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Effect of Vegetable and Fruit peel as a Natural Fertilizer on the Growth of *Vigna Radiata*

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A B S T R A C T

"Biowaste" is considered as municipal solid waste (Bhattacharyya P) including kitchen and garden waste which mainly has vegetable peel and fruit peel used as natural fertilizer for plant growth. Vegetable peel (Luffa acutangula) and fruit peel (Citrus sinesis) were collected from the market. The peels were dried at normal room temperature for a couple of weeks. After that dried peels were taken and was made into powdered form for further uses. Using aqueous and methanol extract of Luffa acutangula and Citrus sinesis phytochemical analysis were carried out. It showeds the presence of (Luffa acutangula) carbohydrates, protein, phenols, tannin, phytosterol and saponins. Carbohydrates, Terpenoids, flavonoids, alkaloids, cardiac glycosides, protein, amino acids, phenols, and saponin revealed the presence of Citrus sinesis. Using the methanol extract of both peels, FTIR analysis possessed the functional group present in the extract. Soil test was carried out using different extracts.

Keywords: *Luffa acutangula*, *Citrus sinesis*, Aqueous and Methanol extract

1. INTRODUCTION

Biowaste is considered as organic and municipal waste. (Gross et al.,2012) Recently Biowaste has become a global challenge because it is associated with some important environmental issues. In India nearly 1000 million tons of organic waste is generated. Kitchen waste is from the peel of fruits and vegetables. Fertilizes are organic or inorganic substances that are meant to enrich the plant growth. Plants depend on the soil nutrients for its plant growth. Different types of fertilizer are applied to crop production. Fertilizers commonly used are sodium nitrates, ammonium salts etc. Ammonium sulphate fertilizer contain sulphur and nitrogen which is applied to alkaline soil to maintain pH of the soil. (Turing ei al., 2006). Fertilizers provide large amount of macronutrients such as nitrogen, phosphorous and potassium to the plant. (Miller, 2014). *Luffa acutangula* is a cucurbitaceous vine that is commercially grown for its unripe fruits as a vegetable. Mature fruits are used as natural cleaning sponges. Its fruit slightly resembles a cucumber or zucchini with ridges. It ranges from central and eastern Asia to southeastern Asia. It is also grown as a houseplant in places with colder climates. An orange is a fruit of various citrus species in the family Rutaceae it primarily refers to *Citrus × sinensis*, which is also called sweet orange. The sweet orange has had its full genome sequenced. This orange originated in a region encompassing Southern China, Northeast India, and Myanmar, and the earliest mention of the sweet orange was in Chinese literature

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND PREPARATION

The vegetable peel of *Luffa acutangula* and fruit peel of *Citrus sinesis* were obtained from the market, Chennai. They were air dried for two weeks and grounded into powder with a mechanical blender. The powdered samples obtained were thereafter stored in clean bottles at room temperature until needed for use.

2.2 EXTRACTION

Powdered samples were subjected to cold percolation extraction by using different solvents such as aqueous and methanol in the ratio 1:10 (plant sample: solvent). All extracts obtained were stored in a refrigerator until required for use.

2.3 PHYTOCHEMICAL ANALYSIS:

The extracts of vegetable peel were analyzed for alkaloids, tannins, glycosides, carbohydrates, proteins and amino acids, flavonoids, saponins using standard procedures (Harborne. 1995 and Raaman. 2006).

2.3.1 DETECTION OF CARBOHYDRATES:

The extract (100ml) is dissolved in 5 ml of water and filtered. The Filtrate is subjected to the following test.

a. **MOLISCH 'S TEST:** To 2 ml of filtrate, add two drops of alcoholic solution α- naphthol. the mixture is shaken well and 1ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand at room temperature. Violet ring indicates the presence of carbohydrate.

b. **BENEDICT'S TEST:** To 0.5 ml of filtrate, add 0.5 ml of Benedict's solution. The mixture is heated on a boiling water bath for 2 minutes. The appearance of red colour indicates the presence of carbohydrates.

2.3.2 DETECTION OF PROTEINS AND AMINO ACIDS:

The extract [100ml] is dissolved in 10 ml of water and filtrate is subjected to tests for proteins and amino acids.

a. **NINHYDRIN TEST:** To 1 ml of the extract, add 2 drops of ninhydrin solution. The appearance of purple colour indicates the presence of proteins.

b. **MILLON'S TEST:** To 2 ml of filtrate, a few drops of millon's reagent was added. The appearance of white precipitate indicates the presence of proteins.

2.3.3 DETECTION OF GLYCOSIDES:

50mg of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on hot water bath and filtered. The filtrate was subjected to Borntrager's test.

a. **BORNTRAGER'S TEST:** To 2ml of extract, 3ml of chloroform is added and shaken. After the chloroform layer is separated, 10% ammonia solution was added. The appearance of red colour indicates the presence of glycosides.

2.3.4 DETECTION OF ALKALOIDS:

To 50ml of the extract few ml of dilute hydrochloric acid was added and filtered. The filtrate is tested carefully with Dragendorff's test.

a. **DRAGENDROFF'S TEST:** To a few ml of filtrate, 1 or 2ml of dragendorff's reagent was added. The appearance of reddish-brown precipitate indicates the presence of alkaloids.

2.3.5 DETECTION OF PHENOLIC COMPOUND AND TANNINS:

a. **FERRIC CHLORIDE TEST:** The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. The appearance of black colour indicates the presence of phenolic compound and tannins.

b. **LEAD ACETATE TEST:** The extract (50mg) is dissolved in distilled water and to this, 3ml of 10% lead acetate solution is added. The appearance of yellow precipitate indicates the presence of phenolic compound and tannins.

c. **ALKALINE REAGENT TEST :** An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. The appearance of yellow colour indicates the presence of phenolic compound and tannins.

2.3.6 DETECTION OF PHYTOSTEROL:

a. **LIEBERMANN BURCHARD TEST:** The (extract 50 mg) is dissolved in 2 ml of acetic anhydride. To this, one or two drops of concentrated sulphuric acid was added slowly along the side of the test tube. The appearance of bluish green colour indicates the presence of phytosterol.

2.3.7 TEST FOR TERPENOIDS:

a. **SALKOWSKI TEST:** To 0.5 ml of extract, 2ml of chloroform and then concentrated sulphuric acid was added in a test tube. The appearance of reddish brown indicates the presence of terpenoids.

2.3.8 TEST FOR SAPONINS:

2 ml of extract was boiled using distilled water in water bath, cooled and filtered. To 2 ml filtrate add 1 ml of distilled water and shaken well for the formation of froth. Presence of froth indicates the presence of saponins.

2.4 PHYTOCHEMICAL ANALYSIS:

The extracts of Fruit peel were analyzed for the presence of phytochemicals like alkaloids, tannins, glycosides, carbohydrates, proteins, amino acids, flavonoids and saponins using standard procedures (Sulekha Gotmare, Jaya Gade 2018).

2.4.1 TEST FOR ANTHROQUINONES:

a. **BORNTRAGER'S TEST:** To 2ml of the extract was boiled with 10 ml of sulphuric acid (conc. $H2so_4$) and filtered while hot, 5ml of Chloroform used to shake the filtrate.1 ml of dilute ammonia was added in the chloroform layer. The appearance of red colour indicates presence of anthroquinones.

2.4.2 TEST FOR TANNINS:

a. **FERRIC CHLORIDE TEST** 2ml of the extract was boiled in 10 ml of water in a test tube and then filtered. To this 3- 4drops of 0.1% ferric chloride was added . The appearance of brownish green or a blue-black coloration indicates presence of tannin.

2.4.3 TEST FOR TERPENOIDS:

a. **SALKOWSKI TEST:** To 2ml of the extract , 2 ml of chloroform was added. To form a layer, concH_2SO_4 (3ml) was carefully added. The appearance of reddish-brown indicates the presence of terpenoids.

2.4.4 TEST FOR FLAVONOIDS:

a. **SODIUM HYDROXIDE TEST:** To 2ml of extract, 1ml of sodium hydroxide was added. A yellow colour indicates the presence of flavonoids.

2.4.5 TEST FOR CARBOHYDRATES:

a. **BENEDICT'S TEST:** To 2ml of extract, 1ml of benedicts reagent was added. A red precipitate indicates the presence of carbohydrates.

2.4.6 TEST FOR ALKALOIDS:

a. **WAGNER'S TEST:** To 2ml of extract and 1ml of wagner's reagent was added. A brown / reddish precipitate indicates the presence of alkaloid.

2.4.7 TEST FOR PROTEINS AND AMINOACIDS:

a. **NINHYDRIN TEST:** To 2ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of amino acids.

b. **BIURET TEST:** To 2ml of extract was treated with 4% Sodium Hydroxide and few drops of 1% Copper Sulphate was added. To violet or pink colour appear the presence of protein.

2.4.8 TEST FOR CARDIAC GLYCOSIDES:

a. **KELLER- KILLANI TEST:** To 2ml of the extract was treated was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring indicates presence of cardiac glycosides.

2.4.9 TEST FOR SAPONINS:

To 2 ml of extract was boiled in distilled water in water bath, cooled and filtered .To 2 ml filtrate add 1 ml of distilled water and shaken well for the formation of froth. Presence of froth indicates the presence of saponins.

2.5 FOURIER TRANSFORM INFRARED SPECTROPHOTOMETERIC (FT-IR) ANALYSIS:

For the FI-IR study, ET-IR spectral system (Shimadzu, IR Affinity l. Japan). Equipped with a DLATGS detector with a mirror speed of 2.8mm/sec. scan range: from 400-4000cm_1 with a resolution of 4 cm_1 was used. The aqueous and methanol extract of *Luffa acutangula* and *citrus x sinesis* samples were used. The extract was evaporated by flash evaporator, and it was pelletized using kBr salt in ratio 1:100. Using the thin pellet, infrared spectra were recorded at the range of 4000-500cm_1(John Coates,2000).

2.6 PLANT ANALYSIS:

2.6.1 SEED GERMINATION:

The certified seeds of *Vigna radiata* having uniform size, colour were chosen for experimental purpose. Thirty healthy seeds were spread uniformly in petri dishes lined with filter paper. The petridishes were treated with an equal volume of different extract and water as control. The seeds were allowed to germinate, and the percentage of germination was recorded.

Percentage of seed germination = No. of seeds germinated/ total no. of seeds x100.

2.7 SOIL ANALYSIS:

2.7.1 PHYSICO CHEMICAL FACTORS

Physico chemical factors such as pH, N,P,K (Molina-Herrera S (2015)Total minerals were analyzed using standard procedures for both control (red soil and sandy soil) before and after plant growth. The mean values of the analysis were presented (Neha Panwar,2015).

2.7.2 POT CULTURE EXPERIMENT

Control soil was filled into plastic bags for the pot culture experiments. Healthy and uniformly sized seeds were collected. The extract was applied to the soil and properly mixed for uniform distribution. Fifteen replicates were maintained for each formulation. After 3 days, seeds of Vigna radiata were sown in pots. Each pot was sown with seeds, and water was poured every day. Results were observed after 30 days of growth.

2.7.3 ASSESSMENT OF PLANT GROWTH AND BIOMASS:

The plant growth biometric observations such as root length, shoot length, and dry weight were recorded at 30-day intervals of growth. Plants were carefully uprooted without any damage to the roots, washed with tap water, and blotted against filter paper. The root length was measured from the collar region to the root tip, and the shoot length was measured from the topsoil level to the tip of the plant in randomly selected 15 plants in each treatment, and their mean value was recorded. Plants taken for measuring root and shoot length were oven-dried at 60°C for 48 hours, and then the biomass in terms of dry weight of the whole plant was analyzed. (Tan Lih Mlin (2015)).

2.7.4 ROOT AND SHOOT GROWTH:

The seeds were sown in soil containing different extracts. The plant samples were collected on the 30th day, and the root length, shoot length, and dry weight were measured, and the data were recorded.

2.8 ESTIMATION OF CHLOROPHYLL:

The chl-a, chl-b, and total chlorophyll in the leaves of Vigna radiata grown under different extracts were analyzed by Arnold's method. Fresh leaves of 1g were ground with 80% acetone, and a pinch of calcium carbonate was added to avoid phaeophytic formation. The homogenate was filtered and centrifuged at 6000 rpm for 15 minutes, and then the supernatant was made up to a known volume. From this, 5ml was taken, and OD was read at 645nm and 663nm. The values were recorded for each plant treated with different extracts.

Chlorophyll-a (mg/g) = (12.7xOD663) - (2.69xOD645) x V/1000x W

Chlorophyll-b (mg/g) = (22.9xOD645) - (4.68xOD663) x V/1000x W

Total chlorophyll (mg/g) = (20.2xOD645) + (8.02xOD 663) x V/1000x W

Where:

V = volume of the sample

W = weight of the sample

The chlorophyll-a, chlorophyll-b, and total chlorophyll content were estimated for treated and normal plants, and results were recorded.

2.9 ESTIMATION OF CAROTENOIDS:

For the estimation of carotenoids, 1gm of fresh leaves was ground with 10ml of 80% acetone. Then it was centrifuged, and the supernatant was made up to 10mL. Then the absorbance was read at 480nm, 645nm, and 663nm. The values were recorded for each plant treated with different extracts. Carotenoid (mg/g) = OD480 – (0.114XOD663) – (0.638xOD645) x V/1000x W. V = volume of the sample. W= weight of the sample.

2.10 PAPER CHROMATOGRAPHY

For paper chromatography, a few grams of leaves in Vigna radiata were collected and crushed in a mortar and pestle, and 10 ml of 80% acetone was added to the paste and centrifuged. The supernatant was collected and used as the extract. Whatman no: 1 filter paper was cut into 20x3cm. A line was drawn at 2cm from the bottom of the paper. Using a capillary tube, leaf extract was spotted on the paper. The spot was allowed to dry. The spotting was done for 3-4 times. The opposite end of the paper was folded and pinned to the lower surface of the cork. The chromatogram was developed using 7:3 petroleum ether:acetone as an extent. After the solvent had risen 12cm, the paper was taken out, and the solvent front was marked, and then it was allowed to dry. The colored spots were marked with a pencil. The Rf value of different color spots on the chromatograph was calculated by the following formula: Distance traveled by solute/Distance traveled by solvent.

3. RESULTS AND DISCUSSION

The vegetable peel of *Luffa acutangula* and fruit peel of (*Citrus x sinesis*) used for the present study were obtained from market, air dried, ground into powder and extracted with aqueous and methanol.

Figure 1: *Citrus Sinesis (*peel*)*

Figure 2: *Luffa acutangula* (peel)

Figure 3: *Citrus Sinesis* (powder)

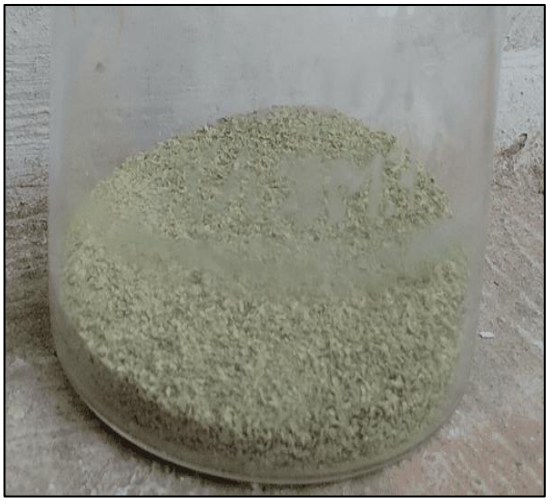

Figure 4: *Luffa acutangula* (powder)

3.1 QUALITATIVE PHYTOCHEMICAL TESTS

The extracts were analyzed for the phytochemical constituents by qualitative methods. In the tests conducted, compounds like phenols, phytosterols, tannins, terpenoids, saponins, carbohydrates, and proteins were found to be present in *Luffa acutangula* and Citrus x sinensis.

The aqueous extract of *Luffa acutangula* showed the presence of carbohydrates, proteins, phenolic and tannins, and saponins. The methanol extract of *Luffa acutangula* showed the presence of carbohydrates, phenolic and tannin, phytosterol, and saponins.

The aqueous extract of *Citrus sinensis* showed the presence of carbohydrates, proteins, tannins, flavonoids, terpenoids, cardiac glycosides, and saponins. The methanol extract of *Citrus sinensis* showed the presence of phenols, cardiac glycosides, flavonoids, alkaloids, tannins, terpenoids, and saponins.

Table 1: **Phytochemical Analysis Of *Luffa acutangula* and *Citrus sinesis* Extracts**

	Phytochemical tests	*Luffa acutangula* (Aqueous extract)	*Luffa acutangula* (Methanol extract)	*Citrus sinesis* (Aqueous extract)	*Citrus sinesis* (Methanol extract)
	Carbohydrate's test				
1	Molisch's test	+	-	-	-
2	Benedict's test	+	+	+	-
	Proteins and Amino Acids test				
3	Ninhydrin test	-	-	-	-
4	Millon's test	+	-		
5	Biuret test	-	-	+	-
	Glycosides test				
6	Borntrager's test	-	-	-	-
	Alkaloids test				
7	Dragendroff's test	-	-	-	-
8	Wagner's test	-	-	+	+
	Phenolic Compounds and Tannins				
9	Ferric chloride test	-	-	-	+
10	Lead acetate test	+	+	-	-
11	Alkaline reagent test	-	-	-	-
	Phytosterols				
12	Libermann-Burchand tests	-	+	-	-
	Terpenoids				
13	Salkowski's test	-	-	+	+
	Flavonoids				
14	Sodium hydroxide test	-	-	+	+
	Cardiac Glycosides				
15	Keller-Killani test	-	-	+	+
16	Saponin	+	+	+	+

3.2 PHYTOCHEMICAL ANALYSIS OF Luffa acutangula AND Citrus sinesis EXTRACTS

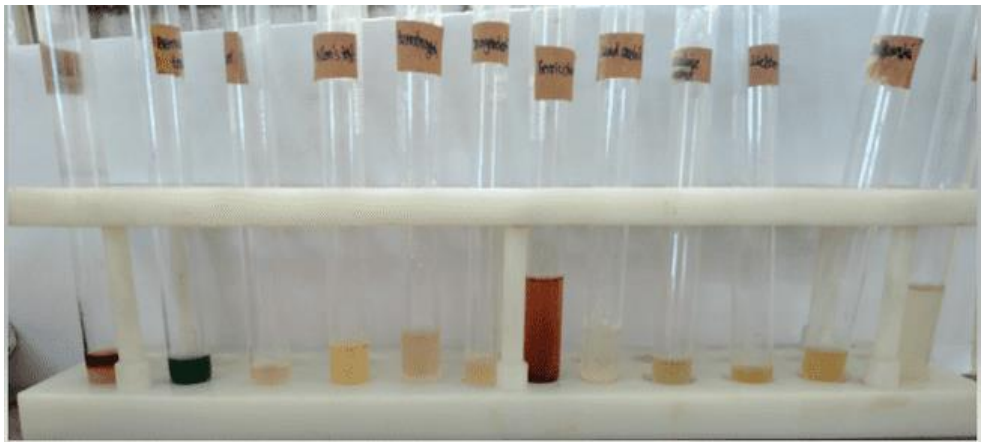

Figure 5: Phytochemical analysis of *Luffa acutangula* (aqueous extract)

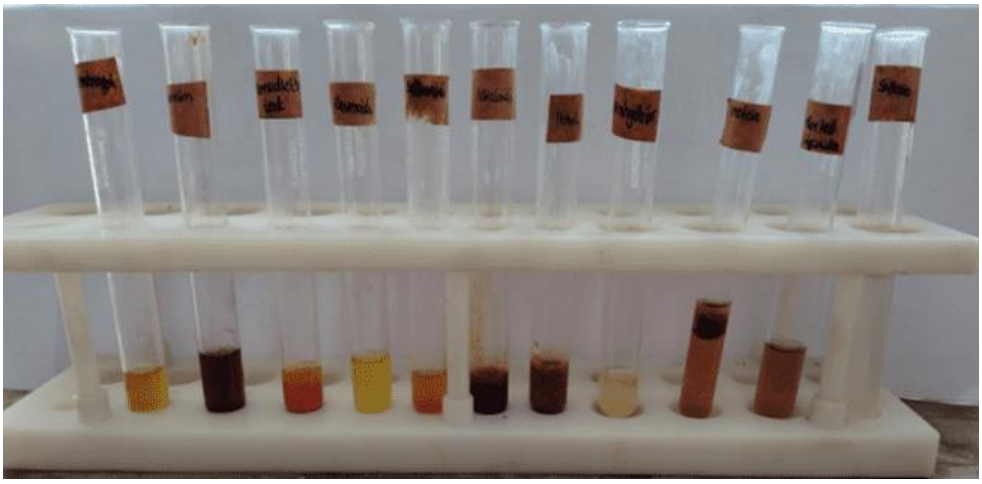

Figure 6: Phytochemical analysis of *Citrus sinesis* (aqueous extract)

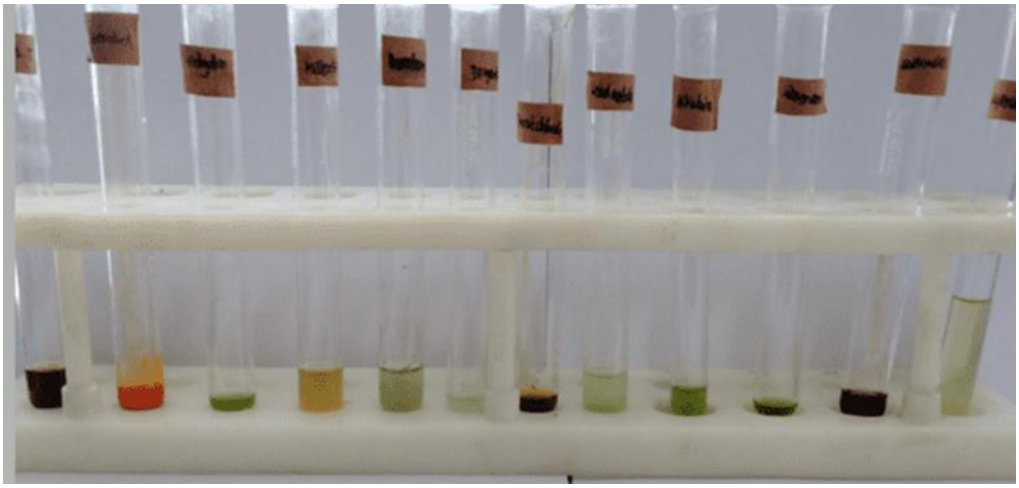

Figure 7: Phytochemical analysis of *Luffa acutangula* (Methanol extract)

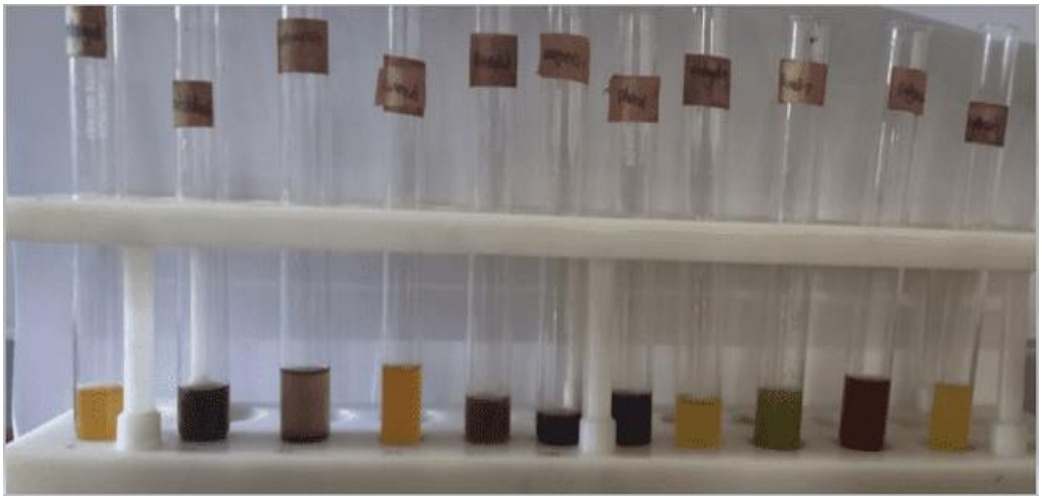

Figure 8: Phytochemical analysis of *Citrus sinesis* (Methanol extract)

3.3 FT-IR ANALYSIS:

Methanol extract of *Luffa acutangula* peel showed sharp peeks at 1373.32 (NO2 stretch), 1710.86 (C=O amide), 2924.09 (-C-H stretch).

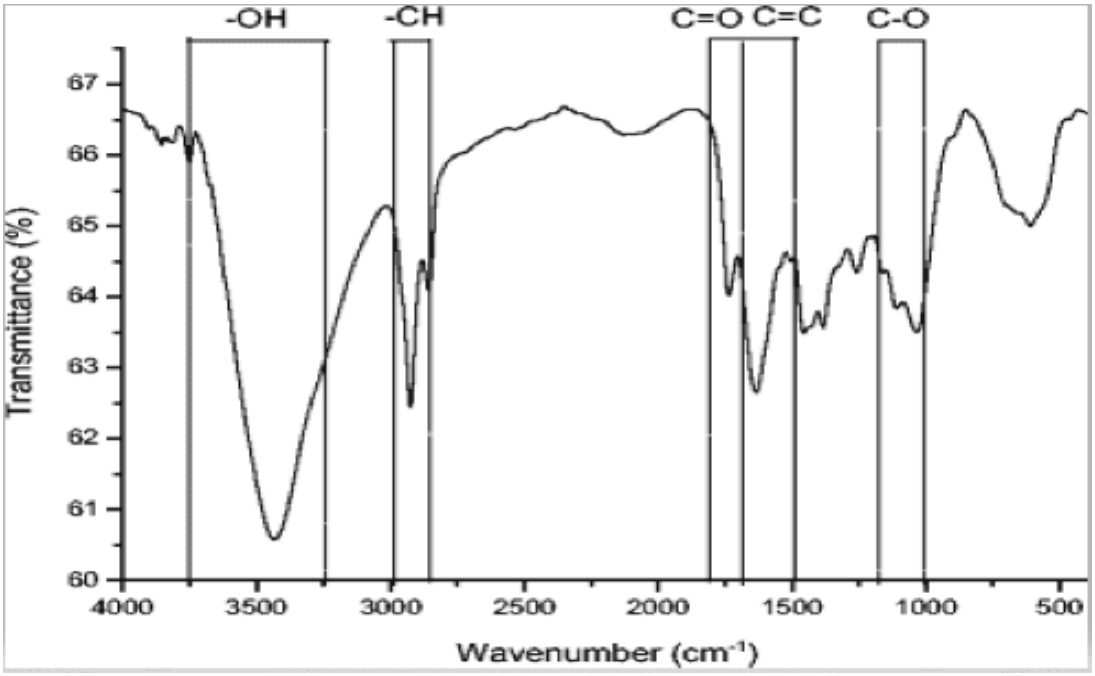

Figure 9: FT-IR spectrum of methanol extract of *Luffa acutangula* peel

Methanol extract of *Citrus sinesis* peel showed sharp peeks at 1605.6 (C=C), 1736.74 (C=O amide), 2911.9 (-C-H stretch).

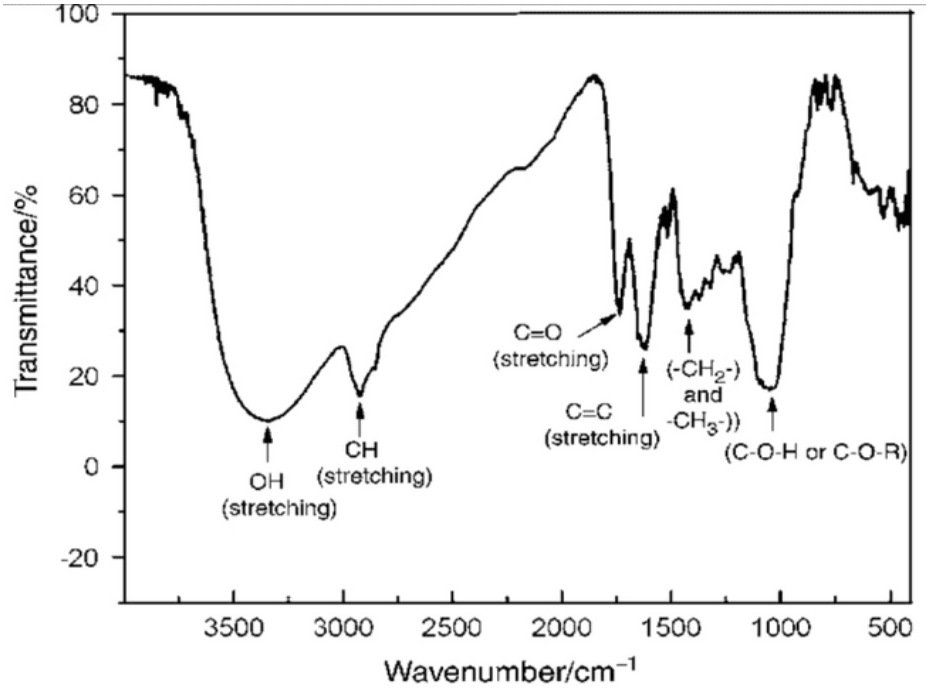

Figure 10: FT-IR spectrum of methanol extract of *Citrus sinesis* peel

3.4 SOIL ANALYSIS:

The result of the Physico-chemical factors of control soil and extract applied soil samples were analyzed for the nutritive values of soil. Nitrogen, Phosphorus, Potassium was mainly observed. After the application of extracts in soil, soil fertility is increased.

Table 2: **PHYSICO-CHEMICAL FACTORS:**

Physico chemical factors	**Control Soil**	**Treatment 1**	**Treatment 2**
PH	6.5	7	7
N (mg/g)	6.0	4.0	5.5
K (mg/g)	6.5	5.5	6.0
P (mg/g)	6.5	8.0	8.0

Treatment 1= *Luffa acutangula* (Peel extract)
Treatment 2= *Citrus sinesis* (Peel extract)

Figure 11: Control

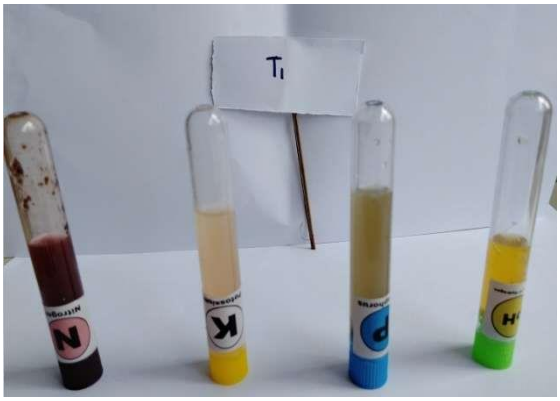

Figure 12: T1

Figure 13: T2

3.5 SEED GERMINATION AND GROWTH OF Vigna radiata

The effect of aqueous extracts of *Luffa acutangula* and *Citrus sinensis* on the growth of Vigna radiata was studied using a pot culture experiment. The results revealed that the aqueous extract of *Luffa acutangula* had a positive effect on Vigna radiata root length (8.9cm), shoot length (25cm), and dry weight (0.47mg) after 30 days of growth. The aqueous extract of *Citrus sinensis*, on the other hand, had an effect on Vigna radiata root length (8.2cm), shoot length (22.7cm), and dry weight (0.30mg) after 30 days of growth."

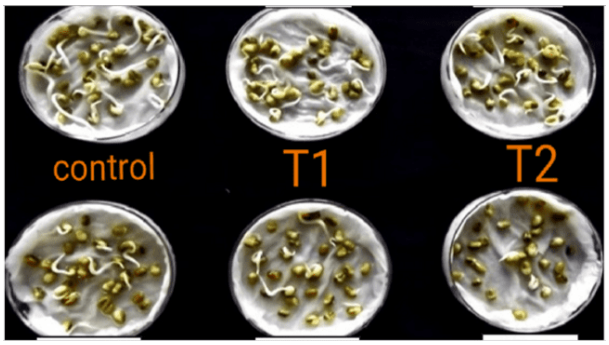

Figure 14: Growth of *Vigna radiata*

Table 3: **PLANT GROWTH ANALYSIS**

Date	Day	Control	T1	T2
Apr 9	1	0.5cm	5cm	3.5cm
Apr 10	2	2cm	5.5cm	4cm
Apr 11	3	5cm	8cm	7cm
Apr 13	5	6cm	10cm	9cm
Apr 17	8	17cm	20cm	18cm
Apr 20	11	20cm	22.5cm	21.5cm
Apr 30	21	22.7cm	25cm	22.5cm

Table 4: **EFFECT OF EXTRACT ON ROOT, SHOOT AND DRY WEIGHT OF *Vigna radiata***

	Root length (cm)	**Shoot length (cm)**	**Dry weight (mg)**
	30^{th} day	30^{th} day	30^{th} day
Control	8.2	22.7	0.30
Aqueous extract of *Luffa acutangula*	8.9	25	0.47
Aqueous extract of *Citrus sinesis*	7.6	22.5	0.36

3.6 ESTIMATION OF CHLOROPHYLL-A, CHLOROPHYLL-B, TOTAL CHLOROPHYLL AND CAROTENOID PIGMENT OF Vigna radiata

The effect of aqueous extract of *Luffa acutangula* and Citrus sinesis on the chlorophyll and carotenoid content of Vigna radiata was studied. The results reveal that the aqueous extract of *Luffa acutangula* showed 0.0085 mg/g of carotenoid pigment, followed by the aqueous extract of Citrus sinesis which showed 0.0035 mg/g, and the control showed 0.0065 mg/g of carotenoid pigment, respectively.

Vigna radiata treated with the aqueous extract of *Luffa acutangula* and Citrus sinesis showed notable amounts of chlorophyll a, chlorophyll b, and total chlorophyll. The aqueous extract of *Luffa acutangula* showed 0.0525 mg/g, 0.1011 mg/g, and 0.1536 mg/g of chlorophyll a, chlorophyll b, and total chlorophyll, respectively. The aqueous extract of Citrus sinesis showed 0.0135 mg/g, 0.0191 mg/g, and 0.0326 mg/g of chlorophyll a, chlorophyll b, and total chlorophyll, respectively. The control showed 0.0325 mg/g, 0.0781 mg/g, and 0.1106 mg/g of chlorophyll pigment, respectively.

3.7 PAPER CHROMATOGRAPHY:

The effect of aqueous extract of *Luffa acutangula* and *Citrus sinesis* on paper chromatography content of *Vigna radiata* was studied. From the sample the leaf pigments chlorophyll a, chlorophyll b, xanthophyll, and carotenoid were separated and observed.

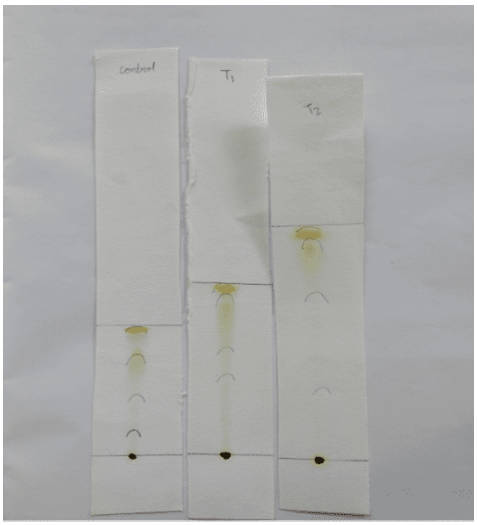

Figure 16: Paper chromatography

Table: 6 Paper Chromatography

Pigments	Chlorophyll a	Chlorophyll b	Xanthophyll	Carotenoid
Control	0.19	0.46	0.76	0.96
Luffa acutangula	0.45	0.61	0.92	0.97
Citrus sinesis	0.29	0.66	0.94	0.98

4. CONCLUSION

From the present study, it can be concluded that the aqueous extracts of *Luffa acutangula* and *Citrus sinensis* can be effectively used as natural fertilizers for plant growth. The proper utilization of their bio-waste is not only useful in increasing soil fertility and plant growth but also in decreasing pollution. Chemical fertilizers can also be replaced by these bio-waste extracts, thus protecting the soil from infertility and chemical hazards. The effect of the extracts on the control showed 89% seed germination, while the aqueous extract of *Luffa acutangula* showed 99%, and the aqueous extract of *Citrus sinensis* showed 82% seed germination. The effect of the aqueous extracts of *Luffa acutangula* and *Citrus sinensis* on the growth of Vigna radiata was studied using a pot culture experiment, and significant improvements in root and shoot length after 30 days were observed between the control and treatment groups.

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