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Comparative efficacy of vermicomposted paper waste and inorganic fertilizer on seed germination, plant growth and fruition of *Cyamopsis tetragonoloba*

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Abstract

The aim of the present study was to assess the influence of vermicompost generated from the paper waste spiked with cow dung slurry on the germination, plant growth and fruition of cluster bean. Two kinds of treatments were studied: (i) vermicast was applied to the soil at the rates of 5, 7.5,10 t ha^{-1} and (ii) amounts of essential nutrients equivalent to those present in the vermicast treatments in inorganic form was amended to the soil. There was a control with only soil without any nutrient supplement. The finding is in contrast to the reports on the beneficial impacts of vermicast on plant growth. In the present study, the inorganic fertilizer treatment exhibited better seed germination and plant growth than the equivalent vermicast treatments. The results indicate that the dose of vermicompost used in the present study was not sufficient to satisfy the nutrient demand of plant species studied. Additional fertilization would have improved the crop productivity.

Key words: Vermicompost, paper waste, plant growth, *Cyamopsis tetragonoloba.*

Introduction

Paper waste generation has been continually increasing over the past years due to increasing population, industrialization, urbanization and literacy. In India, the paper consumption is about 8.5 kg per capita per year and it is 0.81-5.8 % of municipal solid waste (MSW) (Gupta and Garg, 2009; www.indiastat.com). Due to the absence of waste segregating practices, the paper waste is dumped along with all other kinds of waste in open and poorly managed landfills, which is very common practice in most of the cities in India. The improper disposal of this degradable waste may lead to long term threat to the environment and public health, such as the risk of ground water pollution due to leachate seepage, fugitive greenhouse gas emission contributing to climate change and odour pollution caused by non-methane organic compounds, which is direct harassment to adjacent communities (Zhang *et al.*, 2012). Also, open dumping of wastes facilitates the breeding for disease vectors such as flies, mosquitoes, cockroaches, rats, and other pests (CPCB, 2000).

Paper and cardboard have a relatively high heating value, similar to wood, and this energy utilized via incineration can be transformed into electricity (Villanueva and Wenzel, 2007). However, incineration is not very much practiced in India due to lack of awareness and absence of waste segregating practices. The paper waste when mixed with other moist organic waste and inert material reduces its calorific value (Negi and Suthar, 2013). In recent years, due to shortage of raw material, waste paper is preferred for paper production. Also, recycling of paper consumes only 40% of the energy in comparison to the process based on other raw materials (Gupta *et al.,* 1998). However, the paper recycling industries prefer to use imported waste paper because of its better quality in terms of fibre strength and also due to inadequate domestic supply owing to the unorganized collection of waste paper within the country. In addition, the yield from imported waste paper can be as high as 90%, whereas the widely available agro-pulp and wood pulp-based waste paper in India gives yield less than 50% (IPMA, 1996). Nevertheless, if waste papers are segregated at the source itself, it could be the input material for paper recycling units. However, due to lack of efficient waste management service, the paper waste invariably finds its way to the MSW at the end.

The huge generation of these paper wastes can be treated by vermicomposting which convert the waste into useful end product that can be used as a soil amendment (Sinha *et al.,* 2010). Unlike recycling and incineration, the biological composting process is not affected either by quality of waste paper or when mixed with other organic wastes. Processing of paper waste through vermicomposting may provide an answer to the minimization of waste accumulation and also to widespread deteriorated agricultural land due to rampant use of inorganic fertilizers. It is well established that vermicompost application have beneficial impact on soil physical, chemical and biological properties and can increase the germination, plant growth and yield in both natural and agricultural ecosystem (Edwards and Bohlen, 1996). These beneficial effects have been attributed to improvement in soil properties and structure, to greater availability of mineral nutrients to plants (Edwards, 1998). In addition to this, vermicompost contain plant growth regulating components, including plant growth hormones and humic acids that are reported to be responsible for increased germination, growth and yields of plants, in response to vermicompost applications or substitutions, independent of the nutrients they contain (Tomati *et al.,* 1988; Muscolo *et al.,* 1999; Atiyeh *et al.,* 2002; Arancon *et al.,* 2003; 2006).

However, the vermicompost generated from waste paper causes apprehension towards the beneficial impact on plant growth due to the low nutrient content of this substrate. Therefore, an attempt was made to investigate the beneficial impact of vermicompost generated from the paper waste (VC) on the germination, plant growth and yield of cluster bean, a vegetable crop. This plant was chosen due to their drought tolerance which reduces the error due to the other environmental factors. Moreover as it is a leguminous plant, influence of vermicast and inorganic fertilizers on nodules formation and its growth can be revealed. In addition, to evaluate the possible non-nutrient (*i.e.,* hormones and other growth regulating components) dependent effect of vermicast over inorganic fertilizers, all essential nutrients present in the vermicast were supplied in inorganic form (IF) to the plant, and the response of plant to the different fertilizers is briefed in this paper.

Material and methods

Study area: The experiment was conducted at Pondicherry University, Puducherry, India, located on the east coast of Indian peninsula (latitude 11°56' N and longitude 79°53' E). The climate of the experimental site is typical maritime of tropical climate with a disymmetric rainfall. The average annual rain fall is about 1300 mm with 57.25 mean rainy days ((http://port.puducherry.gov.in/Port_data/MetData.htm)), and around 60% of the total rainfall is received during period of October to December through the north-east monsoon.

Treatments: The experiment was set up in 0.4 m^3 size wooden containers lined up with HDPE sheets. These containers were filled with low fertile barren land soil collected inside the Pondicherry University campus to reduce the errors due to previous soil practices. The experimental soil was characterized as sandy loam soil and its physico-chemical properties are shown in Table 1. The experiment was conducted during the *Kharif* season which is best time for sowing the cluster beans in south India. Pusa Navabahar variety was used which is locally available in the experimental area. The vermicompost was generated from the paper waste spiked with cow dung slurry by employing an epigeic species, *Eudrilus eugeniae* Kinberg. The VC was applied to the plant growth containers at the rate of 5, 7.5 and 10 t ha^{-1}. In another set, an equivalent amount of all major and minor nutrients present in vermicompost was supplied as inorganic chemical form to check the efficiency of vermicast over the inorganic fertilizer.

In the IF treatment, the primary nutrients N, P and K, secondary nutrients Ca, Mg and S, and micronutrients of Fe, Mn, Cu, Zn, B, Mo and Cl were applied to an equivalent amount of 5, 7.5 and 10 t ha^{-1} VC treatment. The chemical fertilizers were applied in the form of urea, di-ammonium phosphate, potash, $CaCO_3$, MgO, $Na_2B_4O_7$, $CuSO_4$, $FeSO_4$, $MnSO_4$ and $ZnCl_2$. Besides these treatments, one more set was maintained without any supplementation, as control *i.e.* only soil. The nutrients were supplied in two phases. The first phase was at the time of sowing which comprised half of the total nutrients. The second supplementation of nutrients was done at the time of flowering of plants.

Germination, plant growth and yield characteristics: Two seeds per container, 72 seeds per treatment were sown in all the containers. Seeds were considered germinated when they exhibited radical extension of >3 mm. Counts of germinated seeds were made daily up to eight days to determine the germination rate in terms of germination percentage (GP) and germination value (GV) by method described by Djavanshir and Pourbeik (1976). The GV is used as a comparative index to statistically assess the effects of the treatments. After germination, one plant per compartment was maintained. A separate nursery was also maintained with all VC and IF treatments. Healthy plants from the nursery were transplanted to containers where seeds failed to germinate. Adequate amount of water was provided during the experiment. Weeding was done manually. In few instances when pests were seen, organic pesticides such as neem extract and cow urine were used.

The plant height, length of shoots and roots, number of leaves, stem diameter, number of nodules present in the root and their size, biomass of shoot and root and its dry weight were recorded with randomly collected samples at each week. Leaf chlorophyll a, b and carotenoids content were estimated photometrically by using N,N-di-Methyl formamide (DMF) as extractant (Moran and Porath, 1980). Throughout the study, the disease incidence, day of flowering and number of flowers produced were recorded.

Analytical methods: Soil pH was measured in suspension of 1:2 (v/w) by using Digison™ digital pH meter 7007. The total organic carbon content was measured by modified dichromate redox method according to Heans (1984). The total nitrogen content of the samples was determined by modified Kjeldahl method (Kandeler, 1993) using Kel Plus™ semi-automated digester and distillation units. Inorganic N (N-NH_4^+ and N-NO_3^-) was extracted in 2M KCl solution (1:10 weight: volume) and ammonia content in the suspensions were determined by modified

Table 1. Characterization of vermicast and soil used in this study

Parameters	Vermicompost	Soil
Chemical properties		
pH	7.83	6.30
Total organic carbon (g kg^{-1})	259.60	8.87
Total nitrogen (g kg^{-1})	11.70	2.66
Plant available form of		
Nitrogen (g kg^{-1})	6.21	0.39
Phosphorus (mg kg^{-1})	71.40	4.05
Potassium (g kg^{-1})	1.98	0.40
Sulphur(mg $100g^{-1}$)	0.40	0.54
Calcium (g kg^{-1})	15.90	8.27
Magnesium (g kg^{-1})	7.13	0.09
Boron (mg kg^{-1})	7.28	26.90
Copper (mg kg^{-1})	16.9	5.08
Iron (mg kg^{-1})	208.30	59.90
Manganese (mg kg^{-1})	63.80	45.10
Zinc (mg kg^{-1})	94.10	55.00
Molybdenum (mg kg^{-1})	BDL	BDL
Physical properties		
Dry weight (%)	49.60	94.70
Bulk density (g cm^{-3})	0.24	1.28
Practical density (g cm^{-3})	1.21	2.72
Water-holding capacity (%)	118.00	36.90
Electrical conductivity (mhos cm^{-1})	2.96	0.12
Total porosity (%)	80.08	52.9
Air filled porosity (%)	68.14	46.3

indophenol blue technique with Labindia™ UV- 1201 model UV-Vis spectrophotometer (Bashour and Sayegh, 2007). The nitrate concentration in the extract was measured by Devada's alloy method using Kel Plus™ distillation unit. Inorganic N contents in samples are a measure of N available to plants (Jones, 2001).

Extractable potassium, calcium and sodium were determined using a Flame photometer (Elico™ CL378) after extraction with neutral 1N ammonium acetate solution. Extractable magnesium, boron, copper, iron, manganese, zinc, molybdenum were determined using a ICP-AES (JobinYvon – Ultima 2) by extracting sample/ solution ratio of 1:25 with Mehlich 3 extraction solution (Mehlich, 1984). The same extract was used to determine the extractable phosphorus according to the ammonium molybdate-ascorbic acid method (Knudsen and Beegle, 1988). Mineral S in soil was extracted with 0.0125M $CaCl_2$ solution (ratio of soil: solution, 1:4), and analyzed with a turbidimeter after the addition of $BaCl_2$ which generates insoluble $BaSO_4$ (Bashour and Sayegh, 2007).

Bulk density was measured on undisturbed cores of soil and graduated cylinder method was used for vermicast. The particle density was determined by volumetric flask method (Bashour and Sayegh, 2007). The total and water filled porosity were calculated from the particle and bulk density values of respective samples using standard formula (Carter and Gregorich, 2008). Water holding capacity (WHC) was obtained by water retention of samples filled in perforated base cylinders immersed in water after draining (Margesin and Schinner, 2005). The electrical conductivity (EC) was measured in suspension of 1:2 (v/w) by using EI™ 611E EC meter.

Statistical analysis: The data were analyzed statistically with software SPSS 16 package and subjected to either two-way ANOVA or MANOVA. Comparisons between means were tested with an LSD test. Selection of methods for different parameters were chosen based on the number of observations and number of independent variables for particular parameter.

Results and discussion

Seed germination: In both VC and IF treatments, maximum GV was observed on fourth day since the experiment began and then a decreasing trend was observed as the days progressed. The lowest GV was reported on the eighth day of the experiment (Table 2). The results of germination in terms of GV did not show significant difference with different forms or amount of nutrients applied. However, both forms of fertilizer and amount of nutrient applied were significantly higher than the control treatment (P<0.001) (Table 4). In the VC treatment maximum GP of 88.6% was observed with both 7.5 and 10 t ha^{-1} treatment and maximum GV of 40.0 was also recorded in these treatments.

Table 2. Effect of vermicast and inorganic fertilizer on germination of cluster bean's seed in terms of germination value (GV)

Treatment	Amount	Number of days from start of experiment				
		4	5	6	7	8
Vermicompost	5 t ha^{-1}	24.70	17.27	15.33	11.26	8.62
	7.5 t ha^{-1}	40.00	27.46	20.41	16.01	12.26
	10 t ha^{-1}	40.00	29.38	21.79	16.01	12.26
Inorganic fertilizer	5 t ha^{-1}	40.00	27.46	20.41	16.01	12.26
	7.5 t ha^{-1}	42.91	29.38	20.41	14.99	13.06
	10 t ha^{-1}	40.00	33.44	24.70	18.14	13.89
Control	Nil	2.50	2.64	2.74	4.26	3.47

The VC treatment at dose of 5 t ha^{-1} showed lowest GP and GV values among both VC and IF treatments. In the IF treatment, an increasing trend in GP was observed with increasing dosage of fertilizer application. In this treatment, GP of 88.6, 91.4 and 94.3% was recorded with 5, 7.5 and 10 t ha^{-1} treatment on the eighth day and, a maximum GV of 42.91 was in 7.5 t ha^{-1} treatment. In both VC and IF treatments, maximum seed germination was recorded in 10 t ha^{-1} treatment followed by lower dose of nutrient application. However containers amended with VC had shown lesser germination rate than their respective equivalent inorganic treatments (Table 3).

Other studies on influence of VC on seed germination either showed inhibitory or stimulatory effect with low concentration of VC substitution in growth media. The discrepancy in the response to vermicompost depends on plant species which reacts differently to the concentration of vermicompost application (Atiyeh *et al.*, 2000; Edwards *et al.*, 2004). The different organic substrate used for vermicomposting also changes the quality of vermicast (Zaller, 2007). Ievinsh (2011) reported inhibitory effect similar to the present study on beetroot, beans and pea at low dose of vermicompost generated from cow dung (5-10%), but germination dramatically increased with the increase of vermicompost concentration. The higher germination in IF treatments may be due to nitrate which would have been converted from the urea applied as nitrogen source. This constituents are breakers of dormancy and also stimulator of germination (Egley and Duke, 1985; Hilhorst and Karssen, 2000). Since, the nitrate readily leaches from surface soil by irrigation water, the concentration of nitrate would have declined as the days progressed, and this may be the reason for higher germination at initial days followed by steep decline till the end.

Plant growth: There was no significant difference found in results pertaining to stem diameter, length, fresh weight and dry weight of shoot and root and number of leaves with different treatments. There was significant difference only with number of nodules between different treatments (Table 4). However, there was a differential impact of the vermicast and inorganic fertilizer treatment on all the morphological parameters (Table 3). Since the data gathered were from three leaves stage to maturity, there was a larger difference within the samples. This might be the reason for the insignificant difference in data. In this experiment, the plants which were treated with IF grew faster than the VC treated plants. The highest stem diameter (14.4 mm), shoot length (134 cm), number of leaves (72), fresh weight (182.1 g) and dry shoot weight (52.4 g) was recorded in the plants treated with IF treatment equivalent to 10 t ha^{-1} paper waste VC. Plants treated with VC at the rate of 10 t ha^{-1} showed a maximum of 14.1 mm of stem diameter; 121 cm shoot length, 65 numbers of leaves, 180 g of shoot fresh weight and 57.3 g of shoot dry weight.

Many authors have reported that high dose of chemical fertilizer treatments induce suppression in nodules formation in soybean, chick pea and lupins (van Schreven, 1959; Carroll and Gresshoff, 1983; Harper and Gibson, 1984; Nie, 1989). The finding of the present study contradicts with the previous reports on suppression of nodules formation with IF treatment. There was no significant difference in the number and size of nodules between VC and IF treatment. The results indicates that there was no suppression or stimulation of nodules formation with both VC and IF treatment.

Table 3. Plant growth, leaf pigments, flowering, disease incidence, stunted growth and plant death of cluster bean treated with vermicast and inorganic fertilizer at the end of the experiment

Parameters	Vermicompost			Inorganic fertilizer			Control
	5 t ha^{-1}	7.5 t ha^{-1}	10 t ha^{-1}	5 t ha^{-1}	7.5 t ha^{-1}	10 t ha^{-1}	
Plant growth							
Stem diameter (mm)	10.9	12.8	14.1	11.9	13.1	14.4	11.2
Shoot length (cm)	96.8	118	121	108	119	134	76.8
Root length (cm)	59.2	72.1	83.4	55.7	89.1	50.1	50.2
Number of leaves	59	62	65	51	68	72	42
Number of nodules	28	41	45	38	38	37	16
Shoot dry weight (g)	37.1	47.6	57.3	39.8	43.2	52.4	45.3
Root dry weight(g)	4.78	6.82	7.22	7.53	9.39	12.7	3.21
Leaf pigments							
Chlorophyll a (mg g^{-1})	1.83	1.92	1.87	1.84	2.24	2.29	1.32
Chlorophyll b (mg g^{-1})	1.61	1.63	2.05	1.51	2.12	2.28	1.45
Total chlorophyll (mg g^{-1})	3.44	3.55	3.92	3.35	4.35	4.57	2.77
Carotenoids (mg g^{-1})	0.29	0.46	0.46	0.36	0.36	0.37	0.26
Flowering							
Number of flowers per plant	1.4	3.1	3.6	3.2	4.1	4.4	0.7
Disease incident, plant death and stunted plants							
Number of infected plant	19	28	21	8	14	12	16
Number of plant died	0	1	0	1	0	0	0
Number of stunted plant	0	2	1	1	6	11	11

Table 4. Calculated F-values using two-way ANOVA and MANOVA to study the effect of different fertilizer and amount of application on germination and plant growth parameters of cluster bean

Treatment	Germination GV	Stem diameter	Shoot length	Root length	Number of leaves	Number of nodules	Shoot dry weight	Root dry weight
Type of fertilizer	1.032$^{n.s}$	0.034$^{n.s}$	0.009$^{n.s}$	0598$^{n.s}$	0.149$^{n.s}$	0.360$^{n.s}$	0.034$^{n.s}$	1.999$^{n.s}$
Amount	0.915$^{n.s}$	0.579$^{n.s}$	0.971$^{n.s}$	2.477$^{n.s}$	1.086$^{n.s}$	0.788$^{n.s}$	0.507$^{n.s}$	0.962$^{n.s}$
Type of fertilizer x Amount	0.352$^{n.s}$	0.096$^{n.s}$	0.075$^{n.s}$	0.856$^{n.s}$	0.147$^{n.s}$	0.499$^{n.s}$	0.022$^{n.s}$	0.223$^{n.s}$

*P<0.05, **P<0.01, ***P<0.001, n.s - not significant.

The reason may be that very low amount of nutrient was supplied to the plants in both VC and IF treatment. In addition to this, slow releasing property of vermicast might be lowering nutrient availability to the plants. In the VC treated containers, it was observed that applied VC did not disintegrate till the end of the experiment period. This stable nature of this VC may have slowed down the nutrient release to plant. It might be the reason for lower plant growth in the VC treatment than equivalent IF treatment.

Photosynthetic pigments: Although, photosynthetic pigments such as chlorophyll and carotenoid in the leaves of plants amended with vermicast and inorganic fertilizer highly correlated with amount of nutrient application (Table 3), they were not significantly different (Table 5), probabaly due to the same reasons discussed above. In general, IF application was more effective than respective VC treatment. Lower rate of nutrient application and their poor availability due to the slower releasing property of vermicast may be the reason for lower photosynthetic pigments in this treatment compared to IF treated plants. In the VC treatment, the maximum total chlorophyll content (3.92 mg g^{-1}) was recorded with the dose of 10 t ha^{-1} followed by 3.46 and 3.44 mg g^{-1} with 7.5 and 5 t ha^{-1} treatments, respectively. In case of IF treatment, the maximum total chlorophyll content (4.57 mg g^{-1}) was recorded in 10 t ha^{-1} treatment followed by 4.35 and 3.35 mg g^{-1} with 7.5 and 5 t ha^{-1} IF treatments. The control showed lowest chlorophyll content (2.76 mg g^{-1}) at the end of the experiment period. Carotenoids pigment exhibited similar trend of results. The increasing nutrient availability with increasing dose of fertilizer application can be attributed to the formation of leaf pigments (Mengel and Kirkby, 1987; Shadchina and Dmitrieva, 1995; Ruza, 1996; Tejada *et al.,* 2007).

Disease incidence, plant death and stunted growth: In the first two months of the experimental period many plants were infected with bacterial blight, *Alternaria* fungal infection and whiteflies. As a consequence some plants died and some did not grow normally (Table 3). The observed disease incidence and plant mortality was not significantly different between different forms of nutrient application (vermicast and inorganic fertilizer) (Table 5). The number of stunted plants was maximum in the beginning of the experiment. After a month, all the plants grew well. Inorganic treatment showed more stunted plants in comparison to the VC treatment. In IF treatment a total of 18 stunted plants were recorded, whereas in the case of VC treatment at different doses, two or less stunted plants were recorded.

Effect on flowering: The IF treated plants produced more number of flowers in comparison to the equivalent VC treated plants (P <0.001) (Table 5). A maximum was recorded with 10 t ha^{-1} treatment with both VC and IF treatments. Increasing dose of both fertillizer application showed the trend with maximum number of flowers 10 t ha^{-1}> 7.5 t ha^{-1}> 5 t ha^{-1}> control. The plants grown

Table 5. Calculated F-values using two-way ANOVA and MANOVA to study the effect of different fertilizer and amount of application on flowering, photosynthetic pigments and diseases incident of cluster bean

Treatment	Flowering	Photosynthetic pigments				Infected, died and stunted plants		
	Number of Flowers	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids	No. of infected plants	No. of plant died	No. of stunted plants
Type of fertilizer	21.04***	2.061$^{n.s}$	1.959$^{n.s}$	2.093$^{n.s}$	1.793$^{n.s}$	0.700$^{n.s}$	0.184$^{n.s}$	1.611$^{n.s}$
Amount	17.00***	0.531$^{n.s}$	2.830$^{n.s}$	1.567*	6.642**	1.325$^{n.s}$	2.395$^{n.s}$	0.624$^{n.s}$
Type of fertilizer x Amount	1.316$^{n.s}$	0.384$^{n.s}$	0.895$^{n.s}$	0.596$^{n.s}$	1.873$^{n.s}$	0.040$^{n.s}$	0.184$^{n.s}$	0.424$^{n.s}$

*P<0.05, **P<0.01, ***P<0.001, n.s - not significant.

in the soil treated with 10 t ha^{-1} of both VC and IF showed early flowering than the other treatments. Increase in availability of nutrient in this treatment could be the reason for the higher and early flowering.

Effect on yield: In all the treatments no vegetables were produced. The reason may be due to inadequate availability of minor and trace nutrients to the plants in all these treatments. In this study, low fertile soil collected from barren land was used to minimize the errors due to the previous soil practices in the experimental results. The soil was characterized as very low nutrient soil. Therefore, the growth and yield of plants relied on the applied nutrient either in the form of VC or IF. The trace nutrient applied would have been exhausted thereby impeding the fruiting in all the treatments.

The results obtained from the experiment shows that application of VC generated from the paper waste had no beneficial impact on growth of cluster bean plant. Moreover, the IF treatment exhibited better seed germination and plant growth than the equivalent VC treatment. This indicates that the slow releasing property of VC might have led to depletion of nutrient availability to the plants. In this experiment, increasing dose of both vermicast and inorganic fertilizer improved the germination rate and plant growth parameters. However, the application of inorganic fertilizer did not suppress the formation and growth of root nodules, which is contradictory to the previous reports. There was no significant difference in number and size of nodules with different treatments. In addition to this, the plants did not fructify in all the treatments.

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