The Pharma Innovation Journal 2022; SP-11(8): 341-345

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 341-345

www.thepharmajournal.com
Received: 12-05-2022
Accepted: 17-06-2022

M Priyadharshini
PG Scholar, Department of Agricultural Microbiology, Agricultural College and Research Institute, TNAU, Madurai, Tamil Nadu, India

R Thamizh Vendan
Registrar, Tamil Nadu Agricultural University, Tamil Nadu, India

R Subhashini
Associate Professor, Department of Agricultural Microbiology, Agricultural College and Research Institute, TNAU, Madurai, Tamil Nadu, India

P Kannan
Assistant Professor, Department of Soil and Environment, Agricultural College and Research Institute, TNAU, Madurai, Tamil Nadu, India

Corresponding Author
M Priyadharshini
PG Scholar, Department of Agricultural Microbiology, Agricultural College and Research Institute, TNAU, Madurai, Tamil Nadu, India

Development of enriched potting media for nursery

M Priyadharshini, R Thamizh Vendan, R Subhashini and P Kannan

Abstract
Microbial inoculants are gaining importance for attaining sustainable agricultural production systems**.** A consortium of microorganisms provides an enabling and agreeable choice for maintaining their effective capacity for long enough periods of time, resulting in a good impact on soil microbial activity at the target sites. In this research, microbial consortium was standardized for enriching the cocopeat to use as a potting medium for nurseries. Cocopeat contains 0.41:0.81:1.32% NPK respectively. The cocopeat which was treated with microbial consortium exhibited higher microbial population as well as higher NPK status of 1.3 g kg^{-1}, 1.09 g kg^{-1} and 1.57 g kg^{-1} respectively. From the acquired data, we conclude that the cocopeat enriched with microbial consortium can be a good and sustainable potting medium for nursery crops.

Keywords: Microbial consortium, cocopeat, pro-tray mix

1. Introduction
Increasing crop productivity is of global concern, and will require the development of new technologies. Inputs to optimize crop productivity can be applied through soil and water and a crop will thrive if all inputs are optimal. To achieve optimum crop productivity, the soil should be fertile because plants absorb nutrients from this source. The continuing need for increased crop productivity dictates increasing demands on soil fertility worldwide (Wild *et al.,* 2003) [13]. Extensive use of synthetic fertilizer to maximize productivity often leads to depletion of essential soil nutrients, environmental degradation and adversely affects the soil microbial population (Sani *et al.,* 2020) [11]. Cocopeat is often used as potting medium for raising nurseries. Coco peat increases the porosity of the potting mix and possess excellent properties that make it as a very good soilless medium. Being an organic medium, it has high cation exchange capacity allowing nutrients to be absorbed and released to the plants according to their need. Microbial consortium is combined with cocopeat can be a better potting mix for vegetable nursery crops. Nursery is the main place where we need to produce healthy and vigorous seedlings. In the nursery the growth and quality of the seedlings will be improved with the application of enough nutrients. Bioinoculants provide nutrients and growth hormones to the crop which ensure good growth and development of the seedlings in the nursery. This research is mainly focused on the development of liquid NPK consortium for enriching the cocopeat medium so as produce healthy and vigour seedlings.

2. Materials and Methods
2.1. Estimating the plant growth promoting traits of the selected bacterial cultures
PGPR traits *viz.,* Ammonia production, HCN production, Siderophore production were estimated with the 3 days of incubation. EPS production, Nitrogen fixation, Phosphate solubilization and Potassium release were estimated for the selected cultures after 7 days of incubation. For qualitative assay of phosphate solubilization, Pikovskaya's medium was used (Pikovskaya, 1948) and Aleksandrov's medium was used for assessing the K release (Aleksandrov *et al.,* 1967) [1]. The above estimations were done by following the standard protocols.

2.2. Compatibility test among the bacterial cultures with AM fungi
Commercially using microbial cultures *viz., Azospirillum*, Phosphobacteria (*Bacillus megaterium*) and K release bacteria (*Paenibacillus mucilaginosus*) for N, P and K sources and Arbuscular mycorrhizal fungi for PO_4 mobilization were used in this study. These cultures were obtained from the Department of Agricultural Microbiology, AC & RI, Madurai. The compatibility among the bacterial cultures was tested using cross streak method in nutrient

agar medium. Cross streaked plates were incubated at 37±1^{0}C for 48 h and the zone of inhibition was observed and recorded (Prasad and Babu, 2017) [10].

The compatibility of the selected bacterial cultures and AM fungi was carried out in a pot experiment. Sorghum crop was raised for AM fungi colonization using vermiculite as substrate. *Azospirillum*, PSB and KRB were individually inoculated at the rate of 5ml/kg (containing 10^9 cells/ml) of vermiculite containing 50 g AM inoculum. AM inoculum alone was maintained as control. Spore count was recorded before and after the inoculation of bacterial cultures. 0 and 30 days after inoculation of bacterial cultures, microbial population was examined.

2.3. Formulation of consortium

The three selected compatible cultures were used to develop the microbial consortium in liquid formulation. The bacterial cultures individually were cultured in 500 mL conical flask and incubated for attaining the microbial load of 1×10^9 CFU/mL. The entire quantity of the cultures were mixed with 1.5 lit nutrient broth prepared in 5 lit flat and allowed to grow for a period of one week and bottled. Consortium was stored at room temperature for 60 days. Shelf life of all the cultures in the consortium was recorded and PGPR traits of the consortium was also estimated and recorded.

2.4. Testing the plant growth-promoting traits of the consortium

PGPR traits *viz*., IAA production, Ammonia production, EPS production, HCN production, Siderophore production, Nitrogen fixation (Dobereiner, 1995) [5], Phosphate solubilization and Potassium release were estimated in the developed consortium by following the standard protocols.

2.5. Assessing the quality of the developed consortium

For assessing the quality of the consortium, estimation of the cell count of individual organism (CFU), and contamination level were recorded throughout the storage time up to 60 days.

2.6. Incubation study for enrichment of protray medium

Coco peat being commonly used as pro-tray nursery medium for horticultural crops was used in this experiment. Cocopeat was enriched with the addition of microbial consortium and AM fungi.

- T1: Coco peat (Control)
- T2: Coco peat + microbial consortium + AM fungi

These treatments were evaluated through an incubation study. Microbial consortium and AM fungi were added at 5ml/kg and 10g/kg of potting mix respectively and the moisture content was maintained as 10%. At the end of 30 days of incubation, potting mix was collected from this bag and the pH and nutrient status was estimated.

Available nitrogen (mg kg^{-1}) was estimated using Alkaline permanganate method (Asija *et al.,* 1956), Available phosphorous (mg kg^{-1}) was estimated by Olsen's method (Olsen, 1954), Available potassium (mg kg^{-1}) was estimated using Neutral normal ammonium acetate method (Stanford and English, 1949) [12]. pH and Electrical conductivity (dS m^{-1}) were estimated as per the standard protocols.

2.7. Statistical analysis

The experiment was conducted in a completely randomized block design. The results presented are the mean of five replicates. Sample variability was estimated by the standard deviation of mean. Analysis of variance on the data was calculated at CD 5%.

3. Results and Discussion

Experiments were conducted starting from culture compatibility to the incubation study for pro tray media enrichment. Bacterial cultures *viz.*, *Azospirillum*, PSB and KRB were used to develop the microbial consortium. The plant growth promoting traits were assessed for all the selected bacterial cultures.

3.1. PGP traits of bacterial cultures

Plant growth promotion is defined as the production of plant hormones and other growth related subtances such as providing nutrients, releasing volatile organic compounds in the rhizosphere and suppressing plant disease-causing pathogens and insect pests, either directly or indirectly, according to scientific understanding (Baker *et al.,* 2018). The results of the PGP traits of bacterial cultures are given in the Table 1. *Azospirillum lipoferum* turned the N free malic acid broth from green into blue colour showed the N_2 fixing activity of the culture. P & K solubilization was observed higher in PSB as 86.4 mg P/L followed by KRB (22.7 mg P/L) and *A. lipoferum.* KRB registered high release of potassium 23.8 mg/L. It was significantly higher than the other cultures. PSB registered the K release of 12.9 mg/L, which was significantly higher than the *A. lipoferum* (9.4 mg/L. The results showed that PSB & KRB have the potential of solubilizing/ releasing of phosphorous and potassium. Siderophore production was observed positive in all the three cultures. EPS production was significantly higher in KRB (34.02µg/mL), followed by PSB (15.91 µg/mL) and *A. lipoferum* (12.61 µg/mL). The selected cultures showed positive in Ammonia production, HCN production and siderophore production.

Table 1: Plant growth promoting traits of bacterial cultures

Bacterial cultures	Growth in NFB	PO_4 solubilization (mg/L)	K release (mg/L)	EPS Production (µg/mL)	Siderophore production	Ammonia production	HCN production
Azospirillum lipoferum	+ +	8.9^c	9.4^c	12.61^c	+	+	+
PSB (*Bacillus megaterium*)	-	86.4^a	12.9^b	15.91^b	+	+	+
KRB (*Paenibacillu muciloginosus*)	-	22.7^b	23.8^a	34.02^a	+	+	+
SEd		2.71	0.59	0.79			
CD (0.05)		5.92	1.29	1.72			

Values are mean of five replicates (n=5) and column values followed by different letters are significantly different from each other at p=0.05 with LSD

3.2. Compatibility test of selected cultures and consortium development

Compatibility test were carried out between the bacterial cultures and AM fungi. In a compatibility test, selected cultures had good growth without interfering one another, indicating that they are compatible organisms under pot culture. AM Fungal spores and bacterial counts were recorded before and after the inoculation of bacterial cultures into the vermiculite substrate and tabulated (Table 2).

Table 2: Microbial population and AM fungal spore count in compatibility study

Treatment	No. of AM spores/ g substrate		Microbial population (log cfu/g of substrate)	
	0 Day	30 Days	0 Day	30 Days
Vermiculite + AM inoculum	11	15	-	-
Vermiculite + AM inoculum + *Azospirillum*		20	8.39	8.93
Vermiculite + AM inoculum + PSB		30	8.11	8.69
Vermiculite + AM inoculum + KRB		24	8.28	8.43

Bacterial cultures were added @ 5ml/kg substrate, AM was added @ 50g/kg substrate

In the present study, AM spore count/g inoculum is 11 and the spore count was enhanced after 30 days in all the treatments. The spores count was enhanced to a maximum of 30 spores/g. Addition of *Azospirillum*, PSB and KRB in the substrate enhanced the spore count in the inoculum at higher rate compared without the addition of bacterial cultures. This result demonstrates the compatibility of bacterial cultures with AM fungi. The results are in accordance with the report of Behera *et al.,* (2019) [4]. It was reported that the effect of AM fungi alone and together with 5 different PGPR cultures. Biovolume index of AM fungi alone and AM fungi with *Bacillus subtilis* was 26.56 and 30.14 respectively. Mycorrhizal spore numbers also increased from 100 (AM fungi alone) to 150 (AMF + *B. subtilis*)/ 50 g of dry substrate. The results described the increased spore count with co inoculation of above three bacterial cultures, there by showed the compatibility.

3.3. PGP properties and shelf life of developed consortium

The PGP properties of developed consortium was recorded throughout the storage period of 60 days. The average amount of phosphorous and potassium solubilization was 89.2 mg/L and 26.9 mg/L respectively after 7 days of incubation. IAA production was 2.91 µg/ml. The consortium exhibited positive results for ammonia production, HCN and siderophore production with the incubation of 3 days (Table 3). Estimated population of the individual organisms in consortium at different time intervals are presented in Table 4. In line with this, Minaxi *et al.,* (2012) [7] documented that the *Bacillus sp*. used for the study demonstrated a variety of plant growth-promoting properties, such as 175.3 ppm of phosphate solubilized after 24 hours of incubation, and 54.14 µM α-keto butyrate mg^{-1} protein h^{-1} of ACC deaminase activity in 24 hours of growth. In the presence of 3 mg ml^{-1} tryptophan, it also produced a high quantity of indole-3-acetic acid (39.47 µg ml^{-1}), as well as ammonia and antifungal activity.

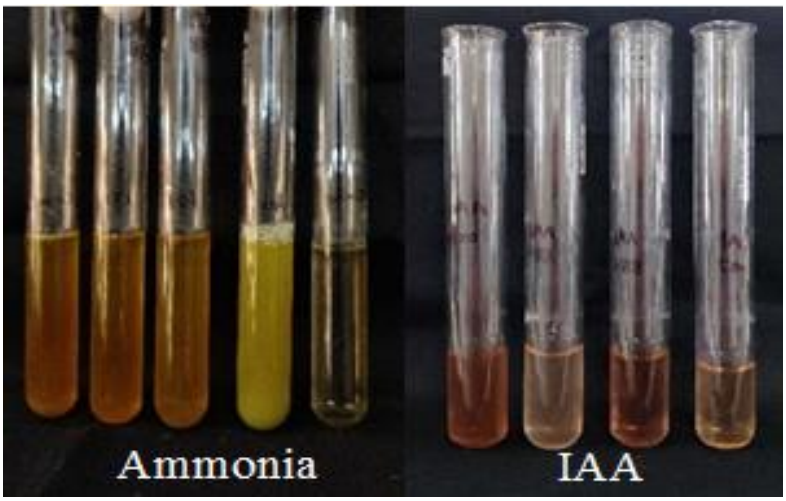

Fig 1: Ammonia production and IAA production of the consortium

Table 3: PGP properties of developed consortium

PGP properties	Results
IAA production	2.91 µg/ml
P solubilization	89.2 mg/L
K release	26.9 mg/L
Ammonia production	+
HCN production	+
Siderophore production	+
Exopolysaccharide production	29.43 µg/ml

+ Positive

Table 4: Assessment of population of individual organisms in the developed consortium

Days	Viable cell population (log CFU/ml)		
	Azospirillum	PSB	KRB
0	9.08	9.38	9.19
10	9.29	9.54	9.36
20	9.42	9.78	9.79
30	9.60	9.90	9.85
40	9.10	9.11	9.14
50	8.76	8.98	8.85
60	8.52	8.79	8.73

Initial population of each organism in the consortium is 10^9 CFU/ml. After 30 days slight increase in cell load was observed and a slight reduction occur when it was 60 days old. The population in day 60 was in 10^8 cell/ml. Slight reduction in pH of the consortium was noticed. Though cell load reduction was observed, which is sufficient to colonize the plants.

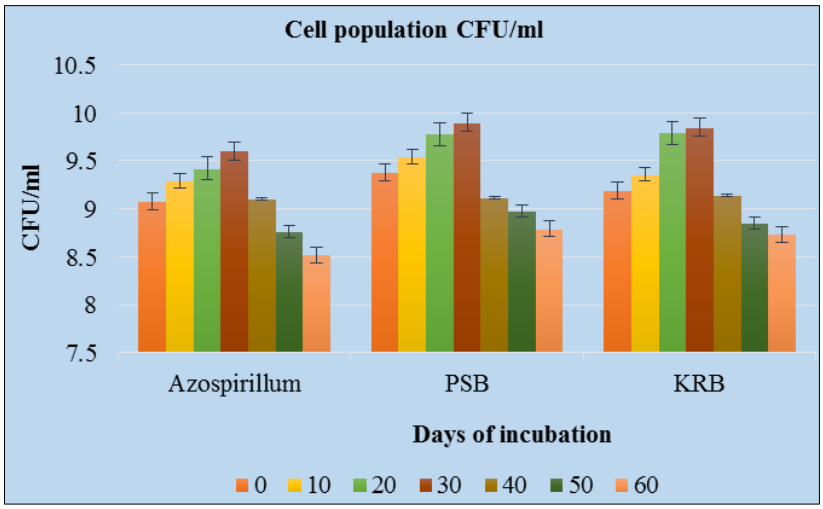

Fig 2: Estimation of viable cell population of consortium

3.4. Incubation study for enrichment of potting media through microbial inoculation

Enrichment of potting mix for nursery was made as per sec 2.6. The best treatment was T2 based on microbial population in potting mix and their available nutrients. Initial population in the consortium was 10^9 CFU/ml of *Azospirillum*, PSB and KRB. After the inoculation of consortium and AM fungi at 5ml/kg and 10g/kg substrate respectively, it was incubated for 30 days. After 30 days the population was assessed in the cocopeat. It was observed that, the increase in the cell load of individual organism is about 1000 – 10,000 cells. Similarly nutrient status in the cocopeat with and without bacterial inoculation was assessed. The results showed the increase in nutrient status of cocopeat medium. Normal cocopeat (T1) contains 0.41 g kg^{-1}, 0.81 g kg^{-1} and 1.32 g kg^{-1} NPK respectively. The cocopeat + microbial consortium mix contains 1.3 g kg^{-1}, 1.09 g kg^{-1} and 1.57 g kg^{-1} NPK respectively. The nitrogen content was approximately increased two-fold and also phosphorous and potassium were increased by 17.2% and 12.9% respectively from the initial content. The results were showed in Fig 3. EC, pH for T1 and T2 was 1.10 (dS m^{-1}), 5.6 and 0.60 (dS m^{-1}), 5.76 respectively. The results of the present experiment showed an increase of microbial population as well as nutrient content of the protray medium after 30 days of incubation with the microbial enrichment. The similar results are expected on plant growth, when enriched cocopeat is used as potting medium in nurseries. Kapadia *et al*., (2021) [9] studied and reported that the inoculation of consortia had a significant influence on the growth-related parameters of the tomatoes. Further the plant growth-related parameters were adversely affected by the higher EC levels of the soil. The plant growth-related parameters were studied at 0.2 dS/m to 8 dS/m under uninoculated and inoculated (microbial consortia) plants.

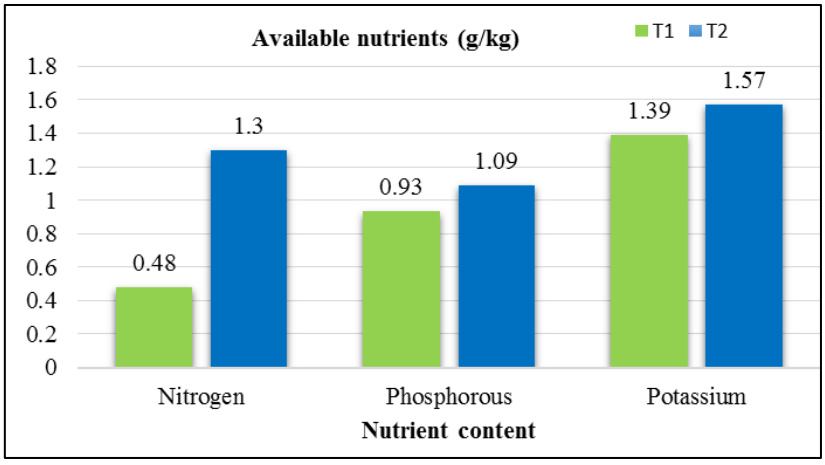

Fig 3: Effect of the microbial consortia on nutrient status of potting media

4. Conclusion

The best potting medium plays an important role in developing vegetable nurseries. When cocopeat alone was used as potting media, it results in better porosity and aeration but when the same was treated with microbial consortium in a definite proportion of *Azospirillum*, PSB and KRB, it showed enhanced characters by increasing nutrient availability along with the existing characters of cocopeat. Hence eventually results in sustainable plant and soil health. This study will be the eye opener for using enriched protray medium for getting

good growth and establishment of the seedlings in the nursery.

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