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AEROBIC DIGESTION OF VEGETABLE AND FRUIT WASTES FOR BIOFERTILIZER PRODUCTION AND ITS IMAPACT ON THE VEGETATIVE DEVELOPMENTAND FRUIT YIELD OF OKRA

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ABSTRACT
Vegetables play an important role in human diet. They are vital sources of essential minerals, vitamins and dietary fiber. Vegetable waste make an extraordinary measure of contamination which contain high part of natural matter that causes genuine ecological and wellbeing issues. Vegetable wastes are produced in large quantities in the markets across the world. The collection, transportation and disposal of these wastes is a big problem. To make use of this waste and to reduce the pollution they can be used to produce Biofertilizers. Biofertilizer is commonly referred as the fertilizer that contains living micro-organisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substance for the plants. Organic biofertilizers are developed from the composted waste materials to supply essential nutrients to plants. The collected vegetables wastes were subjected to decomposition (1month) for producing solid biofertilizer. Since Biofertilizers are one of the best tool that is used in the agricultural field, it is applied as a replacement of conventional fertilizers. The extensive use of chemical fertilizers and pesticides caused number of deaths and illness. Hence the main objective of this study is to build a platform that Biofertilizers obtained from vegetable waste are better than the conventional fertilizers.

KEY WORDS: Vegetables, Vegetable wastes, Organic biofertilizer, Conventional fertilizer.

INTRODUCTION
Vegetables are natural sources of protective food. Vegetables provides carbohydrates, proteins, minerals, vitamins and roughages which constitute the essentials of a balanced diet [1]. Around 1.3 billion tonnes of food is wasted every year in the world. Fruits and vegetables that include tubers and roots showed the highest amount of wastage of any food. Around 8000 tonnes of food and kitchen waste is generated daily in Malaysia. Several vegetables are consumed in India, among them okra is considered one of the most popular vegetable [2] .It is valued for its edible, delicious and nutritious green pods[3]. Okra is one of the most important vegetable crop having rich diet value and also has medicinal and industrial importance[4].

Plants require a number of soil nutrients like nitrogen (N), phosphorus (P) and sulfur (S) for their growth. But, soil nutrient levels can decrease over time when crop plants are harvested, as nutrients are not returned to the soil[5]. Fertilizers are organic or inorganic, natural or synthetic substances that added to soil to enhance plant growth and production. Fertilizers are applied to replace the chemical materials in soil that are utilized by plants during growth and development. Organic fertilizers compose of only plant and animal based material while inorganic fertilizers are produced artificially. Inorganic fertilizers are synthetic and chemical fertilizers that are made up of various formulations to apply to different types of crops.

Food waste can be reduced by converting into useful form organic fertilizers to reduce health problem caused by food wastes disposal. Food wastes can be recycled into organic fertilizer to reduce amount of food waste in landfill and emission of greenhouse gas to surrounding. The use of non-chemical fertilizers and pesticides is one of the common practices that have been introduced with alternative agricultural systems, which includes the use of biofertilizer[6]. Biofertilizer are commonly referred to the fertilizer that contains living microorganisms that their activities will promote soil ecosystem and produce supplementary substance for the plants[7]. Current status of bio-fertilizers development is to give supplement sources and great soil conditions for the developments of harvests when utilized as a live body; to halfway substitute and upgrade the capacity of manure[8].

Hence, the objective of this research was to overcome the problems of the fruit and vegetable waste

accumulation in the environment by utilizing them to produce organic biofertilizer. This study also investigates the effect of the organic biofertilizer under irrigated conditions on the vegetative development and fruit yield of okra.

MATERIALS AND METHODS

MATERIALS

PREPARATION OF FIELD

The field was ploughed twice thoroughly and levelled properly. Bunds and channels were laid and ridges and furrows were formed. The size of the field plot was 6m x 10m.

MANURES

Inorganic fertilizers including urea, super phosphate, muriate of potash were collected from local Agro agency at Avalpoondurai, Erode district.

COLLECTION OF VEGETABLE WASTES

The vegetable wastes were collected from three houses located in solipalayam main street, Avalpoondurai, Erode district.The kitchen waste (i.e., vegetable waste), has been selected as composting material along with the use of fallen tree leaves and as bulking material, for carrying out the study work of aerobic composting. The ratio of vegetable waste and fallen tree leaves and dry grass was taken as 5:2 in order to adjust C/N ratio.

THE COMPOSTING PROCESS IS DONE AS FOLLOWS

30 litre plastic drum was taken. Holes were made randomly all over the sides and bottom of the drum. Holes at the sides helps for aerobic digestion and the holes at the bottom serves for effluent elimination. At the bottom of the drum a layer of coconut husks was spreaded to absorb the water contents. Above coconut husks, fallen dried leaves were spreaded that helps in fast degradation of the vegetable wastes. Then sun dried vegetable wastes (tomato, onion, potato, banana, pomegranate, grapes, etc.,) were added over it. A mixture of butter milk and cow dung were sprinkled for initiating the aerobic digestion process. The contents in the drum were mixed well either using stick (or) by rolling the drum in sideways. Then the drum was closed to prevent the mosquitoes. On every alternative day the butter milk-cow dung mixture was sprinkled and the contents were mixed well. Care should be taken that contents in the drum should not be too wet or too dry. After 45 days of this process, the organic biofertilizer will be ready.

OBSERVATIONS

- The color of the finished product is dark brown.
- It is having an earthy scent and crumbly in nature.

SEEDS

Okra (*Abelmoschus esculentus L.Moench*) was propagated through seeds. The okra seeds were bought from TNAU, Coimbatore.

EXPERIMENTAL DESIGN AND TREATMENTS

The experiment was laid out in "Randomized Block Design" with five treatments. The manures required for respective treatments of plot were weighed and applied to the soil at the time of land preparation.

Experimental code and Design for the study

S.No	Experimental Design	Different Concentration
1	Treatment-0 (T_0)	Soil (Control)-No manure
2	Treatment-1 (T_1)	10g of inorganic NPK fertilizer
3	Treatment-2 (T_2)	10g vegetable waste biofertilizer
4	Treatment-3 (T_3)	30g vegetable waste biofertilizer
5	Treatment-4 (T_4)	60g vegetable waste biofertilizer

METHODS

The present study was carried out in four phases

Phase- I: Determination of soil physicochemical properties [Before sowing and 90 DAS (at the end of the growth period)].

Phase- II: Measurement of growth characters.

Phase -III: Measurement of yield characters.

Phase- IV: Determination of biochemical components like chlorophyll, total carbohydrate and protein.

PHYSICO-CHEMICAL ANALYSIS: (Manivasakam, 1995)

Physico-chemical characteristics were done for Soil and organic biofertilizer.

Phosphate: (A.O.A.C, 1990) [9]

Total Kjeldhal Nitrogen: (A.O.A.C, 1990) [9]

Estimation of potassium (Hanway and Heidal, 1952)[10]

GROWTH PARAMETERS

The effect of different carriers and nutrients on growth and Biomass of the edible okra (Abelmoschus esculentus L.Moench) were conducted. Plant growth and Biomass of the edible crop was recorded every 30days.

SHOOT LENGTH

On the day of final count of the germination test, ten normal seedlings were selected from each treatment and in each replication. The shoot length was measured from the base of primary leaf to base of hypocotyle and mean shoot length was expressed in centimeters.

ROOT LENGTH

Ten normal seedlings used for shoot length measurement were also used for the measurement of root length. It was measured from the tip of primary root to base of hypocotyle and mean root length was expressed in centimeters.

PLANT FRESH WEIGHT (G)
Plant samples were weighed with the electrical weighing balance and recorded.

PLANT DRY WEIGHT (G)
Plant samples were placed in paper bags, these were air dried. After that, the dry weight was weight with the electrical weighing balance and recorded the dry weight.

QUALITATIVE PHYTOCHEMICAL ANALYSIS: (HARBORNE, 1973)[11]

1) Alkaloids: (Wangner's test)
Approximately 0.5g of each extract was diluted with 10ml of acid alcohol and heated to boiling, cooled and then filtered by whatman filter paper then added 2ml of dilute ammonia and 5ml of chloroform. Shaken gently to extract the alkaloidal base, the chloroform layer was extracted with 10ml of Acetic acid and 1ml of wangner's reagent (1.27g of iodine and 2g of potassium iodide) was added to the resultant extract. Formation of cream or reddish brown precipitate indicate the presence of Alkaloids.

2) Flavonoids: (Alkaline reagent test)
To 1 ml of test solution, 5 drops of 5% sodium hydroxide was added. An increase in the intensity of yellow colored solution is seen which becomes colorless on the addition of few drops of 2M Hydrochloric acid.

3) Saponins: (Foam test)
5ml of the test solution was taken in a graduated cylinder and shaken well for five minutes. A stable foam was formed.

4) Tannins: (Ferric Chloride test)
To 2ml of test solution, few drops of 5% ferric chloride solution were added. The blue color indicates the presence of hydrolyosable tannis, while the green color indicates the presence of condensed tannins.

5) Glycosides
1ml of conc. sulphuric acid was taken in a test tube then 5ml of extract and 2 ml of Glacial acetic acid with 1 drop of ferric chloride was added. Reaction shows formation of blue color.

6) Proteins: (Ninhydrin test)
Few drops of Ninhydrin reagent and 1ml of extract were added. Appearance of blue color indicates the presence of proteins.

7) Steroids
2ml of acetic anhydride was added to 0.5g of ethanolic extracts, of sample with 2ml of H_2SO_4. The color change was observed from violet to blue or green that indicate the presence of steroids.

8) Carbohydrates: (Fehlings test)
1gm of sample was added with 1ml of fehlings reagent and kept in a boiling water bath for 5 minutes. A brick red precipitate indicates the presence of carbohydrates.

9) Phytosterols: (Liebermann- Burchards test)
The extract was (2mg) dissolved in 2ml of acetic anhydride and heated to boiling, cooled and 1 ml of concentrated sulfuric acid was added along the sides of the test tube. A brown ring was formed at the junction and the upper layer turned to dark green color indicates the presence of phytosterols.

10) Quinones
A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

11) Carotenoids
1 gm of sample was dissolved in 10ml of chloroform and shaken vigorously then the mixer was filtered and 85% H_2SO_4 was added. The blue color at the interface showed the presence of carotenoids.

12) Phlobatannins
An aqueous extract of the sample was boiled with 1 percent aqueous hydrochloric acid. Deposition of red precipitate as a confirmation for the presence of phlobatannins.

13) Phenols (Ferric chloride test)
A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for the formation of deep blue or black colour.

14) Cholesterol
2ml of the extract and 2ml of the chloroform was added in a dry test tube. 10 drops of acetic anhydride and 2 to 3 drops of concentrated H_2SO_4 was added along the sides of the test tube. A red rose color was changed to blue green color.

15) Terpenoids: (Salkowski test)
Approximately 2mg of dry extract was shaken with 1ml of chloroform and few drops of concentrated sulfuric acid was added along the sides of the test tube. Formation of red brown color at the interface indicates the presence of terpenoids.

QUANTITATIVE ESTIMATION

Estimation of Protein (Lowry *et al.*, 1951)[12]

Estimation of Carbohydrates (Hedge and Hofreiter, 1962)[13]

Chlorophyll Analysis: (NayekSumanta *et al.*, 2014)[14]
500mg of fresh leaf material was ground with a morter and pestle with 10 ml of 80 percent acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved and the residue was re-extracted with 10 ml of 80 percent acetone. The supernatant was saved and the absorbance values were

read at 645 and 663 nm in a spectrophotometer. The Chlorophyll 'a', Chlorophyll 'b' and Carotenoids were calculated using the formula and is expressed in mg/ g fresh weight.

$$C_{chl\text{-}a} = 12.7\ A_{663} - 2.69\ A_{64}$$
$$C_{chl\text{-}b} = 22.9\ A_{645} - 4.68\ A_{66}$$

Carotenoid

The same plant extract used for chlorophyll estimation was also used for carotenoid estimation. The acetone extract was read at 480nm in a UV Spectrophotometer. The carotenoid content was calculated by using the following formula and it is also expressed in mg/g fresh weight.

$C_{X+C} = (1000A_{470} - 1.82\ Ca - 85.02\ C_b) / 198$

A- absorbance; C_a - chlorophyll a; C_b – chlorophyll b; C_{x+c} – carotenoid content.

RESULTS AND DISCUSSION

The present study was conducted by using soil, inorganic fertilizer and organic biofertilizer at different concentrations, such as 10g, 30g and 50g. *Abelmoschus esculentus* was taken for the present study. The results of impact of inorganic and organic biofertilizer on the soil properties, growth, yield and biochemical components of okra (*Abelmoschus esculentus (L.) moench)* are discussed under the following headings.

Physico chemical properties of soil
Growth characters
Yeild characters
Biochemical components in Leaves.

PHYSICO CHEMICAL PROPERTIES OF SOIL (BEFORE SOWING)

Before sowing of the okra seeds, the soil was analysed for p^H, EC, Texture, available N, available P and available K values and the results are given in the table-1.

Table-1 Physico Chemical Properties of Soil (Before Sowing)

PARTICULARS	LEVELS
P^H	7.9
EC (dsm^{-1})	0.09
Texture	Sandy Loam
N (Kg/ha)	90
P (Kg/ha)	43.8
K (Kg/ha)	443

Initial analysis of soil reported that the soil was normal; the texture was sandy loam. The available status of N was low and available P and available K levels were high as per interpretations given by Tamil Nadu Government soil testing centre, Erode.

Table-2 Physico Chemical Properties Of Soil Under Various Fertilizer Treatments (0 DAS).

Soil sample from following treatments	p^H	EC Dsm^{-1}	Texture	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)
T_0 - No manure (control)	8.87	0.03	Sandy Loam	145	34	226
T_1 - Inorganic fertilizer	8.8	0.04	Sandy Loam	162	38.2	246
T_2 - 10g biofertilizer	8.73	0.06	Sandy Loam	188	40.1	313
T_3 - 30g biofertilizer	8.73	0.07	Sandy Loam	192	44.0	310
T_4 - 50g biofertilizer	8.76	0.08	Sandy loam	196	43.6	326

Among different treatments, the control and inorganic fertilizer treated soil showed more alkaline than the other treatments. The increase in pH might be due to the loss of nitrogen as volatile ammonia [15].

EC of treated soils was low on 60 DAS. This might be due to utilization of minerals and their salts by the plants. The promoted mineralization of organic compost brought the nutrients to the ready use form for plants growth [16]. Low EC ensures better degradation of organic wastes [17].

The results obtained in the soil test reported that the organic biofertilizer treated soil samples were rich in the available N, P and K content than the no manure treated soil and inorganic fertilizer treated soil on 60 DAS.

GROWTH CHARACTERS

The studied growth characters include plant height, root length, number of branches and leaves and number of flowers per plant are presented as follows.

Plant Height

Plant height was measured from first cotyledonary node to the tip of the growing point on 60 DAS and the results under different fertilizer treatments are presented in the Figure-1.

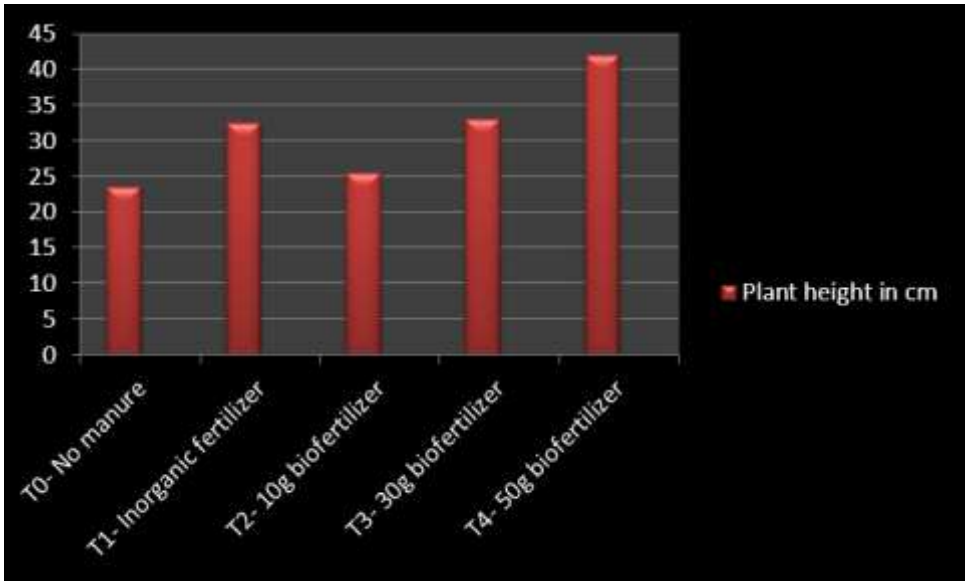

Figure: 1 influence of various fertilizers on plant height.

50g biofertilizer treated plants have registered maximum plant height than other treated plants. Nitrogen resulted in tallest plants has been reported [18].

Root Length

The length of the root was measured after the final harvest and expressed in centimetres. The results regarding the root length of plants treated with different fertilizers are presented in the figure-2.

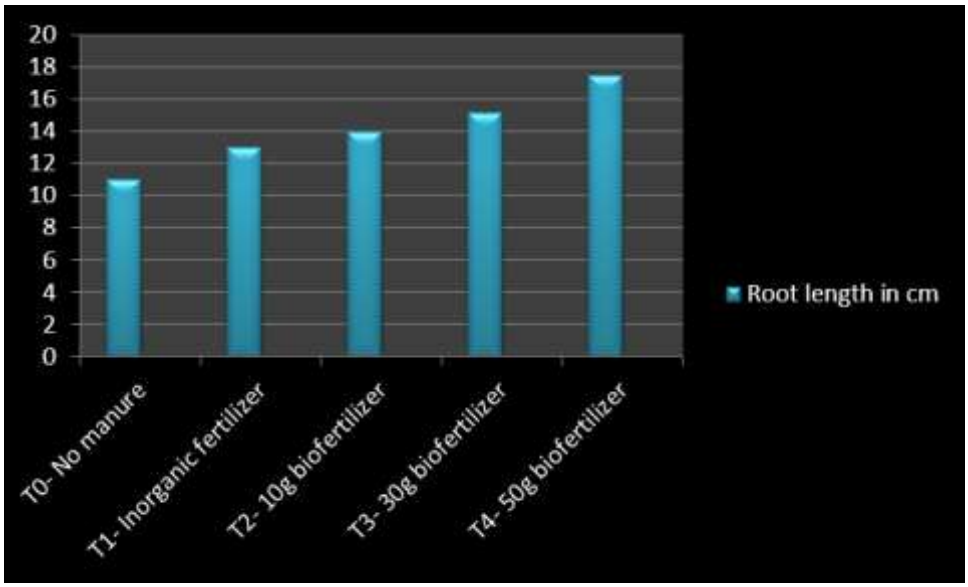

Figure: 2 Influence of various fertilizers on the root length of plants.

Significant increase in the length of the root were observed in organic fertilizer treated plants than the inorganic fertilizer and no manure treated plant.

Number Of Branches And Leaves Per Plant

In each plant the number of branches and leaves were counted until their complete growth period (upto the time of final harvest) and the results are presented in the figure-3.

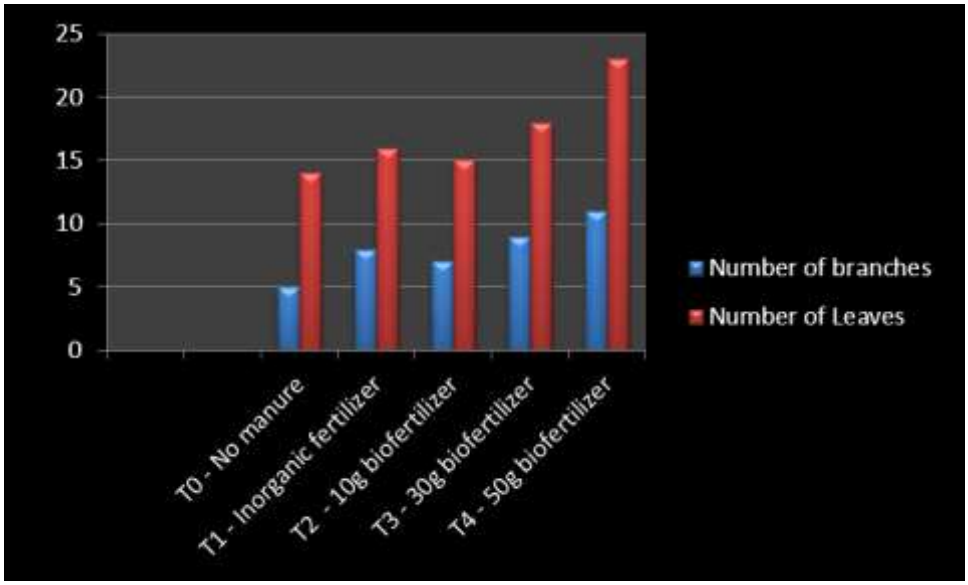

Figure: 3 Influence of different fertilizers on number of branches and leaves.

Organic biofertilizer treated plants have recorded the highest number of leaves when compared to inorganic fertilizer treated and no manure treated plants.Increase in plant growth in terms of number of leaves and plant height as a result of nitrogen is mainly due to its constituent of amino acids and enzymes[19].

Influence Of Organic And Inorganic Fertilizers On Number Of Flowers Per Plant

In each plant, the number of flowers were counted after their opening and the results were presented in the figure-4.

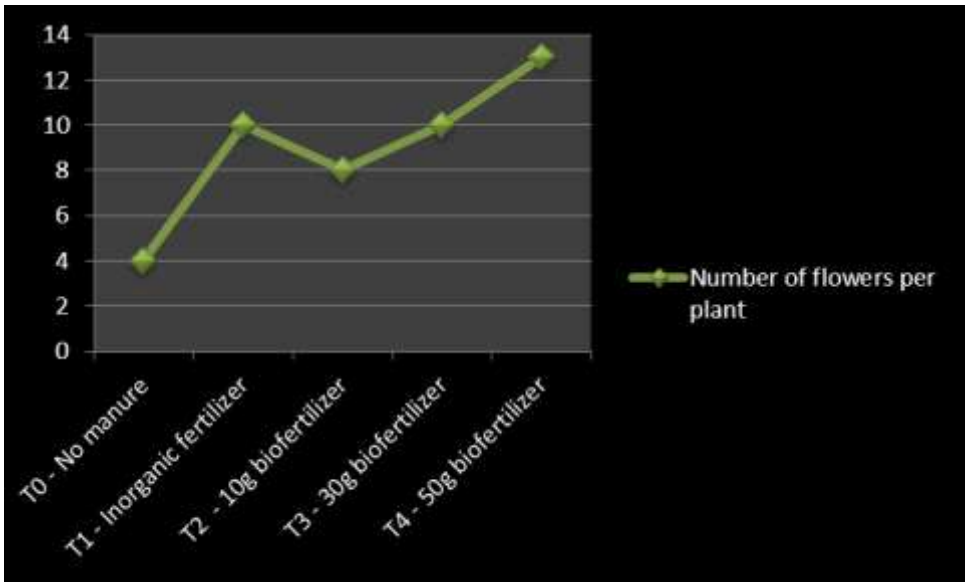

Figure: 4 Effect of various fertilizers on the number of flowers per plant of okra.

Application of organic compost led to statistically significant to improvement in the growth and flowering of crossandra compared to untreated plants [20].Application of nitrogen resulted in the maximum number of florets opening at first. Phosphorous leads information for more vegetative growth to produce quality flowers in golden rod [21].

YIELD CHARACTERS

Yield characters like number of pods per plant, pod length, pod weight, yield per plant were calculated and the results are presented in the tables.

Number of Pods Per Plant

In each plant the tender green pods picked in each harvest were counted and the results regarding the number of pods per plant under various fertilizer treatments are presented in the table – 3.

Table: 3 Effect Of Fertilizers On Number Of Pods Per Plant.

S.No	Treatments	Number of pods per plant
1	T_0 - No manure	6
2	T_1 - Inorganic fertilizer	9
3	T_2 - 10g biofertilizer	8
4	T_3 - 30g biofertilizer	10
5	T_4 - 50g biofertilizer	11

Fertilizer treatments have significant effect on the number of pods per plant. Organic fertilizer treated plants showed highest number of pods when compared to inorganic fertilizer treated plants. Fertilizer application significantly increase the number of fruits in phalsa plants.

Pod Length And Pod Weight

Pod length was measured from the position of pod fusion end to the tapering end of the pod from randomly selected pods in each treatment. Tender green pods were weighed immediately after harvest individually and the mean weight was arrived and expressed in gms.

The results regarding pod length and pod weight under various fertilizer treatments are presented in the figure-4.

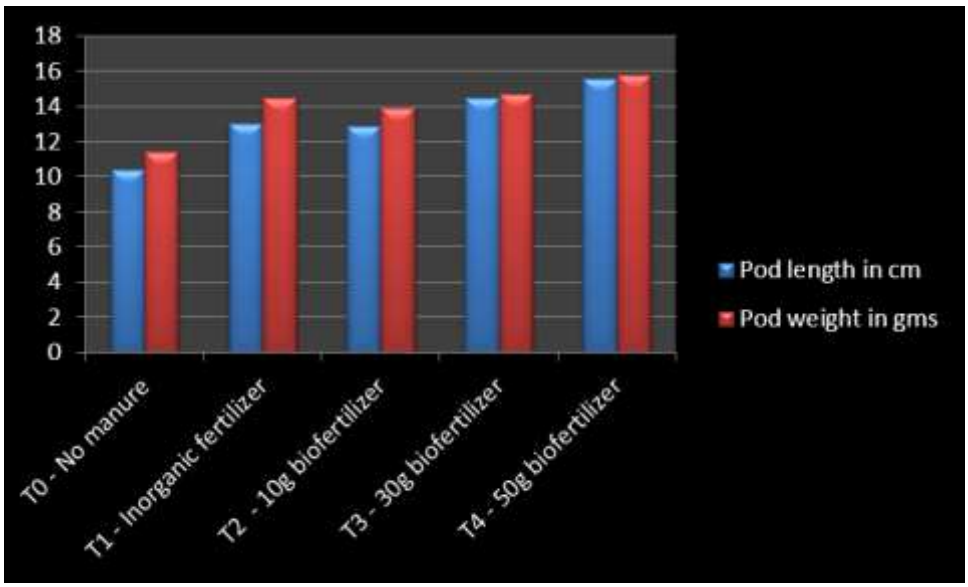

Figure: 4 Influence of Fertilizers on Pod Length And Weight of Okra.

Among different treatments the 50g biofertilizer treated plants have recorded significantly longer pods and higher weight than other treatments.

Yield Per Plant

The tender green pods from each plant were weighed immediately after each harvest. Then all the weights were added and expressed in gms per plant.

The results according to the yield per plant under different manure treatments are presented in the table – 4.

Table: 4 Effect Of Different Fertilizers On Yield Per Plant.

S.No	Treatments	Yield per plant in gm
1	T_0 - No manure	64.7
2	T_1 - Inorganic fertilizer	122.1
3	T_2 - 10g biofertilizer	147.9
4	T_3 - 30g biofertilizer	161.2
5	T_4 - 50g biofertilizer	168.6

50g biofertilizer treated plants have registered significantly maximum yield than other treatments. Inorganic fertilizer treated plants shows increase in yield than no manure treated plants.

BIOCHEMICAL CHARACTERS

Qualitative and qualitative analysis were done in Okra leaves on 60 DAS and the results are presented in the following tables.

Table: 5 qualitative analysis of *Abelmoschus esculentus* on 60th day.

S. No	Parameters	Soil (control)	IOF	10g Biofertilizer	30g biofertilizer	50g 1biofertilizer
1.	Alkaloids	+	+	+	+	+
2.	Flavanoids	+	+	+	+	+
3.	Saponins	-	-	-	-	-
4.	Glycosides	-	-	-	-	-
5.	Protein	+	+	+	+	+
6.	Steroids	-	-	-	-	-
7.	Carbohydrates	+	+	+	+	+
8.	Tannins	-	-	-	-	+
9.	Terpenoids	-	-	-	-	-
10.	Quinine	-	-	-	-	-
11.	Cholesterol	-	-	-	-	-
12.	Phenols	+	+	+	+	+
13.	Carotenoids	+	+	+	+	+
14.	Phylobotannins	-	-	-	+	+
15.	Phytosterols	-	-	-	-	-

(+) indicate present, (-)indicate absent

Table-5 expressed the Qualitative phytochemical analysis. 50g biofertilizer treated *Abelmoschus esculentus* showed the maximum presence of phytochemicals.

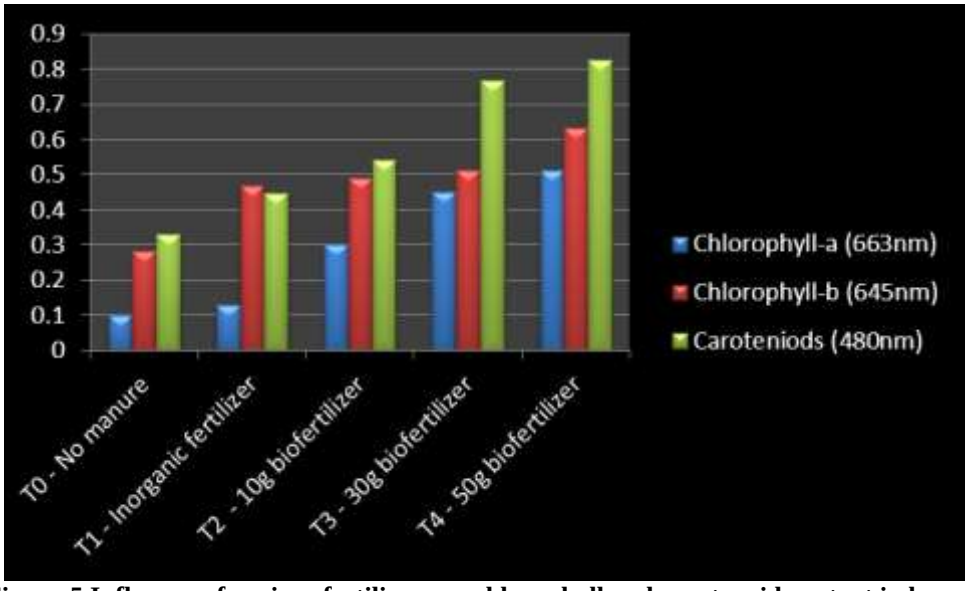

Figure-5 Influence of various fertilizers on chlorophyll and carotenoid content in leaves.

It was found that the chlorophyll content of the 50g biofertilizer treated *Abelmoschus esculentus* (chlorophyll a-0.512, chlorophyll b-0.632, carotenoid-0.824mg/g) and 30g biofertilizer treated *Abelmoshus esculentus* (chlorophyll a- 0.452, chlorophyll b-0.512, carotenoid-0.768mg/g) were found to be maximum when compare to the other treatments.

Table: 6 Quantitative Analysis of *Abelmoschus esculentus* on 60th day.

S.No	Parameters	Protein(µg/ml)	Carbohydrate(µg/ml)
1.	T_0 - No manure	1180	132.6
2.	T_1 - Inorganic fertilizer	1200	158.8
3.	T_2 - 10g biofertilizer	1630	200.0
4.	T_3 - 30g biofertilizer	2120	266.6
5.	T_4 - 50g biofertilizer	2450	325

Table 6 demonstrated the carbohydrate and protein content of *Abelmoschus esculentus*. It was recognized that the level of total carbohydrate and protein content of plant *Abelmoschus esculentus* were highly enchanced by

the 50g biofertilizer treatment that have the value of (325µg/g) and (2450µg/g) respectively. The optimum supply of nitrogen is related to carbohydrates utilization when abundant supply of nitrogen is available, the excess of the synthesized sugars are translocated to the fruits reported by[22]. In our study, we concluded that the plant treated with 30g and 50g biofertilizer have registered better growth and yield property.

CONLUSION

Investigations were undertaken in okra (*Abelmoschus esculentus L. Moench)* to assess the effect of application of organic biofertilizer and inorganic fertilizers on physiochemical, growth, yield and biochemical parameters. The results of the experiments are as follows:

Application of organic biofertilizer resulted in the slight different in p^H values among various treatments on 60 DAS and among various treatments, EC was low in control and high in organic fertilizer treatments. The texture was same in all the treatments. Application of different fertilizers does not change the texture of soil on 60 DAS. The residual available N content on 60 DAS was high in 50g biofertilizer treated soils than the other treatments. It was very low in no manure treated soil. The residual available NPK content 60 DAS was high in soils treated with 50g biofertilizer treated soil than other treatments.

Organic biofertilizer and IOF treatment resulted in the tallest plants and the shortest plants were seen in control. Regarding root length, the plants treated with 50g organic biofertilizer registered the highest values when compared to other treatments and control. The number of branches per plant were high in the fertilizers treated plants than control plants. Number of leaves per plant were high in biofertilizer treated plants. The number of days to first flowering were minimum in the plants treated with fertilizers and more days required for flower initiation in no manure treated plants. The number of flowers per plant was also significantly influenced by the application of all types of fertilizers. There was an improvement over control. Number of pods per plant was increased in the plants treated with 30g and 50g organic biofertilizers than other treatments.50g biofertilizer treated plant have recorded the longest pods than other treatments and no manure treated plants.

Application of organic biofertilizers increased the weight of pods than other treatments. Application of 30g and 50g biofertilizers increased the yield per plant comparably than other treatments.Chlorophyll a and Chlorophyll b content was high in 50g biofertilizer treated plants than other treatments. The total chlorophyll and carotenoid content was high in 50g organic biofertilizer treated plants. The total carbohydrate and protein content was high in biofertilizer treated plants.

15 Qualitative phytochemical tests were tested. It was clearly understandable that the alkaloids, flavanoids, protein and carbohydrates were present in all leaf extract. From the present study, observed that the application of organic biofertilizer enhance the physicochemical properties of soil and growth characters of okra compared to inorganic fertilizer. 50g organic biofertilizer treatment increase the yield attributes and also the biochemical characters compared to other treatments.

Hence it can be concluded that application of increased amount of organic biofertilizers has the potential for improved soil nutrient contents, plant growth, yield and biochemical properties of okra. This experiment demonstrated the effectivess of vegetable wastes which can be used as a organic biofertilizer instead of chemical fertilizer. Hence it is recommended to use vegetable waste as good fertilizer for plant growth.

REFERENCE

1. Maurya, S.K, Amit Aswathi and J.P. Singh,2004. Vegetables of medicinal value, Indian Farmer's Digest. Feb-2004.
2. Kaur,B. Studies on the physical characteristics of local varieties of fresh okra *(Abelmoschus esculentus. L)* cultivars of different maturities available in Northern India, *India,.Fd.Pack.* 1988; 42(3): 37-44.
3. Girase,Y.P., U.D. Chavan and J.K. Chavan,. Mucilage from okra (*Abelmoschus esculentus (L) moench*) cortex Extraction and cultivar Evaluation. *J. Food sci.Technol*. 2003; 40(1): 118-119.
4. Dademal, A.A., J.H. Dongale,. Effect of manures and fertilizers on growth and yield of okra and nutrient availability in lateritic soil of konkan.J.soil and crops. 2004; 14(2): 278-283.
5. Lone MI, Zhen-li H, Stoffella PJ, Yang X. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J Zhejiang Univ Sci B., 2008; 9(3): 210–220.
6. Kanimozhi.K. and Panneerselvam, A. *Der Chemica Sinica*, 2010; 1(3): 138-145.
7. Parr TW, Ferretti M, Simpson IC, Forsius M, Kovacs-Lang E Towards a long-term integrated monitoring programme in Europe: Network design in theory and practice. Environ. Monit. Assess. 2002; 78: 253-290.
8. Susilawati Kasim, Osumanu Haruna Ahmed and Nik Muhamad Abd. Majid., *Effectiveness of Liquid Organic-Nitrogen Fertilizer in Enhancing Nutrients Uptake and Use Efficiency in Corn (Zea mays), African Journal of Biotechnology*, 2011; *10(12): 2274-2281.*
9. A.O.A.C. Association of Official Analysis Chemist. "Official Methods of Analysis of the AOAC". 15th Edison, Wahington, D.C; 1990; 76-79.
10. Hanway, J.J. and H. Heidal,. Soil analysis methods as used in Iowa State College Soil Testing Laboratory, Iowa State College Agric Bull. 1952; 57: 1-13.

11. Harborne JB, Phytochemical Methods. A Guide to Modern Techniques of plant analysis, Chapman and Hall Ltd, London, 1973; 49-188.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, "Protein measurement with the folin phenol reagent", *J Biological chemistry* 1951; 193: 265-275.
13. Hedge JE, Hofreiter BT, In carbohydrate chemistry academic press. Newyork, 1962.
14. Nayeksumanta, Choudhury Imranul Haque, Jaishee Nishika, Roy Suprakash, "Spectrophotometric Analysis of chlorophyll and carotenoids from commonly Grown Fern Species by using various Extracting solvents", *Res ,J, chem.. Sci* 2014; 4(9): 63-69.
15. Hartenstein, R and F. Hartenstein,. Physicochemical changes effected in activated sludge by the earthworm *Eisenia foetida*. *J. Environ. Qual.* 1981; 10: 377-382.
16. Daniel, T., N. Karmegam and K. Alagumalai,. Vermiculture. An approach for sustainable agriculture. *J. Ext & Res.* 1999; 1(2): 16-23.
17. Logakanthi, S.J., B. Rajesh and G.S. Vijayalakshmi,. Biodung – vermiomposting. A Novel method for green waste reyling, *JEP*. 2000; 20(11): 850-854.
18. Rajiv kumar and R.L. Misra,. Response of gladiolus to nitrogen, phosphorous and potassium fertilization. *Journal of ornamental Horticulture.* 2003; 6(2): 95-99.
19. Sehrawat, S.K., D.S. Dahiya Sukhbir and G.S. Rana,. Growth, flowering and orn production in Gladiolus as influenced by NPK application. *Haryana J.Hort.Sci.* 2003; 32(3 & 4): 222-224.
20. Gajalakshmi, S., and S.A.Abbasi,. Neem leaves as a source of fertilizer – cum – pesticide vermicompost. *Bioresour Technol.* 2004; 92(3): 291.
21. Lale,S.R., Effect of marofauna on soil properties in tropical ecosystems.*Agrric. Ecosystems Environ.* 1988; 24: 101-116.
22. Arora, SK., Singh, Inder, M.L. Pandita and I. Singh,. Effect of nitrogen fertilization and plant geomentry on quality indices of tomato *(Lyopersicon esuclentum Mill). Indian journal of Agric. Sci.* 1993; 63(4): 204-207.