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Prospecting inexpensive media for mass production of microbial inoculants at farmers field

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Abstract
Utilization of beneficial and multifaceted microorganism can solve the problem of getting enhanced yield without disturbing the ecosystem. Timely availability of quality microbial inoculants is one of the major concerns of the farming community. Thus, we aimed to design a media based on household wastes for mass culturing of beneficial microbes especially symbiotic nitrogen fixer *Mesorhizobium* spp. and multifaceted Bacillus spp. This work was initiated with the selection of suitable substrate from home waste along with carbon supplements source that can be available at village level. Standard media like Yeast extract mannitol for *Mesorhizobium* and Nutrient yeast salt medium for Bacillus was included as a reference media. Components of newly designed media include boiled rice water, pulse/ vegetables wash and different sources of carbon viz. table sugar, jaggery and glucose. Boiled rice water without any carbon supplementation did not support the growth of targeted microbes. Different concentration of boiled rice water along with dal/vegetables wash and sugar supported the growth of targeted microbes based on visual observation. Among carbon sources, glucose was observed to support maximum growth having optical density of 1.621 and cfu was $170x10^6$. However, The best combination for getting maximum targeted biomass was 58% rice boil water, 10% pulse wash, 10% vegetable wash, 20% water, 2% glucose. The proposed media is highly cost effective and providing high yield of microbial biomass and its combination are available at village level. Designing of media will facilitate the farming community for the production of the microbial inoculants at their site as and when required.

Keywords: Household wastes, boiled rice water, sugar, jaggery, glucose, optical density, C.F.U

Introduction
The challenging task to feed fast growing population is successfully accomplished through intensive use of chemical fertilizer around the world. Such intensive chemical fertilizer application started displaying harmful effects like deteriorating soil and water quality, destroying microorganism and friendly insects, reducing biodiversity (Savci, 2012) [25]. To overcome these emerging challenges, alternative or supplementive resources to supply nutrient to crop are required. Such alternative or supplementive resources are undoubtedly microorganisms, a wheel of agriculture. Biofertilizer are a microbial product containing millions of targeted efficient microorganisms for bionutrient delivery to the crop through seed or soil or root inoculation (Jha and Kumari, 2008) [14]. Biofertilizer means the product containing carrier base (solid/liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization or nutrient mobilization to increase the productivity of the soil and or crop (Yadav, 2006) [36]. Few promising strains are utilized for the biofertilizer production through various methods developed from time to time. *Rhizobium* is a soil habitat Gram-negative bacterium, which can able to colonize the legume roots and fixes atmospheric nitrogen symbiotically. *Bacillus* is a soil habitat Gram-positive bacterium which has the ability to inhibit the growth of other strains and act as a growth inhibitors for other micro-organisms. Use of these microbes as biofertilizers in the field has been reported to increase crop yield. The soil must contain adequate nutrients for biofertilizer organisms to thrive and work. Biofertilizers complement other fertilizers, but they cannot totally replace them. Biofertilizers lose their effectiveness if the soil is too hot or dry. Excessively acidic or alkaline soils also hamper successful growth of the beneficial microorganisms; moreover, they are less effective if the soil contains an excess of their natural microbiological enemies.

Shortages of particular strains of microorganisms or of the best growing medium reduce the availability of some biofertilizers.

The manufacturing process of biofertilizer is selection of suitable strain of the microorganism, Culture of the selected microorganism and its mass multiplication, Inoculant processing and application. The selected microorganism which has been screened for its symbiotic performance must be cultured in large quantities to produce enough inoculant for the field. In media preparations, individual carbon source is not feasible because microbial strains have different nutritional requirements (Tittabutr *et al*., 2005). [30] Initially, YMB medium (Yeast Mannitol Broth) was used for biofertilizer production (Verma *et al*., 2010; Singh *et al*., 2015) [35]. However, literature reported that it is not economically feasible (Tyagi *et al*., 2002) [32].

Most of the researchers have looked for alternative cheap media for producing biofertilizers. A large number of agricultural and industrial by products such as proteolyzed pea husks, molasses and water hyacinth (Gulati, 1979) [12], malt sprouts (Bioardi and Ertola, 1985) [3], deproteinized leaf extracts (Chanda *et al*., 1987, cheese whey (Estrella *et al*., 2004) [4, 7], waste water sludge Rebah *et al*., 2007) [22], Jaggery solution (Jain *et al*., 2000) [13], Potato extract broth (Martyniuk and Oron, 2011) [18], sugar waste (Singh *et al*., 2011) [27] and Dairy sludge (Singh *et al*., 2013) [26]. These products support the growth of rhizobia equal to or better than the known growth in the available media. Most of the microorganisms viz., *Rhizobium*, *Bacillus* can be easily grown in laboratory. However, they are much more difficult to produce in large quantities. To address these problems, one of the approach is to design such a media whose substrate may easily available at farmers level for mass culturing of microbial inoculants. Household waste can be used as a substrate, so that farmers after procuring mother culture of microbial inoculants can mass culture the microbes in their place as and when required. However, criteria for designing should be based on High cost effectiveness, low production problem, availability of media substrate at farmers level and high yield of biomass. Therefore present investigation was undertaken to Search for suitable substrate from household waste and carbon supplement source available at farmer level and studied its colony morphology, colony forming unit, growth, IAA and finally carbohydrate utilization for Standardization of parameter for maximum biomass

Materials and Methods

Origin of isolates

Mesorhizobium and *Bacillus* isolates were obtained from Microbiology Department of Faculty Basic Sciences & Humanities, Rajendra Agricultural University, Pusa, Samastipur, Bihar. These were isolated from different soil of Bihar viz. Chickpea nodule from Tal land and Maize rhizophere of diara soil. These isolates were selected to culture on household waste based media for mass production of microbial inoculants.

Culturing of isolates and Selection of household waste and carbon sources for designing media

Mesorhizobium and *Bacillus* isolates were cultured on selective media for further morphological and colony characteristics. YEM agar media (Fred *et al*., 1932) was used for the growth and culturing of *Mesorhizobium* spp. Nutrient Yeast Salt Medium (NYSM) (Balaraman *et al*., 1982) was used to study colony morphology and also for maintenance of culture on slant of *Bacillus* spp.

Rice boil water was taken in four different proportions. Its composition is as follows (L^{-1}). (1) 18% rice boil water, 10% pulse wash, 10% vegetable wash, 60% water, 2% sugar; (2) 38% rice boil water, 10% pulse wash, 10% vegetable wash, 40% water, 2% sugar; (3) 38% rice boil water,10% pulse wash, 10% vegetable wash, 40% water, 2% sugar; (4) 78% rice boil water, 10% pulse wash, 10% vegetable wash, 2% sugar; agar (18 g) for solidification; then final volume was make-up using distill water; the pH was adjusted to 7.0. Preparation of media was modified for reducing the viscosity by passing the unsterilized media though cheese cloth. The proposed media was designated as Media I, Media II and Media III. The sugar, glucose and jaggery were used as carbon source in media I, II and III for mass culturing of the targeted isolates and rest of the composition were same.

Comparative growth & media selection

Growth of selected *Mesorhizobium and Bacillus* isolates were estimated in three different media (media I, media II, media III) by taking O.D at 620 nm and calculating colony forming unit after incubation at different time interval i.e., 24 hr, 48 hr and 72 hr at 32±2 ^{0}C for selection of suitable treatment for maximum growth. These isolates were further studied for growth parameters, Indole Acetic Acid (IAA) production which acts as growth promoter.

Growth of all isolates were estimated by taking O.D at 620 nm (Tarrand and Gröschel, 1982) [29] after incubation of *Mesorhizobium* and *Bacillus* isolates at 32±2 ^{0}C for 24 hour, 48 hour and 72 hour with shaking in media I, media II and media III. The selection of best treatment was based on the observation of highest growth among all tested treatment of the three designed media.

Colony forming unit represents the scientific estimation of the number of viable (able to live/grow) bacteria in a agar plate. The standard plate count is a reliable method for enumerating targeted isolates. A set of serial dilutions is made, from 10^{-2} to 10^{-6} and 1mLsample of each dilution is placed on the surface of designed Media I, Media II and Media III. YEM media for *Mesorhizobium* and NYSM for *Bacillus* were used for control during study. The number of c.f.u colony was counted after 24 hour of incubation.

The Standard Formula:

$$\frac{\text{Colony count (CFUs) on an agar plate}}{\text{Total dilution of tube (used to make plate for colony count)}\times\text{volume plated}}$$

IAA Production

IAA production by *Mesorhizobium* and *Bacillus* spp was determined by Patten and Glick method (Patten and Glick, 2002) [21]. After culturing the *Mesorhizobium* and *Bacillus* spp. on designed media, isolates were further tested for Indole Acetic Acid production. Loop full of culture was inoculated in 25 mL of Luria's broth amended with 50 µg/mL tryptophan. The culture was incubated at 30+- 2 C on the rotary shaker for 24 hours and centrifuged at 10, 000 rpm for 15 minutes. Then 2mL of supernatant were taken in separate tubes and 2 to 3 drops of O-phosphoric acid added with 4 mL of reagent containing 1 mL of 0.5 M $FeCl_3$ in 50 mL of 85 per cent ($HClO_4$), again incubated for 25 minutes at room temperature and absorbance were recorded at 530 nm. To calculate the concentration of IAA in each sample, a standard curve ranging from 0.01 to 100 µgml−1 of pure IAA was used for comparison. According to the amount of IAAproduced, four

distinct levels of IAA production, low production (<15 µg ml−1), medium production (between 15 and 30 µg ml−1), high production (between 30 and 45 µg ml−1), and very high production (>45 µg ml−1), were considered.

Biochemical characterization

Isolate with high growth rate, high viable cell count and IAA on the different treatments of media I, media II, media III were selected for biochemical characterization based on utilization of 33 different carbon sources, ONGP (O-nitrophenyl ß-D galactopyranoside) and esculin hydrolysis. The carbon sources were Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehlose, Melibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium Gluconate, Glycerol, Salicin, Dulcitol, Inositol, Sorbitol, Mannitol, Adonitol, Arabitol, Erythritol, α-methyl-D-glucoside, Rhamnose, Cellobiose, Melizitose, α-methyl-D-Arabinose, Citrate, Maloante and Sorbose. These test were performed using Hi-media carbo-kit (KB009, Hi carbohydrate kit) i.e., all the 35 wells specific for different carbon sources were inoculated with 50 µL of culture showing turbidity ≥ 0.5 O.D. at 620 nm, and studied for colour change after eight to twenty four hours.

Results and Discussion

Growth pattern of isolates on designed media

Growth pattern of *Mesorhizobium* and *Bacillus* isolates in designed media is placed in (Table 1). The perusal of the data reveals that increasing the level of rice boil water resulted on increase the visual growth of both the targeted *Mesorhizobium* and *Bacillus spp*. It was observed that maximum growth was recorded in the media having 78% of rice boil water. These our results revealed that the 78% of rice boil water was found to be optimum for maximum growth. Similar results were obtained by (Dhingra and Chaudhary, 2009) [6]. However, due to viscosity of media optical density of targeted microbes were not able to be measured and growth pattern was recorded visually. To rectify the problem of viscosity, media were filtered through cheese cloth prior to sterilization for further experimentation.

Growth pattern of isolates on media I, Media II, Media III

Growth pattern of *Mesorhizobium* and *Bacillus* isolates in designed media is placed in (Table 2). *Mesorhizobium* and *Bacillus* isolates were cultured on media I, II and III and multiplication of the *Mesorhizobium* and *Bacillus* spp. was markedly faster, as indicated by higher values of OD_{620}, than in YEM and NYSM. The use of cheap carbon sources and nutritional supplements as substrate is required to reduce the production cost of the biofertilizers. Newly designed media supports better growth of isolates than YEM and NYSM for *Mesorhizobium* and *Bacillus* isolates respectively. The growth was increasing with time of incubation and at the endpoint of 72 hours the growth was highest. Almost a similar study was done by (Mimb *et al*., 2014; Singh *et al*., 2011) [19, 27].

The perusal of data reveals that, the increasing level of rice boil water also resulted in increasing the growth pattern of both the targeted *Mesorhizobium* and *Bacillus* spp. Here also, the highest growth was recorded in the media having 78% of rice boil water. The highest optical density was recorded in the treatment 4 which having 58% rice boil water. However, the optical density of targeted microbes was not able to be measured in treatment 5 due to viscosity which contains 78% rice boil water in media.

Colony Forming Unit

Data of colony forming unit of isolates in Media I, Media II and Media III at 10^{-6} dilution factor is placed in (Table 3). Due to viscosity of treatment 5 which having 78% rice boil water of Media I, Media II and Media III, create problem in measuring optical density so, the another parameter of growth was followed for evaluating the growth pattern in all the treatment of Media I, Media II and Media III.

The perusal of data reveals that in Media I, II and III (in which carbon source is glucose) increasing the level of rice boil water resulted in increasing in the c.f.u value of the targeted isolates. Highest growth in terms of c.f.u was recorded in the treatment having 78% rice boil water, 10% vegetable wash, 10% pulse wash and 2% sugar. The results of Gauri *et al*. (2011) [9] showed that newly designed media supports better growth of *Mesorhizobium* and *Bacillus* spp. than YEM and NYSM respectively. The trend was similar to the growth pattern observed through optical density except that quantitative data on the growth was also generated through viable cell count and growth was maximum in treatment five (T5).

Morphological Characterization

The general microscopic characteristics of the selected isolates showed rod shaped and gram negative in nature which were also earlier reported by Gauri *et al*. (1982); Kloepper *et al*. (1992) [17], and Gilbert and Jack (1993) [10]. Colony morphology of *Mesorhizobium* and *Bacillus* isolates were also examined in different media. Both isolates were characterized according to morphological characteristics such as the appearance, pigmentation, shape, size, margin, and elevation, of the colonies on standard Medium that is, YEM for *Mesorhizobium* and NYSM for *Bacillus* and on the redesigned media i.e, Media I, Media II and Media III. (Table 4).

In case of *Mesorhizobium* the morphology and Colony Characteristics on the standard medium was circular shape, entire margin, convex elevation, wet gummy colony appearance and white pigmentation which was same as the morphological characteristics of colony on Media I, Media II and Media III. In case of *Bacillus* sps. The Morphology and Colony Characteristics on the standard medium was round shape, entire margin, drop like elevation, wet gummy colony appearance and slightly whitish pigmentation which was same as the morphological Characteristics of colony on Media I, Media II and Media III. The selected species were identified as *Bacillus* by comparing with the Media I, Media II and Media III. Similar results were also reported by Verma *et al*. (2016), Vrema *et al*. (2018) [33].

Comparative growth and medium selection

Mesorhizobium and *Bacillus* isolates having origin from different soils were screened for the selection of suitable medium for its maximum growth. These isolates were able to utilize glucose more efficiently than normal YEM medium. Kivanc *et al*. (2006) [16] also carried out such types study and characterized *Rhizobium* species isolated from Bean. The growth pattern of isolates in newly designed media i.e., Media I, Media II and Media III was recorded highest than the control that is YEM and NYSM (Figure 1). Maximum growth was recorded in T4 (treatment 4) of media I, II, and III and Minimum growth was observed in T3 (Treatment 3) of Media

I, II and III. Accordingly T4 (treatment 4) of media I, media II and media III were selected for further study. Among all these three designed media, the media having glucose as carbon source that is Media II gives the maximum biomass yield of the targeted isolates *Mesorhizobium and Bacillus* spp. Similar work has been done by Singh *et al*. (1992) using other substrate for rhizobial growth.

IAA production

Mesorhizobium and *Bacillus* sp. were characterized for their auxin biosynthesis in the absence of precursor. In laboratory study, all the isolates produced auxin (expressed as IAA equivalents) but with variable degree. Microbial production of auxins and their role in plant growth promotion have been reported and reviewed by many researchers (Sarwar *et al*., 1992; Sarwar and Kremer, 1995; Zahir *et al*., 2004) [24, 23, 37].

Screening of isolates for IAA (Indole acetic acid) production has been presented in (Figure 2). Role of *Mesorhizobium* and *Bacillus* as plant growth promoter has been documented (De-Bhasan and Bashan, 2004) [5]. Such augmentation in plant growth is due to production of Indole Acetic Acid hence use of *Mesorhizobium* and *Bacillus* as a PGPR for various crops has been advocated (Bashan and Holguin, 1997) [2]. Thus all *Mesorhizobium* and *Bacillus* isolates were also screened for indole acidic acid. Highest Indole Acetic Acid was recorded in *Bacillus* isolate of Diara soil. The minimum value was observed for *Mesorhizobium* having value 0.967 µg/mL. One of the interesting features was that *Bacillus* isolates exhibit IAA production as indicated by the intensity of pink colour although the amount varied a depending on origin of the strains and the nature of strains.

Numerical strength of microorganism plays an important role in sustaining the soil health and plant growth. Bihar soil is rich in *Mesorhizobium* and *Bacillus* spp. populations. The role of *Mesorhizobium sps.* as a PGPR was also evaluated and isolates exhibit IAA production with values ranging from 1.0 to 2.0µg/ml. This suggests that *Mesorhizobium* was poor in IAA production. The role of *Bacillus* spp. as a PGPR was also evaluated and isolates exhibit IAA production with values ranging from 20.0 to 25.0 µg/ml. This suggests that *Bacillus* spp. was excellent in IAA production.

Biochemical Characterization and Carbohydrate utilization

Carbohydrate utilization pattern of *Mesorhizobium* spp. and *Bacillus* spp. was placed in table 5. Sucrose, maltose, citrate, dextrose, galactose, carboxylic acids like Sodium gluconate and citrate were universally utilized by *Mesorhizobium* and *Bacillus* spp. While *Mesorhizobium* and *Bacillus* strains were not able to utilize malonate. Polymer like Inulin was not utilized by *Mesorhizobium* strain as a carbon and energy source while *Bacillus* strain was able to utilize Inulin. This may also provide advantage to targeted strain for survival competence in the Gram nodule and maize rhizosphere. The enzymes activity like Esculin hydrolysis was positive for both strain while ONGP test was only positive for *Mesorhizobium* strain. *Mesorhizobium* and *Bacillus* strains were able to utilize Salicin and Glycerol as carbon source. Carbon source Sorbitol, Mannitol, malonate, ONPG were specific to *Mesorhizobium* sps. Whereas Carbon Adonitol, erythritol, xylitol, sorbose, inulin, dulcitol were specific to Bacillus spp. Similar biochemical characterization were also demonstrated by various researchers (Tripathi *et al*., 2016; Kaur *et al*., 2006; Geeta *et al*., 2012) [31, 15, 11].

Table 1: Visual observation of *Mesorhizobium* spp. and *Bacillus* spp.

Treatment	Visual observation of *Mesorhizobium* sps.	Visual observation of *Bacillus* sps.
T1- 18% rice boil water, 10% pulse wash, 10% vegetable wash, 60% water, 2% glucose	+	++
T2-38% rice boil water, 10% pulse wash, 10% vegetable wash, 40% water, 2% glucose	+++	+++
T3-38% rice boil water, 10% pulse wash, 10% vegetable wash, 40% water, 2% glucose	++++	++++
T4-78% rice boil water, 10% pulse wash, 10% vegetable wash, 2%glucose;	+++++	+++++

Table 2: Physiological characterization of *Mesorhizobium* spp. and *Bacillus* spp. in Media I, Media II and Media III in terms of OD at 620 nm.

Treatments	T1	T2			T3			T4			T5		
Media	YEM	Media I	Media II	Media III	Media I	Media II	Media III	Media I	Media II	Media III	Media I	Media II	Media III
A. *Mesorhizobium* sps.													
24 hr	0.412	0.312	0.261	0.261	0.936	0.439	0.439	1.011	1.003	0.625	NR		
48 hr	0.705	0.602	0.336	0.336	1.512	0.689	0.689	1.121	1.023	0.915	NR		
72 hr	1.326	1.001	0.911	0.911	1.620	1.389	1.389	1.326	1.621	1.521	NR		
B. *Bacillus spp.*													
24 hr	0.301	0.236	0.294	0.294	0.384	0.356	0.356	0.440	0.440	1.012	NR		
48 hr	0.612	0.381	0.617	0.617	1.134	0.766	0.766	1.188	1.138	1.461	NR		
72 hr	1.012	0.513	0.919	0.919	1.675	1.216	1.216	1.701	1.088	1.701	NR		

T1-YEM for *Mesorhizobium* and NYSM for *Bacillus*
T2-18% rice boil water, 10% pulse wash, 10% vegetable wash, 60% water, 2% glucose
T3-38% rice boil water, 10% pulse wash, 10% vegetable wash, 40% water, 2% glucose
T4-58% rice boil water, 10% pulse wash, 10% vegetable wash, 20% water, 2% glucose
T5-78% rice boil water, 10% pulse wash, 10% vegetable wash, 2% glucose
@ NR- Not recorded due to viscosity problem

Table 3: Population of *Mesorhizobium* spp. and *Bacillus* spp. on media I, media II and media III in terms of Colony forming units (C.F.U.).

Treatments	T_1	T_2			T_3			T_4			T_5		
Time interval	YEM	Media I	Media II	Media III	Media I	Media II	Media III	Media I	Media II	Media III	Media I	Media II	Media III
	(No. of C.F.U.)												
	A. *Mesorhizobium* spp.												
24 hr	25 x10^6	30 x10^6	35 x10^6	30 x10^6	50 x10^6	50 x10^6	40 x10^6	60 x10^6	60 x10^6	40 x10^6	60 x10^6	75 x10^6	45 x10^6
48 hr	70 x10^6	75 x10^6	90 x10^6	50 x10^6	100 x10^6	120 x10^6	75 x10^6	130 x10^6	135 x10^6	70 x10^6	140 x10^6	145 x10^6	70 x10^6
72 hr	170 x10^6	160 x10^6	135 x10^6	110 x10^6	180 x10^6	150 x10^6	130 x10^6	180 x10^6	170 x10^6	140 x10^6	180 x10^6	180 x10^6	145 x10^6
	B. *Bacillus* spp.												
24 hr	20 x10^6	20 x10^6	25 x10^6	25 x10^6	25 x10^6	30 x10^6	35 x10^6	30 x10^6	30 x10^6	45 x10^6	35 x10^6	40 x10^6	50 x10^6
48 hr	40 x10^6	40 x10^6	45 x10^6	35 x10^6	45 x10^6	50 x10^6	50 x10^6	50 x10^6	55 x10^6	55 x10^6	55 x10^6	60 x10^6	60 x10^6
72 hr	70 x10^6	75 x10^6	80 x10^6	75 x10^6	80 x10^6	85 x10^6	75 x10^6	85 x10^6	80 x10^6	80 x10^6	90 x10^6	90 x10^6	85 x10^6

Table 4: Morphology and Colony Characteristics of *Mesorhizobium* spp. and *Bacillus* spp.

***Mesorhizobium* spp.**						***Bacillus* spp.**					
Medium	**Shape**	**Margin**	**Elevation**	**Colony appearance**	**Pigmentation**	**Medium**	**Shape**	**Margin**	**Elevation**	**Colony appearance**	**Pigmentation**
YEM	Circular	Entire	Convex	Wet gummy	White	NYSM	Round	Entire	Drop like	Wet gummy	Slightly whitish
MEDIA I	Circular	Entire	Convex	Wet gummy	White	MEDIA I	Round	Entire	Drop like	Wet gummy	Slightly whitish
MEDIAII	Circular	Entire	Convex	Wet gummy	White	MEDIA II	Round	Entire	Drop like	Wet gummy	Slightly whitish
MEDIAIII	Circular	Entire	Convex	Wet gummy	White	MEDIA III	Round	Entire	Drop like	Wet gummy	Slightly whitish

Table 5: Carbon profiling of *Mesorhizobium* spp. and *Bacillus* spp.

S.N.	Carbon source	***Mesorhizobium* spp.**				***Bacillus* spp.**			
		YEM	Media I	Media II	Media III	NYSM	Media I	Media II	Media III
1	Lactose	-	+	+	+	+	-	+	+
2	Xylose	+	+	+	+	+	-	+	+
3	Maltose	+	+	+	+	+	+	+	+
4	Fructose	+	-	+	+	+	+	+	+
5	Dextrose	+	+	+	+	+	+	+	+
6	Galactose	+	+	+	+	+	+	+	+
7	Raffinose	+	-	+	+	+	+	+	+
8	Trehalose	+	-	+	+	+	+	+	+
9	Melibiose	+	-	+	+	+	+	+	+
10	Sucrose	+	+	+	+	+	+	+	+
11	L-Arabinose	+	+	+	+	+	-	+	+
12	Mannose	+	+	+	+	+	-	+	-
13	Inulin	-	-	-	-	+	-	-	-
14	Sodium Gluconate	-	-	-	-	-	-	-	+
15	Glycerol	-	+	-	-	-	+	-	-
16	Salicin	+	-	+	-	+	+	+	+
17	Dulcitol	-	-	-	-	+	-	-	+
18	Innositol	-	-	-	-	-	+	-	+
19	Sorbitol	-	-	-	-	-	+	-	+
20	Mannitol	-	-	-	+	-	+	-	+
21	Adonitol	-	-	-	-	-	+	-	+
22	Arabitol	-	-	-	-	-	-	-	+
23	Erythritol	-	-	-	-	-	-	+	-
24	a-methyl-D- glucoside	-	-	-	+	-	+	-	-
25	Rhamonose	-	-	-	+	+	-	-	-
26	Cellobiose		-	+	+	+	+	+	+
27	Melezitose		-	-	+		-	+	+
28	a-Methyl-D Mannoside		-	-	-	+	-	+	+
29	Xylitol		-	-	-	-	+	+	-
30	ONPG		+	+	-	-	-	-	-
31	Esculin hydrolysis		-	-	+	+	+	+	+
32	D-Arabinose		+	+	+	-	-	+	+
33	Citrate utilization		+	+	+	+	+	+	-
34	Malonate utilization		-	+	+	-	-	-	-
35	Sorbose		-	-	-	-	-	+	-

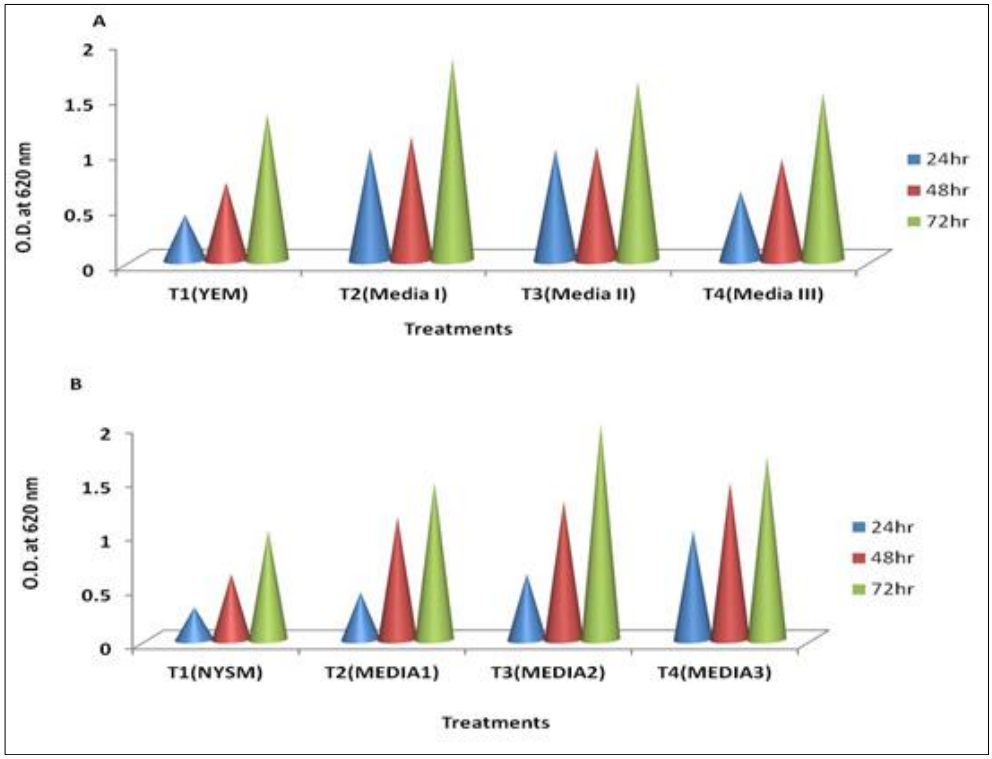

Fig 1: Comparative growth of *Mesorhizobium* spp. and *Bacillus* spp. in different media

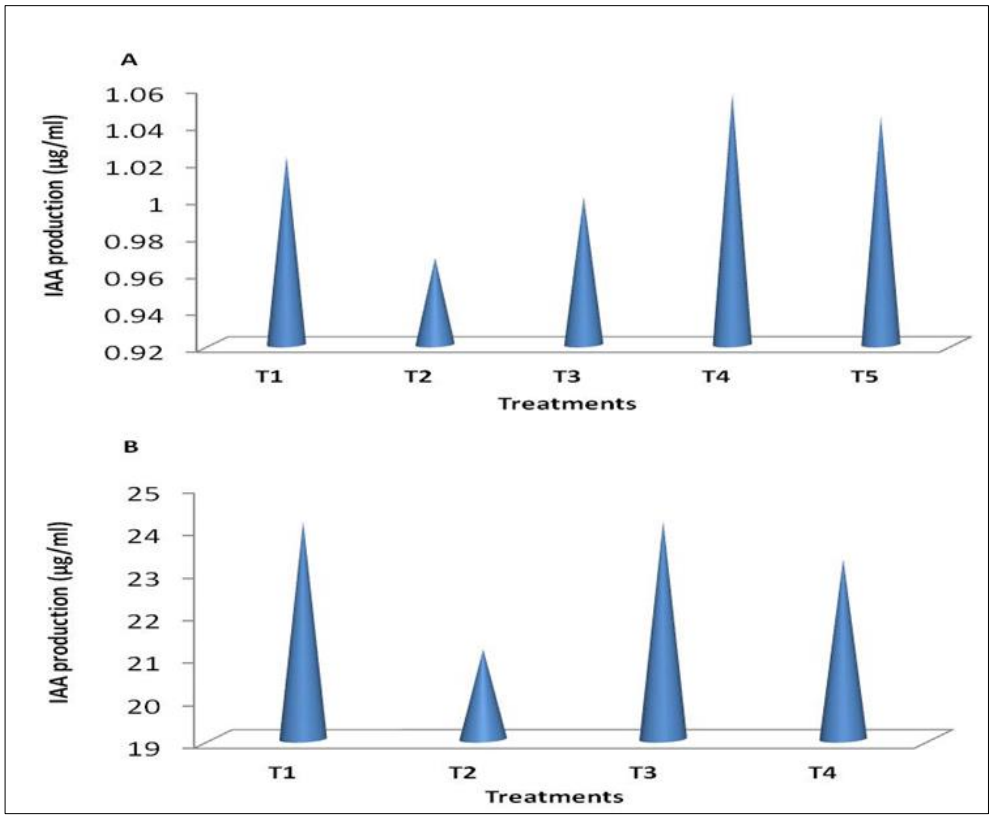

Fig 2: IAA production by *Mesorhizobium* spp. and *Bacillus* spp. in different media.

Conclusion

New inexpensive media based on household waste was designed for mass production of *Mesorhizobium* for cicerbiofertilization and *Bacillus* for multifunctional purposes for cereals and pulses. The growth of targeted microbes in the designed media was much higher than the growth in the media (YEM for *Mesorhizobium* and NYSM for *Bacillus*) used for mass cultivation of targeted agromicrobes. Designing of such media will facilitate the farming community for on site production of the biofertilizer as and when required after procuring mother culture.

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