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Preparation of Alternative Media Source for the Cultivation of Bacteria, Fungi and for Plant Culture Using Fermented Agro waste

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ABSTRACT

Agro waste was collected from street vegetable and fruit vendors from Karad street markets (fruit peel samples and vegetables). All the agro wastes samples were subjected to fermentation and preparation of media. After observation of the results, it was clear that both fungi - *Aspergillus sp.* and *Penicillium sp.* showed excellent growth on all the waste media prepared. The Fermented Banana waste medium and Fermented Cabbage waste medium proved to be the most effective for the growth of *E.coli*, *Micrococcus sp.* and *Bacillus sp.* The 3% concentrated fermented fruit and vegetable waste were found to give satisfactory results for the germination of groundnut seeds. Amongst all the media prepared, Fermented Banana waste, Fermented Cabbage waste and Fermented Banana + Chikoo waste were most effective and supported germination of Groundnut seeds. The Fermented Banana waste and Fermented Banana + Chikoo waste gave rise to shoot formation. It was evident that Fermented Cabbage waste media and Fermented Banana waste media gave excellent results for Bacteria and fungi and micropropagation of groundnut seeds. The present study has revealed that the fermented fruit peel waste and vegetable waste materials contain minerals and nutrients that can meet the nutritional requirements of industrially important fungi, bacteria and plants. Thus, agrowastes can be utilized as an alternative materials in the formulation of culture media for microorganisms and plants and they are less expensive.

Key words : *Alternative media, Agro wastes, Microrganisms, Plant culture*

Introduction

To cultivate and maintain microorganisms under laboratory conditions, providing suitable culture media that offers favorable conditions is very important. Ways of reusing food waste have been constantly developed and improved, as food waste reaches alarming levels today (Ramírez *et al.*, 2020). A number of researches are carried out on use of domestic waste for the production of cheap media. Higher cost of cultivation media is a matter of concern (Adesemoye *et al*., 2005). General media produced using food AND agro wastes are found to be cost effective, and can drastically reduce expenses in the laboratory in cultivation of common bacteria and fungi (Gurav *et al*., 2011). In developing countries, the high cost of culture media hampers practical microbiology classes and scientific research in institutions with insufficient financial resources (Uthayasooriyan *et al.*, 2016; Jadhav *et al.*, 2018; Cruz *et al.*, 2020). The present study focuses on use of fermented fruit peel waste and vegetable waste mate-

rials that can be utilized as alternative materials in the formulation of culture media for microorganisms and plant culture. Thus, agro waste can be useful in producing less expensive culture media.

Materials and Methods

Collection of samples: Agro waste was collected from street vegetable and fruit vendors from Karad markets. The collected waste parts were washed with distilled water and processed further. The waste contained banana and chickoo fruit peels and cabbage and cauliflower.

Preparation of media: A) for cultivation of Bacteria & Fungi (Table 1)

The washed fruit peels and vegetables were crushed separately by using electric blender. Fruit peels and vegetable wastes and kept for fermentation in airtight container. This fermented material was added with 1% of molasses with pH of 4.6 and mixed well and using indigenous microflora it was filtered and further used for media preparation. The 20 ml of filtrate was added to 100 ml of distilled water in separate flasks. The pH was adjusted 7.4 for bacteria and 5.4 for fungi. 2.5-3.0 g of agar powder was added to 100 ml of the liquid as a solidifying agent. Media were then sterilized in autoclave at pressure 15 lbs for 15 minutes, cooled and plates were poured (Gurav *et al*., 2011). Three types of known bacteria *E.coli*, *Micrococcus* sp. and *Bacillus sp* and fungi *Aspergillus* sp. and *Penicillium* sp were inoculated on control nutrient agar, Sabourauds agar, fermented fruit and vegetable waste media. Plates were then incubated as per the organism for 48h.The broths were poured in tubes. Above three types of known bacteria and fungi and were inoculated in control nutrient broth, Sabourauds broth and fermented fruit and vegetable waste media. Tubes were then incubated at 30 ^{0}C for 48h. After incubation, Optical density (OD) was measured on colorimeter at 600 nm with nutrient broth as control to judge the growth and compared with that of conventional media used.

Preparation of Fermented Agro waste media for Plant culture (Table 2)

The fermentation material was prepared as per method described for bacteria and fungi, it was filtered and added with 3% sucrose, 0.1mg/l 2,4 – D (dichlorophenoxy acetic acid) and 0.1 mg/l BAP (benzyl amino purine). The media was added with agar and sterilized at 121 ^{0}C for 15 mins. MS was used was control. The media was observed for seed germination

Results and Discussion

Growth of bacteria and fungi on the agro waste media

i) Results showing the growth of Bacteria on Fermented Agro waste media after 48 hrs of incubation are presented in Tables 3 & 4.

From the above tables, *E. coli* showed scanty growth on any of the waste media prepared.

Micrococcus sp. showed uncountable colonies on all waste mediums except on fermented Cauliflower

Table 1. Media combinations used for bacteria and fungi

Type of media	Amount (10 g each)	Amount of waste(g)
Fermented Vegetable waste media	Cabbage	30g
	Cauliflower	
	Cabbage + Cauliflower	
Fermented Fruit waste media	Banana Peel	30g
	Chikoo Peel	
	Banana + Chikoo Peel	

Table 2. Media combinations used for plant culture

Fermented Vegetable waste media	(10g each) 5% Fermented Cabbage filtrate + Sucrose + Agar + Hormone
	(10g each) 5% Fermented Cauliflower filtrate + Sucrose + Agar + Hormone
	(10g each) 5% Fermented Cabbage & Cauliflower filtrate + Sucrose + Agar + Hormone
Fermented Fruit waste media	(10g each) 5% Fermented Banana filtrate + Sucrose + Agar + Hormone
	(10g each) 5% Fermented Chikoo filtrate + Sucrose + Agar + Hormone
	(10g each) 3% Fermented Banana &Chikoo filtrate + Sucrose + Agar + Hormone

waste medium where scanty growth was obtained. It showed scanty growth on fermented Chikoo waste medium.

Bacillus sp. showed uncountable colonies on all waste mediums except on fermented Chikoo waste medium.

As evident from the above results, *E coli* showed Scanty growthon the agro waste media. *Micrococcus sp.* and *Bacillus sp.*, produced uncountable colonies on all waste media except on Fermented Cauliflower and Chikoo waste medium where scanty growth was obtained. They also showed good growth on Fermented Cabbage + Cauliflower waste media.

Results showing the change in optical density in growth on agro waste media are shown in Table 5 & 6

Results showing the growth of Fungi on Fermented Agro waste media after 48hrs of incubation are presented in Table 7 and 8:

Aspergillus sp. showed growth on all Fermented Vegetable waste media as well as Fermented Fruit waste media. *Penicillium sp.* showed growth on all Fermented Vegetable waste media as well as Fer-

Table 3. Fermented Vegetable Waste Media (Number of colonies):

Name of Test organism	Results (Number of colonies)			
	Control Media (Nutrient Agar)	Fermented Cabbage Media	Fermented Cauliflower Media	Fermented Cabbage + Cauliflower Media
E coli	Uncountable	scanty	Scanty growth	scanty
Micrococcus sp.	Uncountable	Uncountable	Scanty growth	Uncountable
Bacillus sp.	Uncountable	Uncountable	Scanty growth	Uncountable

Table 4. Fermented Fruit Peel Waste Media (Number of colonies)

Name of Test organism	Results (Number of colonies)			
	Control Media (Nutrient Agar)	Fermented Banana Media	Fermented Chikoo Media	Fermented Banana + Chikoo Media
E coli	Uncountable	Scanty growth	Scanty growth	Scanty growth
Micrococcus sp.	Uncountable	Uncountable	Scanty growth	Uncountable
Bacillus sp.	Uncountable	Uncountable	Scanty growth	Uncountable

Table 5. Fermented Vegetable Waste Media (OD): Initial OD at 600 nm: **0**

Name of Test organism	Results (OD at 600 nm)			
	Control Media (Nutrient Broth)	Fermented Cabbage Media	Fermented Cauliflower Media	Fermented Cabbage + Cauliflower Media
E coli	0.3	0.02	0.03	0.03
Micrococcus sp.	0.4	0.51	0.1	0.28
Bacillus sp.	0.3	0.35	0.1	0.2

Table 6. Fermented Fruit Peel Waste Media (OD): Initial OD at 600 nm: 0

Name of Test organism	Results (OD at 600 nm)			
	Control Media (Nutrient Broth)	Fermented Banana Media	Fermented Chikoo Media	Fermented Banana + Chikoo Media
E coli	0.3	0.03	0.02	0
Micrococcus sp.	0.39	0.42	0.1	0.2
Bacillus sp.	0.2	0.3	0.05	0.1

mented Fruit waste mediaIn comparison with control, the fungal growth on the waste media prepared, was similar and showed satisfactory results.

Results for germination of Groundnut seeds grown on Fermented Agro waste media using plant tissue culture are shown in Table 9.

Germination was observed with shoot formation on Fermented Banana waste medium and Fermented Banana + Chikoo waste medium. Germination was also observed on Fermented Cabbage waste medium. No significant germination was observed on the cauliflower and cabbage + cauliflower media of the fermented waste mediums prepared.

Medeiros *et al.* (2020) observed scanty growth on agro waste media results when using an alternative, low-cost medium made with a biocomposite of discarded fruits and vegetables to evaluate the cultiva-

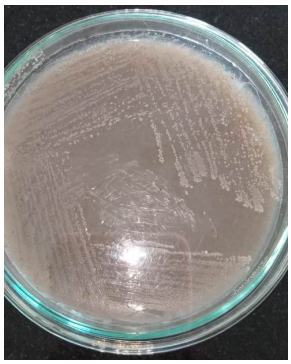

Fig. 1. Colonial growth of *Micrococcus spp* on fermented cabbage waste medium

Table 7. Fermented Vegetable Waste Media

Name of Test organism	Results Control Media (Sabouraud Agar)	Fermented Cabbage Media	Fermented Cauliflower Media	Fermented Cabbage + Cauliflower Media
Aspergillus sp.	Positive	Positive	Positive	Positive
Penicillium sp.	Positive	Positive	Positive	Positive

Positive – growth obtained

Table 8. Fermented Fruit Peel Media:

Name of Test organism	Results Control Media (Sabouraud Agar)	Fermented Banana Media	Fermented Chikoo Media	Fermented Banana + Chikoo Media
Aspergillus sp.	Positive	Positive	Positive	Positive
Penicillium sp.	Positive	Positive	Positive	Positive

Table 9. Fermented Vegetable waste media:

Name of Test sample	Results Control Media (MS media)	Fermented Cabbage Media	Fermented Cauliflower Media	Fermented Cabbage + Cauliflower Media
Groundnut seed	Germination observed with shoot formation.	Germination observed.	No Germination observed	No Germination observed

Table 10. Fermented Fruit Peel Media:

Name of Test organism	Results Control Media (MS media)	Fermented Banana Media	Fermented Chikoo Media	Fermented Banana + Chikoo Media
Groundnut seed	Germination observed with shoot formation.	Germination observed with shoot formation.	No Germination observed	Germination observed with shoot formation.

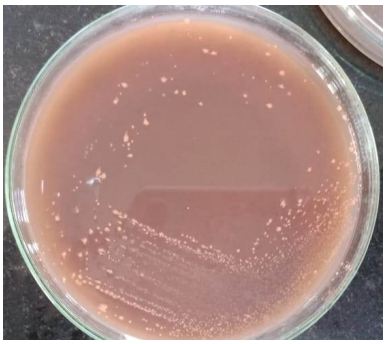

Fig. 2. Colonial growth of *Micrococcus spp* on fermented cauliflower waste medium

Fig. 3. Growth of *Penicillium sp*. on Fermented Cabbage waste media (Left) and Fermented Banana waste media (Right)

tion of microalgae isolated from the Northeast of Brazil (Jadhav *et al*., 2018) analyzed the growth of *E. coli, Serratia* sp. and *Pseudomonas* sp. in nine formulations containing varying concentrations of drumstick (Moringa) seeds and peel, orange peel, potato peel, cauliflower stem, and fenugreek stem. In reported studies; carrot, tomato, cabbage, pumpkin waste was used in formulation of media (Deivanayaki *et al.,* 2012) but in our research we used cauliflower, cabbage and fruit wastes by fermentation for formulation of media.

Conclusion

This present study has revealed that the fermented fruit peel waste and vegetable waste materials contain minerals and nutrients that can meet the nutritional requirements of industrially important fungi, bacteria and plants. They can be utilized as alternative materials in the formulation of culture media for *in vitro* microbial explant cultivation for industrial and research purposes. In solving the problem of the shortage of culture media for laboratory practicals, the result of this research will go a long way in ameliorating this problem. Agro waste can be useful in producing media that are less expensive as compared to synthetic and commercially available media

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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