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Effect of Carrier Materials, Coinoculation and Sterilization on Survival of Plant Growth Promoting Microbes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to investigate diverse carrier materials out of agricultural and environmental waste, that plays a role in maintaining the shelf life of plant growth promoting microbes (PGPM) at room temperature for locally produced inoculants in Malawi. Five different formulations divided into sterilized and unsterilized were prepared, using different carrier materials namely; rice bran plus plant extract (RBP), biochar plus plant extract (BP), Filter mud plus plant extract (FMP), rice bran, biochar and plant extracts (RBCP), filter mud, biochar and plant extracts (FMBP). Carrier materials were packed in polyethylene pack (six per each treatment), thereafter each treatment was divided into sterilized and unsterilized. Each treatment was inoculated with either single or multiple inoculants. Survival of PGPM, was based on colony forming units (CFU) on specific selective media namely: modified yeast extract mannitol, pikovskaya's, Alexandria and

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basal media for nitrogen fixing microbes, phosphate, potassium and zinc solubilising media respectively. Results revealed that encapsulated formulation of based combination formulation of RBCP in both single and multiple inoculants exerted high stable numbers of PGRM along the storage compared to other formulations. The results also show that unsterilized formulations exert high numbers compared to sterilized which is as a result of hydrogen peroxide accumulation during sterilization. The study reveals that filter mud based formulations currently used in both single and multiple inoculants is not favorable for local environments because microbial numbers decrease after 20 days at room temperature. This makes filter mud formulations usage not favorable for rural smallholder farmers with no refrigeration facilities.

Keywords: Carrier materials; agricultural and environmental waste; plant growth promoting microbes; inoculants.

1. INTRODUCTION

The inoculants (biofertilisers) particularly rhizomicrobes have synergistic interaction with roots hence provision of regulatory effects on plant growth and development [1,2]. Biofertiliser have a positive implication on production costs, yield quality and quantity, crop stress regulation and bioremediation [3,4].

Colony Forming Units (CFU) of inoculated plant growth promoting microbes (PGPM) to the soil may increase or decrease depending on environmental factors like moisture, temperature and type of carrier material [5,6,7]. Carrier materials is a substrate that carry viable cells of PGPM to be inoculated in the soil or seed. It has capability for the provision of conducive environment to PGPM to maintain the CFU above the standards. Biological, physical and chemical characteristics of carrier materials has properties similar to the indigenous environment where the microbes are isolated like moisture retention capacity, pH, organic matter content, nutrient levels, etc. [8,9].

Various organic materials such as perlite, biochar, maize bran, biochar, rice bran, karnolite, sodium alginate, peat, clay, begasse, saw dust, wood ashes and plant extracts are some of carrier materials used in inoculants but some of these are expensive, scarce and environmentally unfriendly [7,8,10,11]. Some carrier materials are amended with diverse additives to improve seed adhesion, stabilization of the carrier material, survival of microbes in diverse environment, and easy to inoculate in the field [8,12,9]. Most additives are subjected to trade secrecy as intellectual property. These additives are used because of unique properties that make the product to have higher performance [13,14]. The use of carrier materials and its additives in biofertilisers as inoculation strategy is as old as the history of inoculation [10].

The efficacy of biofertilisers is dependent on several factors and one of which is CFU to be inoculated in the soil, compost or seed. This conforms to the principle of the survivability rate of carrier materials to ensure that the standard CFU is maintained [10,15,16,8,17, 18].

Many investigations have used peat as carrier material for PGPM, but less has been done of using site specific agricultural waste and plant extracts as carrier materials and additives respectively. In the present study, different agricultural and environmental waste were treated as amendment with plant extracts (a trade secret) to develop CM of PGPM consortium and survivability rate of PGPM inoculants.

2. METHODOLOGY

The experiment was carried out at the LOGO-TECH Company. PGPM taken from LOGO-TECH were used in the experiment; *Bradyrhizobium* as nitrogen fixer, *K. pneumonia* as phosphorus solubiliser, *Pseudomonas* as potassium solubiliser, *A. calcoaceticus* as zinc solubiliser were used for the development of single and multiple inoculant formulas.

2.1 Preparation of Carrier Material

Biochar, rice bran, filter mud and plant extracts were collected, milled in a motor miller inserted with 1 mm sieve. Five carrier materials namely; rice bran plus plant extract (RBP), biochar plus plant extract (BP), Filter mud plus plant extract (FMP), rice bran, biochar and plant extracts (RBCP), filter mud, biochar and plant extracts (FMBP) were used. Plant extracts are the recommended ingredient as a sticker and biostimulant under trade secrecy of LOGO TECH Company. Polythene packs were used as packaging material covers. They were further subdivided into two (sterilized and unsterilized) for development of single and multiple inoculants. Sterilization of carrier material was done by autoclaving polythene bags sealed with carrier material at 121°C, carrier material moisture content was adjusted to 40%.

2.2 Preparation of Microbial Consortium

PGRM formulations were prepared using modified yeast extract Mannitol, Alexandria Pikovaskaya's and basal media for nitrogen fixing microbes, phosphate, potassium and zinc solubilising media in the respective order and incubated for 3-4 days using incubator with a shaker at 32°C [19,20]. After incubation period each carrier material (sterilized and unsterilized) was mixed with 20% of broth as single microbe or in a mixture with other microbes with CFU of 1* 10 [8,21]. Multiple formulations were prepared by mixing nitrogen fixing microbes, potassium, phosphate and zinc solubilizing microbes same volume and CFU.

2.3 Storage and Survival Study

Survival rate of PGRM was enumerated using CFU (standard plate count method) of selective media at 0, 5, 10, 15, 20, 30, 90, 150 and 360 days based on a method by Ivan et al. [22] with slight modifications followed by conversion to log10 CFU per gram.

3. RESULTS AND DISCUSSION

Results showed that single inoculants had higher cells on log values for all microbes as shown in Figs. 2 and 3. Regardless of the decrease in CFU, some carrier materials have values above the set standards of one billion viable cells per gram. This could lead to shift from single microbe inoculants due to economic value because same carrier material is used for several PGPM. This is in line with other studies which attribute economic implication of carrier material, method of application and co-inoculation in inoculant production [23,7,14]. Results also showed no significant changes in viable cells of PSM, KSM and ZnSM within the carrier material in single and multiple inoculants. There was high significant reduction in viable cells in multiple and single inoculants for NFM in FMP and FMBP (Figs. 2a and 2b) showing that filter mud provides low favorable conditions for PGPM as observed by other studies [9,11,6,24]. This is due to physical and chemical parameters that have an implication on biological activity and also regulation of temperature [6].

The significant difference in survival of different microbes between single and multiple inoculants was mainly due to competition which in most of the times include temperature, carrier materials and moisture. Diversity of microbes increased the number of microbes per gram which had an implication on temperature, nutrients and by products.

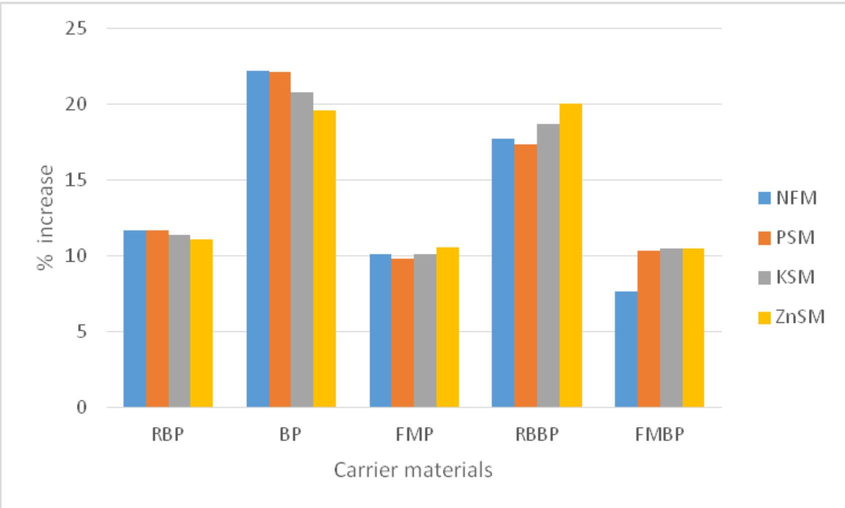

Fig. 1. Average percentage differences between sterilized and unsterilized carrier materials

Results as shown in Figs. 2 and 3 also show percent fluctuation over time of cells on log values under different carriers in single and multiple inoculation is indicative of synergistic effects between microbes and is also dependent on environmental factors like carrier material [25,26]. Ecology of PGPM has an implication on profitability of production methodology due to maximization of raw materials [27].

RBP, BP, and RBCP formulation significantly maintained population density of microbes at the end of storage period at room temperature and combination rice bran and biochar had the highest values (Figs. 2 and 3). This is as a result of combined beneficial characteristics of biochar and rice bran. Biochar is a complex solid material (biological origin) which has made thorough biomass carbonization. It's designed for the reduction of greenhouse gases emission and carbon sequestration in soils for a long time hence its usage has a positive impact on climate change [28]. It's highly porous structure and has positive implication on diversity and interaction of PGPM by promoting mutualism and desiccation by reduced carbon as an energy source and mineral nutrients [29,30,31]. Most of current studies have shown broad spectrum usage of biochar in agricultural land as a sustainable approach to bioremediation [32]. It increases plant growth, biostimulation, soil quality and yielding due to physical, biological and chemical positive changes when applied to soil [32,33,34]. Its effect is also dependent on raw materials and charring condition, application method and rate [28,30,35].

Carrier materials like RBP showing high viable cells in the study provide essential criteria of ideal carrier that are locally found [36,16,6]. Similar results of using different carrier in inoculant production were observed by other studies [37].

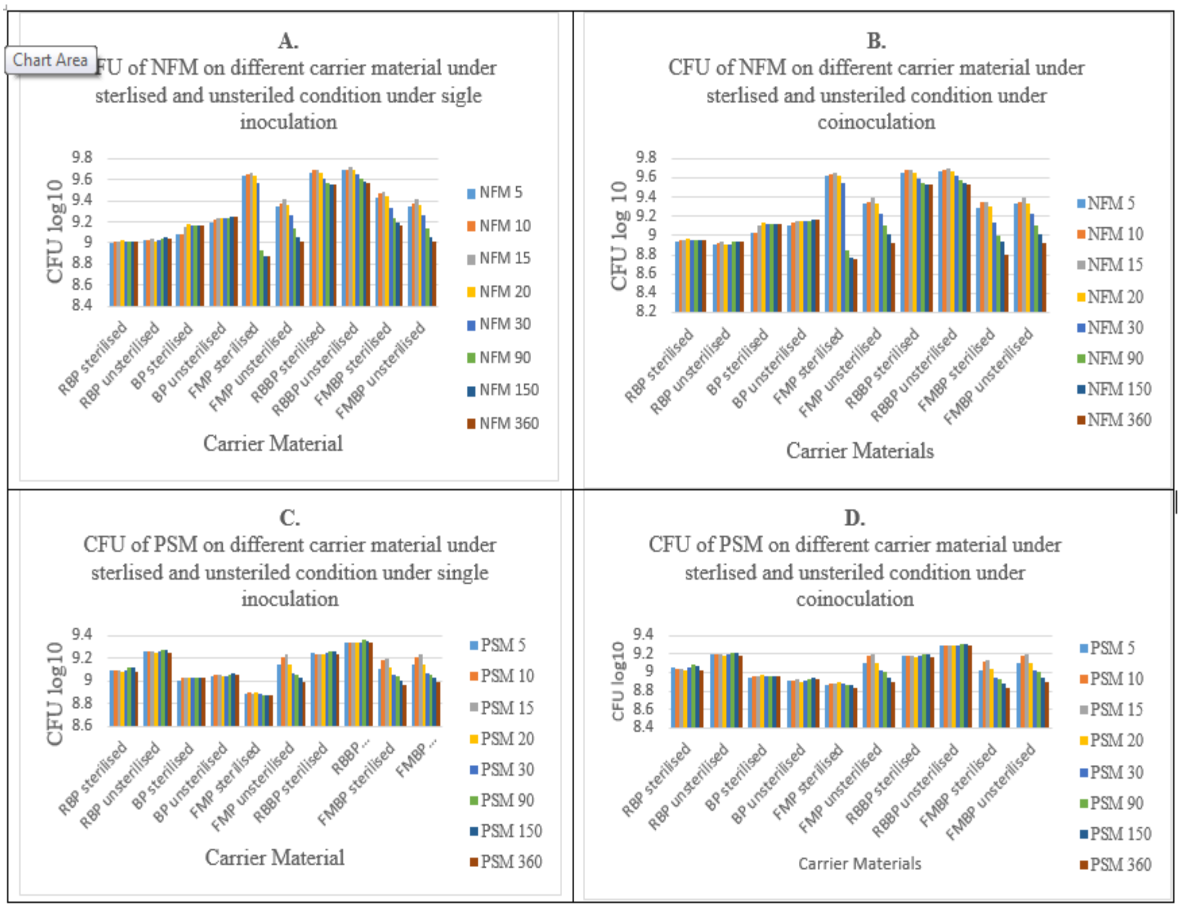

Fig. 1. CFU of PGPM (NFM and PSM) on different carrier materials under sterilized and unsterilized conditions for single and multiple inoculants

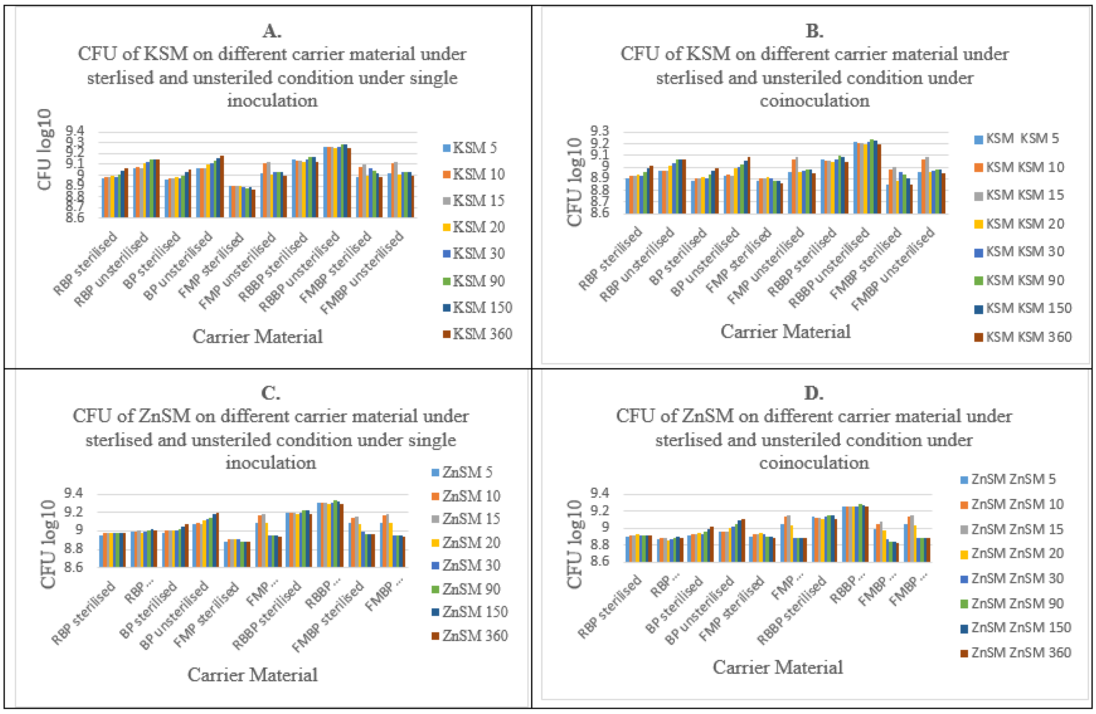

Fig. 3. CFU of PGPM (KSM and ZnSM) on different carrier materials under sterilized and unsterilized conditions for single and multiple inoculants

By showing high CFU within 15 days of storage at room temperature using filter mud and constant CFU if kept at 4 ^{0}c [6] which indicates that filter mud provides conducive environment for commercial farmers with cooling systems [9,38,39]. The quality of carrier material is dependent on delivering standard undistorted number of viable cells, adoptability and economic implications in production. The use of local organic materials like biochar and bran having higher and stable CFU for longtime over filter mud which has been used since 1981 is welcome development for smallholder rural farmers in Malawi [31,40,28,30].

Beside carrier material and consortium development the study also revealed the implication of sterilization in carrier development. The results showed that sterilization had no significant effect on CFU of intended PGPM. Lack of contamination could be attributed to the heat in the motor mill when milling carrier materials which needs additional study. The study results are similar to those obtained by other researchers doing commercial research [24,41] who found that sterilization processes is used for efficiency in inoculant production while carrier material has an implication on quality and quantity of PGPM.

4. CONCLUSION

The study concludes that RBBP is a potential carrier material for inoculant and that sterilization has no implication on contamination but decrease in CFU.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rao. Recent Advances in Biological Nitrogen Fixation in Agricultural Systems. 2014;(2):359-378. doi:10.16943/ptinsa/2014/v80i2/55114
2. Hungria M, Nogueira MA, Araujo RS. Soybean Seed Co-Inoculation with Bradyrhizobium spp . and Azospirillum brasilense : A New Biotechnological Tool to Improve Yield and Sustainability. 2015:811-817.
3. Souza R De, Ambrosini A, Passaglia LMP. Plant Growth-promoting Bacteria as Inoculants in Agricultural Soils. 2015; 419:401-419.
4. Abreu CS De, Figueiredo JEF, Oliveira CA, Santos VL, Gomes EA. Maize Endophytic

Bacteria as Mineral Phosphate Solubilizers. 2016;16(1):1-13.
5. Carriers BI, Vanek SJ, Thies J, Wang B, Hanley K, Lehmann J. Microbial & Biochemical Technology Pore-Size and Water Activity Effects on Survival of Rhizobium tropici in. 2016;8(4):296-306. DOI:10.4172/1948-5948.1000300
6. S, Balume IK, et al. Shelf-life of legume inoculants in Different Carrier Materials Available in East Africa. 2015;23(4): 379-385.
7. Redao C. Biochar as a Potential inoculant Carrier for Plant-beneficial Bacteria; 2016.
8. El-fattah DAA, Eweda WE, Zayed MS, Hassanein MK. Effect of carrier materials , sterilization method, and storage temperature on survival and biological activities of Azotobacter chroococcum inoculant. Ann Agric Sci. 2013;58(2): 111-118. DOI:10.1016/j.aoas.2013.07.001
9. Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). Plant Soil. 2014;378(1-2): 1-33. DOI:10.1007/s11104-013-1956-x
10. Arora NK, Tiwari S, Singh R. Plant Pathology & Microbiology Comparative Study of Different Carriers Inoculated with Nodule Forming and Free Living Plant Growth Promoting Bacteria Suitable for Sustainable Agriculture. 2014; 5:2-4. DOI:10.4172/2157-7471.1000229
11. Chaot W, Alexander M. Carriers for Rhizobium Inoculants. 1984;47(1):94-97.
12. Pindi PK. Liquid Microbial Consortium- A Potential Tool for Sustainable Soil Health. J Biofertilizers Biopestic. 2012;03(04). DOI:10.4172/2155-6202.1000124
13. Bashan Y. Pll S0734-9750(98)00003-2 ELSEVIER. 1998;16(4):729-770.
14. Gopalakrishnan S, Sathya A, Vijayabharathi R, Srinivas V. Formulations of Plant Growth- Promoting Microbes for Field. Published Online. 2016:239-251. DOI:10.1007/978-81-322-2644-4
15. González-andrés F. Formulation of a Highly Effective Inoculant for Common Bean Based on an Autochthonous Elite Strain of Rhizobium leguminosarum bv . Insights Into Its Agronomic Performance. 2019;10(December):1-16. DOI:10.3389/fmicb.2019.02724
16. Sparrow SD, Ham GE. Survival of Rhizobium phaseoli in Six Carrier Materials '. 1983;75:181-184.
17. Srinivasan R, Yandigeri MS, Kashyap S, Alagawadi AR. Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi J Biol Sci. 2012;19(4):427-434. DOI:10.1016/j.sjbs.2012.05.004
18. Adhya TK, Kumar N, Reddy G, Podile AR, Bee H, Samantaray B. Microbial mobilization of soil phosphorus and sustainable P management in agricultural soils. Curr Sci. 2015;108(7).
19. Karpagam T, Nagalakshmi PK. Original Research Article Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil. 2014; 3(3):601-614.
20. Liu M, Liu X, Cheng B sen, et al. Selection and evaluation of phosphate-solubilizing bacteria from Grapevine Rhizospheres for Use as Biofertilizers. 2016;14(4).
21. Mathew L, Sharad V, Divya A, Rishi S, Mandhan P. Cost-effective screening and isolation of xylano-cellulolytic positive microbes from termite gut and termitarium. 3 Biotech. 2017;7(2):1-7. DOI:10.1007/s13205-017-0733-6
22. Ivan P, Júnior F, Rohr TG, Oliveira PJ De, Xavier GR. Polymers as carriers for rhizobial Inoculant Formulations. 2009;(1): 1184-1190.
23. Campbell RM, Anderson NM, Daugaard DE, Naughton HT. Financial viability of biofuel and biochar production from forest biomass in the face of market price volatility and uncertainty. Appl Energy. 2018;230(August):330-343. DOI:10.1016/j.apenergy.2018.08.085
24. Furtak K, Gałązka A. Edaphic Factors And Their Influence On The Microbiological Biodiversity Of The Soil Environment. Published Online 2019:375-384. DOI:10.21307/PM-2019.58.4.375
25. Ma Y. Seed coating with beneficialmicroorganisms for precision agriculture.Biotechnology Advances. 2019;37(7):107423.
26. Nishiyama E, Higashi K, Mori H, Suda K, Nakamura H. The Relationship Between Microbial Community Structures and Environmental Parameters Revealed by Metagenomic Analysis of Hot Spring Water in the Kirishima Area. 2018;6(December). DOI:10.3389/fbioe.2018.00202

27. Id RA martins, Carvalho P, Miranda D. Edible ectomycorrhizal fungi and Cistaceae . A study on compatibility and fungal ecological strategies. Published Online. 2019:1-16.
28. Głuszek S, Sas-paszt L, Sumorok B, Kozera R. Biochar-Rhizosphere Interactions – a Review. 2017;66(2): 151-161.
29. Oelbermann M. conditioned biochar on soil organic carbon in temperate soils using the Century Soil Organic Matter model Evaluation of the long - term effects of pre - conditioned biochar on soil organic carbon in temperate soils using the Century Soil Organic Matter model Matthew Dil and Maren Oelbermann *. Published Online; 2017.
30. Tronto J. Characterization of biochars from different sources and evaluation. Published online 2017:395-403. DOI:10.5935/1806-6690.20170046
31. Głodowska M, Schwinghamer T, Husk B, Smith D. Biochar Based Inoculants Improve Soybean Growth and Nodulation. Published online 2017:1048-1064. DOI:10.4236/as.2017.89076
32. Meiirkhanuly Z. Evaluation of biochar for mitigation of ammonia , hydrogen sulfide , odorous volatile organic compounds, and greenhouse gases emissions from swine manure. Published online 2019.
33. Harter J, El-hadidi M, Huson DH, Kappler A, Behrens S. Soil biochar amendment affects the diversity of nosZ transcripts : Implications for N 2 O formation. Published Online. 2017:1-14. DOI:10.1038/s41598-017-03282-y
34. H AR, I JC, A FD, J MB, C SK. Characterisation and evaluation of biochars for their application as soil amendment Opportunities and constraints for biochar technology in Australian agriculture : Looking Beyond Carbon Sequestration; 2010. DOI:10.1071/SR10058
35. Mary GS, Niveditha PSS, Mary GS. Production, characterization and evaluation of biochar from pod (*Pisum sativum*), leaf (*Brassica oleracea*) and peel (*Citrus sinensis*) wastes. Int J Recycl Org Waste Agric. 2016;5(1):43-53. DOI:10.1007/s40093-016-0116-8
36. Physics R, Kennedy IR, Thies JE. microorganisms used as bio-fertilisers and bio-pesticides Development of high quality carrier materials for ® Eld Delivery of Key Microorganisms used as Bio-fertilisers and. 2000;(March). DOI:10.1016/S0969-806X(99)00480-6
37. Rajasekar K, Daniel T, Karmegam N. Microbial Enrichment of Vermicompost. 2012;2012. DOI:10.5402/2012/946079
38. Plains S, Argaw A. Response of Soybean to Inoculation with Bradyrhizobium spp . in Saline Soils of East African Journal of Sciences (2014) Response of Soybean to Inoculation with Bradyrhizobium spp . in Saline Soils of Shinille. 2015;(October).
39. Bala A, Karanja N, Murwira M, Lwimbi L, Abaidoo R, Giller K. Production and use of Rhizobial inoculants in Africa N2Africa Putting nitrogen fixation to work for smallholder farmers in Africa. 2011;3.
40. Mendes GDO, Zafra L, Vassilev B, Silva R, Ribeiro I. Biochar Enhances Aspergillus niger Rock Phosphate Solubilization by. 2014;80(10):3081-3085. DOI:10.1128/AEM.00241-14
41. Tittabutr P, Teamthisong K, Buranabanyat B, Teaumroong N. Gamma Irradiation and Autoclave Sterilization Peat and Compost as the Carrier for Rhizobial Inoculant Production. 2012;4(12):59-67. DOI:10.5539/jas.v4n12p59

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