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Use of different materials as a carrier for plant growth promoting bacteria

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Abstract

Rhizobium and *Azospirillum* are rhizobacteria which enhance plant growth via nitrogen fixation, nutrients solubilization and hormone production. These activities make them beneficial as microbial bio-fertilizer for legumes and economic crops. In this study, solid-base inoculants i.e. perlite, vermiculite and mixed media were evaluated for their potential use as carriers for *Rhizobium* CIAT899 and *Azospirillum* VAs087. Chemical and physical properties of the carrier materials were performed and the population of the two strains was counted. The results revealed that different carrier showed different effect on growth and survival of CIAT899 and VAs087 during 120 days of incubation. Mixed media gave highest number of both strain (10^8 to 10^9 CFU g^{-1} of carriers) while perlite and vermiculite supported survival at only 10^6-10^7 CFU g^{-1} of carriers during 10 to 30 days of inoculation. On the average the number of CIAT899 was higher in all the carriers than those of VAs087. The number of viable bacteria was decline after reaching the highest number and remained stable, between 10^4 and 10^6 CFU g^{-1} of carriers depended on the type of carrier, after 90 days of incubation. The results indicated that the use of mixed media as a carrier was appropriate for the production of bacterial inoculants.

Keywords: Carrier, Perlite, Vermiculite, Plant growth promoting rhizobacteria

1. Introduction

The increasing global concern regarding economic and environmental costs of the excessive use of chemical fertilizers has been reinforcing the importance of sustainable practice in agriculture. The use of rhizobium and plant growth promoting bacteria (PGPB) is often considered to be sustainable agricultural practices. Rhizobia are bacterial symbionts of legumes that fix atmospheric nitrogen in a process known as biological nitrogen fixation (BNF) [1]. Besides BNF, rhizobia also play role in phosphate solubilization and phytohormone synthesis and can act as non-symbiotic PGPB in non-legume crops. Among PGPB, *Azospirillum* strains have been well recognized as biofertilizers owing to their plant growth promoting activities including BNF. Rhizobium and PGPB have shown promise as bio-fertilizers. Peat is the most commonly used as carrier for commercial bio-fertilizers however, good quality peat is not available in many countries including Thailand. The good carrier material must be non-toxic either to the bacterial inoculants or to the plant itself [2]. Thus, alternative materials able to support high numbers of bacteria are needed. Various kinds of material have been evaluated, including charcoal, vermiculite [3 & 4] and perlite [5]. Vermiculite is a mica-like, hydrated magnesium-aluminum-iron silicate [$(Si_3Al)\ Mg_3(OH)_2O_{10}.Mg_{0.5}.nH_2O$] [6 & 7]. Perlite is a volcanic stone composed of a little-hydrated aluminium silicate [5]. For commercial use, vermiculite and perlite are exposed to an exfoliation process at high temperatures. They can be easily sterilized with no risk of producing toxic substances [5].

Our study aimed to develop inexpensive and suitable PGPR inoculants for use as an alternative to chemical fertilizers. Several carriers of various materials including perlite, vermiculite and mixed media were tested. Investigation of microbial cells enclosed in each carrier by scanning electron microscope (SEM) was also performed. The information from this study could be used to evaluate the potential use of beneficial inoculant for legumes yield improvement.

2. Materials and Methods

The aim of this study was to determine the potential of three different carrier materials for survival of *Rhizobium* and *Azospirillum* strains under room temperature (25-30ºC) for 120 days.

2.1. Microbial strains and culture conditions

The *Rhizobium tropici* CIAT 899 (originating from International Center of Tropical Agriculture, Colombia) was used in this experiment. CIAT899 is a N_2 -fixing microsymbiont of *Phaseolus vulgaris*. CIAT899 was routinely grown in yeast extract-mannitol broth (YMB) (2). Another bacterial isolate used in this experiment was Indole-3-acetic acid (IAA) producing *Azospirillum*, VAs 087 obtained from our previous study [8]. The isolate VAs087 was grown in *Azospirillum* broth medium [9]. The two isolates in each respective medium were placed on a rotary shaker (120 rpm) under room temperature until reaching maximum growth (10^9 CFU mL^{-1}). Stock cultures were maintained in 20% glycerol at -20ºC.

2.2. Carriers and inoculant production

Perlite, vermiculite and mixed media were used as microbial carrier. We have developed mixed media as microbial carrier in our previous study. In brief, mixed media was composed of coconut coir compost, peat moss, leonardite and dolomite [10] & [11]. Chemical properties and water holding capacity (WHC) of all selected carriers were determined prior to the experiment. Each carrier was used for the production of CIAT899 and VAs087 inoculants. All carriers were sterilized at 120ºC for 60 min. Microbial inoculants were aseptically prepared by mixing each sterilized carrier with each bacterial broth culture ($\approx 10^9$ CFU mL^{-1}). The same volume of each broth culture was added to each carrier in order to obtain the same initial number of bacteria, and then sterile water was added until reached the moisture content of 60% WHC of each carrier. For each treatment, three replicates were prepared in 200 mL glass bottle. All the formulations were kept under room temperature for 120 days.

2.3. Bacterial survival in selected carriers

The inoculants were determined for viable cell count (CFU g^{-1}) at 0, 15, 30, 60, 90 and 120 days after incubation. Inoculants were sampled periodically in triplicate (independent container), and viable bacteria were estimated by plating ten-fold serial dilutions on YMA agar and *Azospirillum* medium for CIAT899 and VAs087, respectively. Numbers of selected bacteria were referred to colony forming units (CFU) per gram of dry carrier.

2.4. Observation of Carriers and Microbial Morphology

The morphology of perlite, vermiculite and mixed media were examined by scanning electron microscope (SEM). Microbial morphologies on the surface of all selected carriers were also observed using JEOL (model JSM-5910LV) scanning electron microscope (SEM) at EMR Sc CMU (Electron Microscopy Research and Service Center, Chiang Mai University).

3. Results and Discussion

3.1. Chemical properties of selected carriers

Some chemical properties of the carriers are shown in Table 1. There was a distinct difference among chemical properties of the carriers. The pH of the mixed media was suitable for the growth of microorganisms (6.7) while the pH of perlite (9.9) and vermiculite (8.3) were quite high and could be considered as high alkalinity (pH above 7.5). The nutrients found in mixed media i.e. total nitrogen (0.81%), phosphorous (0.14%), potassium (0.46%), calcium (0.37%) and magnesium (0.25%) indicated that this material contained a good source of nutrients for microbial growth. However major nutrients, carbon, nitrogen and phosphorus in perlite and vermiculite were considered low. Water holding capacity of the three tested carriers was different. The ability for water absorption by mixed media was highest followed by vermiculite and perlite (data not shown). The survival bacteria in carrier with high organic matter (OM) and water holding capacity (WHC) such as peat is normally found to be better than the ones with low OM and WHC [12-14].

Table 1 Chemical properties of selected carriers.

Carrier	pH	EC	Total content (%)					
			OC	N	P	K	Ca	Mg
Perlite	9.9	2.17	0.1	0.02	0.013	0.66	0.59	0.01
Vermiculite	8.3	0.51	0.1	0.04	0.022	0.67	0.04	0.01
Mixed media	6.7	0.27	19.9	0.81	0.14	0.46	0.37	0.25

OC = organic carbon;
N = nitrogen;
P = phosphorus;
K = potassium;
Ca = calcium;
Mg = magnesium

Table 2 Survival of *Rhizobium* strain CIAT899 in three different carriers.

Isolate	Carrier	CFU g^{-1} inoculant					
		Day 0	Day 10	Day 30	Day 60	Day 90	Day 120
Rhizobium	Perlite	2.86×10^{7}	6.37×10^{7}	3.82×10^{7}	1.69×10^{7}	2.27×10^{5}	1.52×10^{5}
CIAT899	Vermiculite	4.02×10^{7}	1.61×10^{8}	4.12×10^{8}	2.04×10^{8}	5.35×10^{6}	1.99×10^{5}
	Mixed media	7.39×10^{7}	1.68×10^{9}	7.59×10^{8}	3.28×10^{8}	5.16×10^{6}	1.69×10^{5}

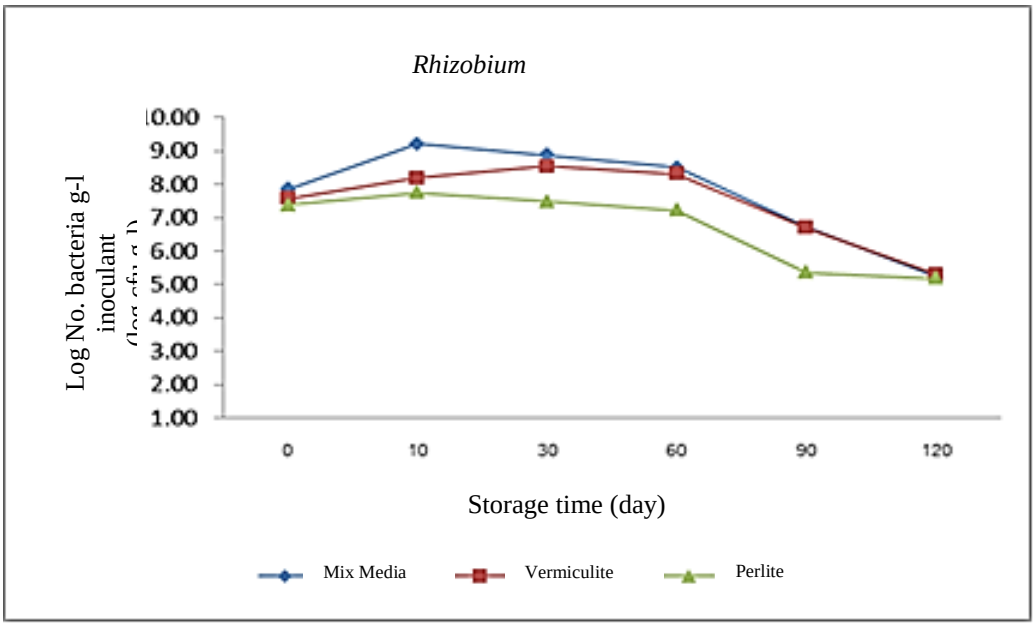

Figure 1 Population density (CFU g^{-1}) of *Rhizobium* strain CIAT899 in different sterilized carriers.

3.2. Survival of CIAT899 and VAs087 in selected carriers

The survival of *Rhizobium* strain CIAT899 and *Azospirillum* isolate VAs087 kept at 25ºC was monitored over a 120-day period in three types of carriers. The initial number of CIAT899 in perlite, vermiculite and mixed media was 2.86×10^{7}, 4.02×10^{7} and 7.39×10^{7} CFU g^{-1}, respectively (Table 2). The lowest number of CIAT899 at the first day of incubation was found in perlite while the highest number of this strain was found in mixed media. The differences in the initial number might be due to different moisture absorbing capacity of the three selected carriers. The water absorbs by mixed media was high followed by vermiculite and perlite. The results suggested that high water adherent capacity of mixed media influenced the high initial population of CIAT899. The present study was in accordance with Arora et al. [14] that coriander husk had the highest water holding capacity and provide the highest microbes population as compared to that of other carriers with low water absorbing ability. The results showed generally that the survival of CIAT 899 at each incubation periods was highest in mixed media

than in perlite and vermiculite (Figure 1). Maximum number of CIAT899 in perlite and mixed media was obtained after 10 days of incubation and after 60 days the number of CIAT899 in all the carriers declined rapidly and the number at 90 days was $1.52x10^5$ to $1.99x10^5$ CFU g^{-1}. Our results were similar to those of Phiromtan et al. [15] which indicated that the survival of bacteria considerably dropped at 7 days and slightly declined during 15 to 30 days and continuously declined until reaching the lowest number at 60 and 90 days after incubation.

On the average, numbers of *Azospirillum* isolate VAs087 in all the carriers were lower than *R. tropici* CIAT899 (Table 2 and 3). Mixed media was also the most suitable carriers for VAs087 with the highest number of 0.8 x 10^8 CFU g^{-1} inoculant after 30 days of incubation. The population of VAs087 in perlite and vermiculite at each incubation time was not much different. However, these numbers of VAs087 were much lower than those of CIAT899 at all the incubation time indicated that *R. tropici* CIAT899 could survive in vermiculite better than *Azospirillum* isolate VAs087. A sharp decrease of bacterial number in all the carriers was obvious at 90 days of incubation. At the end of the storage period (120 days), the numbers of VAs087 was lowest in perlite (1.78×10^4) and highest in mixed media (1.04×10^5) (Table 3). On the average the survival number of VAs087 was much higher in mixed media than in perlite and vermiculite (Figure 2). Our results suggested that different genus of bacteria might survive differently in the same media/carriers. Therefore suitable carriers for each type of bacteria might be different.

During the incubation time, the *Rhizobium* CIAT899 and *Azospirillum* VAs087 showed different population in different carriers. Despite the perlite carrier material, the other two materials were able to provide the *Rhizobium* number up to 10^9 and maintain at 10^8 CFU g^{-1} of carrier until 60 days of incubation. However VAs087 seemed to be more sensitive than CIAT899 (Figure 1 and 2), when perlite and vermiculite were used since the population size was decreased after 10 days of inoculation. Survival of both strains in the mixed media carrier reached the highest number around 10 days after incubation, the number of CIAT899 and VAs087 was 1.68×10^9 and 2.26×10^8 CFU g^{-1} of carrier, respectively. These numbers fulfilled the criteria for acceptable quality of legume inoculants. To maintain high population number, we suggested from our results that after 10 days of incubation or the day of reaching the maximum bacterial number in a carrier, the inoculants should be kept under 4-5°C to maintain high bacterial number. Seven days of inoculum storage under room temperature then transferred it to keep in several tested temperature indicating that the inoculum gave highest number of bacteria when kept at 5°C. This inoculum can be stored up to the maximum of one month at an ambient temperature and up to three months in a refrigerator [15].

3.3. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to visualize the perlite, vermiculite and mixed media particles with and without microbial inoculation. These images are shown in Figure 3 and 4. SEM images of perlite had a crystal-like porous structure while vermiculite had layered structure and micro-pores can be observed among the layers. However mixed media showed various structures of different particle sizes and some layer structure (Figure 3 and 4). The surface of perlite and vermiculite clearly showed the presence of CIAT899 (Figure 3) and VAs087 cells attached on their surface (Figure 4). The cells of CIAT899 seemed to more firmly attached to the surface of these two carriers than that of VAs087. Large numbers of both CIAT899 and VAs087 were present in the mixed media carrier. SEM images confirmed the higher number of viable cells of these two isolates in mixed media than in perlite and vermiculite.

Table 3 Survival of *Azospirillum* VAs087 in three different carriers.

Isolate	Carrier	CFU g^{-1} inoculant					
		Day 0	Day 10	Day 30	Day 60	Day 90	Day 120
Azospirillum	Perlite	1.09×10^7	8.27×10^6	3.06×10^6	2.17×10^6	4.82×10^4	1.78×10^4
VAs087	Vermiculite	7.73×10^6	9.55×10^6	4.70×10^6	2.07×10^6	3.38×10^4	2.03×10^4
	Mixed media	1.64×10^7	2.26×10^8	8.23×10^7	1.41×10^7	1.39×10^5	1.04×10^5

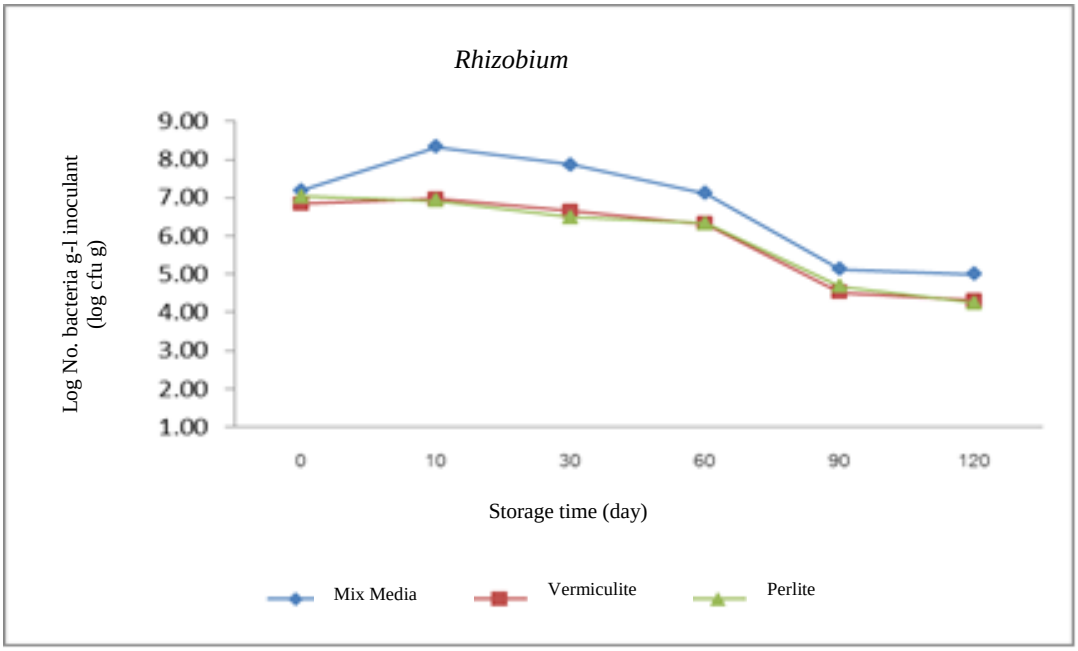

Figure 2 Population density (CFU g^{-1}) of *Azospirillum* strain VAs087 in different sterilized carriers.

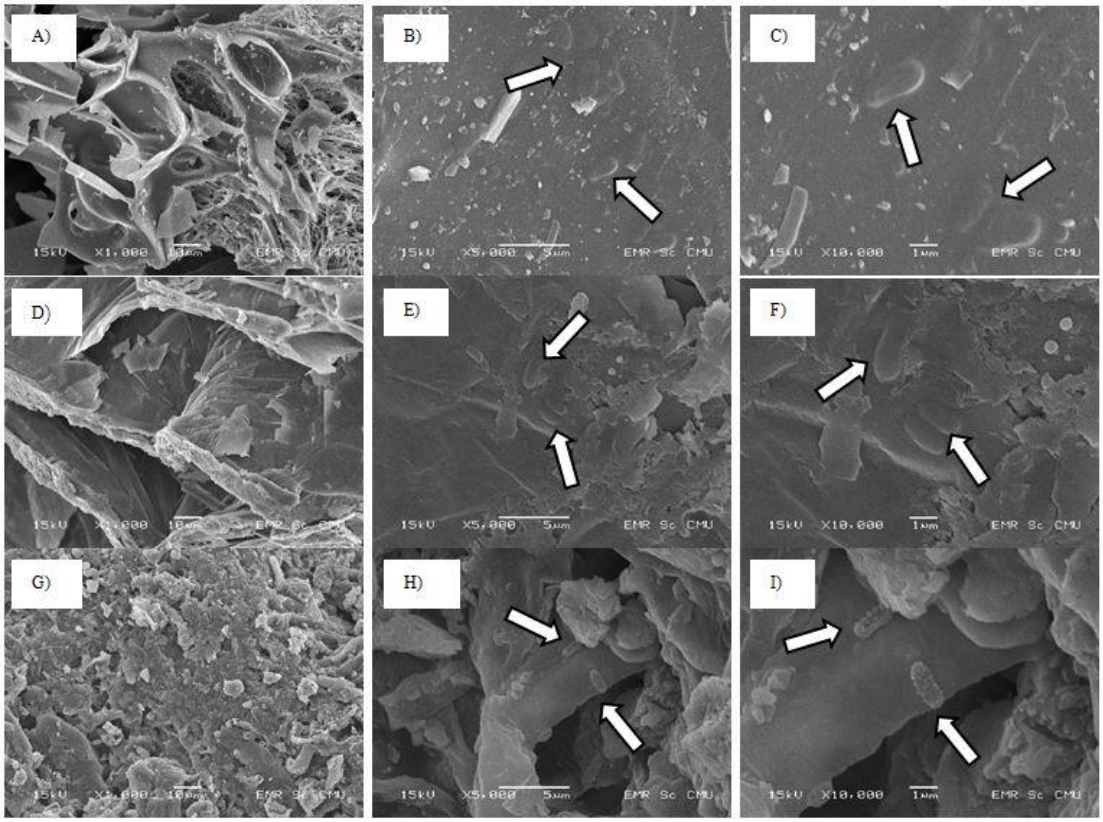

Figure 3 SEM images of uninoculated (A) and inoculated perlite with CIAT899 (B and C); uninoculated (D) and inoculated vermiculite with CIAT899 (E and F); uninoculated (G) and inoculated with CIAT899 (H and I). Arrows indicate some of the attached cells on each inoculated carriers.

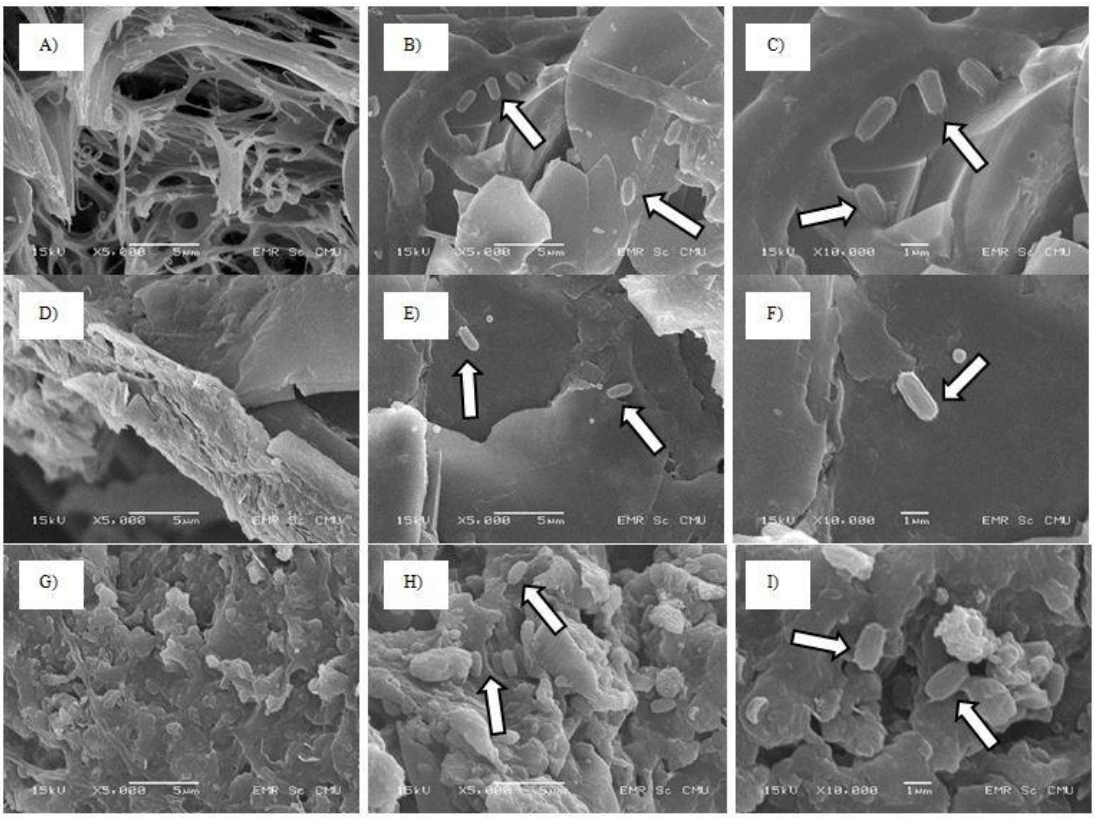

Figure 4 SEM images of uninoculated (A) and inoculated perlite with VAs087 (B and C); uninoculated (D) and inoculated vermiculite with VAs087 (E and F); uninoculated (G) and inoculated with VAs087 (H and I). Arrows indicate some of the attached cells on each inoculated carriers.

4. Conclusion

Perlite, vermiculite and mixed media performed different efficiencies in enhancing and maintaining the population of bacterial isolates due to their different chemical and physical properties. In the three selected carriers, the maximum number of bacteria was reached during 10-30 days of incubation. Mixed media was the best material and suitable for use as bacterial carriers which could raise the bacterial number up to 10^9 CFU g^{-1} inoculant. Maximum population within perlite and vermiculite were around 10^7 and 10^8 CFU g^{-1} inoculant, respectively. So these two carrier materials were at least proved to be an adequate substrate for growth and survival of bacteria during a short period of around 30 days.

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6. References

[1] Laranjo, M., Alexandrea, A., Oliveira, S., 2014. Legume growth-promoting rhizobia: An overview on the Mesorhizobium genus. Microbiological Research 169,2-17.
[2] Somasegaran, P., Hoben, H.J., 1994. Handbook for Rhizobia: Methods in Legume Rhizobium Technology. Springer, Berlin Heidelberg New York, USA, 217–218.
[3] Sparrow, S.D. Jr., Ham, G.E., 1983. Survival of Rhizobium phaseoli in Six Carrier Materials. Agronomy Journal 75, 181-184.
[4] Sangeetha, D., Stella, D., 2012. Survival of Plant Growth Promoting Bacterial Inoculants in Different Carrier. International Journal of Pharmaceutical & Biological Archives 3, 170-178.
[5] Daza, A., SantamarõÂa, C., RodrõÂguez-Navarro, D.N., Camacho, M., Orive, R., Temprano, F., 2000. Perlite as a carrier for bacterial inoculants. Soil Biology and Biochemistry 32, 567-572
[6] Harben, P.W., Kuzvart, Milos., 1996. Vermiculite, in Global geology: London, United Kingdom, Industrial Minerals Information Ltd., 432-437.

[7] Alexandre-Franco, M., Albarran-Liso, A., Gomez-Serrano, V., 2011. An identification study of vermiculites and micas: Adsorption of metal ions in aqueous solution. Fuel Processing Technology 92, 200-205.
[8] Chaiwon, F., Santasup, C., Sringarm, K., Shutsrirung, A., 2013. Antioxidant Activity, Vitamin C Content and Growth of Chinese Kale in Response to High Humus Seedling Media and Beneficial Microorgnisms. Chiang Mai University Journal of Natural Sciences 12, 79-89.
[9] Atlas, R.M., 1996. Handbook of microbiological media., 2nd. Edited by L.C. Parks. CRC Press, Boca Raton, Fla.
[10] Shutsrirung, A., 2012. Selection of Beneficial Microorganisms in Highland Soils for Improvement of Acid and High Arsenic Soils. 2012. Final Report. High Land Research and Development Institution (Public Organization). Chiang Mai.
[11] Shutsrirung, A., 2013. Study and Development of Bio-product Prototype for Reducing Soil Acidity and Arsenic Toxicity in Highland Soils. 2013. Final Report. High Land Research and Development Institution (Public Organization). Chiang Mai.
[12] Iswara, V., 1969. Growth and survival of R. trifoli in coir dust and soybean meal compost. The Madras Agricultural Journal. 59, 52.
[13] Sivasakthivelan, P., Saranraj, P., 2013. Azospirillum and its Formulations: A Review. International Journal of Microbiology Research 4, 275-287.
[14] Arora, N.K., Tiwari, S., Singh, R., 2014. Comparative Study of Different Carriers Inoculated with Nodule Forming and Free-Living Plant Growth Promoting Bacteria Suitable for Sustainable Agriculture. Journal of Plant Pathology and Microbiology 5,2.
[15] Phiromtan, M., Thongchai, M., Srinives, P.,2013. Effect of Various Carriers and Storage Temperatures on Survival of Azotobacter vinelandii NDD-CK-1 in Powder Inoculant. Modern Applied Science 7, 81-89.