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SELECTIVE PLANT-BASED MEDIA CULTURING SOIL ASSOCIATED BACTERIA AND ITS COMPARATIVE ASSESSMENT

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ABSTRACT

Introduction: The food waste pollution increasing across the world. The waste needs to be managed as a raw material for making beneficial products. The most promising is to make use of its available nutrient to grow variety of microbial species which are useful for plant growth. **Method**: In the present study five plants namely onion, carrot, garlic, mango, and orange peel generated as waste was mixed with different compositions of NaCl, yeast extract and soya chunks to make bacteriological plant only based media. The growth count of soil associated bacteria on these media were tested and ascertain for replacement of commercial media mainly obtained from animal origin once compared with mainly standard Azotobacter media.

Result: - Once the 25 compositions of media made via 5 plants and additives, it has been put forward that colonies on onion, garlic and mango waste based are very well grown on media plates and equivalent to the colonies grown on azotobacter media indicated that if we can use these plant waste as media it will assist soil associated bacteria's overall growth and two plant media made by carrot and orange peel found to be low in soil associated bacterial growth. **Conclusion**: Study showcased that soil associated bacteria grows very well on selected plant-based media and thus waste can be used to supplemented the growth of rhizospheric bacteria and overall plant growth can be ensured if such cultured and uncultured grows together.

Key words – Plant only based media, soil bacteria, uncultured bacteria, bacterial growth

1. Introduction

In the development of human, defined use of plant as nutrient source plays an important along with animal source. It is found that edible vegetables produced in agriculture many times get wasted once reaches to market and even in field that generates pollution. In the recent time the

waste of vegetables has increased due to various regions and if not managed effectively it will contribute to environment pollution (Eriksson and Spangberg, 2017 ; Scherhaufer et al., 2018). As per the reports globally one third of food produced for human consumption gets lost or becomes waste (UNEP food waste index report 2021, 2021).

In response various attempts been made to reduce the food waste by influencing consumer behaviors, using proper storage, sustainable coating and by innovative working technique (Roe et al., 2020). In one such approach use of once generated plant waste in cultivation of agriculturally important bacteria which are beneficial to the crop growth once recommended as a nutrient source. Here many workers put forward the concept of plant only based media able to support the growth of soil and rhizosphere bacteria. As we know these media derived from organic sources such as agricultural products, unutilized raw food material and even via food waste. They informed how the nutrient content utilized by bacterial population for growth with better detail.

This plant only based media also been recommended to culture the bacterial microbiota of soil and rhizosphere which was previously uncultured on commercial media and that gives us the chance to obtain essential plant growth promoting support and by which next dimension to grow bacteria has been generated (Sarhan[a] et al., 2019). As recently been put forward that many plant associated bacterial phyla such as *Plantibacter* spp., *Curobacterium* spp., *Agreia* spp., *Rhodococcus* spp. and *Nocardiodes* spp successfully grown which are unculturable on commercial media made by animal based nutrient sources (Sarhan[b] et al., 2019). To record the diversity of grown bacterial population on plant only based media and commercial media use of metagenome analysis and PCR- PAGE analysis has been put forward which showcase exactly which phyla, genus and even species grown substantially or suppress with exact count once reported with Barley endophytic rhizobacteria but applicable in general also (Kremer et al.,2021 ; Mathews et al., 2019).

In a success story use of “inoculum dependent culturing strategy″ has been put forward. They reported the success of plant serial dilutions on to plain water agar plates able to grow specific plant associated bacterial which can then grow profoundly on plant only based media which

gives opportunity to cultivate many novel species in addition to commonly grown species (Duangpaeng et al., 2012 ; Kaewkla and Franco, 2012).

In the present study attempt has been made to cultivate the soil associated bacteria especially from agriculture on various plant-based media once made in with addition of yeast extract, NaCl and soya chunks (Nitrogen source) and compared with Azotobacter media as control to record which plant only based media able to grow equivalent colonies once compared to Azotobacter media.

2. Methodology

2.1 Collection of plant waste

In the study total five plants considered such onion, carrot, garlic, mango, and orange for their peel. These plant parts were collected and dried in hot air oven set at 50°C for 24 hours and then ground to powder using grinder. The powder then transferred in polypropylene bag for easy boiling in media so that it will give only water-soluble content in media and smooth formation of media can be ensured.

2.2 Preparation of media

In the present study onion, carrot, garlic, mango, and orange-based media used to cultivate soil associated bacteria once added as 10 g /litre and in combination with yeast extract, NaCl and soya chunks as given in Table 1 once added with gelling agent agar-agar powder (35 g/liter). The content then transferred in distilled water and boiled for 10 minutes. Upon boiling the media was autoclaved at 121 degree for 15 minutes at 15 lbs pressure. The sterile and cooled media plates were used for pour plate-based inoculation along with Azotobacter media as control (Hi Media).

Table no 1: Preparation of five plant only based formulation for each plant once added with NaCl, Yeast extract, and Soya chunk.

	Yeast Extract	**NaCl**	**Soya chunk**	**Agar**	**Plant powder**
A)	2	5	-	35	10

B)	2	-	-	35	10
C)	-	5	-	35	10
D)	-	5	5	35	10
E)	-	-	5	35	10
Values in g/liter in sterile distilled water					

2.3 Inoculation of soil bacteria

The tomato plant rhizosphere was serially diluted to obtain 10^8 dilution and its 10 µl of water-based dilution loaded on each plant media prepared along with azotobacter media control in triplicates. The plates then incubated at 37 degree for 48 hours to receive well grown colonies having not more than 300 colonies per plates to calculated the growth potential.

2.4 Statistical analysis

In the present study control media set as Azotobacter media (Hi media) which was prominent to grow azotobacter and soil associated bacteria was taken as standard for colony count. The colonies appeared on plant only based media recorded in triplicate and used to compare with Azotobacter media using one way ANOVA and further compared with all compositions also using graph pad prism 5 setting P value < 0.05 for significance changes.

3 Result

3.1 Preparation of plant-based media

In the present study involved five plants namely as onion, carrot, garlic, mango, and orange for its peel-based media as shown in Fig 1-5 showing raw part (peel), powder form its filtrate and final composition of liquid before autoclaving.

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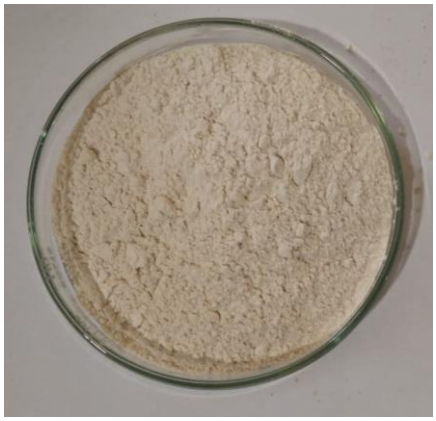
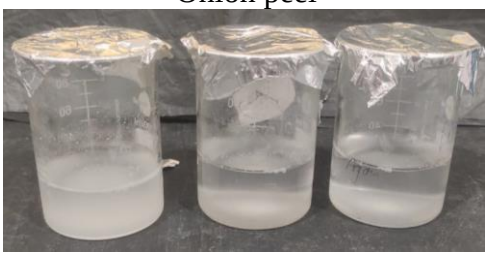
Onion peel



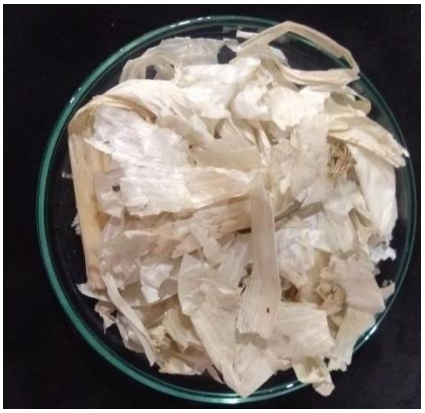
Onion peel powder



Onion peel filtrate

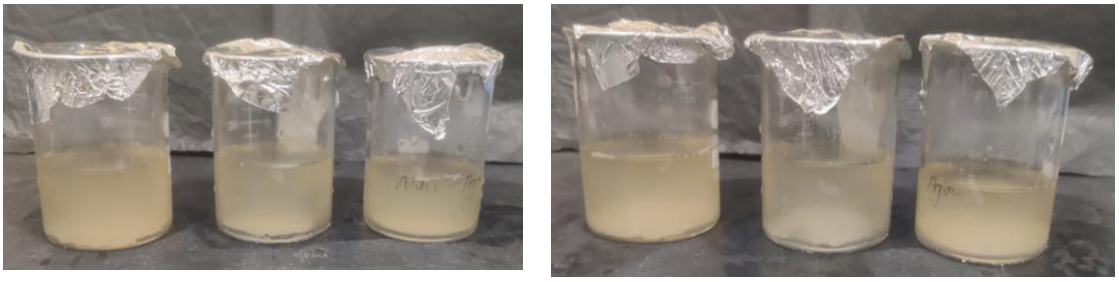
Onion peel media

Fig. 1: Onion peel-based media preparation



Garlic peel

Garlic peel powder

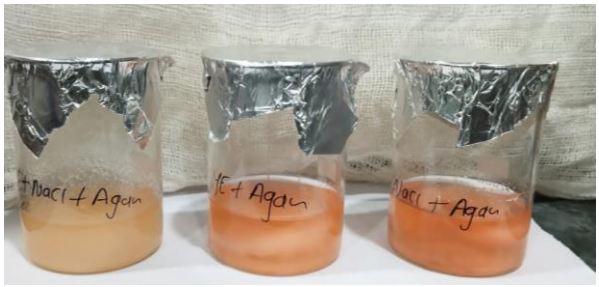


Garlic peel filtrate Garlic peel media

Fig. 2: Garlic peel-based media preparation

Carrot peel

Carrot peel powder

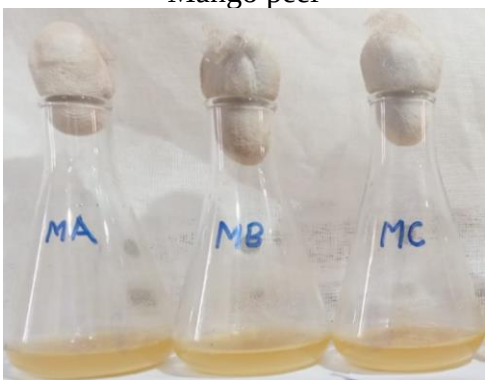


Carrot peel filtrate Carrot peel media

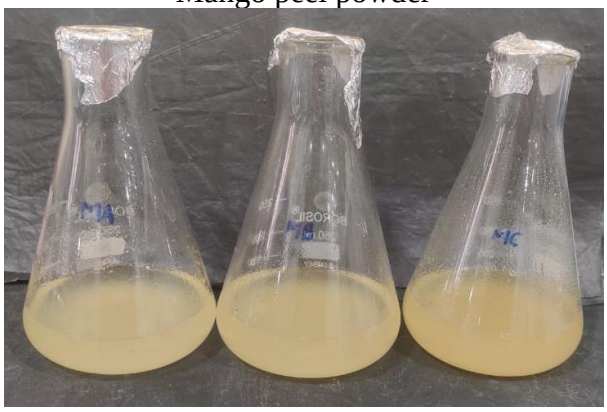
Fig. 3: Carrot peel-based media preparation

Mango peel

Mango peel powder



Mango peel filtrate

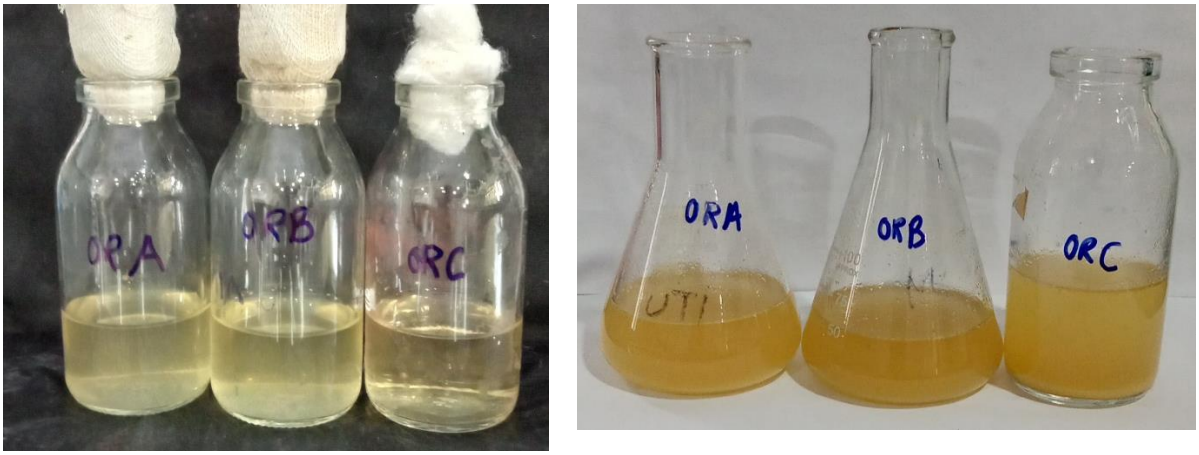


Mango peel media

Fig. 4: Mango peel-based media preparation

Orange peel

Orange peel powder



Orange peel filtrate Orange peel media

Fig. 5: Orange peel-based media preparation

3.2 Colony forming units of soil associated bacteria on plant-based media and Azotobacter media

In the present study total 25 compositions with total five plants peel parts used for culturing ability towards plant growth supporting bacteria. Here the plants media were analyzed for colony forming unit as per formulations and statistically compared to Azotobacter media as given below.

3.2.1 Onion media

In the study with a control set at 10^{-8} dilution-based inoculation of soil sample as Azotobacter media, it has been put forward that the prominent colony count recorded as 285 ± 18 CFU/ plate which was set as standard that every plant media should achieve to record the equivalence in growth potential.

The colonies grown on control (Azotobacter media) showcased in Fig 6. In plant media once we have checked CFU/plate with onion media having five compositions, it has been put forward that only onion media with yeast extract plus NaCl composition able to grow equivalent colonies (300 ± 10) as compared to control with no significance change ($P > 0.05$) indicated its success and other found to be significantly ($P < 0.05$) lower colony count as shown in Table 2 and Fig 7.

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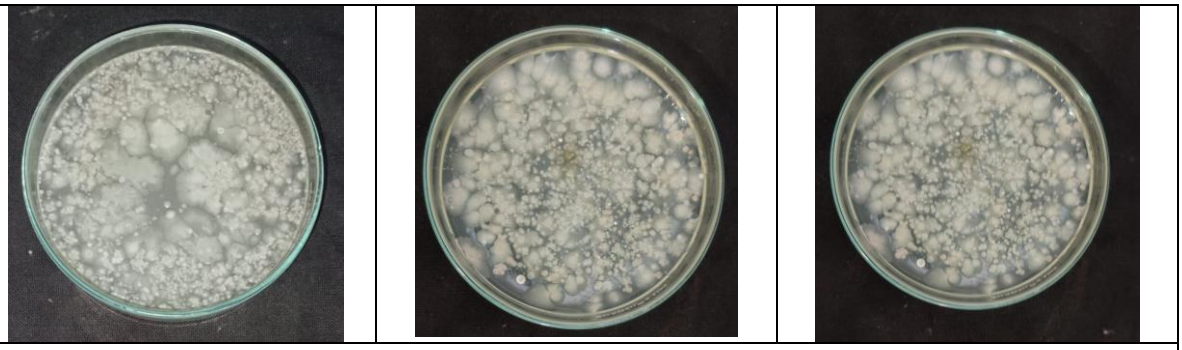


Fig. 6: Azotobacter media showing profound colonies growing on the media in triplicate

Table 2: Colony count per plate recorded for the onion media made with five compositions and along with control with Azotobacter media count

	Onion					
	Azotobacter control	**Yeast Extract + Nacl+Onion**	**Yeast Extract+Onion**	**Nacl+Onion**	**Soya chunk + Nacl+Onion**	**Soya chunk+Onion**
Number of values	3	3	3	3	3	3
Minimum	265	290	150	14	40	70
25% Percentile	265	290	150	14	40	70
Median	289	300	155	19	44	80
75% Percentile	300	310	160	25	55	82
Maximum	300	310	160	25	55	82
Mean	**285±18**	**300±10**	**155±5.0**	**19±5.5**	**46±7.8**	**77±6.4**
Stats	**a**	**a**	**b**	**cf**	**df**	**e**
Std. Error	10	5.8	2.9	3.2	4.5	3.7

Values sharing the same alphabet in stats section for row values indicated statistically non-significant in difference and vice versa

Table 3: Colony count per plate recorded for the carrot media made with five compositions and along with control with Azotobacter media count

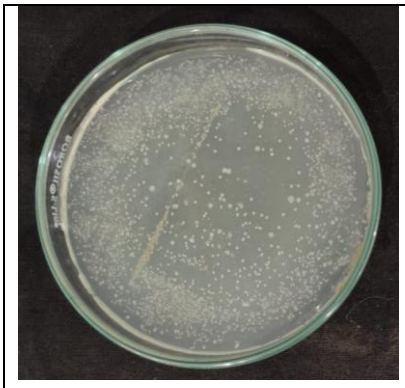
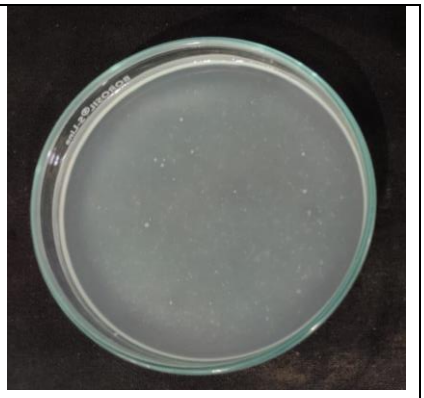
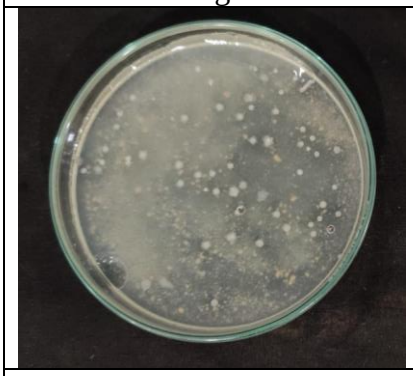
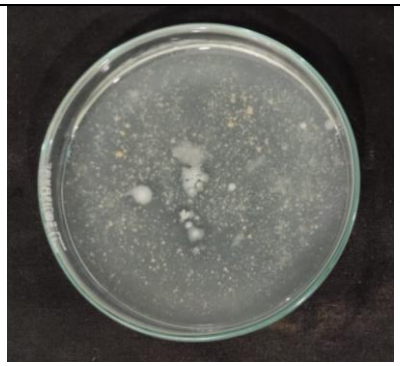
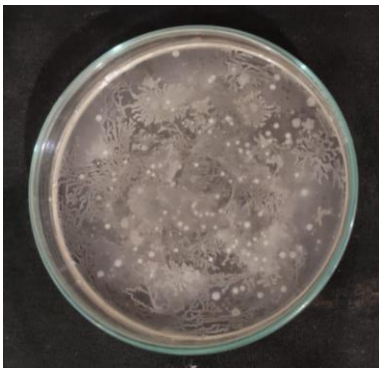
		
Yeast Extract + Nacl + Agar – Agar	Yeast Extract + Agar – Agar	Nacl + Agar – Agar
		
Soya chunk + NaCl + Agar – Agar	Soya chunk + Agar – Agar	

Fig. 7: The soil bacteria especially growing on onion media with different compositions at variable rate

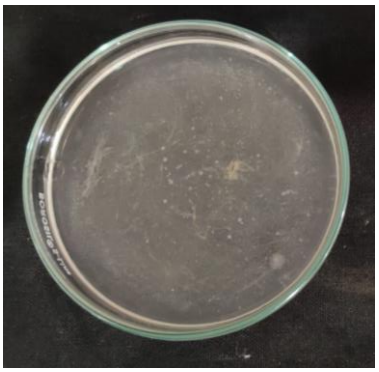
Carrot						
	Azotobacter control	**Yeast Extract + Nacl +Carrot**	**Yeast Extract+Carrot**	**Nacl +Carrot**	**Soya chunk + Nacl +Carrot**	**Soya chunk +Carrot**
Number of values	3	3	3	3	3	3
Minimum	265	60	5.0	190	115	200
25% Percentile	265	60	5.0	190	115	200
Median	289	65	10	200	120	220
75% Percentile	300	70	20	210	127	231
Maximum	300	70	20	210	127	231
Mean	**285± 18**	**65±5.0**	**12±07.6**	**200±10**	**121±6.0**	**217±16**
Stats	**a**	**b**	**c**	**dg**	**e**	**fg**
Std. Error	10	2.9	4.4	5.8	3.5	9.1

3.2.2 Carrot media

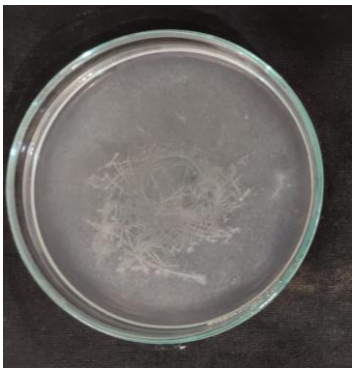
In the carrot media once we have grown soil associated bacteria, it has been observed that carrot media with all five-composition failed to give profound growth supporting nutrient which is not equivalent to commercial media and remained statistically significant ($P < 0.05$) in colony count with low colony count as shown in Table 3 and Fig. 8.



Yeast Extract + Nacl + Agar – Agar



Yeast Extract + Agar – Agar



Nacl + Agar – Agar

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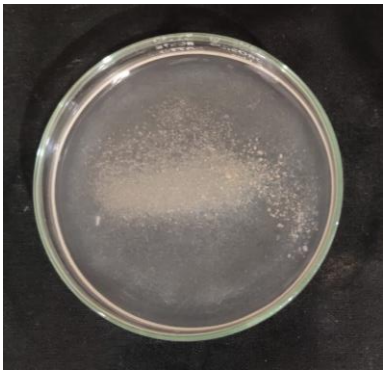


Soya chunk + Nacl + Agar – Agar

Soya chunk + Agar – Agar

Fig. 8: The soil bacteria especially growing on carrot media with different compositions at variable rate

3.2.3 Garlic media

In a garlic media it has been observed that two media compositions made by garlic powder added with NaCl plus yeast extract recorded with 309±12 CFU/ plate and another as only with yeast extract abled to give 297 ± 16 CFU /plate which is equivalent to Azotobacter agar and remain non-significant (P > 0.05) in difference indicated its better use as shown in Table 4 and Fig 9.

Table 4: Colony count per plate recorded for the garlic media made with five compositions and along with control with Azotobacter media count

	Garlic					
	Azotobacter control	**Yeast Extract + Nacl+ Garlic**	**Yeast Extract+ Garlic**	**Nacl+ Garlic**	**Soya chunk + Nacl+ Garlic**	**Soya chunk+ Garlic**
Number of values	3	3	3	3	3	3
Minimum	265	297	280	15	13	40
25% Percentile	265	297	280	15	13	40
Median	289	310	300	18	21	52
75% Percentile	300	320	311	25	30	70
Maximum	300	320	311	25	30	70
Mean	**285 ±18**	**309± 12**	**297± 16**	**19± 5.1**	**21± 8.5**	**54± 15**
Stats	**a**	**ab**	**abc**	**d**	**de**	**def**
Std. Error	10	6.7	9.1	3.0	4.9	8.7

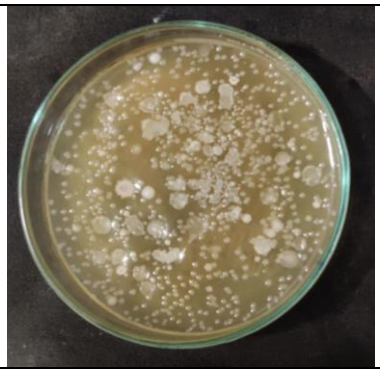
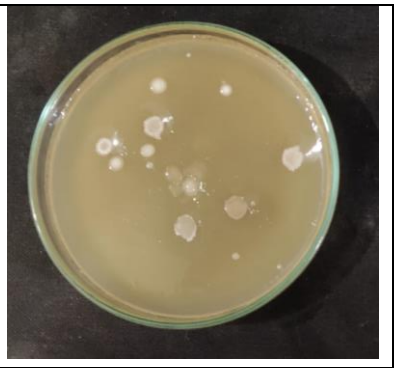
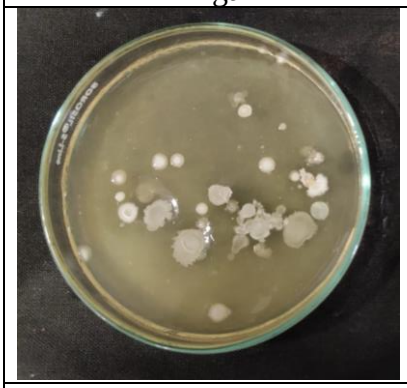
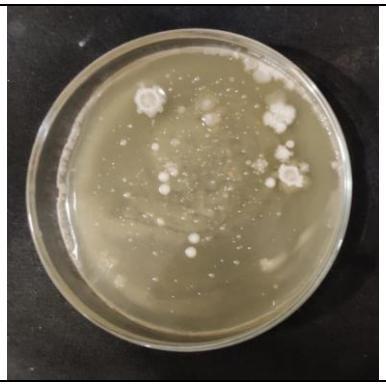
		
Yeast Extract + Nacl + Agar – Agar	Yeast Extract + Agar – Agar	Nacl + Agar – Agar
		
Soya chunk + Nacl + Agar – Agar	Soya chunk + Agar – Agar	

Fig. 9: The soil bacteria especially growing on garlic media with different compositions at variable rate

3.2.4 Mango media

In a mango media soil associated bacteria grown profoundly will with two compositions as mango powder with yeast extract (282 ± 8.2) and another as mango powder with soya chunks plus NaCl (273 ± 7.5 CFU /plate) which is equivalent to Azotobacter media (285 ± 18 CFU / plate) indicated its success as shown in Table 5 and Fig 10.

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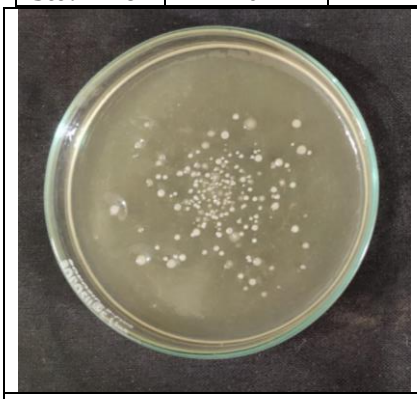
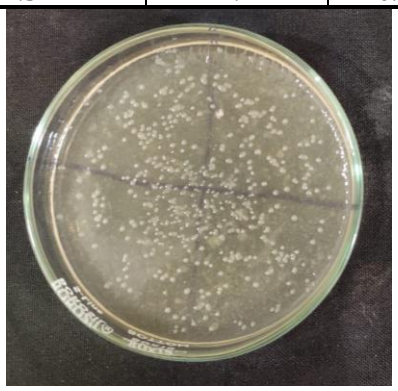
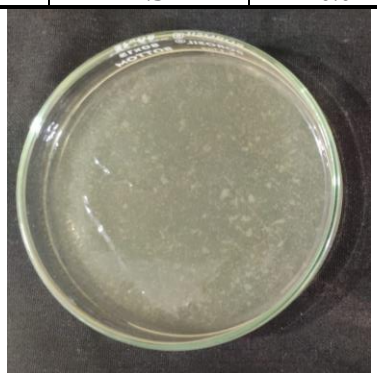
Table 5: Colony count per plate recorded for the mango media made with five compositions and along with control with Azotobacter media count						
	Mango					
	Azotobacter control	**Yeast Extract + Nacl + Mango**	**Yeast Extract+ Mango**	**Nacl+ Mango**	**Soya chunk + Nacl+ Mango**	**Soya chunk+ Mango**
Number of values	3	3	3	3	3	3
Minimum	265	70	275	0.0	265	0.0
25% Percentile	265	70	275	0.0	265	0.0
Median	289	80	280	0.0	273	0.0
75% Percentile	300	95	291	0.0	280	0.0
Maximum	300	95	291	0.0	280	0.0
Mean	**285 ± 18**	**82± 13**	**282± 8.2**	**0.0 ±0.0**	**273 ± 7.5**	**0.0 ±0.0**
Stats	**a**	**b**	**ac**	**d**	**ace**	**df**
Std. Error	10	7.3	4.7	0.0	4.3	0.0



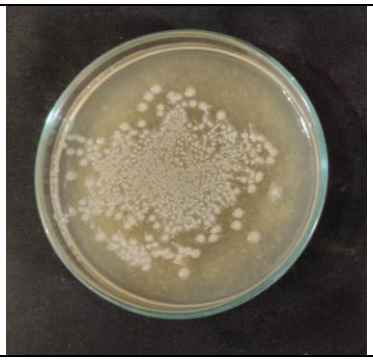
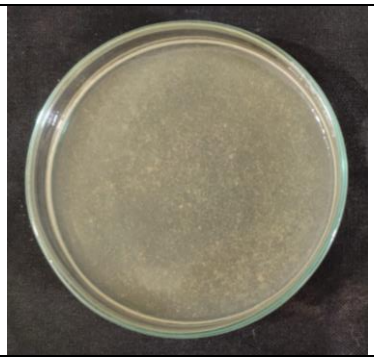
Yeast Extract + NaCl + Agar – Agar



Yeast Extract + Agar – Agar



NaCl + Agar – Agar

		
Soya chunk + NaCl + Agar – Agar	Soya chunk + Agar – Agar	
Fig. 10: The soil bacteria especially growing on mango media with different compositions at variable rate		

3.2.5 Orange media

In the study orange media once cheeked with all five compositions, it has been observed that orange media is not supporting the growth of soil associated bacteria and very low or even no colonies were recorded and thus found to be statistically significance as compared to Azotobacter media ($P < 0.05$) and remained low in count and thus found to low growth promoting as sown in Table 6 and Fig 11.

Hence overall it has been observed that onion media, garlic media and mango media found to be growth stimulatory and equivalent in capacity to promote soil associated bacteria once compared with Azotobacter media.

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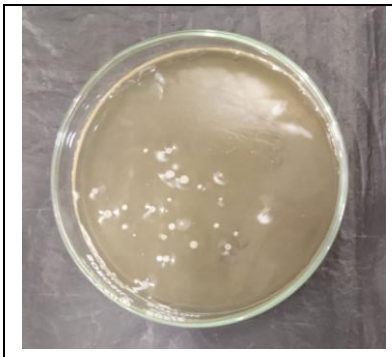
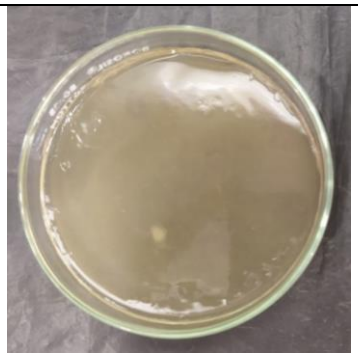
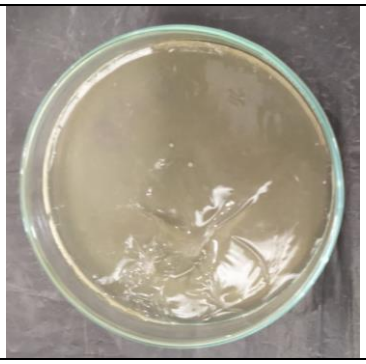
Table 6: Colony count per plate recorded for the orange media made with five compositions and along with control with Azotobacter media count						
	Azotobacter control	**Yeast Extract + Nacl+Orange**	**Yeast Extract+Orange**	**Nacl+Orange**	**Soya chunk + Nacl+Orange**	**Soya chunk+Orange**
Number of values	3	3	3	3	3	3
Minimum	265	20	2.0	1.0	0.0	0.0
25% Percentile	265	20	2.0	1.0	0.0	0.0
Median	289	26	4.0	2.0	0.0	0.0
75% Percentile	300	35	8.0	6.0	0.0	0.0
Maximum	300	35	8.0	6.0	0.0	0.0
Mean	**285 ± 18**	**27 ± 7.5**	**4.7 ± 3.1**	**3.0 ± 2.6**	**0.0 ± 0.0**	**0.0 ± 0.0**
Stats	**a**	**b**	**bcgf**	**bcd**	**edg**	**f**
Std. Error	10	4.4	1.8	1.5	0.0	0.0



Yeast Extract + NaCl + Agar – Agar



Yeast Extract + Agar – Agar



NaCl + Agar – Agar

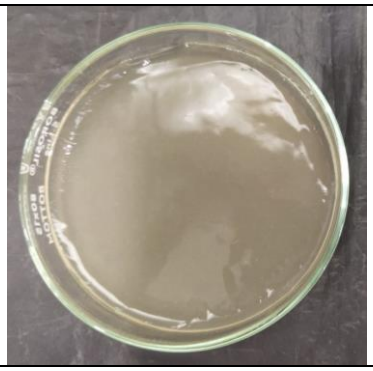
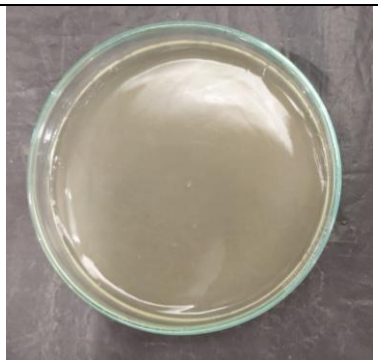
		
Soya chunk + NaCl + Agar – Agar	Soya chunk + Agar – Agar	

Fig. 11: The soil bacteria especially growing on orange media with different compositions at variable rate

Discussion

In the study we have showcased that plant waste could be utilized to grow plant associated bacteria especially those growing in rhizosphere. In a similar study Mourad et al. (2018) reported that plant only based media is useful for rhizobacteria and those can assist in growth of proteobacteria and firmicutes. The possibility of growing environment microbiome reported to be possible with plant media especially once made with soil substrate membrane system that remains culturable with proteobacteria and TM7 especially *Leifsonia xyli* sp. (Ferrari et al., 2005). In the present study we successfully reported that use of NaCl, yeast extract and soya chunks is useful to promote plant-based media to culture azotobacter and soil associated bacteria. In a similar pattern, Cavalcante and Dobereiner (1988) utilized sugar cane juice as nutrient source to supplement the growth of acid tolerant nitrogen fixing bacteria and to isolate novel species like *Saccharobacter nitrocapta*.

In the present study we have reported the use of tea bags for better media preparation which was previously mentioned by Hegazi et al., (2017) where they used it to made media from lucrene powder and that used to cultivate uncultured *Lysobacter sp*. successfully. Lastly as stated by Da Rocha et al., (2010) better rhizosphere isolates dominated by *Acidobacteria* and *Verramicrobia* which was found to be made culturable with potato root exudates supplemented agar medium which recovers 33% more bacterial population than classical agar.

4. Conclusion

In the current scenario pollution is the growing concern for human survival. Plant waste is one of the wastes that need to be tackle in a proper way. Here we have reported that onion, garlic, and mango waste-based media can be utilized to cultivate soil associated bacteria with equivalence once compared to commercial media. Thus, in future we can replace the synthetic or animal origin media with plant-based media to cultivate range of plant growth promoting bacteria and with low cost and for better sustainability in future.

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