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Influence of Various Substrates in Vermicomposting Its Application on Different Plants (*Abelmoschus esculentus, Capsicum annum and Trigonella foetum-graceum)*

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Abstract- It is estimated that India produces about 4.3 million compost per year. Composting is nothing but a biological process of converting solid waste into organic matter or fertilizers, which can be useful for the plants. Vermicomposting is an ecological box that is an environmentally friendly technique that employs earthworms to convert solid waste into manures, which also contains hormones and enzymes that may act as plant growth hormones. Hence, the present study focuses on Vermicomposting's process using different substrates (Sugarcane waste, Sheep manure, Paper waste, and Sewage sludge) by the mixture of two different earthworm species, *Eisenia fetida* and *Eudrilus eugeniae*. Further, their physicochemical characteristics were evaluated. Different microorganisms, like Phosphate solubilizing bacteria (PSB), Nitrogen-fixing bacteria (NFB), and Microflora, were isolated from the gut of earthworms to show the activity of plant growth promoting microbes present in earthworms. The isolated bacterial strains were characterized biochemically and molecularly identified. Finally, the vermicompost was applied as a fertilizer to study plant growth.

Keywords- Vermicomposting, TKN, TOC, lady's finger, chilli, and fenugreek

I. INTRODUCTION

Dumping solid waste in an open environment is a majorly rising problem in the modern world, especially in developing countries like India [1]. An increase in the human population, industrialization, and agricultural activities have increased waste materials accumulation.

The solid waste management is a great challenge for the scientist to reduce the wastes. To use a sustainable way to reduce waste, such as earthworms, is an environmentally friendly method [2]. Vermicomposting is an eco-biotechnological process in which soil contains hormones and enzymes that stimulate plant growth and inhibit plant pathogens. Earthworms play a crucial role in soil biology by serving as versatile nature's bio-reactor to effectively harness. The beneficial soil microflora, converting organic waste into valuable products such as bio-fertilizer, biopesticides, vitamins, enzymes, antibiotics, and pertinacious worm bio-mass [3]. Phosphate solubilizing bacteria (PSB) can hydrolyze organic and inorganic phosphorous from insoluble compounds [4,5]. Phosphorous is one of the significant essential macronutrients for plants and becomes unavailable to plants due to its unavailability. PSB converts these insoluble phosphates into soluble form through acidification, chelation, exchange reactions, and gluconic acid [6,7]. Nitrogen-fixing bacteria are microorganisms present in the soil or plant roots that can change atmospheric nitrogen into stable nitrogen compounds that plants can use. There are two forms of bacteria, free-living bacteria and mutualistic bacteria [8]. Vermicompost technology has been a promising method to meet the organic manure requirement in irrigated and rain-fed areas. It has tremendous prospects in converting agro-wastes and city garbage into valuable agricultural input. Thus, various economic uses can be obtained from organic wastes and garbage that can prevent pollution. In this process, earthworms get multiplied, and the excess worms also get converted into vermiprotein, which can be used as poultry, fish feed.

The present work was to evaluate the effect of different substrates on vermicompost production using the vermitechnology. In the study, two different earthworms (*Eisenia fetida* and *Eudrilus eugeniae)* were studied with different substrates (Sugarcane waste, Sheep manure, Paper waste, and Sewage sludge), and their effects were evaluated on the plant growth. During the process, different physicochemical parameters like pH, organic carbon, and total nitrogen for a period of 45 days. The microbial interactions with vermicomposted substrates, Phosphate solubilizing bacteria, Nitrogen Fixing Bacteria, and Fungi were isolated from earthworms' gut. Vermicompost soil stimulates the root, leaves, and stems the plant's height by facilitating the nutrient absorption and thereby favouring higher yield. Further, the vermicomposted soil was used to study the growth of Lady's finger, Fenugreek, and Chilli on different vermicompost substrates.

II. MATERIALS AND METHOD

PREPARATION OF VERMICOMPOSTING TRAY-COLLECTION OF EARTHWORMS, SUBSTRATES, AND BEDDING MATERIALS

The species of earthworms such as *Eudrilus eugenia* and *Eisenia foetida* were procured from Stella Maris College Nursery. Sugarcane waste was collected from Sugarcane shop at Triplicane, treated sewage sludge from Nesapakkam Sewage Treatment plant, paper waste from Stella Maris College, Chennai, and sheep manure from Thrivallur district. Sawdust and Coir pith was used as bedding material and collected from Triplicane, Chennai. The Vermi Bed was prepared according the standard protocol [9,10].

EXPERIMENTAL DESIGN OF VERMICOMPOSTING PIT

In the experimental design, various bedding materials such as gravel, sand, soil. Leaflets, rice straws, banana leaves, grasses, coir pith and sawdust, and different substrates such as dung of cow and buffalo, sheep manure, sewage sludge, and waste sugarcane and paper. Sixteen combinations of treatment with the size of 30 cm × 11 cm × 5 cm and covered with drapery with micro-holes were performed and denoted as C1, C2, C3, S1. S2, S3, S4, S5, S6. C1, C2, and C3 served as control. The first set up of vermibed consists of gravel, soil, coir pith, sugar cane waste, cow dung, buffalo dung, rice straw, leaflets, and sheep manure in the ratio of 4:1. The second experimental setup consists of gravel, soil, coir pith, hay, sawdust, cattle manure, sewage waste, and paper waste in the ratio of 4:1 as in Figure S1 and Figure S2 [11]

Figure S1: Vermicomposting setup

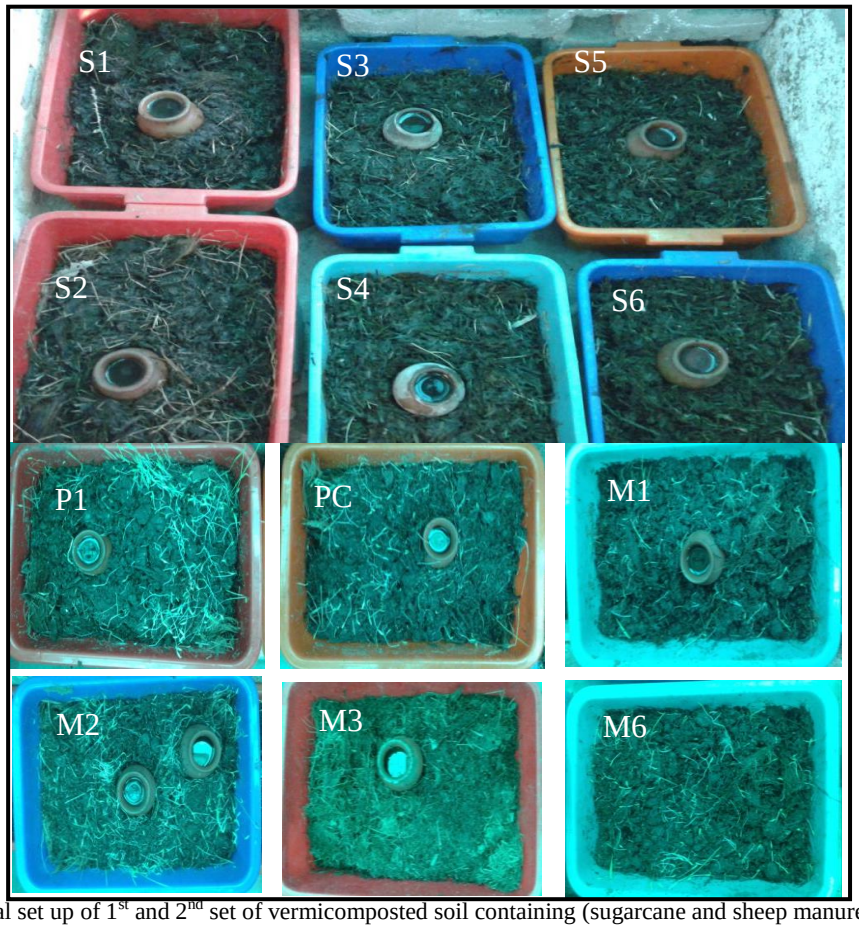


Figure S2: Experimental set up of 1st and 2nd set of vermicomposted soil containing (sugarcane and sheep manure) and (paper waste and sewage waste) (A) P1 (B) PC (C) M1 (D) M2 (E) M3 (F) M4 (G) M5 (H) M6 (control)

PHYSICAL PARAMETERS AND NUTRIENT ANALYSIS OF THE VERMICOMPOST

Physical properties such as pH, temperature, moisture content was determined. Nutrient analysis such as total organic carbon using walkey and black method, total Kjeldahl method was determined [12,13]

ISOLATION OF BACTERIAL STRAINS FROM THE GUT OF EARTHWORMS

Earthworms (*Eudrilus eugeniae*) collected from the vermicomposting unit were washed and cleaned externally with 70% ethanol. The midgut of the earthworm was excised, and gut content (1g) was suspended in 10ml of sterile 0.85% NaCl solution, serially diluted (10-1 to 10-7) and plated on Pikovskaya agar medium, N2 free glucose medium, and Sabouraud dextrose agar and incubated at 37°C for 24 -48hrs [14,15]

EFFECT OF TRICHLOROPHOSPHATE(TCP) ON BACTERIAL STRAIN

TCP's effect in Pikovskay's medium using different concentrations tricalcium phosphate such as (25mg/l, 50mg/l, 75mg/l, 125mg/l) [16] was studied at 540nm.

CHARACTERISATION STUDIES

Morphological identification such as Gram's staining and motility test and biochemical identification such as catalase, urease, IMViC, and TSI were performed. Further genomic DNA extracted, amplified, sequenced, and the bacterial strains were identified, a phylogenetic tree was constructed [17,18].

III. RESULTS

COMPOSTING MATERIAL USED IN THE STUDY

In the present study, a different experimental setup was used for each substrate used for vermicomposting. Figure 1 shows the control substrate without earthworm, and there is no degradation observed. Figure 2 shows an experimental setup using sugarcane waste, sheep manure, paper waste, and sewage waste.

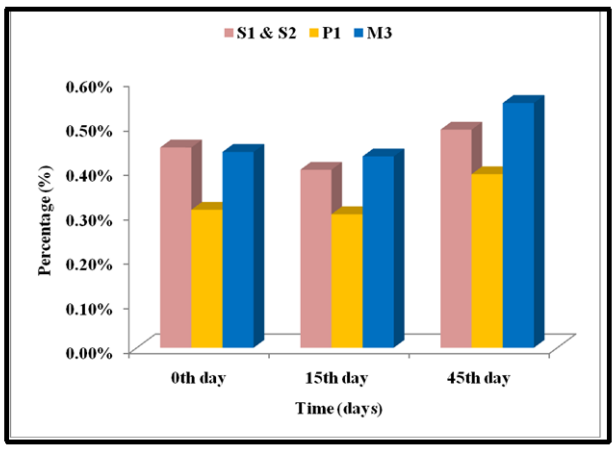


Figure 1 Total organic carbons of the vermicompost substrates

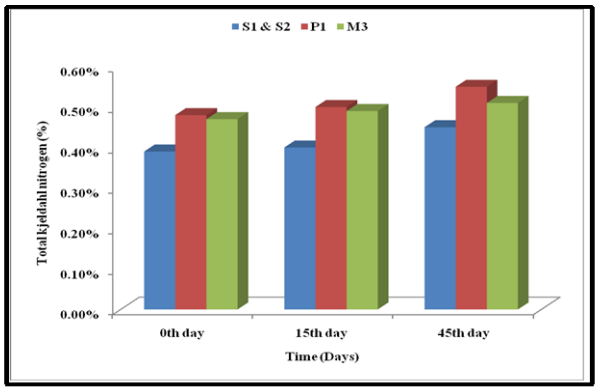


Figure 2 Total kjeldahl nitrogen of the vermicompost substrates

PHYSICOCHEMICAL PARAMETERS OF VERMICOMPOST

The present study temperature of the 1st set of the vermicomposted substrate (sugarcane waste and sheep manure) was studied. In S1 and S2, the temperature on 0th day showed 28° C. At the end of 45th day, the temperature increased to 26° C.The temperature of control (C2) was 29° C on the 0th day, at the end of 45th day, it was 28° C. In the 2 rd set of vermicomposted substrates (Paper waste, sewage sludge) the Control (PC) showed 29° C on the 0th day and decreased to 27°C at the end of 45thday respectively. The temperature of M1 was 29°C on the 0th day and decreased to 26°C on the 45thday. The temperature of M5 was 28°C on the 0th day and increased to 31°C at the end of the 45thday. The temperature of M6 was 28°C on the 0th day and increased to 32°C at the end of the 45thday (Figure S3).

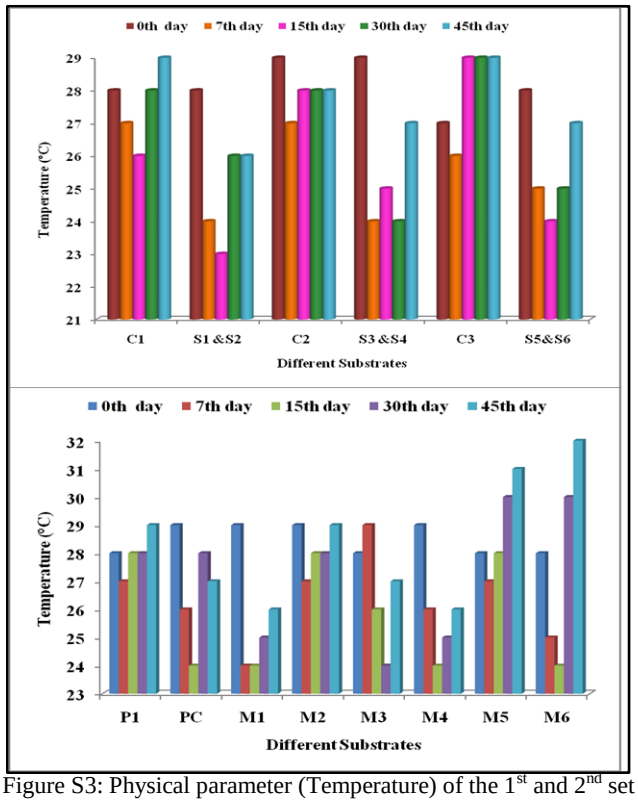


Figure S3: Physical parameter (Temperature) of the 1st and 2nd set of vermicomposted soil

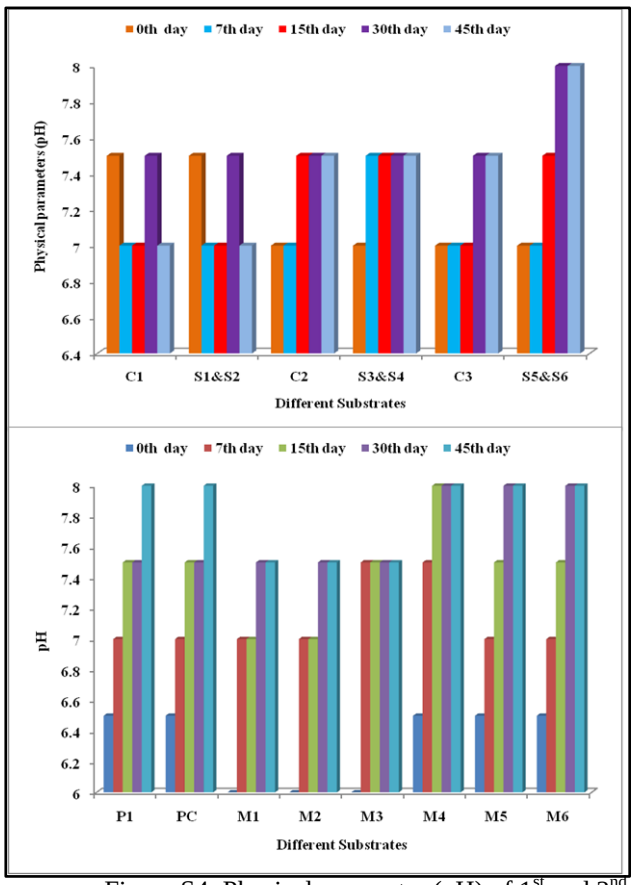
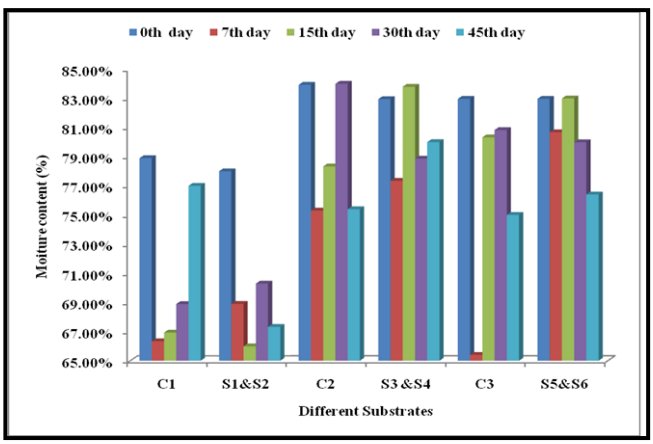


Figure S4: Physical parameter (pH) of 1st and 2nd set of vermicomposted substrate (paper waste and sewage waste)

There was not much variation in pH in the first experimental setup (sheep manure and sugarcane waste). There was a slight fluctuation in the pH; the highest pH recorded was pH eight, and the lower pH was 7. The pH of S5 and S6 was eight, which is the highest, and pH seven was almost recorded in all the vermicompost setup. pH 8, which is the highest in PC, P1, M4, M5, M6 at the 45th day, and lowest pH recorded, was 6.5 in almost all the samples on the 0th day (S4). The moisture content was checked for all the vermicompost substrates, and S5 is more moisture than other substrates and lower in S1 & S2. In paper and sewage waste, the moisture content was more in M4, followed by M3 and lower in PC (control) (Figure S5).



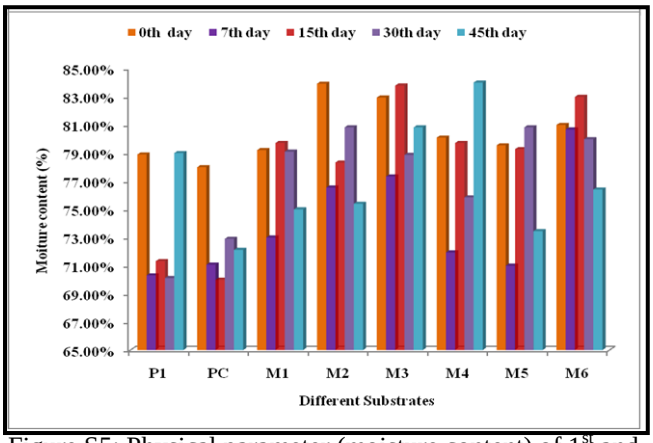


Figure S5: Physical parameter (moiture content) of 1st and 2st set of vermicomposted substrates

NUTRIENT ANALYSIS OF VERMICOMPOST

In the present study, the total organic carbon content and nitrogen content of the Vermicompost substrate was determined, and it was found that S1, S2, P1, and M3, which gave better plant growth was estimated. The total organic carbon in S1 and S2 was 0.49% on the 45th day. The total organic carbon in P1 was 0.55% on the 45th day (figure 1). The total Kjeldahl nitrogen of S1 and S2 was 0.46% on the 45th day. The TKN in P1 and M3 was 0.55% and 0.51% on the 45th day. The highest organic carbon values were obtained in M3, followed by S1 and S2, and the lowest values were obtained in P1, as illustrated in figure 6. The highest values of TKN were obtained in P1, followed by M3, and the lowest values were obtained in S1 &S2 was illustrated in figure 2.

EFFECT OF VERMICOMPOSTED SUBSTRATES ON LADY'S FINGER

Lady's finger is the family Malvaceae, native West Africa, Ethiopian, and South Asia. The plant is cultivated in tropical, subtropical, and warm temperature region around the world. This species is an annual and perennial growing to 2m tall. The leaves are 10-20 cm long and broad, palmately lobed with 5-7 lobes. The flowers are 4-8 cm in diameter, with five white to yellow petals, often with a red or purple spot at each petal base. The fruit is a capsule up to 18cm long, containing numerous seeds. Figure 8 shows the growth of Abelmoschus esculentus.

Germination percentage was studied; S1, S2, S3, and S6 showed a 100% germination rate while C3 showed 33.30%, which was recorded to be the lowest (figure 3). Figure 4 shows the germination percentage of Abelmoschus esculentus. The plant's height was recorded it was found that plants are grown in S3, and S4 resulted in maximum height and followed by plants grown in S1 and S2. The lowest height of the plant was observed in control (C1). The number of the leaves was measured in test and control plant seedlings; it was found that the maximum number of leaves was seven on S3 and S4; the lowest was five in control (without substrate). The total numbers of fruits in each plant grown in vermicompost substrate were counted, and the mean value of each plant was calculated. 9 fruits were

produced in S1 followed by eight fruits in Substrate 2. The lowest production of the fruit was observed on C3 (Two fruits). The fruit produced by the plant is shown in figure 5 and Figure S6.

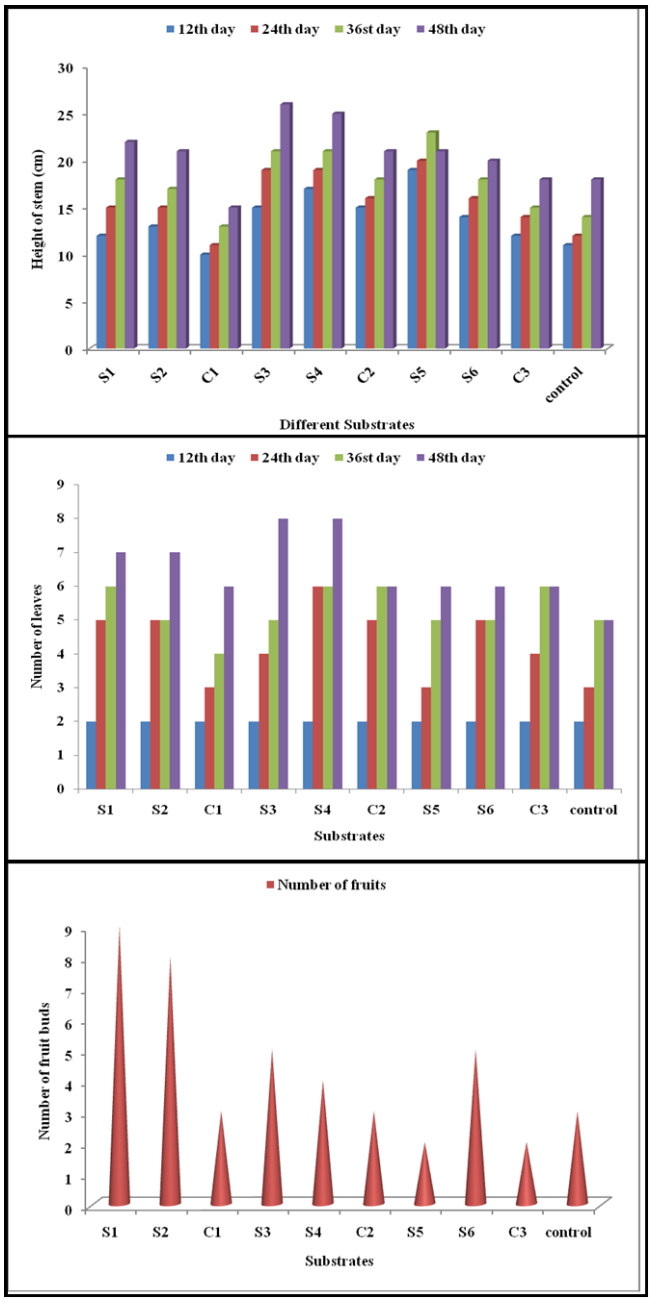


Figure S6 Height of Stem, number of leaves and fruits produced by lady's finger using different substrate.

Figure 3 Seedling growths of Abelmoschusesculentus

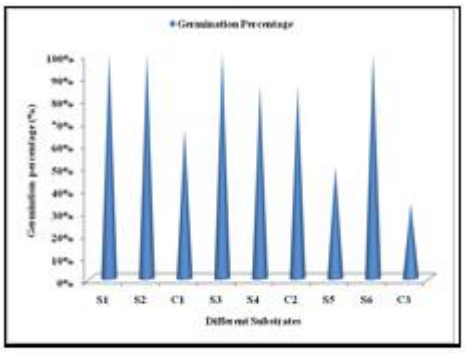


Figure 4 Germination percentage of *Abelmoschus esculentus*

Figure 5 Fruits produced in S1 and S2 substrate

EFFECT OF VERMICOMPOSTED SUBSTRATES ON CHILLI

The chilli is the fruit of plants from the genus Capsicum, members of the nightshade family, Solanaceae. This is an early, tall spreading hybrid yielding dark green, medium-large fruits (14 cm x 1.4 cm). The fruits are straight, smooth, pungent, maturing to attractive deep red color. The seedling growth of the Capsicum annum plant was measured in control and vermicompost soils. The plants grown in vermicompost soils showed higher growth than the control (figure 6 and Figure S7). The germination percentage was studied and illustrated in figure 7. Different height of the plant and the number of leaves produced were measured, and it was shown that a significant difference in plant height was observed on a plant grown in test and control. Plants that were grown in S1 and S2 resulted in the maximum height of the stem. The lowest height of the plant was showed in control (without substrates). At the flowering stage, the maximum number of leaves was 19 on S5 and S6. The lowest leaves (13) were in control (without substrate)

Figure 6 Seedling growth of chilli plant on 48th day

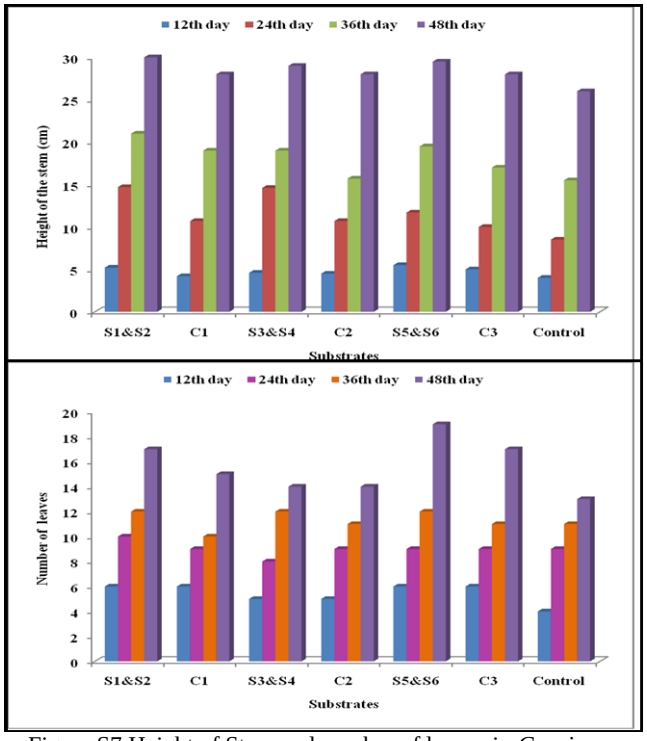


Figure S7 Height of Stem and number of leaves in *Capsicum annum* using different substrate.

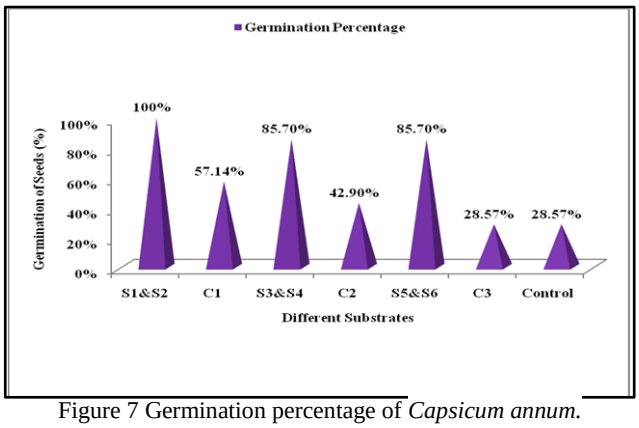


Figure 7 Germination percentage of *Capsicum annum.*

EFFECT OF VERMICOMPOSTED SUBSTRATES on *Trigonellafoenum gracecum*

Fenugreek is an annual plant in the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop, and its seeds are a common ingredient in dishes in South Asia. The seedling growth of the Capsicum annum plant was measured in control and vermicompost soils. The plants which were grown in vermicompost soils showed higher growth than the control. The growth of the plant on 12th day before and after harvest was shown in figure 8 and Figure S8.

The stem's height using the 2nd set of the vermicomposted substrate (Paper waste and sewage sludge) was measured, and a significant difference in plant height was observed on a plant grown in test and control. Plants that grow in M6 resulted in the maximum height of the stem and followed by plants grown in P. Lowest height of the plant was in control (PC). Likewise, the number of leaves and height of the roots were also measured. A significant difference in plant height was observed on a plant grown in test and control. Plants that grow in M3 resulted in the maximum height of the stem and followed by plants grown in P. Lowest height of the plant was in control (PC). Figure S9 shows the growth of fenugreek.

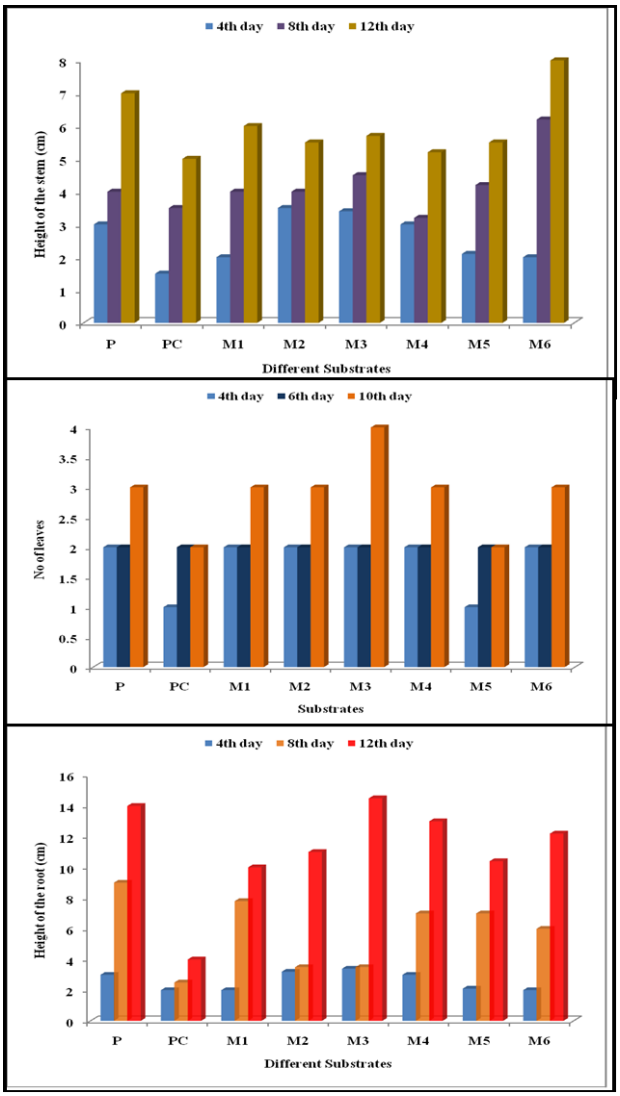


Figure S8 Height of stem, number of leaves and root height of *Trigonellafoenum-gracecum* using different substrate.

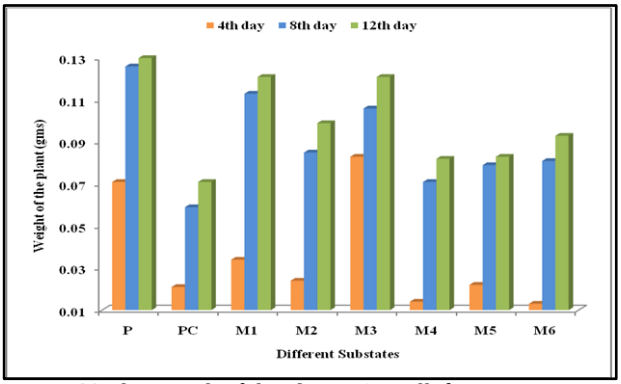


Figure S9 The growth of the plant *Trigonellafoenum-gracecum* with increase in weight of the plant using different substrate.

The weight of a single plant and the whole weight of the fenugreek was weighed and showed in Figure S10. A significant difference in plant weight was observed on a plant grown in test and control. Plants that were grown in P1 (0.179 g) showed maximum weight and followed by plants grown in M3 (0.121 g) [19]. The lowest height of the plant was in control (PC). The whole plant's maximum weight was obtained in P, which was 112.7 g and the lowest yield was in control (PC), which was 60.489 g.

Figure 8 Seedling growth of Fenugreek plant before and after harvesting

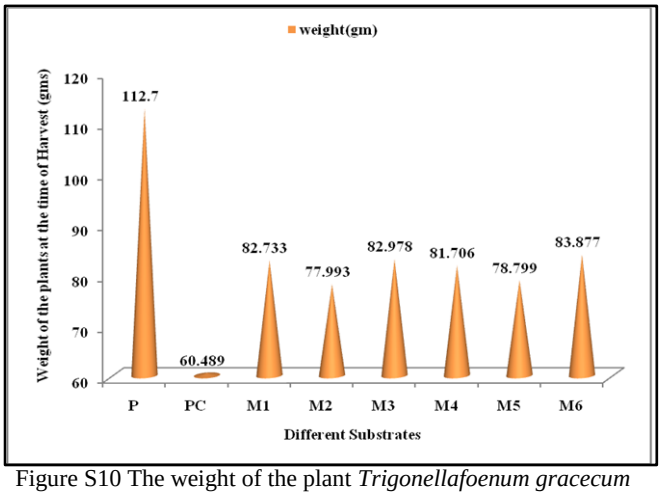


Figure S10 The weight of the plant *Trigonellafoenum gracecum* different substrate.

IV. DISCUSSION

Current trends in agriculture are centered on reducing the use of inorganic fertilizers by organic manure and the application of biofertilizers such as vermicompost and PSB and NFB biofertilizers. [20]reported that during vermicomposting, the pH declines from 9.3 to 8.7. In the present study, maximum pH (8 to 8.5) was observed in S5 & S6, P, M3, and M5. [21] reported that the activity, metabolism, growth, respiration, fecundity, and growth period from hatching to sexual maturity of earthworms are greatly influenced by temperature. The present study showed that the temperature was more in M2, M3, S1, and C3. [22] demonstrate that after six months of vermicomposting, the end product's nitrogen content was high. In the present study, the highest values of nitrogen (0.55%) were obtained in the 45th day, and the lowest values were obtained as 0.45%. [23]reported significantly increased growth and yields of field tomatoes (*Lycopersicon esculentum*) and Peppers (*Capsicum anuumgrossum*) produced from vermicompost cattle manure, food waste, recycled paper significantly increases in the yield of fruits. The present work also proves that Lady's finger in the first set of vermicompost substrates (Sugarcane waste, Sheep manure) at the end of the 48th day. The Height of the Stem was maximum up to 26cm, and the number of leaves was 8. The fruit was obtained maximum in S1 and S2, which beared nine fruits. The germination percentage was maximum in S1, S2, S3, and S4 (100%) and lower in control (without Substrates). [24] reported that *Capsicum anuum* grown in vermicomposted food and recycled paper waste and inorganic fertilizers has a significant increase in shoot length and leaf area. The present work also proves that Chilli in the first set of vermicompost substrates (Sugarcane waste, Sheep manure) at the end of the 48th day. The Height of the Stem was maximum upto 30cm; the number of leaves was 17 in test substrates. Fenugreek plant grown in the second set of vermicompost substrates, plant height, number of leaves, leaf area, root length (after harvesting), wet weight (after harvesting) were significant in Vermicompost substrates P and M3 and less in PC (control), wet weight (after harvesting) yield per plant, as well as yield per plot, was maximum for the P and M3. [25] reported that some species of bacteria, Pseudomonas, bacillus, and Rhizobium, are the most potent phosphate solubilizers, which can mineralize and solubilize. In the present study, the bacterial strain isolated belongs to the genus *Bacillus, Bacillus pumilus*, which released a high amount of phosphate. [26] isolated *Arthobacter sp*. and *Xanthomonas sp*. from the soil showing the phosphate solubilizing ability. In the present study, the isolated strain belongs to the genus *Arthobacter*, which accumulated a measurable amount of phosphate.

CONFLICT OF INTEREST

The authors hereby declare that they had no conflict of interest

ACKNOWLEDGEMENT

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