4.45±0.09	1.8±0.36	2.0±0.19	3.1±0.16
pH	7.8±0.3	3.5±0.66	7.1±0.35	6.5±0.15
Total C (%)	85.5±3.98	51.7±4.2	46.5±3.76	57.9±3.61
Total N (%)	1.1±0.3	1.8±0.14	2.9±0.7	2.7±0.3
Total P (%)	2.1±0.06	1.2±0.12	1.3±0.21	3.9±0.23
Total K (%)	0.18±0.04	1.5±0.06	1.3±0.09	0.3±0.04
C:N	77	28.7	16	21

Table- II: Survival of bacteria in carrier materials after 30, 60 and 90 days

Carrier Material	After 30 days (Total count * 10^8 CFU g^{-1})	After 60 days (Total count * 10^8 CFU g^{-1})	After 90 days (Total count * 10^8 CFU g^{-1})
Biochar	44.11±2.56	19.98±2.4	9.20±2.26
Humic acid	18.37±5.53	10.25±2.44	3.51±0.61
Compost	37.06±1.71	15.41±3.05	7.11±1.81
Peat	46.20±4.59	21.32±3.33	11.18±1.93

Table III. Effect of different inoculated carriers on plant growth attributes

Treatments	Root length Cm	Shoot length cm	Root dry wt. g/plant	Shoot dry wt. g/plant	No of nodules	Nodule dry wt. mg/plant
Control	13.8c	29.5b	1.7d	3.5e	11c	3.55c
Inoculation	17.6b	37a	2.41c	4.2d	13c	4.21b
Inoculated biochar	19.2ab	37.4a	3.12a	5.5b	17ab	5.77a
Inoculated humic acid	16.9b	37.2a	2.08c	6a	14bc	4.32b
Inoculated compost	22.6a	39.3a	3a	5.6b	18a	5.83a
Inoculated peat	17.8b	43.7a	2.83b	4.9c	15abc	4.6b

Table IV. Effect of carrier based inoculum on NPK content of shoot and grain

Treatments	Shoot			Grain		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	3.13d	0.283e	2.53b	3.96b	0.223f	2.01c
Inoculation	3.5c	0.308d	2.69ab	4.89a	0.296e	2.78b
Inoculated biochar	4.23a	0.352b	2.84a	5.38a	0.373a	3.26a
Inoculated humic acid	3.7bc	0.307d	2.68ab	4.91a	0.316d	2.98ab
Inoculated compost	4.13a	0.364a	2.83a	5.46a	0.35b	3.24a
Inoculated peat	3.8b	0.333c	2.74a	5.19a	0.333c	3.11a

Table V.Effect of inoculated carriers on yield and physiological parameters

Treatments	Grain yield (g pot^{-1})	100 grain yield (g pot^{-1})	Photosynthetic rate (μmol m^{-2} s^{-1})	Transpiration rate (μmol m^{-2} s^{-1})
Control	1.89c	4.02e	7.74c	1.29c
Inoculation	2.04c	5.04d	8.05bc	1.56c
Inoculated biochar	2.33a	6.0b	9.87a	2.61a
Inoculated humic acid	2.12abc	5.16d	8.56a	2.17b
Inoculated compost	2.32ab	5.7c	8.30bc	2.49b
Inoculated peat	2.06bc	6.4a	9.80a	2.88a

Table-VI: Effect of carrier based inoculation on P mineralization and its availability

Treatments	Quantitative	
Parameters	P mineralization (ppm3.82)	Available P (ppm)
Control	16.3d	8.61d
Inoculation	22.3c	10.53c
Inoculated biochar	32.7a	13.98a
Inoculated humic acid	26.3bc	10.77c
Inoculated compost	34.1a	13.82a
Inoculated peat	30.9ab	12b