APPLICATION OF WATER HYACINTH VERMICOMPOST ON THE GROWTH OF *Capsicum annum*

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ABSTRACT:

The water hyacinth has been developed into biofertilizer by vermicomposting through two methods. Samples have been collected from Kanakkan Yeri, Pondicherry, India. The earthworm chosen for this study was *Eudrilus eugeniae.* Vermicompost has been prepared using *Eudrilus eugeniae*. In the present study, two methods were followed. In one method, water hyacinth waste was collected composted by using *Eudrilus eugeniae*. In the other method, the cellulose present in water hyacinth was hydrolyzed enzymatically and composted by using *Eudrilus eugeniae.* The vermicompost was collected from both the methods and used for analyzing enzymes, physicochemical parameters, level of macro and micronutrients. The efficacy of the prepared vermicompost has been studied on the vegetable plant *Capsicum annum*. Germination time, growth of the plant, number of the leaves has been studied. Finally, it has been compared with the plants which were grown using chemical fertilizers (NPK).

KEYWORDS:

Vermicompost, water hyacinth, Biofertilizer, *Capsicum annum.*

INTRODUCTION:

The practice of vermiculture is at least a century old but it is now being received worldwide with diverse ecological objectives such as waste management, soil detoxification, regeneration and sustainable agriculture. [1]. Water hyacinth is one of the worst weeds of the world [2]. It has failed all attempts of eradicating it by chemical, biological and mechanical [3]. Vermicompost, a very potential organic input for agriculture, contains beneficial microorganisms, both major (N, P, K) and micronutrients, enzymes and hormones [4]. Adding of vermicompost to soil improves the chemical and biological properties of soil and hence improves its fertility [5]. Vermicompost helps to improve the soil quality. So that it helps for the plants to grow very rapidly without use of any chemical fertilizers. By that environmental problems will be solved. Water hyacinth helps to absorb heavy metals from polluted water. Later on it will create eutrophication and that problem also resolved by vermicomposting. It restores microbial population which includes nitrogen fixers, phosphate solubilizers etc. It provides major and micro- nutrients to the plants also improves soil texture and water holding capacity of the soil, which provides better aeration to soil, thereby improving root growth and multiplication of beneficial soil microorganisms. It also decreases the use of pesticides. Improves structural stability of the soil, thereby preventing soil erosion and enhances the quality of grains/ fruits due to increased sugar content. In the present study, two methods were followed. In one method, water hyacinth waste was collected composted by using *Eudrilus eugeniae*. In the other method, the cellulose present in water hyacinth was hydrolyzed enzymatically and composted by using *Eudrilus eugeniae.* The vermicompost was collected from both the methods and used for analyzing enzymes, physicochemical parameters, level of macro and micronutrients.

MATERIALS AND METHOD

(1) Vermicompost By Two Methods

(1.1) Method 1 (V1)

The water hyacinth waste was collected from nearby lake, Kanakkan Yeri in Pondicherry, India. The collected wastes were allowed to partial decomposition for 25 days. The waste was mixed with of cow dung and added to mud pot which was meant for vermicompost preparation. Water was sprinkled every day. The stirring of the mud pot was carried out every day to remove methane and the other gases from the mud pot. The initial degradation was carried out for 4 days. On fifth day earthworms (*Eudrilus eugeniae*) were added in the mud pot. Everyday water was sprinkled on the mud pot. After 45 days vermicompost was obtained and it was used for further analysis and efficacy study.

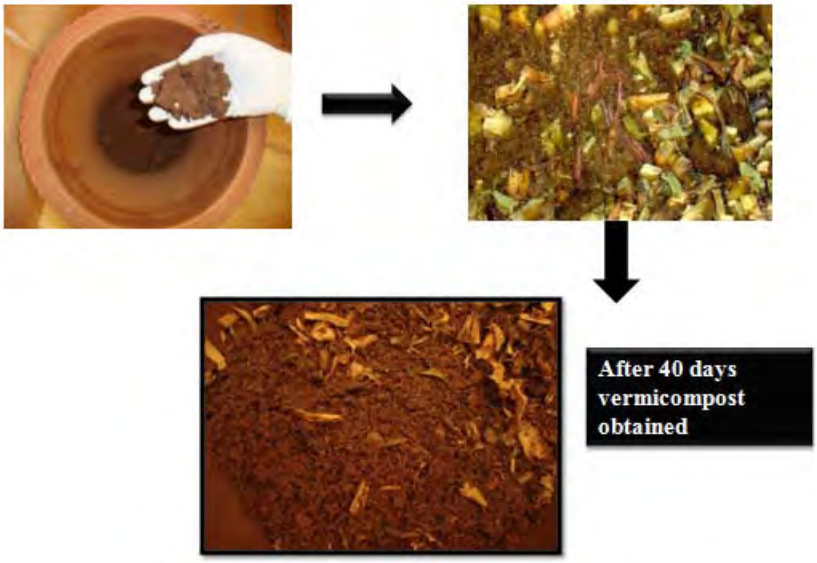

Fig. 1 Vermicompost obtained by method 1 (V1)

(1.2) Method 2 (V2):

The water hyacinth waste was collected and was allowed to partial decomposition for 25 days. Then the cellulose present in water hyacinth was degraded with *Trichoderma reesei*. After the degradation of the cellulose, the wastes were mixed with cow dung, added to mud pot which was meant for vermicompost preparation. Water was sprinkled every day. On fifth day earthworms (*Eudrilus eugeniae*) were introduced in the mud pot. Everyday water was sprinkled. After 15 days vermicompost was obtained and it was used for further study.

(1.3) Enzymatic Hydrolysis In Method 2 [6]:

For enzymatic hydrolysis of water hyacinth with *Trichoderma reesei*, inoculums (2mL) was inoculated into an Erlenmeyer flask (250mL) after sterilization containing hydrolysis medium (50mL), which comprised: water hyacinth, 2g; and Mandel & Sterberg's mineral media (Sternberg D, 1976), 50mL [$(NH_4)_2$ SO_4, 1.4g/L; KH_2PO_4, 2.0g/L; $CaCl_2$, 0.3g/L; $MgSO_4.7H_2O$, 0.3g/L; $FeSO_4$. $7H_2O$, 5.0g/L; $MnSO_4.H_2O$, 1.6mg/L; $ZnSO_4.7H_2O$, 1.4mg/L; and $CoCl_2$, 2.0 mg/L]. Initial pH of the medium was adjusted to 5.0 before being autoclaved at121°C for 15 min. Culture in Erlenmeyer flask was incubated at 30°C for 5 days. Culture was filtered through muslin cloth. Filtrate culture was centrifuged at 7200 rpm for 10 min and supernatant was analyzed for cellulose and pellet has been used for vermicompost.

Fig. 2 Vermicompost obtained by method 2 (V2)

(2) Analysis Of Nutrient Status:

Nutrient status was analyzed for vermicompost. Nutrient analysis was carried out for N, P, K, Fe and Cu

(3) Nitrogen Estimation [7]:

0.5 gm of the sample was taken in aluminum foil and then was put in a microkjeldhal flask. The catalyst mixture was added and digestion was carried out. Sample was heated on flame for 10-30 min till charred. The flask was rotated until the organic matter destruction and till gray colored solution obtained. Digested sample was then diluted with 10 ml of distilled water and 5ml was taken in condensation flask, it was then heated till solution boils. At the end titration was carried out with 0.1 ml HCl by adding phenolphthalein indicator. End point - purple to pink.

Calculations:

%N = (A-B) X N of HCl X 1.4/ wt. of the sample.

A= ml of HCl used, B= ml of HCl used for blank

(4) Phosphorus Estimation [7]:

1 gm of dried sample was taken and 200 ml of 0.002 N H2SO4 was added in it and mixture was stirred for half an hour. Solution was then filtered through Whatman filter paper no. 42. 5 ml of filtrate was taken and 2 ml of ammonium molybdate along with 05 drops of SnCl2 was added in it. Total volume of the mixture was made to 100 ml with distilled water and absorbance was taken at 690 nm. Standard graph was plotted and readings were extrapolated.

Calculation:

% Available Phosphorus = mg/l of sample/50.

(5) Estimation Of Physicochemical Parameters:

(5.1) pH [8]:

5ml of finely powdered vermicompost was taken in a volumetric beaker and 50ml of distilled water was added and the pH was measured by pH meter.

(6) Estimation Of Nutrient Content:

(6.1) Estimation Of Total Nitrogen [9]:

10ml of vermicompost was measured in a measuring cylinder. Transfer to 30ml digestion flasks and add 1.9g potassium sulphate, 80mg mercuric oxide and 2 ml of concentrated H_2SO_4 to the digestion flasks. Boiling chips were then added and the samples were digested till the solution becomes colourless. After cooling the digest, diluted them with a small quantity of distilled ammonia – free water and transferred to the distillation apparatus. The kjeldahl flask should be rinsed with successive small quantities of water. 100ml conical flasks containing 5ml of boric acid solution with a few drops of mixed indicator with the tip of the condenser dipping below the surface of the solution were placed. Now add 10ml of sodium hydroxide thiosulphate solution to the test solution in the apparatus. Distill and collect the ammonia on boric acid. Rinse the tip of the condenser and titrate the solution against the standard acid until the first appearance of violet colour, the end point. A reagent blank was run with an equal volume of distilled water and subtracted the titration volume from that of sample volume.

(7) Estimation Of Total Phosphorus [8]:

Dissolve 0.2 g of pure KH_2PO_4 in 400ml of distilled water and add 25ml of 7N H_2SO_4 and make upto 1000ml to get 50ppm of phosphorus (50μg/ml). Phosphorus standards ranging from 0 to 20ppm were prepared. Pipette out 5.0 ml of this solution and made up the volume to 50ml. This contains 50ppm of phosphorus. Into a 25ml volumetric flasks pipette out 5.0ml of this solution and add 2.5ml of Bartons reagent and made up the volume to 25ml. Intensity of the colour of each standard was measured on the colorimeter and a standard curve was drawn. 1ml of vermicompost was taken in standard flasks and added 15ml of the triple acid mixture. The samples were digested over heated stand bath, made up the volume to 500ml with distilled water. Into 25ml volumetric flasks pipetted out 10ml of aliquot and added 2.5ml of Bartons reagent and the volume was made upto 250ml with distilled water. Waited for few minutes and the intensity of yellow colour developed were read at 470nm in a spectrophotometer.

(8) Estimation Of Total Potassium [8]:

1.0 ml of the vermicompost from the 100ml triple acid mixture and the volume was made upto 50ml with distilled water. Potassium contents were feed directly to the flame photometer after adjusting the flame photometer to zero with blank and standardizing with 100 ppm of potassium solution with 100 galvanometer readings. The galvanometer readings were noted. From the standard curve drawn the corresponding ppm was read. From the ppm, the percentage of potassium was calculated.

(9) Estimation Of Iron, Manganese, Zinc And Copper [10]:

1.0 ml of vermicompost was taken into microkjeldahl flasks and added 12ml of triple acid (Triple acid**:** 9:2:1 concentrated nitric acid, concentrated sulphuric acid and concentrated perchloric acid), digested the samples over heated sand bath, made upto 100 ml with distilled water. Feed the contents directly to the atomic absorption spectrophotometer with the nm of 248.3, 213.9, 279.5 and 324.8, the corresponding iron, manganese, zinc and copper were respectively estimated. Read the corresponding ppm from the standard curve drawn.

(10) Enzymes Involved In the Degradation of Complex Organic Material into Simple Compounds:

(10.1) Amylase:

1.0ml of starch solution and 1.0ml diluted enzyme was added together in a test tube, and then incubated it at 27°C for 15 minutes and then reaction was stopped by the addition of 2.0ml of dinitrosalicyclic acid reagent. The solution was heated in a boiling water bath for 5 minutes. While the tubes are warm, 1.0ml potassium sodium tartrate solution was added and then cooled in running tap water and made up the volume to 10ml by addition of 6.0ml water. Prepare a standard graph with 0-100µg maltose .Read the absorbance at 560nm and terminated the reaction at zero time in the control tubes. The amylase activity was calculated using the standard graph. Activity was expressed as mg of maltose produced during 5 minutes incubation with 1% starch.

(10.2) Cellulase:

0.45ml of 1% carboxymethyl cellulose (CMC) solution was added at a temperature of 55°C and 0.05ml of enzyme extract was taken and incubated the mixture at 55°C for 15 minutes. Immediately after removing the enzyme substrate mixture from the bath 0.5ml dinitrosalicyclic acid reagent was added and the mixture was heated in boiling water bath for 5 minutes. While tubes are warm, 1.0ml of sodium potassium tartrate solution was added and then cooled to room temperature. Water was added to make 5.0ml of volume and measured the absorbance at 540nm. A standard graph was plotted with glucose in the concentration range 100µg to 500 µg/ml. Enzyme activity was expressed as the mg glucose released per minute per mg protein.

(10.3) Invertase:

1ml of vermicompost and 1ml of 0.3M sucrose solution in a test tube and incubated it at 37^0C for 15 minutes and then reaction was stopped by the addition of 2.0ml of dinitrosalicyclic acid reagent. The solution was heated in a boiling water bath for 5 minutes. While the tubes are warm, 1.0ml potassium sodium tartarate solution was added and then cooled in running tap water. Read the absorbance at 560nm and terminated the reaction at zero time in the control tubes. Prepare a standard graph with 0-100µg glucose. The activity was calculated using the standard graph. Activity was expressed as mg of glucose produced during incubation with 0.3M of sucrose.

(11) Determination Of Total Carbohydrate By Anthrone Method [11]:

A standard was prepared by taking 0.2, 0.4, 0.6, 0.8, and 1mL of the 10 ml of standard glucose to 100 ml with distilled water. Test was prepared by taking 0.5 ml and 1ml of vermicompost. Volume is made to 1ml in all the tubes including the sample tubes by adding distilled water. 4mL of anthrone reagent was added in all the tubes. Heated for 8 minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the vermicompost.

Calculation:

Amount of carbohydrate present in 100mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

(12) Estimation Of Protein [12] :

Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the diluted 10ml of the weighed accurately 50mg of bovine serum albumin and dissolved in distilled water and made up to 50ml in a standard flask to 50ml with 0.1N NaOH in a standard flask , into a series of test tubes. Pipetted out 0.5 and 0.1ml of the vermicompost in two other test tubes and made up the volume to 1.0ml in all the test tubes. A tube with 1.0 ml of water serves as the blank and added 5.0 ml of alkaline copper solution to each tube. Mixed well and allowed to stand for 10 minutes and then added 0.5ml of Folin-ciocalteau, mixed well and incubated at room temperature in the dark for 30 min. Blue colour was developed. Readings were taken at 660nm. A standard graph was drawn and the amount of protein in the samples was calculated.

(13) Study On The Effect Of Vermicompost On Growth of *Capsicum annum*

The stem cuttings of *Capsicum annum* were grow in two different pots T_1, T_2, T_3 and T_4

T_1 - control (without vermicompost).

T_2 - vermicompost prepared by method 1.

T_3 - vermicompost prepared by method 2.

T_4 - Chemical Fertilizer NPK

After treatment, stem cuttings were carefully removed from the soil without any damage and washed in running water to remove soil particles. The length of the root, shoot and leaf counts were done.

RESULTS:

Table.1

Biochemical parameters in vermicompost V1.

BIOCHEMICAL PARAMETERS	CONTROL	VERMICOMPOST BY METHOD 1 (V1)
pH	6.2	6.9
Amylase (mg/g)	10	25
Cellulase (mg/g)	18	38
Invertase (mg/g)	3.2	5.3
Nitrogen (%)	0.16	0.46
Phosphorous (%)	0.56	1
Potassium (%)	0.82	1
Iron (%)	0.72	1.26
Copper (%)	0.18	1.27
Proteins (mg/g)	4.2	57
Carbohydrates (mg/g)	45	33.5

TABLE.2

Biochemical parameters in vermicompost V2.

BIOCHEMICAL PARAMETERS	CONTROL	VERMICOMPOST BY METHOD 2 (V2)
pH	5.9	7.2
Amylase (mg/g)	9.5	22
Cellulase (mg/g)	10	23
Invertase (mg/g)	4.6	7.3
Nitrogen (%)	0.28	0.56
Phosphorous (%)	0.56	0.98
Potassium (%)	0.92	1.26
Iron (%)	0.88	2.5
Copper (%)	0.29	1.97
Proteins (mg/g)	5.6	37
Carbohydrates (mg/g)	44	37

TABLE. 3

Study on the effect of vermicompost on growth of *Capsicum annum.*

	Control	Vermicompost 1	Vermicompost 2	NPK Fertilizer
Root Length (Cm)	2.5	3	2	3
Shoot Length (Cm)	4.5	7	10	5
Number Of Leaves	4	5	6	4

CONCLUSION:

Investigations were carried out to explore the potential of selected earthworm namely *Eudrilus eugeniae* in the degradation of water hyacinth leaves and to convert the waste into a vermicompost. The physicochemical parameter pH was tested and was found that the earthworms were sensitive to pH and that they can grow only in pH 6.9 to 7.2.The enzymes amylase and cellulase considerably increased in V2 compared to V1 .But the concentration of invertase is higher in V1 compared to V2.The macronutrient test also confirmed the presence of high concentration of potassium, phosphorous and nitrogen in method V2 compared to method to method V1. The micronutrients copper and iron was also higher in V2.The carbohydrate and protein concentration of both the methods was found by the biochemical test which indicated that carbohydrate decreased in both methods, but was found to be lesser in V2 indicating the important role of removing cellulose, thus the earth worm is able to consume the plants easily when tested with plant *Capsicum annum.* The protein concentration was 57 mg/g in method V1 and 37 mg/g in V2.The presence of protein indicates the enzymes produced by the micro organism. The vermicompost of water hyacinth was treated with plant *Capsicum annum* and their growth parameters was observed. Root length of V2 was 4cm, shoot length was 10cm, number of leaves was 6 for V2 treatment. Thus it is concluded that V2 method is more efficient compared to V1 method and plant grown using NPK.

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