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Effect of vermicompost, vermiwash and microbial inoculants on growth of *Abelmoschus esculentus* L.

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Abstract
"Earthworms are popularly known as the "farmer's friend" or "nature's plowman". Earthworms influence microbial community, physical and chemical properties of soil. In the present study, vermicompost (using fruits waste, cow dung and earthworms), bacterial inoculants (*Rhizobium*, *Azospirillum*) are environmental friendly and low cost. The physicochemical parameters such as p^H, Moisture, Temperature, Nitrogen, Phosphorus, Magnesium, Potassium were tested. The micronutrients of the vermicompost and vermiwash were analyzed using Atomic Absorption Spectrophotometer. The seedling of *Abelmoschus esculentus* were transplanted in five pots of equal size, which were noted as (T1, T2, T3, T4 and T5). The seedling pots were treated with vermicompost, vermiwash and the uninoculated pot was denoted as control. The vermicompost and vermiwash could enhance the morphological parameters such as height of the plant, number of leaves, number of roots, shoot length and root length were analyzed at 45^{th} day.

Keywords: fruit waste, vermicompost, vermiwash, microbial inoculants, *Abelmoschus esculentus* L

Introduction

"Sustainable agriculture" can be ensured in future with the help of organic farming systems which includes various processes of biological origin such as compost and vermicompost. It is an established fact that earthworms act upon various kinds of wastes including sewage sludge, animal wastes and crop residues etc. Composting and vermicomposting are appropriate technologies which convert waste to wealth. Vermicomposting is increasingly becoming popular as an organic farming and solid waste management technique and it produces two vital biofertilizers, vermicompost and vermiwash.

Vermicompost as an excellent plant growth medium and influence the developedment of plants and promote leaf length, root length and number of leaves [1]. Various species of earthworm feed on the waste and their gut act as the bioreactor where the vermin casts are produced. Biological [2] degradation of organic waste by earthworms and microorganisms produces vermin compost.

Waste is a valuable raw material located at a long place and it can be converted into useful products by making use of an appropriate processing technology. There has been a phenomenal growth in the quantum diversity of solid waste materials arising out of domestic, commercial, industrial and agricultural products that comprise of both biodegradable and non-biodegradable materials. In India about 7000 million tons of wastes are generated annually and agricultural waste alone has been estimated to be about 320 million tons.

It is a dual process by which earthworms of various species derive nourishment from the microbes which are flourishing in the organic waste as well as degrade the complex organic waste into smaller particles in their gizzard, a part of their alimentary canal [3].

Earthworms are popularly known as the "farmer's friend" or "nature's plowman". Earthworms influences microbial community, physical and chemical properties of soil. The primary decomposers of organic matter are microorganisms. Microbial activity in the earthworms gut, cast [4] and soil is very essential for the breakdown and release of nutrients in available form to plants. The microorganisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter. Vermicomposting is a suitable system for studying microbe earthworm interactions. Microbial activity is stimulated by favourable conditions like moisture content, pH and high concentration of mucus in the anterior part of the gut, in the midgut this enhanced microbial activity results in the digestion of soil organic matter and the digestion are partially absorbed in the posterior part of the gut. Epigeic species which consume considerable amounts of raw organic matter have a range of enzymatic activities, mainly originating from ingested microflora [5]. For instance the presence of fungal endophytes substantially increased the nutritional value of grass leaves for *E. fetida*.

Vermicomposting process of fruit waste in combination with cow dung, microbial inoculants such as *Rhizobium*, *Azospirillum* and nutrient analysis of the vermicompost produced.

Materials and Methods

Sample Collection

Fruits wastes were collected from the markets in Mannargudi town, Thiruvarur District, Tamil Nadu, South India and shade dried for a week. The dried fruit wastes were mixed with dry cow dung 1:1 ratio (w/w) and allowed for pre decomposition for 30 days. The pre decomposed substrate was divided into five combinations of feed mix.

Physicochemical properties of worm worked compost [6]

The physical properties of worm worked compost such as pH, temperature, moisture and content were evaluated.

Determination of pH7

5gm of worm worked compost was weighed which was suspended in 10 ml of distilled water, shaken for 30 minutes and pH of the supernatant was measured using pH meter.

Determination of Temperature [8]

Temperature was an important physical factor. The temperature was measured by using standard centrigrade thermometer gratitude from 0° to 100˚C.

Collection of Vermiwash [9]

Vermicompost was obtained from waste fruit with cow dung manure. Vermicomposting was done for 30 days using earthworms. 60 ml of vermiwash was continuously collected per day from the 5 kg organic waste that was vermicomposted. 4 kg of paddy field soil were used for the study. The vermicompost and vermiwash were hand mixed with the soil.

Microbial Population

The compost samples were cultured to determine bacterial and fungal population using the following methods. Culture media used

1. Nutrient agar medium (NA)
2. Mortins Rose Bengal agar medium (RBA)

The total number of bacteria and fungi were estimated by 'Serial dilution plate method' [10]. It is assumed that each developing colony in the plate from a single cell or spore. To develop the microbial colonies from the sample suspension "pour plate method" was followed. For each dilution of each group of microorganism three replicates were maintained. The petridishes were poured with appropriate agar medium ie, NA, RBA respectively for bacteria and fungi. The medium was allowed to set and the plates were incubated in inverted position at room temperature (27˚C) for the following periods, for bacteria 1 day and fungi 3 days [11].

Enumeration of Microbes

The colonies on the plates were counted with the help of colony counter on first day, third day, seventh day of incubation for bacteria and fungi respectively.

Calculation

$$\text{Microbial population present} = \frac{\text{Average number of colonies and dilution factor}}{\text{Per gram oven dried sample Moisture factor}}$$

Pot Culture Experiment

The seedling of *Abelmoschus esculentus* L. were transplanted in five pots of equal size. Worm worked compost was used as the culture medium. The pots were provided with water facilities. Pot culture experiment was conducted at PG and Research Department of Microbiology, STET Women's College, Mannargudi, Tamil Nadu.

Treatment

There were five treatments from combination of

T1 – Vermicompost + Vermiwash
T2 – Vermicompost + Vermiwash + *Rhizobium*
T3 – Vermicompost + Vermiwash + *Azospirillum*
T4 - Vermicompost + Vermiwash + *Rhizobium* + *Azospirillum*
T5 - Control (Substrate only)

The replication for each treatment were performed. All the pots were arranged in a randomized design. The pots were maintained in the open shade at the temperature 27-30˚C.

Morphological Parameter (15th, 30th, 45th day)

The plants were removed from the each pot and studied for the following (15th, 30th, 45thday) morphological parameters. They were

- Height of the plant(in cm)
- Number of leaves (per plant)
- Shoot length(in cm)
- Root length(in cm)
- Number of flowers (per plants)
- Yield (seed in gram)

Statistical analysis [12]

Random sampling was used in the entire test, the results obtained in the present investigation were subsjected to statistical analysis like mean X and standard deviation (SD) by using the formula

Mean $(\bar{x})$ =Sum of all values of the variable
N=Number of Observation

- Where add together all values of variable x and obtain X, divide the total by the number of observation
- The standard deviation (SD) was calculated by the formula

$$\text{S.D.} = \sqrt{\frac{\sum(x-x)^2}{n-1}}$$

Where
$\bar{X}$ - Arithmetic mean
x - Sum of all the values are variable
N - Total number of observation

Find the deviation of each value from the mean (x-x) square the deviation and take the total of square deviation divide the total by number of observation.

Result

In vermicompost, the physico-chemical properties such as pH (6.7), moisture (70%), temperature (34˚C), Nitrogen (28%), Magnesium (27%), Phosphorus (23%), and Potassium (45%) were observed. The effect of vermicompost on the growth of *Abelmoschus esculentus* L was studied and compared with control.

In vermiwash, pH (6.4), moisture (60%), temperature (25˚C), Nitrogen (25%), Magnesium (24%), Phosphorus (21%), Potassium (42%) were observed. The effect of vermiwash on the growth of *Abelmoschus esculentus* L. was studied and compared with control.

The micronutrients were analysed by using (AAS) Spectrophotometer. The micronutrients found to be rich in vermicompost. Vermiwash contain lowest content of micronutrients.

Table 1: Analysis of Physicochemical parameters of vermicompost and vermiwash

S. No	Physico-chemical parameters	Vermicompost	Vermiwash
1	pH	6.7	6.4
2	Moisture (%)	70%	60%
3	Temperature (˚C)	34˚C	25˚C
4	Nitrogen (%)	28	25
5	Magnesium (%)	27	24
6	Phosphorus (%)	23	21
7	Potassium (%)	15	42

The microbial count observed in the 80th day of vermicompost. In the microbial count observed during 80th day of vermicompost, maximum bacterial count was analysed using colony counter. The bacterial population was 78.9 ± 0.43 $\times 10^8$/g. The maximum fungal count was recorded in 70.3 ± 5.20 $\times 10^5$/g.

In vermiwash, the microbial count observed during final day of vermiwash, maximum bacterial count was analysed by using colony counter. The bacterial population was 73.5 ± 0.30 $\times 10^8$/g. The maximum fungal count was recorded in 70.0 ± 0.11 $\times 10^5$/g.

Table 2: Microbial Population of Vermicompost and Vermiwash (Cfu/ml)

S. No	Microorganisms	Vermicompost	Vermiwash
1	Bacteria	78.9±1.43	73.5±0.30
2	Fungi	70.3±5.20	70.0±0.11

Analysis of morphological parameters (*Abelmoschus esculentus* L)

In 45th day,treatments with vermicompost + vermiwash + *Rhizobium* + *Azospirillum* (T4) applied plant was increased yield concepts such as height of the plant was 15.9 ± 2.32cm, vermicompost + vermiwash + *Azospirillum* (T3) showed 13.6 ± 6.72cm height, vermicompost + vermiwash + *Rhizobium* (T2) observed the height i.e 13.3 ± 5.32cm, vermicompost + vermiwash (T1) showed the height 10.2 ± 3.29cm and followed by control 5.1±1.0cm.

In 45th day,same result was also obtained in treatments with vermicompost + vermiwash + *Rhizobium* + *Azospirillum* (T4) applied pots showed better response in leaves of plant was 16.10 ± 4.33cm, vermicompost + vermiwash + *Azospirillum* (T3) showed 14.8 ± 5.25cm leaves, vermicompost + vermiwash + *Rhizobium* (T2) observed the leaves i.e 11.5 ± 5.19cm, vermicompost + vermiwash (T1) showed the leaves 9.2 ± 3.12cm, and substrate only followed by control 5.1 ± 1.0cm.

In 45th day, same result was also obtained in treatments with vermicompost + vermiwash + *Rhizobium*+ *Azospirillum* (T4) applied pots showed maximum response in shoot length of plant was 18.11 ± 4.32cm, vermicompost + vermiwash + *Azospirillum* (T3) showed 15.9 ± 5.23cm shoot length, vermicompost + vermiwash + *Rhizobium* (T2) observed the shoot length i.e 14.6 ± 5.11cm, vermicompost + vermiwash (T1) showed the shoot length 12.4 ± 3.9cm and followed by control 11.3±1.12cm.

In 45th day, among the overall treatments with vermicompost + vermiwash + *Rhizobium* + *Azospirillum* (T4) applied pots showed maximum response in root length of plant was 13.12 ± 4.21 cm, vermicompost + vermiwash + *Azospirillum* (T3) showed 11.8 ± 6.25cm root length, vermicompost + vermiwash + *Rhizobium* (T2) observed the root length i.e 12.5 ± 5.10cm, vermicompost + vermiwash (T1) showed the root length 9.5 ± 3.7cm and followed by control 7.1 ± 1.3cm.

In 45th day, among the overall treatments with vermicompost + vermiwash + *Rhizobium*+ *Azospirillum* (T4) applied pots showed maximum response in number of roots 10.11 ± 2.0cm, vermicompost + vermiwash + *Azospirillum*(T3) showed 6.22 ± 2.8cm number of roots, vermicompost + vermiwash + *Rhizobium* (T2) observed the number of roots i.e 7.64 ± 0.3cm, vermicompost + vermiwash (T1)showed the number of roots 4.05 ± 2.2cm and followed by control 3.4 ± 1.4cm.

Among the overall treatments in 45th day, treatments with vermicompost + vermiwash + *Rhizobium*+ *Azospirillum* (T4) showed maximum response in yield seed in grams per plant was 50.54 ± 2.52mg/g, vermicompost + vermiwash + *Azospirillum* (T3) showed 30.63± 1.53mg/g of seeds, vermicompost + vermiwash + *Rhizobium* (T2) observed the seeds i.e 22.62 ± 1.13mg/g, vermicompost + vermiwash (T1) showed the seeds 21.54 ±1.10mg/g and followed by control 18.23± 1.05mg/g.

Table 3: Effect of vermicompost and vermiwash on morphological parameters of *Abelmoschus esculentus* L on 45th day

Treatments	Height of the plant (cm)	Number of leaves/plant	Shoot length (cm)	Root length (cm)	Number of roots	Yield seeds in Gram
T1	10.2±3.29	9.2±3.12	12.4±3.9	9.5 ±3.7	4.05±2.2	50.54±2.52
T2	13.3±5.32	11.5±5.19	14.6±5.11	12.5±5.10	7.6±0.3	30.63±1.53
T3	13.6±6.72	14.8±5.25	15.9±5.23	11.8±6.2	6.22±2.8	22.62±1.13
T4	15.9±2.32	16.10±4.33	18.1±4.32	13.12±4.4	10.11±2.0	21.54±1.10
Control	5.1±1.0	5.1±1.0	10.3±1.12	7.1±1.3	3.4±1.4	18.23±1.05

Phytochemical analysis of *Abelmoschus esculentus* L

Chlorophyll analysis

In 45th day, among the overall treatments with vermicompost + vermiwash + *Rhizobium* + *Azospirillum* (T4) was showed higher content of chlorophyll-a (0.325 mg/g), chlorophyll-b (0.323 mg/g) and total chlorophyll (0.648 mg/g). Vermicompost + vermiwash + *Azospirillum* (T3) showed 0.190 mg/g of chlorophyll, vermicompost + vermiwash + *Rhizobium* (T2) observed the chlorophyll i.e 0.170 mg/g, vermicompost + vermiwash (T1) showed the chlorophyll 0.104 mg/g, control showed lowest content of chlorophyll (C)- 0.126 mg/g.

Carbohydrate Analysis

The total carbohydrate content was estimated on the growth of plant leaves. Among the five treatments, treatments with vermicompost + vermiwash + *Rhizobium* + *Azospirillum* (T4) showed higher carbohydrate content 18.0mg/g, T3- showed 16.4mg/g of carbohydrate, T2- observed the carbohydrate i.e 15.8mg/g, T1- 12.3mg/g and lowest carbohydrate content was observed control (C) 7.0mg/g.

Protein Analysis

The protein content was estimated on the growth of plant leaves vermicompost+vermiwash+*Rhizobium*+*Azospirillum* (T4) showed highest protein content 4.620mg/g, T3- showed 4.367mg protein, T2- observed the protein i.e 3.560mg/g, T1- showed the protein 2.163mg/g and control (C) was lowest protein content 1.150mg/g.

Carotenoids Analysis

Among the overall treatments, (T4) was showed maximum response in carotenoids of plants (0.698mg/g), T3- was showed better response in carotenoids of plants (0.588mg/g), T2- observed the carotenoids i.e (0.467mg/g), T1- showed the carotenoids (0.342mg/g) and the control (0.310mg/g.

Hence our study was clearly highlighted that the treatment of earthworm and bacterial inoculants could enhance the morphological parameters such as height of the plant, number of leaves, root length, shoot length, yield (seed in gram) and phytochemical constituents such as chlorophyll, carbohydrate, protein and carotenoids.

Discussion

Now a days, farmers are interested in reducing dependence on chemical inputs, so rather than chemical control such as agriculture practices, sanitation, biological control, soil solarization etc, could be exploded to play an important role in Integrated Pest Management (IPM) systems, especially in case of vegetable production. A model describing the several steps required for a successful IPM has been developed. Farm technologies that sustain high productivity levels without impairing the soil health and water resource are the need of the day. However in the name of sustainable and environment friendly agriculture, some typical approaches of farming, collectively termed as organic farming are being promoted in the country advocating sole use of organic or biological nutrient resources and other inputs with complete exclusion on fertilizers and agrochemicals [13].

Vermicompost have considerable potential for improving plant growth significantly, when used as components of horticulture soil or container media. Nevertheless, there appear to be major differences between the effects of the vermicomposts and composts were used in our study, in terms of their influence on plant growth, depending upon the source of the parent waste material used in their production. These differences in growth responses could be due in part to fundamental differences between the composting and vermicomposting processes which use quite different microbial communities, with composting tending to result in the release of mineral nitrogen in the ammonium form, whereas vermicomposting releases most of the nitrogen in the nitrate form. The vermiwash and vermicompost improve the trace element content of the soil. However the combination of these biofertilizers was more effective in improving soil micronutrients content. Bio-fertilizers (vermicompost and vermiwash) contribute macronutrients and micronutrients in amount that is required by plans [14].

The intensive agricultural practices prevalent in the modern times are heavily dependent on the application of chemical inputs including fertilizers, 162 which affect soil health and fatigue in the long run. During the last few decades, a significant increase in growth and yield of agronomically important cereal crops and improved soil fertility, in response to inoculation with PGPR has been reported. Rice (*Oryza sativa L.)* is one of the most prominent food crops globally, and represents the staple diet for almost half of the human population of the world. It is estimated that there will be about 8 million people by the year 2020 of which 760 million tons of rice. Wheat is the stable food for 35% of the world's population. It provides more calories and proteins in the diet than any other crop. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality [15].

Vermicompost has an ability to fight soil-borne plant disease such as root rot. Humus also increase water permeability and more efficiently [16] use in soil moisture. It is found that nitrogen concentrations are higher in vermicompost than in aerobic compost piles. There are other agronomic benefits of composts application, such as high levels of soil-borne disease suppression and removal of soil salinity. One study reported that mean root disease was reduced from 82% to 18% in tomato and from 98% to 26% in capsicum in soils amended with compost.

Vermiwash is a liquid that is collected after the passage of water through a column of worm action. It is a mixture of excretory products and mucus secretion of earthworms along with micronutrients from the soil organic molecules. It contain nitrogen as nitrogenous excretory product and growth promoting hormones and essential enzymes and infuses resistance in plants. It is applied in foliar spray. This is transported to the leaf, shoots and other parts of the plants in the natural ecosystem. It contains various enzymes cocktail of protease, amylase, unease and phosphatase. These are beneficial for growth and development of plant and stimulate the yield and productivity of crops and also microbial study of vermiwash [17] found that nitrogen fixing bacteria like *Azotobacter*, *Agrobacterium* and *Rhizobium* and some phosphate solubilizing are also found in vermiwash.

Conclusion

In the present study, vermicompost (using fruits waste, cow dung and earthworms), bacterial inoculants (*Rhizobium*, *Azospirillum*) are environmental friendly and low cost. The physicochemical parameters such as pH, Moisture, Temperature, Nitrogen, Phosphorus, Magnesium, Potassium were tested. The micronutrients of the vermicompost and vermiwash were analyzed by using Atomic Absorption Spectrophotometer. The seedling of *Abelmoschus esculentus* were transplanted in five pots of equal size, which were noted as (T1, T2, T3, T4 and T5). The seedling pots were treated with vermicompost, vermiwash and the uninoculated pot were denoted as control. Then the vermicompost and vermiwash could enhance the morphological parameters such as height of the plant, number of leaves, number of roots, shoot length and root length were analyzed at 45th day.

The vermicompost and bacterial inoculants could be recommended to farmers to insure the public health and a sustainable agriculture. Then the effects of vermicompost and vermiwash from each treatment were observed in the growth of the *Abelmoschus esculentus*. From the results, the plants inoculated with the treated compost (Vermicompost +Vermiwash+ *Rhizobium* + *Azospirillum*) showed maximum effect.

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