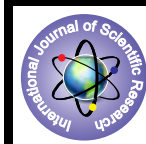


Effects of biofertilizer enriched vermicompost on the growth parameters of *Vigna unguiculata* (L.)



Biotechnology

KEYWORDS : *Cassia auriculata*; *Eudrillus eugeniae*; *Gliricidia sepium*; *Tephrosia purpurea*; *Vigna unguiculata*.

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ABSTRACT

The present study aimed to identify the productivity of cow pea plant with biofertilizer enriched vermicompost. The values of maximum root length, shoot length, number of root nodules, internodes, number of appeared flowers, number of pods appeared, pod length, pod wet weight and number of seeds found higher on 70th day with VC (TP + CA) + MI × 108 treated plants. The level of C/N and C/P ratio found maximum in leaf litter of GS + CD (1:1) and Leaf litter of (CA + GS) + CD (1:1:2) treated vermicompost. The total numbers (140.16) and total biomass (61.36 g) of the recovered worms found higher in leaf litter of (TP + CA) + CW (1:1:2) in treated litters. The microbial counts found higher in leaf litter of (TP + CA) + CD (1:1:2) treatments. The percentage occurrence of nutritional factor, chlorophyll content and biochemical parameter found higher in (TP + CA) + CD (1:1:2) treated plants. It has concluded that, the enrichment of vermicompost with biofertilizer has enhanced the various physiological properties in *Vigna unguiculata*.

1. Introduction

Pollution by organic materials in the environment is becoming an ever-increasing problem to mankind due to the growth of human population, industries and their needs. On the other hand, the extensive use of chemical fertilizers has led to the depletion of soil fertility, leading to loss of crop productivity in addition to environmental pollution. These two problems can very well solved by utilizing earthworms. The vermicompost thus derived is a rich in available nutrients, soil beneficial microbes and plant growth promoting substances (Daniel and Karmegam, 2000). On the other side, the biofertilizers (PSM, PPB and nitrogen fixing bacteria) also proved to have the crop plant growth enhancements (Darzi et al., 2012). But the studies related to the biofertilizer enriched vermicompost in plant growth studies too limited. Keeping this in view, the present study aimed to identify the productivity of cow pea plant with biofertilizer enriched vermicompost.

2. Materials methods

2.1. Vermicompost preparation

Vermi beds prepared as follows: Group 1: Leaf litter of *Tephrosia purpurea* + Cowdung (1:1) + 20 numbers of *Eudrillus eugeniae*; Group 2: Leaf litter of *T. purpurea* + Cowdung (1:1); Group 3: Leaf litter of *Cassia auriculata* + Cowdung (1:1) + 20 numbers of *Eudrillus eugeniae*; Group 4: Leaf litter of *C.auriculata* + Cowdung (1:1); Group 5: Leaf litter of *Gliricidia sepium* + Cowdung (1:1) + 20 numbers of *Eudrillus eugeniae*; Group 6: Leaf litter of *G.sepium* + Cowdung (1:1); Group 7: Leaf litter of *T. purpurea* + *C.auriculata* + Cowdung (1:1:2) +20 numbers of *Eudrillus eugeniae*; Group 8: Leaf litter of *T. purpurea* + *C.auriculata* + Cowdung (1:1:2); Group

9: Leaf litter of *C. auriculata* + *G.sepium* + Cowdung (1:1:2)+ 20 numbers of *Eudrillus eugeniae*; Group 10: Leaf litter of *C. auriculata* + *G.sepium* + Cowdung (1:1:2); Group 11: Leaf litter of *T. purpurea* + *G.sepium* + Cowdung (1:1:2)+ 20 numbers of *Eudrillus eugeniae*; Group 12: Leaf litter of *T. purpurea* + *G.sepium* + Cowdung (1:1:2). The vermicomposting trials carried out in the rearing room with the relative humidity and the temperature of 75-85 percent and 27±2°C respectively. The substrate turned (mixed) once in a week and maintained up to 90 days. The experiment carried out with three replicates for each substrate with proper control as indicated in the experimental design (Daniel and Karmegam, 2000).

2.2. Physico-chemical parameter analysis of vermibed substrates

The vermibed substrates i.e., the worms-worked (WW) and the worms-unworked (WUW) substrates analyzed for various physico-chemical parameters such as p^H, Electrical conductivity (EC), organic carbon, total nitrogen, total phosphorus, total potassium, total sodium, total calcium, total magnesium zinc, copper, iron and manganese using standard procedures (Prakash and Karmegam, 2010). The C/N and C/P ratio also calculated using the standard protocols (Paul et al., 2011).

2.3. Enumeration of Microbial population

The total colony forming units (CFU) of bacteria, fungus and actinomycetes in vermibed substrates at the beginning of the experiment (Initial) and at the end the experiment (Worm-worked and Worm-unworked substrates) enumerated using standard plate count method (Daniel and Karmegam 2000).

2.4. Isolation of *Rhizobium* sp., *Bacillus* sp., and *Aspergillus* sp.

Bacillus sp. and *Aspergillus* sp. isolated from the rhizosphere soil and *Rhizobium* sp., isolated from the root nodule of the cow pea plant and identified using standard protocols (Daniel and Karmegam 2000).

2.5. Pot culture studies

Pot culture studies carried out as follows: T1: Control - Sand + Red soil (1:1); T2: Sand + Red soil (1:1) + MI-1 $\times 10^8$; T3: Sand + Red soil (1:1) + VC(T.P + C.A); T4: Sand + Red soil (1:1) + VC(T.P + C.A) + MI-1 $\times 10^8$; T5: Sand + Red soil (1:1) + VC(C.A + G.S); T6: Sand + Red soil (1:1) + VC(C.A + G.S) + MI-1 $\times 10^8$; T7: Sand + Red soil (1:1) + VC(T.P + G.S); T8: Sand + Red soil (1:1) + VC(T.P + G.S) + MI-1 $\times 10^8$. After 35 days of sowing root length, shoot length, number of root nodules calculated and number of internodes, number of flowers appeared, pod length, pod weight, number of seeds per pod calculated on 70th day. Further, the level of NPK, total chlorophyll content, chlorophyll a, chlorophyll b, total carbohydrate, total protein and total fat identified for the T1: Control - Sand + Red soil (1:1); T2: Sand + Red soil (1:1) + MI-1 $\times 10^8$; T4: Sand + Red soil (1:1) + VC(T.P + C.A) + MI-1 $\times 10^8$; T6: Sand + Red soil (1:1) + VC(C.A + G.S) + MI-1 $\times 10^8$; T8: Sand + Red soil (1:1) + VC(T.P + G.S) + MI-1 $\times 10^8$ (which showed maximum growth parameters) using standard protocols (Prakash and Karmegam, 2010).

3. Results and discussion

The values of Physico-chemical characteristics of worm-unworked (WUW) and worm worked (W W) vermicompost represented in table 1. The values of maximum (7.14 \pm 0.02) and the minimum (6.9 \pm 0.02) pH range identified with the worm unworked (WUW) Leaf litter of CA + CD (1:1) and worm worked leaf litter of GS + CD (1:1) treatments this might be due to the neutralization of the ammonium ions by humic acid which produced during decomposition process (Bisen et al., 2011). The analysis of the EC (1.42 \pm 0.02 dS/m), N₂ (1.52 \pm 0.0%), P (1.13 \pm 0.02%), K (0.60 \pm 0.02%), Mg (1.04 \pm 0.02%), Fe (0.23 \pm 0.02%) and Cu (33.88 \pm 0.05 ppm) showed highest values with the (TP + CA) + CD (1:1:2) treatments with the worm worked leaf litters. But the minimum values of EC (0.93 \pm 0.02 dS/m), Fe (0.09 \pm 0.02 %) and Cu (27.91 \pm 0.04 ppm) identified with the TP + CD (1:1) worm un-worked leaf litters. Similarly, minimum levels of P (0.59 \pm 0.02%), K (0.19 \pm 0.02 %) and Ca (0.96 \pm 0.02%) identified with the CA + CD (1:1) treatments with the worm un-worked (WUW) leaf litters. The values of electrical conductivity have shown considerable increase in the worm-worked vermicompost compared to the worm-unworked compost. The level of soluble salt increased due to the controlled availability of the free ions and mineralization process in the earthworm guts [Priya and Prabha, 2011]. The level of C/N and C/P ratio found maximum as 64.16 and 74.59 in leaf litter of GS + CD (1:1) and leaf litter of (CA + GS) + CD (1:1:2) treated vermicompost (Fig.1b). The worm-worked vermicompost of the earthworm species *E. eugeniae* showed highly significant reduction of organic carbon, organic matter content, and C/N and C/P ratio when compared to the worm un-worked composts. Because of the combined action of microorganisms and the earthworms a large portion of the organic matter in the initial substrate lost as CO₂ by the end of the vermicomposting period. Govindan (1998) pointed out that the production of the mucus and nitrogenous excrements enhances the level of nitrogen in the vermicompost and this helps in bringing down the ratio of carbon to nitrogen, which is essential in the humification process. The total numbers (140.16) and total biomass (61.36 g) of the recovered worms found higher in leaf litter of (TP + CA) + CW (1:1:2) in treated litters (Fig.1a). The microbial counts of bacterial population (376 CFU $\times 10^7$ /g), fungal counts (249 CFU $\times 10^3$ /g) and actinobacterial counts (286 CFU $\times 10^4$ /g) found higher in Leaf litter

of (TP+CA)+CD(1:1:2) treatments (Fig 1c). The physico-chemical characteristics of pH, electrical conductivity, organic carbon, N, P, K and other elements such as Ca, Na, Mg, Fe, Cu, Zn organic matter, C/N and C/P ratios of *T. purpurea* and *C. auriculata* leaf litter efficient than other treatment; but the leaf litter of *T. purpurea* and *G. sepium* is also effective for vermicomposting operations. The reduction of p^H in the litter of *T. purpurea* and *C. auriculata* might due to the production of CO₂ and organic acids by microbial activity during the process of bioconversion of the different substrates. Similarly, Daniel and Karmegam (2000) reported the effect of various organic materials using *E. eugeniae* and *E. foetida*. The increasing of nitrogen content during the vermicomposting achieved by the excretion of worms as mucous and nitrogenous materials. Increased level of P during vermicomposting is due to the mineralization and mobilization of the earthworm gut derived phosphatase activity (Le Bayon and Binet, 2006). The elevated level of potassium in the vermicompost indicates the accelerated conversion of the organic material as available forms (Suthar, 2008). The increased level of macro and micronutrients in the vermicomposts in conformity with the results of earlier works (Daniel and Karmegam., 2000). The lowering of C: N ratio during vermicomposting is achieved by the nitrogenous mineralization process as nitric oxide compounds and combustion of carbon substrate during respiration process (Ndegwa and Thompson, 2000). Combination of the leaf litters showed enhancement of bacteria, fungi and actinobacteria counts this probably due to the lesser chemical toxic and decreased particle size (<5mm) nature of the leaf litter. The results of the nutritional, chlorophyll and biochemical constituents in various vermicompost enriched bifertilizer treatments represented in table 3. The values of maximum root length (28.26 \pm 0.28 cm), shoot length (85.66 \pm 0.38 cm), number of root nodules (98.33 \pm 1.57), internodes (17.00 \pm 1.06), number of appeared flowers (46.33 \pm 1.50), number of pods appeared (44.00 \pm 1.02), pod length (18.66 \pm 0.18 cm), pod wet weight (5.03 \pm 0.17 g) and number of seeds (5.03 \pm 0.17) found higher on 70th day with VC (T.P + C.A) +MI $\times 10^8$ treated plants (Table 2). The pot culture studies have shown that, the root and shoot parameters all higher in pots supplied with biofertilizer enriched vermicompost this might due to uptake of readily available micro and macro nutrients in the vermicompost and its associated microbes (Ach-Sah and Lakshmi Prabha, 2013). The percentage occurrence of maximum nitrogen (2.75 \pm 0.05%), phosphorous (0.83 \pm 0.04%) and Potassium (1.94 \pm 0.06%), chlorophyll-a (0.803 \pm 0.04 mg/g), chlorophyll-b (0.593 \pm 0.087 mg/g) total chlorophyll (1.500 \pm 0.78 mg/g), carbohydrate (59.98 \pm 3.07%), protein (25.90 \pm 7.09%) and fat (1.13 \pm 0.92%) content found in T2 treated plants. Biochemical parameters of chlorophyll, total carbohydrate, total protein and total fat contents found higher in biofertilizer enriched vermicompost treatments. Increased amount of chlorophyll contents seems to correlate the increased photosynthetic properties (Khomami and Moharam, 2013). In general the absorbed nitrogen, along with carbohydrates synthesized by the leaves, which ultimately led to the formation of nitrogenous substances such as amides, proteins, enzymes etc., would have helped to build up new tissues (Latrach et al., 2014). *Rhizobium* sp. inoculation significantly enhanced the plant height and dry matter as compared to un inoculated treatment at various stages of crop growth. In the present study, this is reflected through the enhancement of NPK value of the *V. unguiculata* plants in the vermicompost enhanced biofertilizer treated pots over than the control.

4. Conclusion

The above observations concluded that, the biofertilizer (Microbial inoculums of *Rhizobium* sp., *Bacillus* sp., and *Aspergillus* sp.) enriched vermicompost (*T.purpurea* + *C.auriculata* + Cowdung 1:1:2) has enhanced the various physiological properties in *V. unguiculata*. This technique can save the farmers of India from the problem posed by high cost of fertilizers by offering a com-

paratively inexpensive sustainable agriculture.

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Table 1. Physico-chemical characteristics of worm-un worked (WUW) and worm worked (W W) vermicompost

Parameters	Leaf litter of TP + CD (1:1)		Leaf litter of CA +CD (1:1)		Leaf litter of GS+CD (1:1)		Leaf litter of (TP+CA)+CD (1:1:2)		Leaf litter of (CA+GS)+CD (1:1:2)		Leaf litter of (TP+GS)+CD (1:1:2)	
	WUW	W W	WUW	W W	WUW	W W	WUW	W W	WUW	W W	WUW	W W
pH	7.06±0.03	6.97±0.02	7.14±0.02	7.04±0.03	7.06±0.02	6.9±0.02	7.08±0.02	6.94±0.02	7.08±0.02	7.05±0.02	7.06±0.02	6.99±0.02
E.C (dS/m)	0.93±0.02	1.08±0.02	1.07±0.02	1.04±0.03	0.97±0.02	1.08±0.02	1.06±0.02	1.42±0.02	1.05±0.02	1.17±0.02	1.00±0.02	1.30±0.02
O.C (%)	46.41±0.06	28.0±0.08	47.10±0.08	29.9±0.06	48.40±0.08	28.8±0.08	47.5±0.07	24.47±0.07	47.92±0.08	28.73±0.78	47.29±0.08	26.54±0.08
N ₂ (%)	0.88±0.02	1.29±0.02	0.83±0.02	0.90±0.41	0.75±0.02	1.10±0.02	0.90±0.02	1.52±0.02	0.90±0.02	1.29±0.02	0.85±0.02	1.34±0.02
P (%)	0.69±0.03	0.94±0.02	0.59±0.02	0.95±0.03	0.74±0.02	0.97±0.02	0.69±0.02	1.13±0.02	0.66±0.02	0.96±0.02	0.70±0.02	1.01±0.02
K (%)	0.25±0.02	0.36±0.02	0.19±0.02	0.33±0.28	0.28±0.02	0.40±0.02	0.30±0.02	0.60±0.02	0.26±0.02	0.45±0.02	0.27±0.02	0.52±0.02
Ca (%)	0.98±0.02	1.24±0.03	0.96±0.02	1.04±0.03	1.04±0.02	1.29±0.02	0.97±0.02	1.35±0.02	0.99±0.02	1.42±0.02	0.98±0.02	1.69±0.02
Na (%)	0.36±0.03	0.26±0.02	0.41±0.02	0.25±0.02	0.48±0.02	0.37±0.02	0.45±0.02	0.24±0.02	0.37±0.02	0.27±0.02	0.40±0.02	0.25±0.02
Mg (%)	0.87±0.02	0.90±0.02	0.89±0.02	0.89±0.02	0.84±0.02	0.88±0.02	0.96±0.02	1.04±0.02	0.79±0.02	0.84±0.02	0.87±0.02	0.97±0.02
Fe (%)	0.09±0.02	0.15±0.02	0.13±0.02	0.16±0.02	0.11±0.02	0.16±0.02	0.80±0.02	0.23±0.02	0.15±0.02	0.16±0.02	0.98±0.02	0.24±0.02
Cu (ppm)	27.91±0.04	28.77±0.09	29.99±0.05	30.07±0.05	28.11±0.26	29.40±0.84	30.0±0.10	33.88±0.05	28.70±0.04	29.60±0.05	29.79±0.04	32.17±0.04
Zn (ppm)	40.82±0.05	39.78±0.04	41.19±0.05	39.19±0.05	42.06±0.05	39.91±0.05	41.33±0.04	36.91±0.04	41.99±0.05	39.66±0.04	41.69±0.02	38.09±0.02

TP: *Tephrosia purpurea*; CA: *Cassia auriculata*; GS: *Gliricidia sepium*; CD: Cowdung

Table 2. Effect of vermicompost enriched microbial inoculums in root and shoot parameters of cow pea plant

Parameters	Root length (cm)		Shoot length (cm)		Number of root nodules		Number of internodes		Number of flowers appeared		Number of pods appeared		Pod length (cm)		Pod wet weight (gm)		Number of seeds per pod
	70	35	70	35	70	35	70	35	70	35	70	35	70	35	70	35	70
Control	2.3±0.09	4.7±1.09	6.04±1.92	13.9±3.07	14.87±3.87	30.13±5.01	-	6±1.09	-	17±2.98	15±3.09	-	1.6±0.645	-	1.7±0.64	-	6±1.04
MIX10 ⁸	4.8±0.96	9.96±1.98	16.16±1.09	30.26±1.93	18.33±2.10	33.33±1.01	-	12±2.01	-	28.33±1.03	25±2.01	-	9.76±0.92	-	2.76±1.01	-	10±1.01
VC(TP+CA)	9.30±1.30	18.2±3.07	27.09±1.59	60±12.98	27.09±5.09	52.08±4.09	-	10±3.96	-	25±3.08	22±1.09	-	12.5±3.13	-	2.9±0.97	-	9±0.87
MIX10 ⁸	15.36±0.33	28.26±0.28	50.16±0.44	85.66±0.38	50.33±1.53	98.33±1.57	-	17.00±1.06	-	46.33±1.50	44.00±1.02	-	18.66±0.18	-	5.03±0.17	-	17.00±1.05
VC(CA+GS)	7.03±0.24	15.9±2.09	23.94±2.87	53.90±6.02	24.18±3.87	49.90±7.19	-	8.84±2.09	-	21.97±4.09	19.02±2.09	-	10.25±1.94	-	2.5±0.91	-	8±2.07
MIX10 ⁸	14.16±0.31	24.36±0.30	46.36±0.41	74.96±0.40	49.33±1.56	81.33±1.50	-	16.00±1.05	-	40.33±1.51	38.00±1.01	-	17.06±0.22	-	4.32±0.21	-	14.00±1.03
VC(TP+GS)	10.67±2.09	18±1.09	26.09±4.90	57±8.34	23.09±7.72	51.40±6.09	-	10±4.98	-	23±6.03	20±3.98	-	11.7±1.971	-	2.8±0.03	-	9±1.07
MIX10 ⁸	15.56±0.34	27.06±0.32	48.96±0.43	83.36±0.37	50.33±1.48	92.33±1.52	-	17.00±1.07	-	44.33±1.54	42.00±1.03	-	17.96±0.19	-	4.79±0.23	-	16.00±1.04

VC: Vermicompost; MI: Microbial Inoculum; TP: *Tephrosia purpurea*; CA: *Cassia auriculata*; GS: *Gliricidia sepium*

Table 3: Nutritional, chlorophyll and biochemical constituents in various vermicompost enriched bifertilizer treatments

Treatment	Plant Nutritional factor			Chlorophyll content (mg/g)			Seed biochemical Parameter		
	N	P	K	a	b	Total	Carbohydrate (%)	Protein (%)	Fat (%)
Control - Sand + Red soil (1:1)	1.54±0.03	0.43±0.05	0.67±0.08	0.526±0.078	0.090±0.002	0.646±0.06	41.83±6.09	16.74±2.76	0.68±0.05
Sand + Red soil (1:1) + MI-1X10 ⁸	2.30±0.02	0.62±0.02	1.02±0.19	0.593±0.05	0.166±0.056	0.863±0.04	50.43±5.08	19.67±11.09	0.78±0.03
Sand + Red soil(1:1)+ VC(T.P+C.A)+ MI-1X10 ⁸	2.75±0.05	0.83±0.04	1.94±0.06	0.803±0.04	0.593±0.087	1.500±0.78	59.98±3.07	25.90±7.09	1.13±0.92
Sand + Red soil (1:1)+ VC(C.A+ G.S)+ MIX10 ⁸	2.57±0.06	0.72±0.03	1.85±0.02	0.658±0.01	0.365±0.087	1.096±0.90	56.90±6.08	24.72±7.09	0.90±0.48
Sand + Red soil (1:1)+ VC(T.P+G.S)+ MIX10 ⁸	2.66±0.03	0.78±0.08	1.88±0.05	0.718±0.04	0.380±0.023	1.170±0.57	58.28±7.09	25.17±4.09	0.97±0.94

VC: Vermicompost; MI: Microbial Inoculum; TP: *Tephrosia purpurea*; CA: *Cassia auriculata*; GS: *Gliricidia sepium*

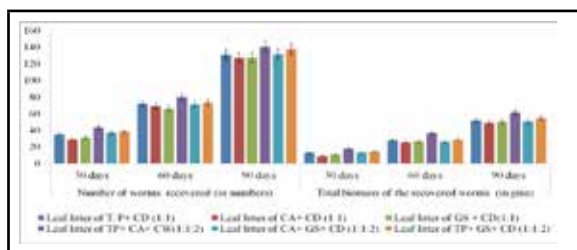


Fig 1a) Total number of earthworms and biomass in various leaf litter treatments

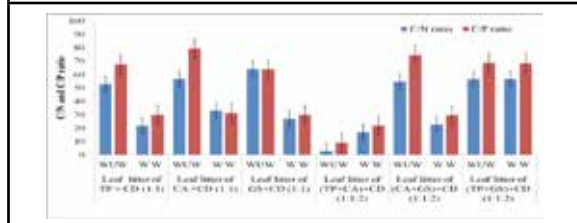


Fig 1b) Ratio of C/N and C/P in different leaf litter treatments

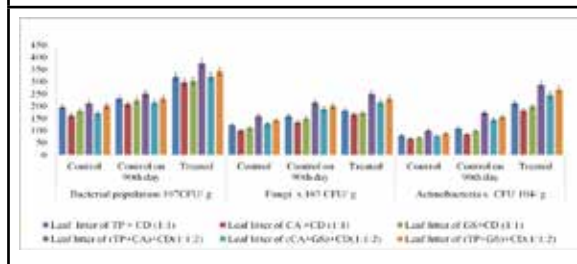


Fig 1 c) Total Counts of bacteria, fungi and actinobacteria in different leaf litter treatments

REFERENCE

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