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Studies on the Effect of Vermiwash on the Growth of *Pisum sativum* L. and *Cicer arietinum* L.

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Abstract

The present work was carried out by employing *Eudrilus eugineae* to produce vermiwash by utilizing kitchen waste. Vermiwash collected was sprayed on to priorly germinated seeds of *Pisum sativum* and *Cicer arietinum* and planted on plastic pots containing sterilised sand and soil. The growth study was monitored for 28 days. Physiochemical characteristics and biochemical composition of soil amended with vermiwash and those deprived from vermiwash was studied by standard protocols. Soil amended with vermiwash had an alkaline pH, higher electrical conductivity and was rich in organic carbon, sodium, potassium, iron, copper and zinc. *P. sativum* and *C. arietinum* plantlets grown under vermiwash treatment showed better growth in terms of shoot and root length, internodal length, number of leaves and biomass over control. However, the root length in vermiwash amended plantlets of *C. arietinum* was shorter, suggesting that soil had good amount of nutrients that were easily available.

Keywords: Vermiwash, *Pisum sativum, Cicer arietinum*, growth parameters, physiochemical characteristics, biochemical composition.

Introduction

The Green Revolution in India, which was heralded in the 1960's, was a mixed blessing. Ambitious use of agrochemicals boosted the food production but also destroyed the agricultural ecosystem[1]. Rising demand on fertilizer is closely related with the rising demand for crop as the population increases. With the increase in health awareness among people, organically cultured fruits and vegetables are getting attention and are in demand[2].The practice of vermiculture is now being revived worldwide due to its environmental friendly approach with diverse ecological objectives, such as soil detoxification, regeneration, waste management, and sustainable agriculture[3]. Beside the compost produced, recent interest has been brought up due to the possible use of the liquid by-products from this green technology. Earthworms can consume large quantities of organic wastes rapidly and process them, serving like an aerator, crusher and degrader in decomposition system.

Vermiwash is a liquid leachate collected by allowing excess water to saturate the actively vermicomposting substrate in such a way that the water washes the nutrients from vermicast excreted by the earthworms as well as the earthworm's body surface[4]. The vermiwash has the valuable nutrients and the microorganisms present in the drilosphere[2]. The present investigation was an attempt to study the effect of vermiwash application on the growth of *Pisum sativum* and *Cicer arietinum*, so as to promote field trials, also to be utilized as an effective bio-fertilizer to obtain good yield, minimize the usage of chemical fertilizers and thus help in growth of Indian agricultural economy.

Methodology

Vermicomposting

In the present study, plastic bucket of 18″ height and 16″ diameter was used. Two holes were punched on the sides, about 3cms above the bottom of the bucket and closed with coir, to facilitate the draining of excess water. The bin was placed on a small plastic stool, to maintain elevated position within a wider plastic tub.The bottom of the bin was covered by coconut coir, up to 15cms. Dried leaves were broken into pieces and mixed with cow dung and water, so that the leaves are moistened and no excess draining of water occurs. The mixture was placed on the coir bed and left for 20 days for 'primary decomposition' or 'composting' to occur[5]. Moisture was maintained by sprinkling of water and proper mixing was done once in 2-3 days. Earthworms belonging to the species *Eudrilus eugeniae* (~100) were procured from the Vermiculture Unit of St. Aloysius College, Mangalore, Karnataka.

On 21st day, the collected earthworms were introduced into the vermibin to carry out the 'secondary decomposition' or vermicomposting. The kitchen waste (organic waste) was collected daily (~500g/day) and added onto the vermibin. Water was sprinkled into the vermibin once in two days, to ensure moisture content of about 30-40%.

Vermiwash collection

The water liberated out from the earthworm body into the surrounding, during composting and the water added manually that seeps through the vermicasts, is the vermiwash which was collected in the tub placed below the bin. Vermiwash was collected on weekly basis and brought to the lab for further studies.

Experimental design

Red and black soil was obtained from Alva's College Campus, Vidyagiri, Moodbidri, Karnataka. Twenty plastic pots were filled with ~1kg of moistened sterilised soil comprising of the mixture of red and black soil, with sand in the ratio 4:1. A total of ten pots were designated for each plant species. Out of which, five were labelled as CONTROL and five as TEST.

Thirty seeds each of *Pisum sativum* and *Cicer arietinum* were soaked in water overnight and three seeds each of both the plant species were inoculated into the labelled pots respectively. The seeds were watered to promote germination and growth. After 3 days of inoculation, the seed with healthy germinated plant was retained in each pot and the rest were removed. 100ml of the collected vermiwash was sprinkled onto the pots labelled as TEST and 100ml of regular tap water was sprinkled onto the pots labelled as CONTROL.

The vermiwash treatment was given twice on a weekly basis, with a time interval of 3 days. The growth of the plants was monitored regularly and the growth parameters were recorded every week.

Soil Sample Preparation

Soil samples for further analysis were collected on the 7th, 14th, 21st and 28th day just before the application of fresh vermiwash, each from CONTROL and TEST pots by random sampling method. The soil samples collected were designated as SC_1, SC_2, SC_3, SC_4, and ST_1, ST_2, ST_3, ST_4 respectively for CONTROL and TEST based on the week of collection of samples. The soil samples collected were homogenized by taking 1g of soil sample in 10ml of distilled water.

Physiochemical analysis of soil samples

pH and Electrical conductivity: pH and electrical conductivity of the soil samples were measured by digital glass electrode pH meter (Elico LI 129) and conductometer (Elico CM 180)[6].

Biochemical analysis of soil samples

Quantitative analysis of important elements and ions: Organic carbon content in the soil samples was estimated by Loss on ignition method[6]. The concentration analysis of elements like sodium and potassium was carried out by flame photometry technique and that of iron, copper and zinc was carried out by Atomic Absorption Spectroscopy. Analysis of nitrate and phosphorous was carried out by spectrophotometric method[6].

Measurement of plant growth parameters

Growth parameters like number of leaves, length of the shoot, and internodal distance of the plants grown in pot culture was measured every week using standard scale and tabulated.Other parameters like root length and biomass were measured using standard scale and weighing balance and recorded after the completion of the vermiwash application study on the plants, i.e., on 28th day of the inoculation of germinated seeds, and the results were tabulated.

Results

The pH and electrical conductivity of the soil samples amended with water and vermiwash are tabulated and have exhibited variation. The soil amended with vermiwash had an average alkaline pH of 7.06 (Table 1). The average EC of the soil samples amended with vermiwash used for growing *Cicer arietinum* and *Pisum sativum* was 0.444mS and 0.424mS respectively (Table 2).The amount of various important elements and ions present in the soil samples amended with water and vermiwash for a period of 28 days were presented in table 3 and 4. The average organic carbon percentage was found to be 7.08 and 7.11 in soil amended with vermiwash, growing *P. sativum* and *C. arietinum.* 615.62mg/Kg and 615.61mg/Kg was the average amount of sodium present and an average of 364.67mg/Kg and 363.44 mg/Kg potassium was estimated in the vermiwash amended soils growing *C. arietinum* and *P. sativum* respectively (Table 3; Table 4).

An average of 515.08x0.01mg/Kg and 511.32x0.01mg/Kg iron content, 9.24x0.01mg/Kg and 8.83x0.01mg/Kg of copper and 36.70x0.01mg/Kg and 36.60x0.01mg/Kg of zinc was found in the soils used for growing *P. sativum* and *C. arietinum*, amended with vermiwash. Phosphorous and nitrate in the soils amended with vermiwash was lower than the soil amended with water. 25.38 mg/Kg and 25.08 mg/Kg was the average amount of phosphorous present, 282.52x0.1mg/Kg and 282.87x0.1mg/Kg nitrate was estimated in the soil samples amended with vermiwash, *P. sativum and C. Arietinum*, respectively (Table 3; Table 4).

The observations regarding the variations in growth parameters like length of shoot and root, number of leaves, internodal distance and biomass of the plants grown on soil amended with vermiwash and on control soil are presented in Figure 1 and 2 respectively, for *P. sativum* and *C. arietinum.* The average length of the shoots obtained in *P. sativum* and *C. arietinum*, under vermiwash treatment was 33.3cms and 34.2cms. Average length of root obtained in *P. sativum* , under vermiwash treatment was 9.66cms . The average length of the root obtained in *C. arietinum*, under vermiwash treatment was 9.30cms and 10.48cms under control condition.

The average length of the internode observed in *P. sativum*, under vermiwash treatment was 7.46cms and 4.34cms under control condition (Figure 1). The average length of the internode obtained in *C. arietinum* under vermiwash treatment was 6.0cms and under control condition was 4.99cms (Figure 2). The mean number of leaves in *P. sativum* and *C. arietinum* was found to be 13 and 71 in the plants treated with vermiwash (Figure 1; Figure 2), higher when compared to the number of leaves found in plants grown

without vermiwash. The average dry biomass of vermiwash treated *P. sativum* and *C. arietinum* was found to be 1.64g and 2.80g (Figure 1; Figure 2).

Discussion

The study revealed important information about the impact of vermiwash on soil and on the growth of *Pisum sativum* and *Cicer arietinum.* The pH of the soil, amended with vermiwash were increased to alkaline pH over a period of 28 days of experiment over control. Higher average pH clearly indicates that the vermiwash has high amount of H^+ ions which is responsible for the alkalinity of soil, making them highly suitable for microorganisms to thrive efficiently. An increase in the conductivity of soil, on regular application of vermiwash signifies that the vermiwash is helpful in adding movable ions into soil, making them easier for absorption by plants. Increase in soil conductivity and pH on application of vermiwash was supported by one of the earlier work in which the vermiwash was obtained from *Eisenia fetida*[7].

The higher amount of organic carbon in soils amended with vermiwash, showed that application of vermiwash for longer duration of time, improves organic carbon content in the soil. An increase in the concentration of sodium and potassium was observed in the soils amended with vermiwash. The continuous application of vermiwash resulted in decreased phosphorous content possibly because the phosphorus was in soluble form and was ready to be absorbed by the soil and taken up by the plants. Reduction of soil nitrogen content is attributed to the conversion of nitrogen into nitrate by the nitrogen-fixing microorganisms present in the soil and also added by the vermiwash. An investigation by Manyuchi et al, showed reduction in nitrogen, phosphorous content and increase in potassium content in the soil on application of vermiwash[7].

The increase in the copper content suggests that the vermiwash is capable of amending copper into the soil, for easy uptake by microorganisms and plants. Increase of copper content in the soil on increasing the application time of vermiwash was also reported in a previous study[8]. Zinc has less mobility than phosphorus in the soil, so incorporation into the root zone is very important, which is carried out by the microflora present in the soil and in the vermiwash. 0.151mg/L iron content in the vermiwash obtained from *Eudrilus eugeniae* utilized for composting agricultural and domestic wastes, depicts that good amount of iron is added into the soil amended with vermiwash[9]. The present study results are also comparable to these reports. The higher average shoot length and internodal length of the plants under vermiwash treatment indicates that the plants have better and faster growth when compared to that of the plants grown without vermiwash. The higher average root length of the plants under vermiwash treatment indicates that the plants had deep penetrating roots, with more of lateral roots, in case of *Pisum sativum* and the lower average length of roots of *Cicer arietinum* indicates that nutrients were available easily to the plants amended with vermiwash on the surface level, eliminating the necessity for the roots to penetrate deep in the soil in search of nutrients. Similarly, in another study[10] on the effect of vermiwash from *Eisenia fetida,* on growth of *Vigna radiate, Vigna mungo* and *Sesamum indicum* resulted in the increased root length, shoot length, leaves, number of twigs,flowers, pods, grains and biomass as well as showed high level of macro and micro nutrients like calcium, sulphur, phosphorous, potassium, organic carbon, iron, copper, manganese and zinc over control, thus concluding that the high grain yield was due to high level of macro and micronutrients available in the vermiwash. The application of vermiwash resulted in a significant increase in number of leaves in plants, over that of control, resulting in the presence of more chlorophyll, leading to increase in photosynthesis and healthy growth of the plants. The higher biomass of the plants treated with vermiwash showed that the vermiwash provided the necessary nutrients which were available in less quantity to the plants grown under

controlled condition. There are few other studies on effect of vermiwash on the biomass and biomolecule increment on cereals and vegetable crops such as significantly higher yield of spinach and onion[11], okra[12], lobia, radish[13], paddy, maize, millet[14], cowpea, rice[15], bhendi[4] and curry leaves var. Suvasini[16]. Earthworm excreta (vermicast) is a nutritive organic fertilizer rich in micronutrients, humus, beneficial soil microbes; nitrogen fixing, phosphate solubilising bacteria, actinomycetes and growth hormones auxin, gibberellin and cytokinins. Both vermicompost and its body fluid (vermiwash) are proven as growth promoters and protectors for crop plants[17]. Vermiwash was found to contain enzyme cocktail of amylases, proteases, phosphatase and urease. In a study, vermiwash obtained from *Eudrilus eugeniae* was able to inhibit growth of *Staphylococcus aureus, E. coli, Bacillus subtilis, Salmonella aboni, Pseudomonas aeruginosa, Candida albicans* and *Aspergiluus flavus*[18]. Microbial analysis has revealed that vermiwash contains nitrogen fixing bacteria like *Azotobacter* sp., *Agrobacterium* sp. and *Rhizobium* sp. and some phosphate solubilising bacteria[19]. The overall effects result in healthy plants with profuse growth and yield.

The present work was carried with the objective of utilizing the kitchen waste at the individual house level to produce nutrient rich vermicompost and vermiwash and avoid the waste from accumulating at the landfills[20,21]. The present study shows that the extract from earthworms offer a valuable resource which could be effectively exploited for increasing the production of economically important crops like *P. sativum* and *C. arietinum*, through field trials and by emphasizing on the exploitation of the various potentials of vermiwash by further studies on the utilization of domestic wastes, agricultural wastes and different types of industrial wastes for the production of large amount of vermiwash, by employing statistical analysis and conducting field trials to boost chemical-free organic farming in future and reduce the harm to the soil, improving soil quality and thus leading to agricultural economic upliftment of the country.

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Tables and Figures

Table 1: pH of soil samples collected from rhizosphere

Plant name	Soil Sample / Day	Day 0	Day 7	Day 14	Day 21	Day 28
Pisum sativum	**Control**	6.30	6.24	6.20	6.25	6.40
	Test	6.47	6.58	7.23	7.32	7.70
Cicer arietinum	**Control**	6.20	6.45	6.30	6.15	6.33
	Test	6.30	6.50	7.04	7.57	7.89

Table 2: Electrical conductivity of soil samples collected from rhizosphere

Plant name	Day / Soil sample	Day 0	Day 7	Day 14	Day 21	Day 28
Pisum sativum	Control (mS)	0.335	0.358	0.378	0.410	0.421
	Test (mS)	0.357	0.389	0.422	0.457	0.493
Cicer arietinum	Control (mS)	0.346	0.378	0.399	0.432	0.451
	Test (mS)	0.373	0.405	0.440	0.484	0.517

Table 3: Quantitative analysis of elements in soil samples under study, from the rhizosphere of *Pisum sativum*

Elements/ ions (mg/Kg)	Soil samples									
	Day 0		Day 7		Day 14		Day 21		Day 28	
	SC0	ST0	SC1	ST1	SC2	ST2	SC3	ST3	SC4	ST4
Organic Carbon (x 0.01) (%)	6.30	6.27	6.41	6.94	6.49	7.25	6.52	7.38	6.59	7.56
Sodium	600.00	600.45	603.31	609.26	602.12	617.55	602.57	622.11	603.24	628.70
Potassium	352.91	352.45	350.22	354.05	351.72	360.00	351.02	371.68	351.24	379.03
Phosphorous	25.07	26.00	25.14	25.89	25.12	25.22	25.11	24.96	25.12	24.84
Nitrate (x 0.1)	287.98	287.02	287.47	285.00	287.45	282.36	287.32	281.15	287.25	277.07
Iron (x 0.01)	475.90	475.60	476.10	483.20	476.20	501.00	476.40	542.30	476.40	573.30
Copper (x 0.01)	7.20	7.23	7.27	7.97	7.25	8.33	7.28	10.63	7.30	12.02
Zinc (x 0.01)	33.90	33.89	33.92	34.25	33.98	35.86	34.07	38.62	34.11	40.89

Table 4: Quantitative analysis of elements in soil samples under study from the rhizosphere of *Cicer arietinum*

Elements/ ions (mg/Kg)	Soil samples									
	Day 0		Day 7		Day 14		Day 21		Day 28	
	SC0	ST0	SC1	ST1	SC2	ST2	SC3	ST3	SC4	ST4
Organic Carbon (x 0.01) (%)	6.31	6.30	6.38	6.96	6.47	7.27	6.54	7.41	6.60	7.63
Sodium	601.00	600.02	601.81	607.62	602.21	615.51	602.46	623.34	602.53	631.60
Potassium	352.71	352.60	351.32	353.95	351.38	361.08	351.12	373.68	351.21	382.03
Phosphorous	25.01	24.97	25.11	25.69	25.10	25.35	25.13	24.67	25.13	24.72
Nitrate (x 0.1)	287.35	287.20	287.38	284.90	287.42	281.86	287.30	281.25	287.35	279.15
Iron (x 0.01)	475.80	475.80	476.30	481.30	476.30	500.20	476.40	535.00	476.40	564.30
Copper (x 0.01)	7.20	7.21	7.25	7.82	7.26	8.37	7.28	9.65	7.29	11.10
Zinc (x 0.01)	33.87	33.88	33.97	34.55	34.09	35.78	34.06	37.74	34.10	41.07

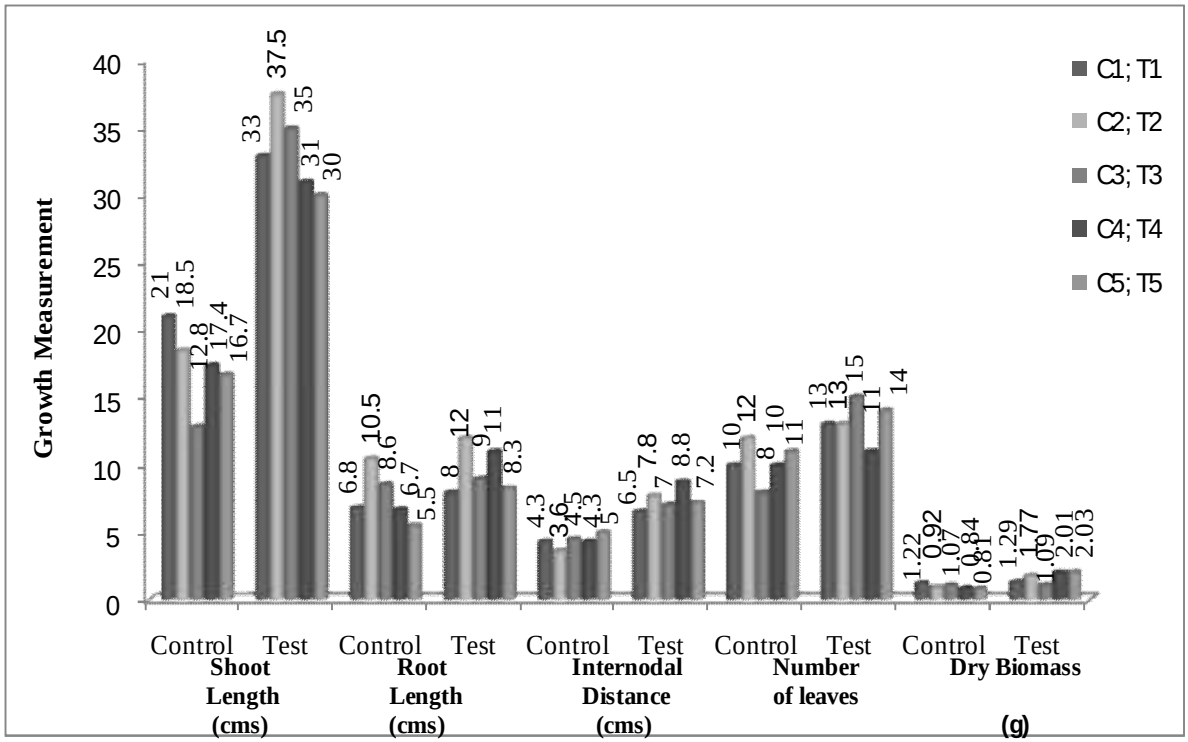

Fig. 1: Effect of vermiwash on growth of *Pisum sativum*

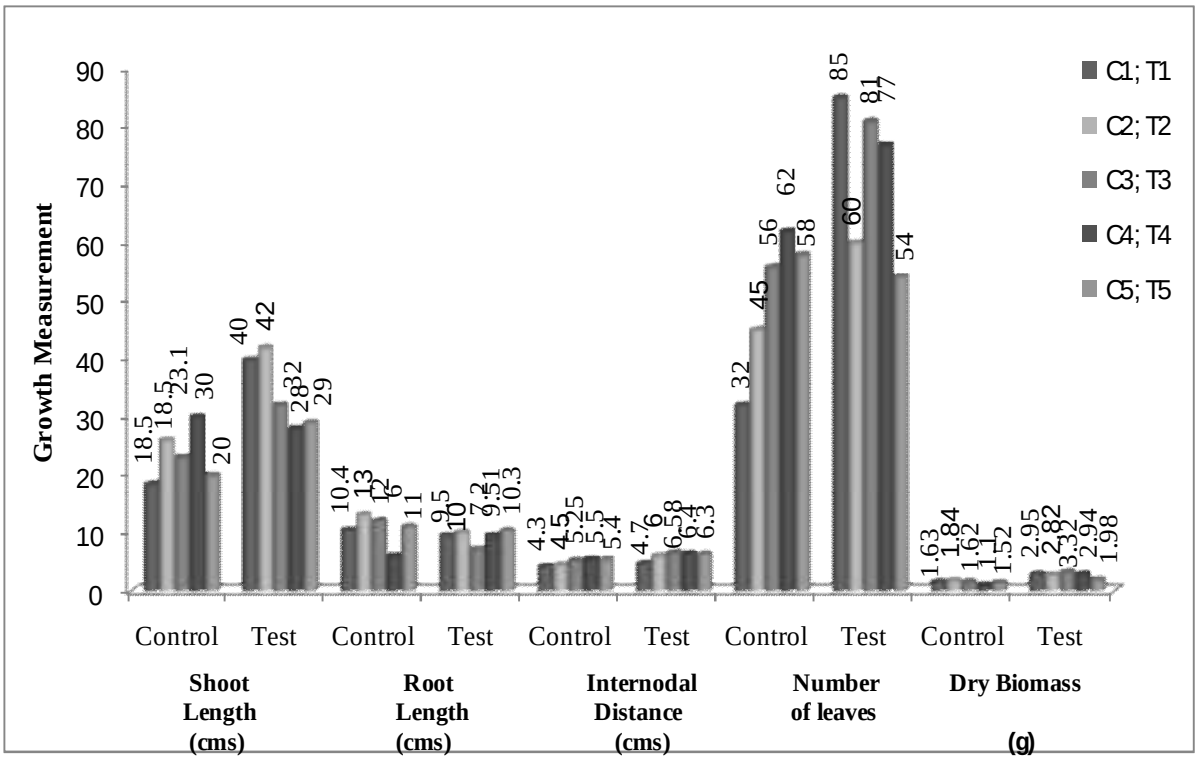

Fig. 2: Effect of vermiwash on growth of *Cicer arietinum*