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Research Article

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Effect of Vermiwash of *Eudrilus Eugeniae* on the Growth of Chilli Plant *Capsicum Annum L.*

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Abstract: Vermiwash is a rich combination of chemical compounds, macronutrients, and micronutrients that helps in the effective growth of plants. The current examination is focused on evaluating the effect of vermiwash produced with the help of earthworm species Eudrilus eugeniae in the growth of Chilli Plant (Capsicum annum). This study is outlined in a way to understand the impact of vermiwash in the growth of Chilli Plants, especially when they are sprayed with different proportions of the vermiwash produced by the *E. eugeniae*. This experiment assesses the physicochemical qualities and large-scale supplements present in vermiwash prior to the vermiwash application and a relative report was prepared on the impact of vermiwash based on different plant growth parameters. Plots were planted with *C. annum* plants and measurements of the studied parameters (shoot length, leaf length, and the number of leaves) were noted for 90 days with plants being observed in 20 days intervals. Our study results show that plants treated with the vermiwash exhibit a reduction in natural carbon(C) and an increase in macro-nutrients like nitrogen(N), Phosphorous (P), and Potassium (K). Also, there is an observed increase in the different growth parameters of the plant implying that vermiwash can help boost the growth characteristics of the plants which correspond to the ongoing sustainability trends. In conclusion, this study recommends that the liquid extract vermiwash prepared using the micro-organism *E. eugeniae* can be a powerful and quality bio-fertilizer that helps in increased uptake of supplements by plants resulting in higher development and more yield.

Keywords: Vermiwash, *E. eugeniae*, *C. annum*, physicochemical characteristics, macronutrients, bio-fertilizer, vermicompost.

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1. INTRODUCTION

India produces around 3,000 million metric huge loads of natural wastes yearly which are discarded by sea unloading, burning and land application. Wastes from home-grown, agribusiness, metropolitan and mechanical sources are the fundamental driver of natural soil contamination[1]. The cycle of vermiculture cultivating includes the use of earthworms (regular flexible bioreactors) to tidy up the climate with savvy squandering the board innovation for feasible agribusiness (otherwise called worm culture)[2]. Vermicompost, a product of decomposing the organic wastes by nightcrawlers, intervened in natural waste recycling, which is wealthy in supplements and has developed advanced substances (bio-manures)[3]. Vermicomposting is an eco-accommodating and practical innovation of disintegrating natural matter and creating natural compost that has a rich supplement level. It additionally influences soil pH, microbial growth and soil compound exercises well[4]. This approach is considered as one of the best methods for effectively utilizing waste disposal[25] and is gaining the attraction of farmers, consumers, and even the government. Different types of materials can be utilized for vermicomposting, like animal-plant wastes, industrial wastes like paper, sugar stick or cotton wastes. Health benefits of the vermicompost is reliant on the wastes used to produce them, for example: vermicompost created from Dairy cattle and Pig's Fertilizer induces increased germination and development of plants when compared to other bedding materials[5-8]. In the recent days, commercial vermiculturists have started to promote a product called as Vermiwash that contains enzymes along with the bodily fluids of earthworms that can stimulate the plant growth and yield of the crops when applied with vermiwash[9]. This vermiwash is a liquid concentrate collected after water passes through various layers of worm culture. This liquid containing excretory secretions of earthworms, is rich in plant supplements like micronutrients, macronutrients and plant growth promoting substances. This vermiwash is a good plant tonic that can be used as a foliar spray. Owing to their rich mixture of plant supplements, many vermiculturists are considering vermiwash production for benefitting their business[10]. Vermiwash can be used to enhance the growth of many plant species irrespective of their vegetation. One such plants is Chilli (*C. annuum* L.), a significant spice crop cultivated worldwide. It belongs to the genus Capsicum under the Solanaceae family. Chilli plant is a shrub seen with solid white flowers and dim green- or purple-coloured leaves that grow up to 1.5 m in stature. Chilli plant is cultivated all through warm mild, tropical and subtropical nations and is believed to be originated from South America. Chilli or hot pepper is a fundamental spice used in almost every Indian food because of its sharpness, taste, engaging scent, and flavour. Chilli fruits are rich in nutrient C, A and E. Chilli is renowned for its pleasant aromatic flavour, pungency and high colouring substance oleoresin and it is broadly utilized in culinary, drug and refreshments businesses all through the world. The current study is carried out to assess the physicochemical investigation of vermicompost applied soil and the impact of vermiwash of *E. eugeniae* on the development of plants (C. *annuum*).

2. MATERIALS AND METHODS

2.1 Experimental Animal: (E. Eugeniae)

E. eugeniae is an earthworm species and it is commonly known as the African night crawler (Figure 1). It is a large worm that rapidly grows. An adult earthworm can be from 10 mm long and 1 mm wide to 3 m long and over 25 mm wide; there will be eight setae on each section and are arranged in four sets of ventro-lateral form. The clitellum is on the fourteenth to eighteenth segments with a pair of female pores on the fourteenth segment and a pair of male pores at the joint of the 17th and 18th segments. The alimentary canal is a straight tube from the mouth to the anus. Four pairs of pseudo heart are commonly seen in the vascular system. The cocoons of *E. eugeniae* are soft, greyish white in colour, have an irregular oval shape with one side being flatter than the other. Each cocoon has long, sharply pointed, fibrous tips at the two ends. They harden rapidly with the hull becoming strong and leathery with the colour changing to orange-brown. The brown colour intensifies with time, becoming dark brown right before earthworms' hatch from cocoons. The incubation period covers 16-17 days with each cocoon producing 2-3 hatchlings after incubation.

2.2 Description of The Experimental species

Earthworms are invertebrates and vary in size. Earthworms vary in their habitat and moist conditions are usually necessary for earthworms to survive. Earthworm is a segmented worm commonly living in the soil that feeds on live and dead organic matter. They are hermaphrodites - having both male and female sex organs in the same animal. More number of chemoreceptors are located near their mouth and digestive system is differentiated into a buccal cavity, pharynx, oesophagus, crop, gizzard, and intestine. The muscular walls of the pharynx draw in food and pharynx secretes mucus. When food moves into the oesophagus, calcium is pumped in to maintain calcium levels between the blood and the food. Digestion of the food happens with the strong muscular contractions along with the help of mineral particles ingested with the food. The intestine secretes enzymes such as pepsin to digest proteins, amylase to digest polysaccharides, cellulose to digest cellulose, and lipase to digest fats. The digested food is absorbed by blood vessels present in the intestinal walls and transported throughout the body. Earthworms feed on variety of organic materials like dead leaves and other dead plant materials. They are also able to eat some living plants, dead animals, manure, and tiny animals in the soil.

2.3 Procurement And Maintenance Of The Earthworms

The epigeic worm species *E. eugeniae* was used for the preparation of vermiwash. The adult worms were safely procured from a local vermicompost vendor in a wet gunny fabric sack with native soil substrate and were kept in vermibeds made of cows' dung for mass culture of *E. eugeniae,* much similar to the collection and maintenance of Earthworms as mentioned by Vasanthi (2018)[3]. These vermibeds were prepared in an earthen cement tank (40cm tall with 58.5 cm in diameter) with cow dung and soil, maintaining an optimum temperature of 25-27°C within the vermicompost unit constructed for this experiment. The culture was periodically monitored throughout the study and was sprayed with water to retain the moisture for better survival and growth of the earthworms. Mature clitella worms from this stock culture was utilized for this experiment.

Fig 1 Image showing Mature E. eugeniae worms used in the vermiwash production

2.4 Collection Of Leaf Litter

Partially disintegrated leaf litter was powdered manually with the help of thick wooden bars. The powdered materials were sieved through a sieve net of about 1mm x 1mm to acquire a medium with particle sized under 1 mm as suggested by Reinecke and Venter[24] and was sprinkled with water regularly to get the materials decomposed. Additionally, this waste was turned up and down for proper air circulation and quicker decomposition. This trial proceeded for 15 days.

2.5 Construction Of Vermiwash Unit

A vermiwash unit was designed according to Ismail (1997)[11] with few modifications. A plastic barrel of 15 litre capacity is used with an opening made on one side and a vertical limb of the "T" joint tube is fixed in a way that half of the tube is inside the barrel. A tap is fixed to one of the horizontal end of the I joint tube and the other is closed with a net. The entire setup is set over the trip stand keeping the tap open. The barrel's bottom is filled with smooth pebbles up to 20cm height followed by 10 cm of clean, coarse gravel as one layer and another layer of clean sand or nursery soil filled for another 10cm on top of that forming the vermiwash filtration. Above the soil layer, shade-dried and powdered cow dung is added. Then water is allowed to course through these layers and drain from the tap to ensure there's no blockage of water and filtration layers are set. This forms the base of the filter unit. Over this, 30-45 cm layer of moist loamy soil is placed. Water is then added in small quantities enough to keep the substrates with 80% of moisture. To this, 500 adult earthworms *Eudrilus sp.* was released. Cow manure and other organic materials are added on top of this layer and was kept moistened every day.

2.6 Vermiwash Collection

In the vermiwash unit designed according to Ismail (1997)[11], the tap is closed and water is sprinkled on top of the unit. Elutes from the unit is prepared by sprinkling about 5L of water for 16 days. Water percolating slowly through the filter unit carries the nutrients from freshly formed worm castings present within the barrel. This elute water (referred as vermiwash) is then collected from the tap in separate containers for further use.

2.7 Experimental Plot

The experiment was conducted to study the effect of vermiwash on the growth and flowering of *C. annum* plants. The plant saplings were taken from the nursery and transplanted in different plots, like in Ansari's AA study (2008)[10]. Each experimental plot was 7.5 cm long and 20 cm wide with a 2kg capacity. The saplings were rooted with a space of 3cm at a depth of 3cm in each plot treatment and adequate moisture is maintained in these plots.

2.8 Concentration Of Vermiwash

Different concentrations of vermiwash are prepared by mixing water in the different ratios and are termed as Control (0% vermiwash), Treatment 1 (25% vermiwash), Treatment 2 (50% vermiwash), Treatment 3 (75% vermiwash), Treatment 4 (100% vermiwash). These treatments are used as a foliar spray to chilli plants[11]. Chilli (*C. annuum*) saplings were cultivated in five plots for experimenting with these different treatments of vermiwash.

2.9 Soil Analysis

Soil testing was completed for samples taken from the control treated plot before starting the experiment and later it was performed for the samples taken from vermiwash treated plots, after completion of experiment to evaluate and analyse the nutrients and physicochemical (physical and chemical) properties of the soil[12].Standard tests were carried out to determine the following parameters: (i) Soil pH, (ii) Electrical conductivity (EC), (iii) Organic carbon (OC), (iv) Nitrogen (N), (v) Phosphorus (P) and (vi) Potassium (K).

2.10 Collection Of Experimental Data

During the entire study period of 90 days, different parameters like Shoot length, Leaf length and number of leaves per plot were recorded. In ten, randomly chosen plants per plot, growth parameters were measured from the base to the tip with the help of a ruler(±0.5cm) like Getnet and Raja's study[13]. These measurements were recorded for the 10th day, 30th day, 50th day, 70th day, and 90th day. Both Shoot length and Leaf length is expressed in centimetres (cm) and count of the leaves produced by those plants were also noted.

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters

As per the analysis carried out to evaluate the fore mentioned physicochemical parameters, soil from the control plot had a pH of 7.50 with Electrical Conductivity (EC) being 0.15(dS/m), Total Organic Carbon (OC) being 1.35%, available Nitrogen(N) being 2.6%, available Phosphorus(P) being 0.59% and available Potassium(K) present being 5.8%. After the application of vermiwash, the physicochemical properties estimated to a pH of 7.71, EC of 0.17(dS/m), OC of 1.22%, available N of 2.87%, available P of 0.72%, available K of 6.2% (Figure 2).

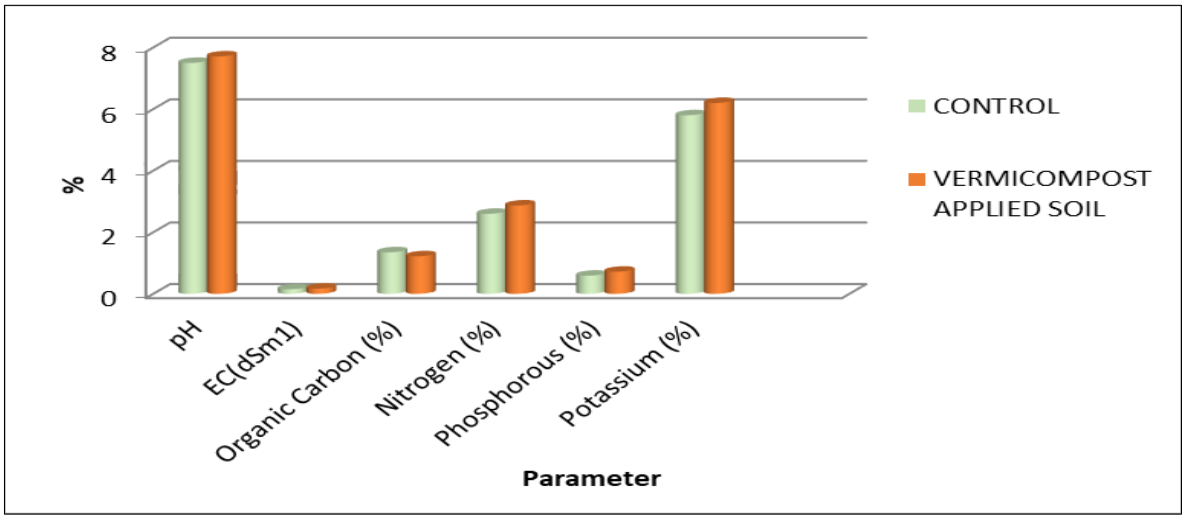

Fig 2 Graph showing the analysis of physical and chemical parameters of control soil and vermicompost applied samples

Table 1: Analysis And Determination Of Physical And Chemical Properties Of Soil Samples

Parameters	Control Soil Sample	Treated Soil Sample[1]
Soil pH	7.50	7.71
Electrical Conductivity (dS/m)	0.15	0.17
Organic Carbon (%)	1.35	1.22
Total Nitrogen (%)	2.60	2.87
Total Phosphorous (%)	0.59	0.72
Total Potassium (%)	5.80	6.20

[1] Treated Soil Sample refers to the vermiwash applied soil over a period of 90 days considered for analysis at the end of the study.

The level of pH is seen increased in the vermiwash treated soil when compared with control with a difference of 0.21 increase in the former. The increase in the pH value indicates the participation of microbes in the degradation of organic wastes through aerobic metabolism. The current results can be seen in accordance with the studies of Gajalakshmi and Abassi (2004) reported which shows that earthworms are sensitive to changes in pH and prefer conditions of neutral reaction[14]. The Electrical Conductivity observed was 0.15 dS/m in the control where treated soil had 0.17 dS/m. Although, it is not a significant increase, this is in relation to the fact that there are number of mineral salts released due to the decaying of organic matter. The Organic Carbon content was 1.35% in control and is decreased to 1.22% showing that earthworms modify the substrate particles promoting the carbon loss through microbial respiration and mineralization. This in agreement with the study reported by Bai and Vijayalakshmi (2000) which concludes that increased EC and reduced carbon in vermiwash is observed after inoculation of earthworm *E. eugeniae*[15]. Coming to the nitrogen percentage, it is 2.6% in the control and 2.87% in the vermiwash treated soil. This increase in the N content can lean on the fact that earthworms aid the increase in Nitrogen availability in soil due to the loss of organic carbon (OC), as reported by Tripathi and Bhardwaj (2004), in the form of mucus, nitrogenous excretory substances, growth stimulatory hormones and enzymes from the gut of earthworms[18]. Likewise, Potassium was 6.2% and Phosphorous was 0.72% in the treated soil which clearly is higher than the control soil. Studies by Vasanthi et. al., (2018) suggested that the enhanced phosphorous level in vermiwash applied soil may be due to mineralization of phosphorous during the vermicomposting process[12]. The increase in Potassium (K) can be attributed to the changes in the distribution of K between its non-exchangeable and exchangeable forms. This is in accordance with studies reported by Suthar (2007) suggesting that the earthworm processed organic matter contained higher concentration of exchangeable potassium due to the microbial activity during vermicomposting process[1].

3.2 *Analysis of parameters*

3.2.1 *Shoot length (cm)*

Shoot length recorded for the 10th day was 11.32 cm for control, 11.84 cm in 25%, 12.04 cm in 50%, 13.0 cm in 75% and 14.58 cm in 100% treatments. After 20 days interval (on 30th day), recorded values were 24.0 cm, 25.72 cm, 26.32 cm, 27.82 cm, 29.68 cm for the control, 25%, 50%, 75% and 100%. On 50th day, it was 30.7 cm, 32.68 cm, 33.54 cm, 34.28 cm, 36.26 cm in 0%, 25%, 50%, 75% and 100% treatments. For 70th day, the recordings were 36.48 cm, 38.76 cm, 40.54 cm, 43.56 cm respectively in 25%, 50%, 75% 100% when contrasted with control 35.96 cm. On the 90th day, the shoot length was 37.0 cm, 39.72 cm, 40.76 cm, 41.92 cm, and 44.03 cm for the different proportions of vermiwash (as represented in Figure 3). The length of the shoot recorded was 44.03 cm, with 100% vermiwash, at the end of the study and it is a significant increase from the control plot recorded value 11.32 cm. It can be observed that throughout the experiment, for different ratio of vermiwash applied, there's a significant growth seen in the shoot length of the plant. The results reported by Karmegam N (1999) and Maya M (2015) for the growth and yield of *Phaseolusaureus* (Green gram) resp. with the use of vermicompost and vermiwash, where the shoot length was recorded at the start of the experiment for the control and all treatment groups for comparison with the values recorded after the experiment, show that there is a remarkable difference seen in the length of the shoot[16,17], proving the impact of vermiwash on the growth of the plant.

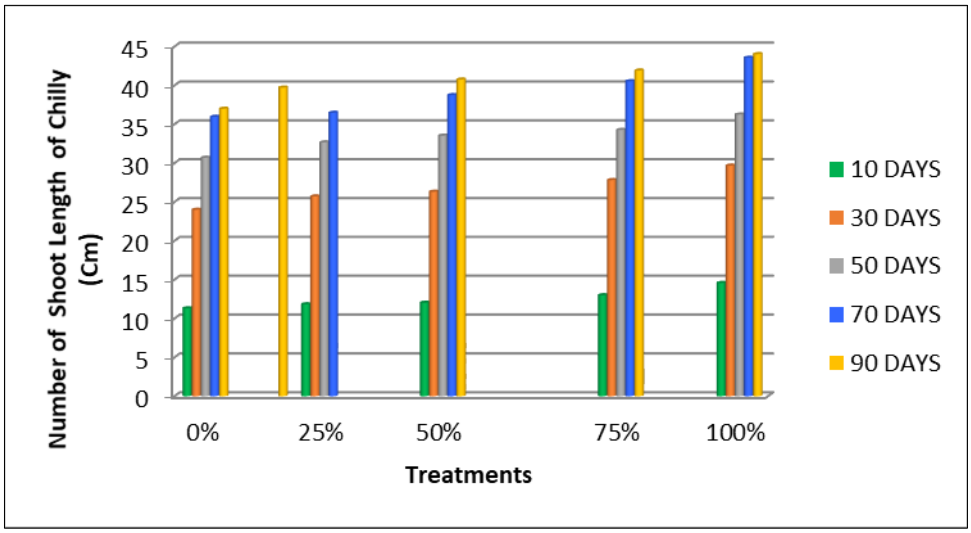

Figure 3 Graph showing effect of vermiwash on the shoot growth of the C. annum plant over 90 days period

Table 2: Effect Of Treatment On Shoot Length Of Chilly Plant In A Period Of 90 Days Study

Treatments	Number Of Shoot Length Of Chilly Plants (Cm)[2]				
	10 Days	30 Days	50 Days	70 Days	90 Days
CONTROL (0%)	11.32±0.0.4	24±0.31	30.7±0.80	35.96±1.49	37±1.85
25%	11.84±0.62	25.72±2.12	32.68±1.31	36.48±1.06	39.72±2.4

[2] Values of the shoot length measured as mean ± S.E. of three replicates.

50%	12.04±0.93	26.32±1.75	33.54±1.67	38.76±1.94	40.76±1.22
75%	13±1.2	27.82±1.9	34.28±1.0	40.54±1.7	41.92±2.3
100%	14.58±0.68	29.68±0.62	36.26±0.85	43.56±0.93	44.03±0.91

3.2.2 Leaf length (cm)

The various concentrations of vermiwash treated chilli plants were estimated for the leaf's length on the 10th day and respectively recorded as 2.3 cm, 2.8 cm, 3.4 cm, 4.0 cm, and 4.6 cm in 0%, 25%, 50%, 75% and 100 %. In the next 20 days (i.e., on 30th day), the length was again recorded, and it was observed that there was an increase in the length of leaves in all concentrations when compared to the control. Again, an increase in the leaf length was seen on 50th day as 6.88 cm, 7.16 cm, 8.26 cm, 8.98 cm, 9.6 cm in 0%, 25%, 50%, 75% and 100% concentration of the vermiwash application. On the 70th day, the observed measurements were 7.26 cm, 7.92 cm, 8.34 cm, 9.01 cm and 9.62 cm long, again showing an increase in the length of the leaf. Progressive increase in the length of the leaves was noted in all the proportions of the vermiwash on 90th day. It can be noticed that in control just 8.4 cm in leaves length was seen while 8.56 cm, 9.0 cm, 9.20 cm,9.82 cm was found in, 25%, half 75%, 100% proportions (Figure 4). This result shows that even the length of the leaf is impacted with the application of vermiwash showing that the growth of the overall plant is enhanced. This is in accordance with results obtained by Getnet and Raja (2013) whose study reported that with the application of vermicompost on the cabbage plants, their leaf length had increased compared to the untreated cabbage plants[13].

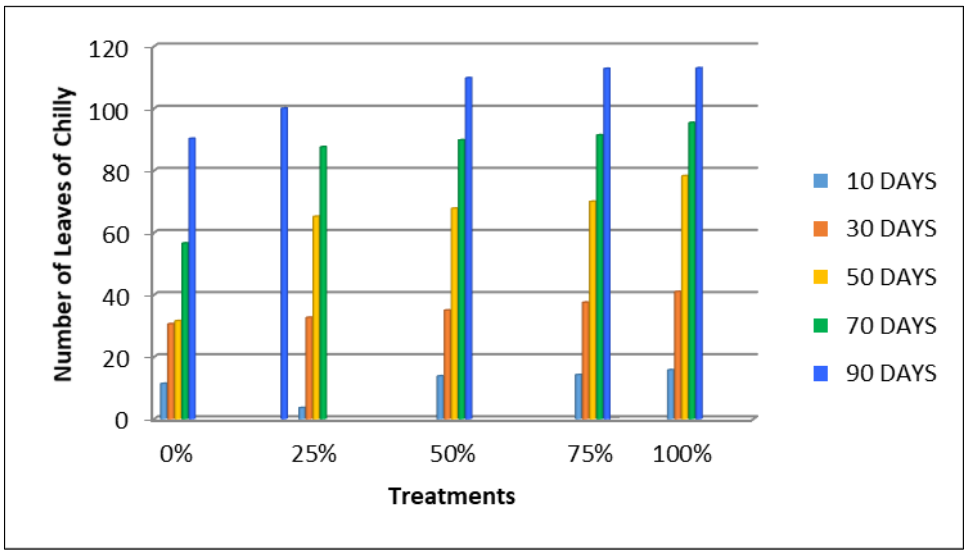

Figure 4 Graph showing effect of vermiwash on the leaves count of a C. annum plant over 90 days period

3.2.3 Number of leaves per plant (n)

Analysing the number of leaves produced by the Chilli plants with the application of vermiwash, they were 3.61, 13.82, 14.23, 15.8 on the 10th day of the experiment. In the next 20 days, the numbers recorded were 30.6 in control, 32.68 in 25%, 35.0 in 50%, 37.53 in 75% and 41.0 in 100 % showing a good increase in the leaves. The further increases in the number of leaves were noted on the 70th day with the count being 87.6 in 25%, 89.8 in 50%, 91.4 in 75% and 95.4 in 100%. It can be noticed that on the 90th day, increase in the number of leaves produced in the plants is still happening with count going up to 100 in 25%, 109 in 50%, 112.8 in 75% and 113 out of 100 % vermiwash treated plants (Figure 5). At the end of the experiment, the number of leaves kept increasing denoting that the plant growth was stimulated using vermiwash.

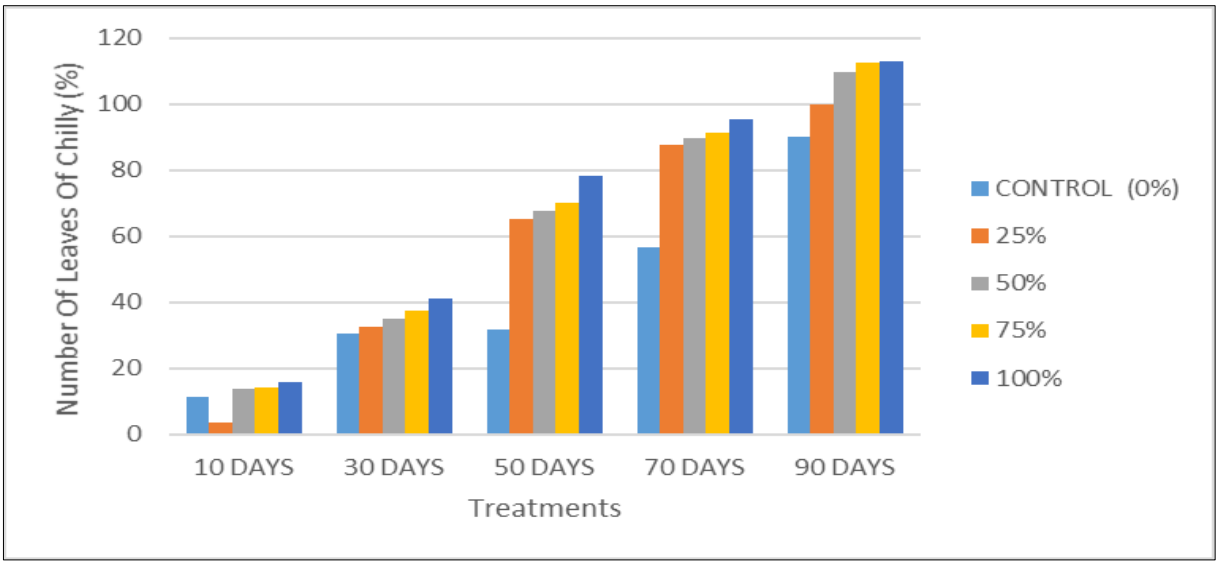

Fig 5 Graph showing effect of vermiwash on the leaf's growth of C. annum plant over 90 days period

Table 3: Effect Of Treatment To Determine Number Of Leaves Produced In A Chilly Plant In A Period Of 90 Days Study

Treatments	Number Of Leaves Of Chilly Plants (Cm)[3]				
	10 Days	30 Days	50 Days	70 Days	90 Days
CONTROL(0%)	16.4±0.11	30.6±0.13	31.6±0.14	56.6±0.15	90.27±0.17
25%	8.61±0.15	32.68±0.17	65.2±0.0.1	87.6±0.22	100±0.30
50%	15.82±0.02	35±0.08	67.8±0.11	89.8±0.18	109.8±0.09
75%	16.23±0.01	37.53±0.07	70±0.09	91.4±0.11	112.8±0.17
100%	18.8±0.04	41±0.31	78.26±0.80	95.4±1.14	113±1.85

[3] Values of the number of leaves given as mean ± S.E. of three replicates.

The vermiwash may possess cytokines, auxins, amino acids and vitamins, enzymes possibly derived from microbes associated with earthworms. Vermiwash at a higher dilution can increase the growth rate and enhance the number of leaves. The results obtained in the current study have corroborated the results of Shivasubramanian and Ganesh Kumar (2004)[9] on marigold and with Lalitha et al (2000)[22] related to Okra. Highest leaf yield was recorded of vermiwash spray. Bio foliar induced promontory effect due presence of growth inducing substances, essential amino acids, vitamins and plant nutrients. The results are in accordance with the Singh V et. al., (2001)[23] who have reported that 15.17 (%) leaf yield was increased by the foliar application of salicylic acid in mulberry.

Figure 6 Image showing the Control Plants and Vermiwash treated Plants (in order)

4. CONCLUSION

Various varieties of earthworms produce natural manure from agriculture and livestock wastes. Earth's waste is abundant in nitrogen, potassium, and phosphorus, as well as many micronutrients, enzymes (such as proteases, cellulose, amylases, and lipase), plant growth hormones, and some nitrogen-fixing bacteria that strengthen vermicomposting nutrient value. It also improves the soil structure, the ability to maintain the environment, soil texture, and reduces soil erosion, thereby producing good, harmless, and tasty food from the soil. It is a cost-efficient and easy method that can be followed easily by the rural farmers for their profit. As per this study, the impact of vermiwash on the chilli plant had provided a better overall growth result. In any given condition, it is to be accepted that vermiwash can become a viable bio-manure when directly sprayed onto the growth of plants. It was also observed that the plants treated with vermiwash were diseases resistant and no worms were seen on leaves and other parts of the plants. Vermiwash showed the potential application in sustainable development of agriculture with respect to its origin, cost effectiveness, easy availability, time saving, reproducibility, reliability, and eco-friendliness.

5. AUTHOR CONTRIBUTION STATEMENT

Dr. Vasanthi Padmanaban conceptualized, discussed the methodology & results in the final manuscript, and analysed the results. The author read and approved the final version of the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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