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Advances in Applied Science Research, 2014, 5(3):243-248

ISSN: 0976-8610
CODEN (USA): AASRFC

Influence of probiotics supplemented vemicompost on growth and chlorophyll content of cowpea *Vigna unguiculata L.*

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ABSTRACT

The present study aims at understanding the effect of probiotic amended vermicompost on the growth and chlorophyll content of cowpea (Vigna unguiculata L.) plant. The vermicomposts were prepared using different wastes such as cow dung, leaf litter, flower waste and onion garlic waste to grow cowpea (V. Unguiculata L.) plants under field conditions. Various probiotics, Lactobacillus sporogenes, Essential microbes and Saccharomyces cerevisiae were used to enrich the vermicompost. After 60 days of germination, the plants were harvested and various morphological characteristics such as number of leaves, leaf length, leaf width, shoot length, number of shoot branches, root length, branches of roots, root nodules were measured. Our results indicate that the type of waste used for vermicomposting and the type of probiotic influences germination, growth parameters and chlorophyll levels in V. unguiculata L. Our results indicate that no significant increase in chlorophyll a was recorded in cowdung and leaf litter groups. In flower waste and onion garlic waste groups significant increase was recorded in chlorophyll a. No significant increase was seen in chlorophyll b in flower waste groups except in essential microbes amended groups. A highly significant increase was recorded in onion garlic waste groups. Total chlorophyll increased in cowdung + L. sporogenes, leaf litter + essential microbes, flower waste + essential microbes and onion garlic waste + essential microbes amended groups. Among plant parameters- number of leaves, leaf length, shoot length, number of root branches were highest in leaf litter amended with L. sporogenes. Leaf width and number of shoot branches were higher in onion garlic waste amended with S. cerevisiae. Root length was highest in onion garlic waste amended with L. sporogenes. Number of root nodules was the same in flower waste + essential microbes and onion garlic waste + S. cerevisiae amended groups. The results are discussed in the light of increasing plant production by using probiotics. Different wastes affect the seed germination of the test crop significantly. The study revealed that vermicompost amendment increased crop growth and is recommended for raising seed crops. The nutrients in vermicompost may determine the formation of chlorophyll in leaves and thereby the energy entrapment in photosynthesis [11].

Key words: Cowpea seeds (*Vigna unguiculata L.*), Earthworms (*Eudrilus eugeniae*), Probiotics (*Lactobacillus sporogenes*, Essential microbes and *Saccharomyces cerevisiae*), Vermicompost

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INTRODUCTION

In today's era, heavy doses of chemical fertilizers and pesticides are being used by the farmers to get a better yield of various field crops. Excessive use of fertilizer will cause environmental pollution and will destroy the balance of the ecosystem [24]. Farmers need to raise the crops by organic farming that will reduce the costs and will decrease the negative impact on the environment. In addition, organic farming will reduce the burden of environmental pollution that is caused while manufacturing these synthetic fertilizers [32]. Hence a switch over to organic forming using earthworms to recycle wastes is the need of the day. Vermicompost serves as a soil conditioner and has emerged as a means of maintaining soil productivity.

Vermiculture is a mixed culture containing soil bacteria and an effective strain of earthworms [27]. Earthworms have efficiency to consume all types of organic rich waste material. Vermicompost is the microbial composting of organic wastes through earthworm activity to form organic fertilizer which contains higher level of organic matter, organic carbon, total and available nitrogen, phosphorus, potassium and micronutrients, microbial and enzyme activities [13; 31; 28]. Microbes in the environment significantly influence the biogeochemical cycle of phosphorus. The organic phosphorus compounds are decomposed and mineralized by enzymatic complexes like phosphatases produced by microbes.

Chlorophyll is involved in the absorption and transfer of light energy and electron transfer, all of which are vital processes in photosynthesis. Chlorophyll content can change in response to biotic and abiotic stresses such as pathogen infection and light stress. Thus, quantification of chlorophyll provides important information about the effects of environment on plant growth. The present study aims at assessing the effect of application of different probiotics amended vermicompost on the growth of cow pea *V. unguiculata L.*

MATERIALS AND METHODS

Collection of Cowpea seeds
Cow pea (*Vigna unguiculata L*) belongs to the family Fabaceae. The seeds were obtained from Agriculture University, Tamil Nadu, Coimbatore, India.

Experimental site and design
Cow pea seeds (*V. unguiculata L*) were selected to find out the efficacy of vermicompost on plant growth. The cultivation was carried out in the Devi Chamber garden at Pannimadai, Coimbatore, Tamil Nadu, India. The field for cultivating cowpea was prepared during the month of November 2012. Four replicate plots of 2.5 feet were used for the experiment. Vermicompost from different wastes such as cow dung, leaf litter, flower waste and onion garlic waste were selected. In each group *L. sporogenes*, Essential microbes and *S. cerevisiae* added vermicompost were taken. Fifty seeds were sown in each of the plots and the seedlings were watered regularly up to 60 days.

Measurement of plant growth characteristics
After 60 days of germination various morphological growth parameters, such as number of leaves, leaf length, leaf width, shoot length, number of shoot branches, root length, branches of roots and root nodules were measured.

Estimation of chlorophyll
One gram of fresh leaf sample was collected from cow pea plants, transferred to a motor and 20ml of 80% acetone was added and homogenized. The homogenate was centrifuged at 5000rpm for 5minutes. The supernatant was collected and using spectrophotometer, maximum absorbance was read at 645nm for chlorophyll a, and at 663nm for chlorophyll b and 652nm for total chlorophyll. The quantity of these pigments present was calculated [7].

RESULTS

Of the various vermicomposts, number of leaves and leaf length were maximum in leaf litter + *L. sporogenes* added groups. Leaf width was highest in leaf litter + *S. cerevisiae* added groups. Shoot length was maximum in cowdung + *S. cerevisiae* added groups. Number of shoot branches was maximum in onion garlic waste + Essential microbes, root length was maximum in onion garlic waste + *L. sporogenes* added groups. The number of root nodules was highest in leaf litter + *L. sporogenes* added groups.

The number of leaves was maximum in Leaf Litter + *L. sporogenes* (40 ± 1.30cms) and minimum was noted in leaf litter supplemented groups. Leaf length was lowest in cowdung + Essential microbes supplemented groups (6.28 ± 0.18cms) and highest in leaf litter + *L. sporogenes* (8.68 ± 0.59cms) respectively. Leaf width was higher in leaf litter + *S. cerevisiae* (7.66 ± 0.57cms) and onion garlic waste + *S. cerevisiae* (7.58 ± 0.91cms), lower leaf width was recorded in cowdung + *S. cerevisiae* added groups (4.94 ± 0.66cms).

Shoot length was 244.44 ± 5.97cms in cowdung + *S. cerevisiae* 219.78 ± 4.78cms in onion garlic waste + *S. cerevisiae* and 200.08 ± 7.92cms in onion garlic waste + Essential microbes added groups respectively. Number of shoot branches was maximum in onion garlic waste + *L. sporogenes* 22 ± 1.99cms and minimum level in onion garlic waste (14 ± 0.80cms) added groups.

Branches of root were more in leaf litter + *L. sporogenes* (26 ± 1.52cms) and flower waste + Essential microbes (20 ± 1.58cms) and minimum in cowdung + Essential microbes (13 ± 1.30cms) added groups. Root nodules were maximum in leaf litter + *L. sporogenes* (8 ±1.30cms) and minimum in cowdung (5 ±1cms) added groups.

Chlorophyll a content were maximum in cowdung + *L. sporogenes* (0.21 ± 0.06 µg/l) and minimum in cowdung + Essential microbes (0.11 ± 0.06 µg/l) added groups. Chloropyll b content was highest in leaf litter + *L. sporogenes* (0.38 ± 0.41 µg/l) and flower waste + *S. cerevisiae* (0.33 ± 0.21 µg/l), lowest in leaf litter + Essential microbes (0.09 ± 0.05). The total chlorophyll content was highest in flower waste + Essential microbes (0.42 ± 0.11µg/l) and onion garlic waste + Essential microbes (0.41 ± 0.08 µg/l) added groups, lowest in onion garlic waste (0.15 ± 0.06 µg/l) added groups.

DISCUSSION

In the present study on cowpea (*Vigna unguiculata L.*) plant growth greatly increased with the application of probiotics added vermicompost. Germination percentage was maximum (100%) in seven experimental groups (Table-1) and minimum (93.3%) was seen in onion garlic waste, onion garlic waste + *L. sporogenes* and cowdung groups. The substitution of vermicompost in soil has always been associated with increasing germination percentage and yield of vegetable even at low substitution rates and is independent of nutrient supply in various experiments [8]. Better germination in vermicompost compared with control has been reported in several vegetable and ornamental seedlings [12; 15].

Comparing bio digested slurry and vermicompost [20] and [21] showed that the fresh and dry matter yield of cowpea (*Vigna unguiculata. L*) were greater when soil was amended with vermicompost. Vermicompost applied at a rate of 5t ha^{-1} have also been reported to significantly increase yield of tomato (*L. esculentum L.*) (5.8t ha^{-1}) in farmers field compared with control (3.5t ha^{-1}) [25]. Vermicompost has been recognized as a potential soil amendment [4] and contains high proportion of humic substances and microbial components which enhances plant growth and causes suppression of disease [25]. Suppression of plant disease such as botrylis has been reported in strawberries, green beans, grapes, geranium, leaf spot on tomatoes and powdery mildew on apple [18; 1; 36].

Maximum number of leaves with higher leaf length was seen in leaf litter on supplementation with *L. Sporogenes*. Maximum leaf width was recorded in leaf litter supplemented with *S. cerevisiae*. Leaf numbers and leaf area have been reported to increase in vermicompost tea treated plants [6]. Shoot length was higher in *S. cerevisiae* supplemented groups. Number of shoot branches showed variable results. Humic, fulvic and other organic acids extracted or produced by microorganisms in vermicompost tea could induce plant growth [5]. Aqueous extracts contain compounds with molecular structure and biological activity analogous to auxins [15].

Supplementation of *L. sporogenes* increased root length in all the four experimental groups. Water extractable growth regulators or phytohormones from vermicompost has a positive effect on initial root development [14]. Root nodules showed various results in the experimental groups. Root initiation and root biomass has been attributed to organisms essential for maintaining vigorous plant growth capable of withstanding environmental stress [9].

Table-1. Effect of various vermicomposts amended with probiotics *Lactobacillus sporogenes*, Essential microbes and *Saccharomyces cerevisiae* on seed germination chlorophyll a, b and total chlorophyll content of cowpea (*Vignaunguiculata L.*)

Parameters	Cowdung groups				Leaf litter groups				Flower waste groups				Onion garlic waste groups			
	CD	CD + L. s	CD + EM	CD + S. c	LL	LL + L. s	LL + EM	LL + S. c	FW	FW + L. s	FW + EM	FW + S. c	OGW	OGW + L. s	OGW + EM	OGW + S. c
Seed Germination (%)	93.3	96.7	100	100	96.7	100	100	100	98.3	93.3	98.3	100	93.3	93.3	96.7	100
Chlorophyll µg/l(a)	0.41 ± 0.59	0.21 ± 0.06^{ns}	0.11 ± 0.06^{ns}	0.12 ± 0.05^{ns}	0.09 ± 0.3	0.7 ± 0.02^{ns}	0.05 ± 0.03^{ns}	0.14 ± 0.06^{ns}	0.07 ± 0.02	0.13 ± 0.04*	0.16 ± 0.03*	0.15 ± 0.05*	0.05 ± 0.04	0.07 ± 0.05^{ns}	0.15 ± 0.05*	0.15 ± 0.04**
Chlorophyll µg/l (b)	0.14 ± 0.09	0.18 ± 0.07^{ns}	0.19 ± 0.09^{ns}	0.25 ± 0.06^{ns}	0.17 ± 0.04	0.38 ± 0.41^{ns}	0.09 ± 0.05*	0.23 ± 0.10^{ns}	0.13 ± 0.05	0.24 ± 0.10^{ns}	0.26 ± 0.08*	0.33 ± 0.21^{ns}	0.10 ± 0.06	0.19 ± 0.07*	0.26 ± 0.08**	0.24 ± 0.07**
Total Chlorophyll µg/l	0.32 ± 0.20	0.38 ± 0.10^{ns}	0.31 ± 0.03^{ns}	0.29 ± 0.12^{ns}	0.26 ± 0.04	0.25 ± 0.06^{ns}	0.30 ± 0.31*	0.37 ± 0.14^{ns}	0.20 ± 0.07	0.37 ± 0.12*	0.42 ± 0.11**	0.38 ± 0.13*	0.15 ± 0.06	0.27 ± 0.04**	0.41 ± 0.08**	0.39 ± 0.11**

*NS : Statistically not significant, * significant, ** highly significant. Each value represents the mean (±SD) of 5 observations, **Significant at 5% level, * Significant at 1% level*

Table-2. Effect of various vermicomposts amended with probiotics *Lactobacillus sporogenes*, Essential microbes and *Saccharomyces cerevisiae* on growth parameters of cowpea (*Vigna unguiculata L.*)

Growth Parameters	Cowdung groups				Leaf litter groups				Flower waste groups				Onion garlic waste groups			
	CD	CD + L. s	CD + EM	CD + S. c	LL	LL + L. s	LL + EM	LL + S. c	FW	FW + L. s	FW + EM	FW + S. c	OGW	OGW + L. s	OGW + EM	OGW + S. c
Number of leaves	30 ± 1.92	25 ± 1.34**	25 ± 1.81**	29 ± 1.14^{ns}	27 ± 3.24	33 ± 1.30**	30 ± 2.39^{ns}	28 ± 3.45^{ns}	31 ± 2.30	33 ± 1.52^{ns}	33 ± 1.48^{ns}	31 ± 1.82^{ns}	32 ± 0.84	32 ± 1.902^{ns}	32 ± 2.97**	30 ± 0.84**
Leaf length (cm)	7.2 ± 0.56	8.1 ± 0.29*	6.28 ± 0.18**	7.84 ± 0.77^{ns}	7.86 ± 0.81	8.68 ± 0.59^{ns}	8.42 ± 0.74^{ns}	8.42 ± 0.36^{ns}	7.88 ± 0.71	7.24 ± 1.13^{ns}	7.86 ± 0.90^{ns}	7.82 ± 0.87^{ns}	7.04 ± 0.46	7.76 ± 0.52*	8.62 ± 0.72^{ns}	8.34 ± 0.38**
Leaf width (cm)	5.34 ± 0.56	5.44 ± 0.38^{ns}	5.66 ± 0.25^{ns}	4.94 ± 0.66^{ns}	5.03 ± 0.46	6.82 ± 0.48**	6.38 ± 0.39**	7.66 ± 0.57**	6.92 ± 0.98	6.12 ± 1.21^{ns}	6.72 ± 1.48^{ns}	6.8 ± 1.00^{ns}	6.38 ± 0.46	6.52 ± 0.73^{ns}	6.8 ± 0.46^{ns}	7.58 ± 0.91*
Shoot length (cm)	189.7 ± 5.95	214 ± 4.41**	214.2 ± 3.95**	244.44 ± 5.97**	181.26 ± 7.30	246.2 ± 2.46**	161.18 ± 6.27**	179.04 ± 2.28^{ns}	150.22 ± 10.29	163.44 ± 7.59*	174.12 ± 7.31**	189.32 ± 3.85**	197.7 ± 8.43	203 ± 5.68^{ns}	200.08 ± 7.92^{ns}	219.78 ± 4.78*
Number of shoot branches	22 ± 3.05	26 ± 2.28*	25 ± 1.14^{ns}	19 ± 1.22^{ns}	24 ± 0.84	18 ± 3.03**	19 ± 1.52**	25 ± 1.48^{ns}	25 ± 1.58	22 ± 2.07*	20 ± 2.07**	27 ± 1.34^{ns}	30 ± 1.58	25 ± 1.14**	35 ± 1**	34 ± 4.64^{ns}
Root Length (cm)	18.56 ± 0.59	19.66 ± 0.50*	19.46 ± 0.69^{ns}	19.14 ± 0.97^{ns}	15.84 ± 0.41	19.12 ± 0.66**	18.68 ± 1.07**	16.66 ± 1.74^{ns}	17.3 ± 1.38	17.94 ± 1.91^{ns}	17.72 ± 2.03^{ns}	19.1 ± 1.24^{ns}	13.98 ± 0.80	21.82 ± 1.99**	20.6 ± 5.49*	17.76 ± 0.95*
Number of Root branches	16 ± 1.34	15 ± 0.71^{ns}	13 ± 1.30*	16 ± 1.14^{ns}	17 ± 0.84	26 ± 1.52**	24 ± 1.92**	21 ± 2.77*	16 ± 1.92	15 ± 2.07^{ns}	20 ± 1.58**	19 ± 1.92*	21 ± 1.52	18 ± 1.14**	18 ± 1.14*	18 ± 2.07NS
Number of Root Nodules	5 ± 1	6 ± 0.84^{ns}	6 ± 1.14^{ns}	7 ± 1.64*	7 ± 1.92	8 ± 1.30^{ns}	6 ± 2.41^{ns}	7 ± 1.92^{ns}	8 ± 2.30	6 ± 1.64^{ns}	8 ± 1.14^{ns}	7 ± 1.58^{ns}	7 ± 1.14	7 ± 0.84^{ns}	7 ± 1.30^{ns}	8 ± 1.14^{ns}

*NS : Statistically no significant, * significant, ** highly significant. Each value represents the mean (±SD) of5 observations, *Significant at 5% level, * Significant at 1% level*

Increase in plant hight, number of leaves, buds have been reported by [26] in chilli pepper plant *Capsicum annum*. Plant height, number of leaves and flowering buds increase after application of vermicompost. However, vermicompost dose beyond 15% dose does not increase productivity of crop. 10-40% vermicopost has been reported to show greatest plant growth responses (Atiyeh *et al*., 2002). Vermicompost from cattle, pig manure and food waste has been reported to increase rate of germination, growth and flowering of a range of ornamental and vegetable seedlings [8]. Vermicompost and its components have also shown to benefit plant growth in poor light textured soil which was attributed to high rate of nitrogen mineralization as a result of high cation exchange capacity (CEC), slow and gradual release of nitrogen with minimum losses due to leaching [17]. There is also evidence that humic acids extracted from vermicompost stimulated increase in the number of roots giving the plant ability to scavenge nutrients from the vermicompost added soil for growth and development [2]. Amendment with vermicompost from bio digested slurry, increased the fresh and dry matter yield of cow pea [20]. Soils amended with vermicompost has the ability to retain moisture, improve soil structure and cation exchange capacity, have a higher rate of plant growth hormones and humic acids, higher microbial population and activity and less root pathogens and soil borne diseases [30; 29] and overall improvement in plant growth and yield [19; 3]. Amendment with solid vermicopost at 2.5% and 10% significantly stimulated pine seedling height but not aerial biomass in *Pinus pinaster* [22]. Inhibition of aerial and root biomass with 25% vermicompost substitution was also reported.

Chlorophyll represents the principal class of pigments responsible for light absorption and photosynthesis. Photosynthesis is a complex process that is sensitive to environmental factors such as macro and micro nutrients [23]. Nutrients such as NPK, Mg, Fe and Co which are readily available through vermicompost are used in the formation of chlorophyll which is required for light harvesting and subsequent conversion into chemical energy via photo assimilation [35]. Highest total chlorophyll content was reported in 20% vermicompost and arbuscular fungal inoculum treatments [34]. Fungal symbiosis with the roots will cause increase in nutrients absorption, micronutrient uptake, and enhance resistance to pathogens and increase yield and plant growth [33]. Application of vermicompost increased photosynthetic pigment and leaf gas exchange in red chilli *Capsicum annum* [10]. Chlorophyll content after application of vermicompost in red chilli was 24% for chemical fertilizer, 2.9% for 20% vermicompost dosage and 2.1% in control which indicates that chlorophyll content is enhanced [26].

CONCLUSION

Vermicomposting helps to convert various wastes into value added material thereby reducing the cost of using chemical fertilizers. Vermicompost are by themselves are rich in total nitrogen, phosphorus and potassium administration of potassium further enhances the nutrient level in the compost. Based on the results of the present student it is concluded that using earthworm various wastes can be composted and the compost used for plant growth. Further the amendment of probiotic bacteria and fungi can enhance the nutrient level in the compost. This compost can provide better growth of plant such as *Vigna unguiculata L.* Vermicompost from various wastes also influences chlorophyll content, chlophyll a and chlorophyll b.

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