Vegetable Waste as an Alternate Plant Tissue Culture Media for Laboratory and Industry

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ABSTRACT:
The clean production concept shows a good utilization potential for solid vegetable waste. It could achieve a reduction of investment and raw materials and can contribute to waste minimised plant production. Thus rendering cheap and environmentally safe alternative to commercial media. At present study was aimed at finding the potential of vegetable waste as a culture media. Composition of the media and the efficiency of the media to support plant growth were tested. Vegetable waste of Cabbage, Beetroot and Onion were collected and dried in the sun light for 2 weeks. Dried vegetable waste was grinded into fine powder. These vegetable waste media (alternative plant tissue culture media) was prepared and coconut water was added for natural hormone (cytokinin). The alternative media pH was 5.6 to 5.7 before sterilization. The vegetable waste powder was analysed for Macronutrients which include potassium (268 mg/kg), sodium (520 mg/kg), calcium (1591 mg/kg), while the micronutrients were analysed copper (5.421 mg/kg), iron (1101 mg/kg) and zinc. The growth was comparable to that obtained on routine commercial media.

KEYWORDS: Vegetable waste, BCO, plant tissue culture media, cost effective.

INTRODUCTION:

The waste was present two types biodegradable solid waste and non-biodegradable solid waste. The Biodegradable solid waste can be primarily classified into two categories[1].

A growth medium is powder designed to support the growth of the plant. The commercially available media are costly. Routine practical required large amount of media on regular basis for plant tissue culture experiments.

Availability of low cost media rich in nutrients, giving comparative results is the need of the day. The search for alternative, cheap media for use in laboratory agents for routine plant tissue culture experiments, is going on[2].

India generates about million tonnes of municipal solid waste every year from cities. Due to the rapid expansion of cities with massive migration of population from rural to urban centres, as well as considerable increase in per capita generation of waste with each passing day, generation of municipal waste has continuously been increasing. Over 90% of these of wastes are used for unscientific land filling or uncontrolled dumping on outskirts of towns and cities, which have serious implications in global warming. Existing municipal solid waste management systems in India, which includes storage, collection, transportation, segregation, processing and disposal of waste is poorly developed[3].

Today's society, in which is a great demand for appropriate nutritional media for cost effective. Decreasing availability of raw materials together with much concern about environment pollution. The vegetable waste is dumped from the all places. The decreasing the level of waste and useful products will be taken for plant growth. Proper management of waste is major problem in most of the areas. Composting is one of the oldest and simplest method of organic waste[4].

Waste to energy has been employed widely to generate from waste materials, as well as to reduce the volume of waste substantially. The vegetable wastes are used as a compost for soil. The alternative technique for vegetable waste. The new method to use preparation plant tissue culture media. Earlier it is used in vegetable waste alternative media for microbiology. Then my research work is alternative plant tissue culture media for growing plant[5].

Plant growth used for micro and macronutrients. Macronutrients are nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), Sulphur (S), magnesium (Mg) and sodium (Na). Present in micronutrients for plant growth boron (B), chlorine (Cl), manganese (Mn), iron (Fe), copper (Cu), nickel(N), molybdenum (Mo), cobalt (Co)[6].

The vegetable waste are present micro and macronutrients. The present study was aimed at finding the potential waste as a culture media. Composition of media and the efficiency of media to support media pH was checked before and after sterilization period. The plant growth was comparable to that obtained on routine commercial media[2].

To determination of macro and micronutrients in plant tissue culture is an important measure used to analysed plant nutrition status and to evaluate. The possible need for laboratory usage. The micro and macronutrients are using spectrochemical techniques such as flame atomic absorption spectroscopy (FAAS). Microwave plasma atomic emission spectrometer (MP-AES) magnetically microwave energy was used for determination (P, K, Ca, Mg, B, Co, Mo, Cu, Fe, Mn, Si and Zn. This instrument is used to quantifying plant nutrients. Calibration curves were constructed were constructed using approximately 500 samples were analysed[6].

Normally the methods for macro and micronutrients atomic absorption spectroscopy (AAS). The vegetable waste was used for cabbage, beetroot, onion and cabbage. The media pH was 5.4 before sterilization. Medium supported growth of plant. Plant growth was compared routine commercial media. The media was formulated as an alternative to the costly commercial media that is used for routine experiments in the laboratory. Thus it could replace the media for practical such as plant growing methods. This has cut down the expenditure on media, significantly. Similarly, the efficiency of the media as production media for plant growth needs to be checked. Attempts have been made at replacing the industrial media by cheap alternative vegetable based or agriculture based media for production of plant growth[2].

MATERIALS AND METHODS:

Raw Material and Processing:

The raw materials of vegetable waste: a) Beetroot peels, b) Cabbage peels, c) Onion peels. Dried the peels were 2-3 weeks in the sun light, separately. The peels were grinded to powders in appropriate proportion were used to prepare media[2]. UV/VIS spectrophotometer- Pharmacia Biotech (NOVASPEC 11), Atomic Absorption Spectrometer-Buck 210 VGP, Flame Photometer 04, pH meter- Hannah instruments 211, Shaker- Taiyo shaker SR-1, Conical flasks, Volumetric flask, gas cylinders. The vegetable waste was collected from nearby market and hotels. These waste include Onion peels, Beetroot peels, Cabbage peels. The peels were dried 2-3 weeks in sunlight.

Grinding:

The vegetable wastes were (Beetroot peel, Cabbage peel and Onion peel). These wastes were grinded into fine powder. In the waste powder used to prepare the alternative media for plant tissue culture laboratory.

Fig. 1 Beetroot waste powder

Fig. 2 Cabbage waste powder

Fig. 3 Onion waste powder

Preparation of Alternative Media:

1 g of the Beetroot powder + 0.5 g Cabbage powder + 0.5 g Onion powder + 100ml distilled water. The mixture was allowed to 30 min and then used. Added 0.5 ml of natural cytokinin (coconut water) + 2g of agar. Semisolid media was prepared using plant tissue culture laboratory.

Sampling:

Samples were collected in market and separated. Then which were used for plant tissue culture laboratory.

Drying:

Drying was done under moderate temperatures under shake where air circulation was possible. The vegetable waste samples were placed in bottles. The samples are mixed to use in plant tissue culture laboratory.

Sample Extract Preparation:

Vegetable waste sample was extract by chemical methods. (P Akeng'a *et al,* 1987). 0.7ml of concentrated H_2SO_4 was mixed with 8.6ml of HCl then 800ml of distilled water added in 1000ml volumetric flask. The solution was topped up to the mark with distilled water. 10g of the vegetable waste powder sample was placed in plastic bottles and 50ml of the extracting solution added then taken to a reciprocating shaker where it was shaken for one hour. The solution was prepared by whatman No.42 filter paper and the filtrate stored.

Flame photometer method:

1000 ppm standard solution should have 1000mg of element in 1000ml salt solution of the element, thus 250ml standard solution should be made up a quantity of salt of the element in mg equivalent to (250 * Molecular weightt of salt / Atomic weight of element) to contain 250mg of element.

Table 1. Estimation quantity of salt required

Element	Atomic weight of the element	Salt	Molecular weight of the element	Salt concentration(mg)
Na	22.99	Nacl	58.44	635
K	39.10	Kcl	74.56	477
Ca	40.08	$CaCo_3$	100.09	624

Preparation of standards:

Sodium and Potassium:

635 mg and 477 mg of Nacl, Kcl were weighed and dissolved in 250 ml of double distilled water. The samples were diluted to 1:100 to get 1mg. Na/100ml and 1mg K/100ml, which is equivalent to 10 ppm.

Calcium:

624 mg were weighed $CaCo_3$ and dissolved in 250ml double distilled water. The samples were double to 1:10 to get 10mg. Ca/100 ml which is equivalent to 100 ppm.

Procedure:

- Switch on the compressor and adjusted the pressure to 10lb.
- Checked the gas (LPG) flow and switched on instrument.
- Auto ignition regulated the blue flame (the flame should be single cone shape).
- Calibrated the standards used calibration function.
- After standardization the instrument is ready for concentration estimated unknown sample.
- The concentration of samples can be measured directly without any standardisation, following the calibration curve generation in calibrated function.

Determination of Macronutrients:

Determination of Potassium[7]:

Stock solution of potassium standard (1000 mg/l) was prepared by dissolving 1.9068g of dry KCl in distilled water and volume made up to 1000ml. the stock solution was diluted to produce working standards of concentration range 1-100 mg/l. Each working standard was run in the emission spectrophotometer and the intensity for each standard was recorded. The calibration curve was constructed to determine the concentration of potassium in the vegetable waste sample. The blanks were also determined in the same way and subtraction done where necessary.

Calculations: K, Ca, Na (mg/kg) = (a-b) * v * f * 1000 / (1000 * w)

Where:
a = concentration of K, Ca, Na in the sample extract
b = concentration of element in the blank extract
v = volume of the extract solution
w = weight of the soil sample
f = dilution factor

Determination of Calcium:

Stock solution of calcium standard (1000 mg/l) was prepared by dissolving 2.4973g of dry $CaCO_3$ in 200ml of distilled water containing 5ml of concentrated HCl. The solution was heated to drive out CO2 and after cooling it was made up to 1000ml. the stock was diluted to produce working standards of concentration range 1-100 mg/l. The spectrometer was set at the operating wavelength of calcium. The standards and sample were aspirated into the flame as was with potassium and from the calibration curve constructed of calcium was obtained.

Determination of Sodium:

Stock solution of sodium standard (100 mg/l) was prepared by dissolving 0.2542g of dry NaCl in distilled water and was made up to 1000ml. the solution was diluted to obtain produce working standards of concentration range 1-100 mg/l. The standards and sample solution were aspirated into the flame after selecting the operating wavelength of sodium. From the constructed calibration curve the concentration of sodium was determined.

Determination of Micronutrients:

Determination of Zinc:

Stock solution of Zn (100 mg/l) standard was prepared by dissolving 0.4398g of $ZnSO_4.7H_2O$ in distilled water and diluted to 1000 ml. The stock was diluted to obtain working standards of range 0.5 mg/l. the sample solution and standards were aspirated in the flame. The calibration curve was used to determine the concentration in ppm of zinc.

Calculations:

Cu, Fe, Zn (mg/kg) = (a-b) * v* f * 1000 / (1000 * w)

Where:
a = concentration of Cu, Fe, Zn in the sample extract
b = concentration of element in the blank extract
v = volume of the extract solution
w =weight of the vegetable waste sample
f = dilution factor

Determination of Copper:
Stock solution of Cu standard (100 mg/l) was prepared by dissolving 0.3930g of $CuSO_4.5H_2O$ in distilled water then diluted to 1000 ml. an intermediate of 10 mg/l was prepared then diluted further to produce a working range of 0.5 mg/l. the spectrometer was set at the operating wavelength of copper and the sample solution and standards aspirated in the flame. From the calibration graph constructed the concentration of sample were obtained.

Determination of Iron:
Stock solution of Fe standard (100 mg/l) was prepared by dissolving 0.1g of clean untarnished iron in 10ml of warm 10% H_2SO_4. After cooling it was diluted to 1000 ml. The stock solution was diluted to obtain a working range of 0-20 mg/l. the spectrometer was set at the operating wavelength of iron. From the calibration graph constructed the concentration of the sample was obtained.

Determination of Vegetable Waste Sample pH:
10g of the vegetable waste sample was placed in long beakers and 20 ml of de-ionized distilled water was added. The suspension was stirred for 30 minutes and left to stand for 1hour. The pH meter was calibrated by carefully immersing the combined electrode into the clear supernatant solution.

RESULTS AND DISCUSSION:

Alternative Plant Tissue Culture Media:
BCO media supported the growth of plant, various explant in the plant. There was no significant variation in the plant growing morphology of explant. The seed was grow better in BCO media. On semi solid media, the plant growth was faster as compared to regularly used media. In the alternative media, faster root and shoot elongation was observed. Good growth was obtained after 3days. Thus, root and shoot elongation was observed after 7days in BCO media. This shows that BCO media is very suitable for growth of plants. There is no contamination after 5days.

Fig. 4 Alternative plant tissue culture media

Observation

Table 2. Alternative media observed results

DAYS	OBSERVED RESULTS
1	No contamination
2	No contamination
3	No contamination
4	No contamination
5	No contamination

Inoculation of Alternative Media:
The alternative media was prepared. The media was inoculated and stored. These alternate media were stored in dark and its light for 12 hours, respectively. The plant growth was obtained. The seed was inoculated in the BCO media. Similar results were obtained using BCO media in plant tissue culture as reported by[8].

Shoot and root elongation:
The growth was obtained using BCO media had shoot and root elongation. The BCO media proved its efficiency as a cheap media for routine plant tissue culture media.

Fig. 5 Initial growth

Fig. 6 Shoot and root elongation

The alternative BCO media is designed to support the growth of plant. The commercially available media are very costly. The alternative media is inoculated after 3days initial growth was obtained. Then, after 7^{th} day shoot and root elongation is obtained. The plant growth was obtained after 14^{th} days. There is no contamination for BCO media. The BCO media after 21 days were utilised for hardening the plant. Thus the plant was transferred to the soil for plantation[9].

Different media concentration:

The alternative media was prepared using different concentrations for inoculation to be used for the growth of the plant. The different formulation was assessed to check its effectiveness against the growth of the plant.

Table 3. Different media concentrations

Vegetable waste samples	M1	M2	M3	M4	M5	M6	M7	M8	M9
Beetroot g)	1.0	0.5	0.5	1.0	-	1.0	-	0.5	1.5
Cabbage g)	0.5	1.0	0.5	-	1.0	1.0	1.5	-	0.5
Onion (g)	0.5	0.5	1.0	1.0	1.0	-	0.5	1.5	-

M: Media concentrations (1 to 9)

DIFFERENT BCO MEDIA CONCENTRATIONS (BCO media)

Fig. 7 M1 **Fig. 8 M2** **--Fig. 9 M3** **Fig. 10 M4**

Fig. 11 M5 **Fig. 12 M6** **Fig. 13 M7** **Fig. 14 M8**

Fig. 15 M9

The results were obtained (M1, M2, M3, M4, M5, M6, M7, M8, M9) different media formulation. The higher growth was obtained for Media2. The formulation had the BCO media concentration range (0.5g, 1g, 0.5g), respectively[10].

Alternative Explant Used for BCO Media:

The mulberry explant was chosen as an alternate. It is very useful for the production of silkworm. Mulberry plant was inoculated for alternative media and adding natural cytokinin observed shoot elongation. These plant were grown by shoot elongation was observed on 14th day. Alternative media grows faster than the normal plant tissue culture media.

Fig. 16 Alternate explant (mulberry)

Shoot and root elongation is obtained after 23 days. The alternative media is faster than the normal plant tissue culture laboratory. These media also used as an alternative plant tissue culture industry. The alternate explant(mulberry) growth is faster than the commercial media as reported by [11].

DETERMINATION OF MACRONUTRIENTS:

Detection of potassium:

The potassium standard was prepared. Then vegetable waste sample powder was used as a test solution. The potassium was obtained at 766nm. The potassium present in the Beetroot sample (T1=78 mg/kg), Cabbage sample (T2=37 mg/kg), Onion sample (T3=163 mg/kg). The higher amount of potassium present in Onion waste sample.

Table. 4 Potassium standards and test sample

Concentration (ppm)	Absorbance (766nm)
Blank	2.2
20	20.9
40	40.7
60	60.5
80	80.2
100	100.6
T1	105.5
T2	102.3
T3	185.6

Detection of calcium:

The calcium standard was prepared. Then vegetable waste sample powder was used as a test solution. The calcium was obtained at 622nm. Then calcium present in the Beetroot sample (T1=371 mg/kg), Cabbage sample (T2=448 mg/kg), Onion sample (T3=772 mg/kg). The higher amount of calcium present in onion sample.

Table 5. Calcium standards and test sample

Concentration (ppm)	Absorbance (622nm)
Blank	0.042
20	0.053
40	0.063
60	0.074
80	0.081
100	0.088
T1	0.729
T2	0.242
T3	0.838

Detection of sodium:

The sodium standard was prepared. Then vegetable waste sample powder was used as a test solution. The sodium was obtained at 589nm. Then sodium present in the Beetroot sample (T1=192 mg/kg), Cabbage sample (T2=93 mg/kg), Onion sample (T3=235 mg/kg). The higher amount of sodium present in Onion waste sample.

Table 6. Sodium standards and test samples

Concentration (ppm)	Absorbance (589nm)
Blank	0.113
20	0.002
40	0.004
60	0.005
80	0.007

100	0.011
T1	0.687
T2	1.975
T3	0.803

Determination of Micronutrients:

Detection of zinc:

The zinc standard was prepared. Then vegetable waste sample powder was used as a test solution. The zinc was obtained at 213nm. Then zinc present in the Beetroot sample (T1=20 mg/kg), Cabbage sample (T2=3 mg/kg), Onion sample (T3=6 mg/kg). The higher amount of zinc present in Beetroot waste sample

Table 7. Zinc standards and test samples

Concentration (ppm)	Absorbance (213nm)
Blank	0.031
20	0.037
40	0.067
60	0.115
80	0.137
100	0.158
T1	0.907
T2	0.414
T3	0.712

Detection of copper:

The copper standard was prepared. Then vegetable waste sample powder was used as a test solution. The copper was obtained at 324nm. Then copper present in the Beetroot sample (T1=0.421 mg/kg), Cabbage sample (T2=2 mg/kg), Onion (T3=3 mg/kg). The higher amount of copper present in Onion waste sample.

Table 8. Copper standards and test samples

Concentration (ppm)	Absorbance (324nm)
Blank	0.01
20	0.03
40	0.04
60	0.057
80	0.069
100	0.083
T1	0.714
T2	0.512
T3	0.321

Detection of iron:

The iron standard was prepared. Then vegetable waste sample powder was used as a test solution. The iron was obtained at 259nm. Then iron present in the Beetroot sample (T1=183 mg/kg), Cabbage sample (T2=385 mg/kg), Onion sample (T3=636 mg/kg). The higher amount of iron present in Onion waste sample.

Table 9. Iron standards and test samples

Concentration (ppm)	Absorbance (259nm)
Blank	0.001
20	0.0025
40	0.004
60	0.0065
80	0.0075
100	0.01
T1	0.871
T2	0.788
T3	0.577

Macronutrients Present in Samples (mg/kg):

The vegetable waste samples are checked for the amount of plant nutrients present. The levels of Potassium, Calcium, Sodium and Zinc, Copper, Iron present in the mg/kg of sample were listed in (Table 4.9).

Table 10. Macronutrients present in the samples (mg/kg)

Sample	Potassium (K) (mg/kg)	Sodium (Na) (mg/kg)	Calcium (Ca) (mg/kg)
Beetroot waste	78±0.112	192±0.578	371±0.453

Cabbage waste	37±1.21	93±1.72	448±3.21
Onion waste	163±1.47	235±1.38	772±2.88

The levels of potassium were present in sample were found to be 163 mg/kg, 78 mg/kg, 37 mg/kg. Thus, the amount of potassium higher in Onion sample than Beetroot sample than Cabbage sample.

The levels of sodium were present in sample were found to be 235 mg/kg, 192 mg/kg, 93 mg/kg. Thus, the amount of sodium higher in Onion sample than Beetroot sample than Cabbage sample.

The levels of calcium were present in sample were found to be 772 mg/kg, 448 mg/kg, 371 mg/kg. Thus, the amount of sodium higher in Onion sample than Cabbage sample than Beetroot sample.

Table 4.10 Micronutrients present in the samples (mg/kg)

Sample	Zinc (Zn) (mg/kg)	Copper (Cu) (mg/kg)	Iron (Fe) (mg/kg)
Beetroot waste	20±1.23	0.421±0.025	183±3.63
Cabbage waste	3±0.23	2±0.052	385±1.29
Onion waste	6±1.37	3±0.041	636±1.99

The levels of zinc were present in sample were found to be 20 mg/kg, 6 mg/kg, 3 mg/kg. Thus, the amount of zinc higher in Beetroot sample than Onion sample than Cabbage sample.

The levels of copper were present in sample were found to be 3 mg/kg, 2 mg/kg, 0.421 mg/kg. Thus, the amount of copper higher in Onion sample than Cabbage sample than Beetroot sample.

The levels of iron were present in sample were found to be 636 mg/kg, 385 mg/kg, 183 mg/kg. Thus, the amount of iron higher in Onion sample than Cabbage sample than Beetroot sample.

Table 4.11 The pH measurements obtained from the vegetable waste samples

Sample	Beetroot sample	Cabbage sample	Onion sample
pH	5.57	5.45	5.43

The pH was optimum for the normal growth of the plant. These optimum level of vegetable sample was found to be pH-5.6-5.7. It can be used for alternative (BCO media) plant tissue culture media.

Table 4.12 The wavelength of emitted light specific for specific element

Element	Wavelength(nm)	Colour of flame
Potassium	766	Violet
Calcium	622	Orange
Sodium	589	Yellow

The flame photometer run for the vegetable waste sample was analysed. The wavelength and the colour of flow obtained for Potassium (Violet-766nm), Calcium (Orange-622nm), Sodium (Yellow-589nm).

Table 4.13 Cost analysis of the media

Media type	Cost in Rs/ (1kg or 1litre)	
	Commercial media	BCO media
Solid media	900	200
Liquid media	780	150

Vegetable waste collection = Rs.50
Grind waste powder = Rs.50
Nutrients analysis = Rs.100

BCO media (vegetable waste) prepared total cost analysis for Rs.200. Compared to commercial media price, BCO media price is less. It is available at affordable price.

The media was formulated as an alternative commercial media that is used for the routine experiments in the laboratory. Thus it could replace the media for practical work. Similarly, the efficiency of the media can be used as a production for various plants. Thus Replacing the industrial media could render it cheaper, alternative vegetable based or agri-based media for production of plants.

CONCLUSION:

Vegetable waste was used to formulate a cost effective media. Media with micro and macronutrients was formulated and named BCO media. Concentrations of the all the macro and micronutrients under study were

within the required levels for normal plant growth. The growth of different explant was comparable with regular media. The higher amount of yield present in the vegetable waste media. When compared with commercial media to BCO media cost effective and faster growth of the plant. The macro and micronutrients were assessed to production of normal plant growth. Cost analysis showed the BCO media to be highly cost effective. BCO is good alternative media for laboratory use. It can be used at industrial level for various plant growing based productions.

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