



## Growth And Production Of *Eudrilus Eugeniae* Cultured In Different Ratios Of Partly Decomposed Cowdung And *Camellia Sinensis* (Stain Removed) Powder Waste

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### Abstract

The rate of cocoon production, cocoon incubation time and hatching ability the earthworm *Eudrilus eugeniae* were observed in 100, 75, 50 and 25 per substrate ratio prepared from mixed cow dung and *Camellia sinensis* (stain removed) powder waste materials cow dung and *Camellia sinensis* (stain removed) powder waste materials and soil for 45 days study. The worms kept in 25, 50, 75 and 100 PSR medium observed gradual increase weight at the end of 45<sup>th</sup> days. However, the per cent weight observed in 25 PSR medium was relatively very high when compared to other medium. The worm kept in 75 PSR medium for 45 days produced relatively more cocoons (1086 as total and 0.482 cocoons/ worm/ day) than the worms kept in other 50, 100 and 25 PSR medium (693, 661 and 446 total and 0.308, 0.230, 0.0198 cocoon/ worm/ day). Among the MCDCS medium studied, the worm kept in 75 PSR medium of 1086 cocoons and were hatched out in to the 667 young-ones at the rate of 0.614 with 61.4% hatching after a period of 13 days of incubation time. However maximum of 110.76 hatching success was noticed in the cocoons collected from the earthworm exposed the 25 PSR (110.76) medium. Soil parameters such as pH, Ec, Macro nutrients (N, P & K) and Micro nutrients (Fe, Mn, Zn & Cu) present in the PSR sample of MCDCS before and after using them by the earthworm *Eudrilus eugeniae* observed. The pH observed in the samples of 25, 50, 75, 100 PSR medium (7.8, 7.8, 7.7, 8.9) after 45 days showed a significant increase over their values observed before earthworm invasion medium (7.6, 7.7, 7.2, 7.6). The Ec level on the contrary showed a significant decreased over its initial values in partly decomposed cowdung, 25 and 50 PSR samples obtained after cultured medium. Over all, the level of K and Mn present in MCDCS medium were relatively high when compared to other nutrients. The earthworm while cultured drastically lowered levels of Phosphorus and Iron, the PSR medium obtained. The MCDCS vermicompost tested *Vigna radiata* plant exhibit higher seedling growth rate and percentage of germination than control plants. It could be inferred that the substrate ratio with 75% is considered to be good medium for the culture of earthworm under laboratory condition and also *Vigna radiata* seedling growth study.

**Keywords:** *Eudrilus eugeniae*, *Camellia sinensis*, *Vigna radiata*, Cocoon, PSR, Vermicompost, MCDCS, Macro nutrients, Micro nutrient.

### INTRODUCTION

Vermiculture means scientific method of breeding and raising earthworms in controlled conditions. It aims at creating improved conditions artificially so that earthworms multiply in shortest possible time and space. Earthworm farming (vermiculture) is another bio-technique for converting the solid organic waste into compost (Chirashree Ghosh, 2004).

Earthworms play a vital role in the soil environment where they contribute to the complex process of decomposition while affecting aeration water transport and soil structure (Van hook, 1974). Higher content of earthworm biomass protein can serve as feed for live stocks (Sabine, 1983).

The *Eudrilus eugeniae* worm is about 20-25 cm in length 5-7 mm in diameter and weighs around 5.6 g. The clitellum makes its appearance by about 24-30 days and becomes fully developed copulation takes place during night by about 30-32 days. Cocoons are laid a day after copulation. The cocoons are oval in shape, pointed at both ends. It is about 6.00 mm in length, 3 mm wide and weighs about 16.0 mg a mean production of 1.3 cocoons/worm/day was reported by Viljoen and Reinecke (1993). Incubation is for about 16.6 days (27°C) with 80% hatching success and 2.6 mean number of hatching per viable cocoon.

Tea wastes need to be mixed with other nitrogen rich organic wastes in order to provide nitrogen, other nutrients and inoculums of micro-organisms. The addition of nitrogen rich organic wastes such as cow dung and kitchen wastes vermicomposting is an eco-friendly and cost-effective technology in which the earthworms are used as bioreactors to convert organic materials into valuable compost.

Population dynamics, productivity and energy flow in earthworms cannot be fully understood unless the life cycle of earthworms is known. Earthworm's growth, maturation, cocoon production and reproductive potential are not only influenced by environmental conditions but are also strongly affected by quality and availability of food. Studies on the life cycle of earthworms are also necessary for effective vermiculture (Bhattacharjee and Chaudhuri, 2002). They have further concluded that earthworms could serve as useful biological indicators of contamination.

Green gram is one of the important pulse crops in India since ancient times. It is widely cultivated throughout the Asia including India, Pakistan, Bangladesh, probably recently. Green gram is protein rich staple food it has 25% protein, which is almost three times that of cereals.

The present study, the mixed cow dung and partly decomposed *Camellia sinensis* (stain removed) powder waste MCSCD used as raw material is composting and its effects on growth parameter of *Eudrilus eugeniae* like cocoon morphology and analysis of cocoon development hatching success, dynamics of cocoon production. Hence to establish the quality of vermicompost obtained from MCSCD after using them by *Eudrilus eugeniae* a pilot study has been conducted to know the impact of vermicasts on the growth and yield of *Vigna radiata* cultivated in pots under laboratory conditions and nutrient status thereby achieving cost effective agriculture crop production.

## MATERIALS AND METHODS

### Collection and maintenance of earthworms

Specimens of 500gm adult epigeic earthworms, *Eudrilus eugeniae* were obtained from the vermiculture unit of Periyar Maniyammai University, Tanjavur. They were kept in large trays with a substrate medium, containing 50 % partly decomposed cow dung and 50 % soil and maintained under shade place.

### Collection of soil

Dry soil was taken from the A.V.C college campus for this study. It is manually powdered stone marter.

### Collection of *Camellia sinensis* (stain removed) powder wastes

*Camellia sinensis* powder waste (stain removed) was collected from A.V.C college hostel and tea shop in Mannampandal.

### Partial decomposition of *Camellia sinensis* (stain removed) powder wastes

Circular plastic tank free from earthworm with size free invasion was used for the *Camellia sinensis* (stain removed) powder. The tank was filled with dry *Camellia sinensis* (stain removed) powder wastes and poured with sufficient water. The tank was closed with polythene sheets to avoid water evaporation and a possible release of foul smell. Water was poured regularly in the tank was closed again with same polythene sheet for proper decomposition. Once in three days, the decomposing materials were thoroughly mixed using a wooden rod to ensure uniform decomposition. The decomposed wet powder was sun dried and sieved through 1mm<sup>2</sup> sieve net to obtain a medium with a particle size less than 1 mm as suggested by Reniecke and Venter (1985).

### Preparation of substrates for cocoon Preparation of feeding materials

Three different type of feeding material namely partly decomposed cowdung (CD) and partly decomposed stain removed *Camellia sinensis* powder waste (CSW) and mixed in cowdung and *Camellia sinensis* powder waste (MCDCS) 1:1 were used.

### Experimental design

The vermibeds were prepared using plastic bags and watering was done regularly in moist medium. Four sets of five media with per cent substrate ratio (PSR) 100, 75, 50 and 25 were prepared using dry soil and MCDCS powdered waste materials (1:1 cowdung; stain removed *Camellia sinensis*) powder waste materials with volume basis and mixed well. Three liters of substrate in each percent ratio taken in on earthen sufficient volume of water was added into ensure optimum moisture condition as suggested by Martin (1982). To assess the rate of cocoon production in the selected media 10 adult earthworms were introduced into each cultivable plastic bag. Fifth, sixth and seventh sets of control (cowdung (CD) and partly decomposed stain removed *Camellia sinensis* (CS) and soil alone as substrate. Experiments with 10 adult earthworms in each were also maintained simultaneously along with these media. Regular watering was made to provide optimum moisture condition to the earthworms. Cocoon produced by earthworms were collected and recorded once in 15 days. Rate of cocoon production was calculated daily.

### Study on Hatching growth

All media used in the cocoon study after one month will be renewed with mixed cowdung and partly decomposed *Camellia sinensis* (stain removed) for incubation time, hatching success and hatching growth study. Cocoons collected at 15 days intervals for 45 days from earthworm *Eudrilus eugeniae* after 45 days of exposure to different PSR media would be placed separately in plastic cups containing the respective PSR medium and daily observed their incubation time and hatching ability until all cocoon are hatched out into hatching. The hatching collected from the above cups were placed in the plastic cups containing respective medium. Incubation time and hatching ability as all cocoons were also be assessed.

## Effects of vermicompost of mixed cowdung and partly decomposed *Camelliasinensis* (stain removed) powder waste on the germination percentage and seedling growth of *Vigna radiata* (MCDCS)

### Experimental details

Twenty polythene bags were taken and divided into four sets of 5 pots. The first, second and third were filled each with 3 liters of 25, 50 and 75 PSR of mixed cowdung and partly composed *Camellia sinensis* powder waste materials respectively. The fourth set of 5 polythene bags were filled each with 3 liters of soil as control.

### Seed materials

*Vigna radiata* seed purchased from the local seed farm at Mayiladuthurai.

### Seedling growth (cm/seedlings)

Twenty seedlings were randomly selected from each treatment for recording the seedling growth. The growth of the 10 days old *Vigna radiata* seedling root were measured by using a centimeter scale and the values were recorded.

### Macro and Micronutrient analysis

The levels of pH and electrical conductivity (EC) were measured in the samples of partly decomposed cowdung and partly decomposed stain removed *Camellia sinensis* powder waste and mixed cowdung and *Camellia sinensis* powder waste (1:1) and its different PSR (before and after during period of earthworm cocoon production). Macronutrients such as Total Nitrogen, Total Phosphorus & Total Potassium and Micronutrients such as Zinc, Copper, Iron and Manganese were also estimated for the samples at the soil testing Laboratory, Indian Rice Research Institute Aduthurai, Tanjavur.

### Statistical analysis

The rate of cocoon production of earthworms (cocoon / worm / day) was calculated in each medium and statistical comparisons were made between control and experimental data. Statistical comparisons were made between control and experimental data of germination percentage and root length of seedling under different mixed cowdung and partly decomposed *Camellia sinensis* (stain removed) powder waste materials using student 't' test.

## RESULTS AND DISCUSSION

### Growth of earthworm

The rate of cocoon production and weight gain / loss of *Eudrilus eugeniae* kept in 0, 25, 50, 75 and 100 PSR (per cent substrate) media prepared from partly decomposed cowdung *Camellia sinensis* powder waste (stain removed) 1:1 with soil and 100 PSR media cowdung and 100 PSR media cowdung and *Camellia sinensis* powder waste (stain removed) for 45 days were given in table 1 and 2 (figure 1 and 2) respectively. The worms kept in 25, 50 and 75 PSR mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials media and pure *Camellia sinensis* (stain removed) powder waste media for 45 days showed gradual increase in their body weight until germination of this study and their respective percent weight values were 139.55, 38.03, 52.34 and 108.77 (table 1). *Eudrilus eugeniae* showed maximum mean individual biomass ( $31.31 \pm 11.47$ ) on 25 PSR MCDCD. 100 PSR cowdung and *Camellia sinensis* (stain removed) powder waste materials higher quality than other medium. The increase of *E. eugeniae* body weight observed in current reproductive study is important observation noted in this study. From the result it is inferred that the cowdung and *Camellia sinensis* (stain removed) powder waste substrate materials used for this culture practice is generally suitable for biomass production. However, the studies of Sabitha, (2013) using cowdung and elephant dung was found to be most suitable feed material. Normally, the use of inorganic fertilizers also has a positive impact on earthworm numbers. This is probably an indirect effect of the increased crop biomass production and consequent increases in organic residues (Edwards *et al.*, 1995).

### Hatching and cocoon production

The production of cocoon by the *Eudrilus Eugeniae* in 100, 75, 50 and 25 prepared from mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials with soil and cowdung and *Camellia sinensis* (stain removed) powder waste materials for 45 days is given in table 2. The worms kept in soil alone for 45 days, though showed improvement in their size and laid cocoon. However the worms kept in 75 PSR medium for 45 days produced relatively more cocoons (1086 as total and 0.482 cocoons/worm per day) than the worms kept in other PSR medium. The value of incubation time and hatching such as of cocoon collected at 15 days intervals for 45 days for the earthworm *Eudrilus eugeniae* after 45 days previously exposed to 25, 50, 75 and 100 PSR mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials were given in table 3. Among the mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials studied, the worm kept in 75 PSR medium of 1086 cocoons and were hatched out into 667 young-ones at the rate of 0.614 with 61.4% hatching after a period of 13 days of incubation time. However, maximum of 110.76 hatching success was noticed in the cocoons collected from the earthworms exposed to 25 PSR medium. This result was in confirmation with the results of Sabitha (2013) and Ramalingam (1997) and where they respectively used for (25, 50, 75 and 100) PSR of mixed cowdung and elephant dung and for (25, 50, 75 and 100) PSR of cowdung media observed more cocoons in 25 and 50 PSR media than other media. This it is inferred that the medium with 25 and 75 PSR of mixed cowdung and *Camellia sinensis* (stain removed) powder waste is considered as a good substrate among the four mediums for *Eudrilus eugeniae* under laboratory condition for the production of cocoon studies. Ndegwa and Thomson, (2000), Tripathi and Bhardwaj, (2004) and Gajalakshmi *et al.*, (2005) have revealed that the kind, probability and quality of tested four feed mixtures to evaluate neem leaves potential to be used as feeding substrate (100% horse manure, 100% freshly ground neem leaves, 50% horse manure).

**Effect of vermicompost of mixed partly decomposed cowdung and *Camelliasinensis* (stain removed) powder waste materials on the growth of plant *Vigna radiata***

**Germination studies**

Germination percentage of *Vigna radiata* seedlings as influenced by the application of various doses of vermicompost such as control 100, 75, 50 and 25PSR are presented table 5. The highest germination percentage (94%) is recorded in 25,50 and 75 PSR MCSCD vermicompost and lowest germination percentage (90%) recorded in *Vigna radiata* seedling grown without vermicompost. Germination the critical phase in the lifecycle of a crop plant is subjected to numerous environmental factors (Dixit, 1994).

**Seedling growth**

The growth of the 10 days old *Vigna radiata* seedling was measured by using centimeter scale and the values are recorded in table 5 (Figure 5). The seedling growth observed after 10 days was maximum 75 PSR ( $3.14 \pm 1.4684$  cm/seedling) MCSCD vermicompost and minimum recorded in *Vigna radiata* seedling grown without vermicompost treatment. From this result it is inferred that the vermicompost's obtained from mixed partly decomposed cowdung and *Camellia sinensis* (stain removed) powder waste materials induces the green gram in a positive manure to germination and growth of seedling due to more amount of macro and micro nutrients present in them. The application of vermicompost increased the seed germination percentage of groundnut when compared with control. The highest germination percentage was observed in 200g of vermicompost which resulted the highest germination percentage of radish when compared to the control (Buckerfield, *et.al.*, 1999). Vegetable and ornamental seedling result showed earlier and better germination in a vermicompost compared with control (Gutierrez-Miceli *et al.*, 2007). Alves and Passoni (1997) reported that vermicompost increased the germination index of the Brazilian tree (*Licanialo mentosa*) and in a previous study we observed the use of vermicompost.

**Macro and micronutrients analysis before and after earthworm cultivable medium**

The levels of soil parameters such as pH, Ec, macro and micronutrients present in partly decomposed cow dung (CD), partly decomposed *Camellia sinensis* powder waste (CS), 25, 50, 75, 100 PSR of mixed cowdung and *Camellia sinensis* powder waste (MCDCS) before and after earthworm cultivation medium are given table 5. The pH values observed in the samples of 25, 50, 75, 100 PSR media after cultivation for 45 days showed a significant increase over their values observed before *Eudrilus eugeniae* invasion of medium. The Ec level on the contrary showed a significant decrease over its initial values in partly decomposed cowdung, 25 and 50 PSR samples obtained after earthworm invasion medium. The soil samples obtained from the above mixed cowdung and *Camellia sinensis* (stain removed) powder waste medium after invasion earthworm media showed an increase in the levels of N, P, Fe, Mn & Zn over their initial values. But the samples obtained from mixed cowdung tea waste media showed an increase K, Mn, Zn & Cu levels and decrease in P (except 25 PSR MCDCS medium) levels of earthworm cultivation medium. The maximum increase in level of potassium in 25 PSR sample of MCDCS. It is important to note here that the present study earthworm drastically improved the levels of P, Zn and Mn. Another important observation noted is that Phosphorus and Iron present in mixed Cowdung *Camellia sinensis* (stain removed) powder waste medium played a major role on the earthworm growth and yield of cocoon. From these results it is inferred that the soluble salts were greatly used by this earthworm culture while growing for its better yield of cocoon. Like the present work, Sabitha (2013) also reported a significant increase in the P, K in the worm worked Cowdung and Elephant dung mixture using *Lampito mauritii*. The present analysis through there is a change in pH during vermicomposting, the pH level observed in all the cases was at safe level (6-7) as suggested by Brady (1999). Likely Ramalingam (1997) has also noticed an increased levels of macronutrients such as N, P, K, Ca and Mg in the vermicompost obtained from individually and in combination with different organic wastes such as coir waste, press mud, water hyacinth, farm wastes, farm yard manure and big gas slurry of press mud and cattle dung using then by *Lampito mauritii* and *Eudrilus eugeniae* individually.

**Table 1: Variations in the weight of earthworm during cocoon production of *Eudrilus eugeniae* kept in Soil, Cowdung, *Camellia sinensis* powder waste and different PSR media of mixed cowdung *Camellia sinensis* (stain removed) powder waste (MCDCS) 1:1.**

PSR	0 Day	45th Day	% Change
Soil alone	11.49 ± 1.19	11.04 ± 2.21	-3.91
25 MCDCS	13.07 ± 0.90	31.31 ± 11.47	139.55
50 MCDCS	13.67 ± 1.34	18.87 ± 5.00	38.03
75 MCDCS	11.71 ± 1.34	17.84 ± 2.79	52.34
100 MCDCS	12.54 ± 4.17	12.69 ± 1.96	1.18
100 Cow dung	11.72 ± 0.63	18.54 ± 2.00	60.89
100 <i>Camellia sinensis</i> Powder waste	8.32 ± 2.3	17.37 ± 4.54	108.77

**Table 2: Rate of cocoon production of adult earthworm (*Eudrilus eugeniae*) kept in different percentage substrates (PSR) of *Camellia sinensis* (stain removed) powder waste (MCDCS) 1:1 for (26.09.2013 to 10.11.2013).**

PSR	Total cocoons collected in 5 pots	Total cocoon / pot	Cocoon/day	Cocoon/day/worm
Soil alone	12	2.4	0.05	0.005
25 MCDCS	446	89.2	1.98	0.198
50 MCDCS	693	138.6	3.08	0.308
75 MDCS	1086	217.2	4.82	0.482
100 MCDCS	661	132.2	2.93	0.293
100 Cowdung	1751	350.2	7.78	0.778
100 <i>Camellia sinensis</i> powder waste	626	125.2	2.78	0.278

**Table 3: A comparison of the incubation time and hatching success of cocoons collected at 15 days intervals in 45 days from the earthworm *Eudrilus eugeniae* after 45 days previously exposed to different PSR media of partly decomposed mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials (MCDCS).**

PSR	Total cocoons collected in 5 spots	Incubation	Hatchling	Hatchling/cocoon	Hatching success %
Soil alone	12	14	4	0.333	33.33
100 Cowdung	1751	13	2113	1.206	120.6
100 <i>Camellia sinensis</i> powder waste	26	13	600	0.958	95.87
100 MTWCD	661	14	480	0.726	72.61
75 MTWCD	1086	13	667	0.614	61.41
50 MTWCD	693	14	733	1.057	105.77
25 MTWCD	446	14	494	1.107	110.76

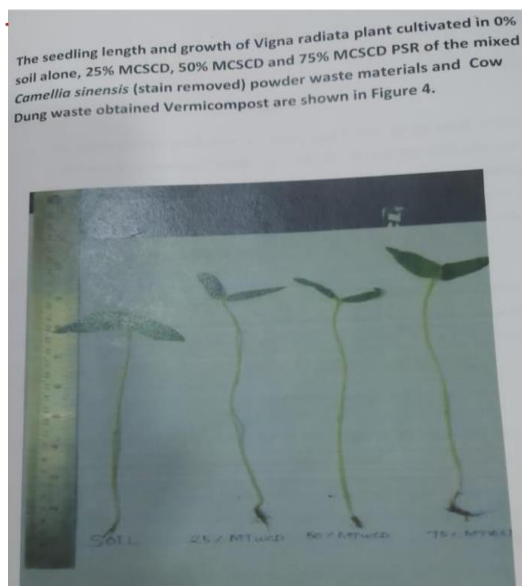
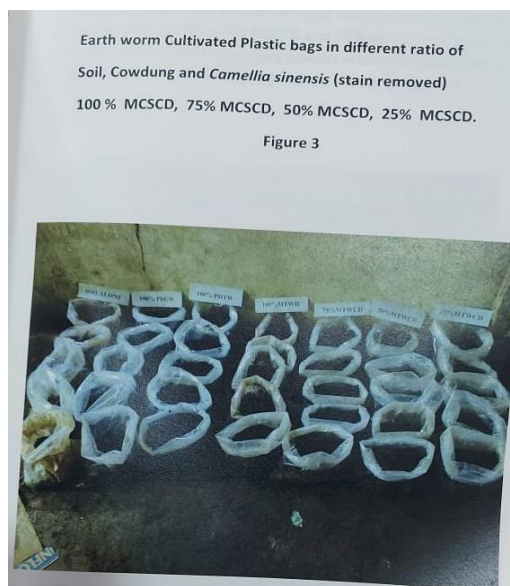
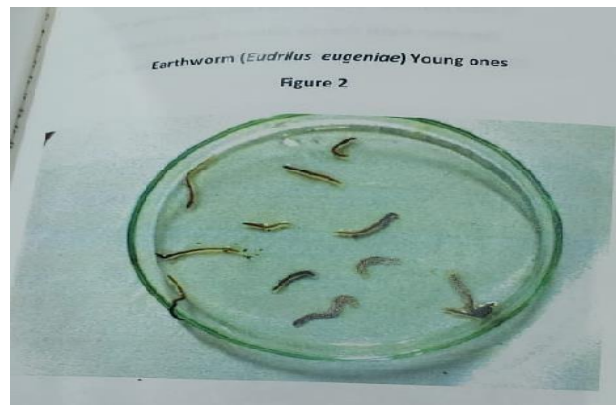
**Table 4: Variations in the levels of pH, Ec, macro and micronutrients obtained from samples of partly decomposed *Camellia sinensis* (stain removed) powder waste, cowdung (CD) and different PSR of mixture of partly decomposed tea waste and cowdung (MTWCD) before and after earthworm culture medium.**

Parameters	soil		Partly Decomposed <i>Camellia sinensis</i> (stain removed) powder waste		Partly Decomposed Cow dung		Different ratios of mixed cowdung and <i>Camelliasinensis</i> (stain removed) powder wastes PSR ( per cent substrate ratio)							
	Initial	Final	Initial	Final	Initial	Final	25		50		75		100	
							Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>pH</b>	7.12	6.7	7.7	7.0	7.6	7.6	7.6	7.8	7.7	7.8	7.2	7.7	7.6	8.9
<b>EC (dsm-1)</b>	0.62	0.9	0.60	0.9	0.72	0.6	0.60	0.2	0.62	0.3	0.38	0.4	0.40	0.5
<b>Nitrogen (%)</b>	49.0	77	63.0	63.0	70.0	62	77.0	77.0	70.0	70.0	49.0	49.0	70.0	72.0
<b>Phosphorus (%)</b>	32.5	19.5	39.5	15.6	45.0	35.10	45.5	32.5	32.5	27.3	26.0	19.2	39.3	18.3
<b>Potassium (%)</b>	132.5	132.5	140	100	140	85	100	140	125	115	85	100	115	120
<b>Iron (%)</b>	2.4	2.6	2.0	2.5	4.0	2.0	2.6	2.8	2.7	2.0	2.8	2.0	4.0	2.4
<b>Manganese (ppm)</b>	3.6	3.5	3.2	3.0	3.0	3.0	3.6	3.6	1.8	3.2	1.8	3.5	2.0	3.2
<b>Zinc (ppm)</b>	0.8	0.9	0.6	0.7	0.7	0.6	1.4	0.2	0.4	0.2	0.2	0.4	0.6	0.7
<b>opper (ppm)</b>	1.2	1.2	1.2	1.2	1.2	1.2	1.0	1.2	2.0	3.0	3.0	1.0	1.0	1.2

**Table 5: Mixed cowdung and *Camellia sinensis* (stain removed) powder waste materials (MCDCS) their effect (vermicompost) on seed germination and growth of *Vigna radiata*.**

Treatments	Germination percentage(%)	Seedling Length(cm/seedling)
Soil alone (control)	90	16.56 ± 0.8639
25% MTWCD	94	19.48 ± 1.0703(17.63)*
50% MTWCD	94	20.64 ± 0.6829(24.63)*
75% MTWCD	94	24.06 ± 0.8039(45.28)*

Mean ± SD of 5 *Vigna radiata* plants\* significant (P< 0.05) over control Values in parenthesis indicate per cent increase over respective control values.



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