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Efficacy of Vermicompost against fertilizers on *Cicer* and *Pisum* and on population diversity of N_2 fixing bacteria

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Abstract: *Vermicompost is a very important biofertilizer produced through the artificial cultivation of worms i.e. Vermiculture. Vermicompost is enriched with all beneficial soil bacteria and also contain many of the essential plant nutrients like N, P, K and micronutrients. It increases soil aeration, texture and jilt. In this work, study is being carried out to find out the effect of different fertilizers such as DAP, FYM and Vermicompost on various morphological parameters and on the in vitro growth of bacterial colonies and its diversity in relation to two important leguminous plants such as Pisum sp. and Cicer sp. Results showed that plant grown in Vermicompost pretreated soil exhibited maximum increase in all morphological parameters such as root length, shoot length, number of root branches, number of stem branches, number of leaves, number of flowers, number of pods and number of root nodules in four months sampling in comparison to untreated, FYM treated and DAP treated soils. Further in Vermicompost pretreated soil, number of N_2 fixing bacterial colony was maximum and showed highest diversity indices (1.6 and 0.99 and 2.0 and 0.99 for Cicer sp. and Pisum sp. respectively) than FYM, DAP and untreated control. Thus not only does the Vermicompost stimulate plant growth but also it increases the N_2 fixing bacterial population in soil and also its diversity.*

Key words: *Vermicompost, Pisum sp., Cicer sp., Morphological parameters, Bacterial diversity*
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Introduction

Vermiculture means artificial cultivation of worms (Sultan, 1997). It is the basic culture that is employed for the production of vermicompost *i.e.*, conversion of the organic waste into organic fertilizer. In recent years, vermicomposting has emerged as an efficient technology for recycling wide range of organic waste into good quality compost with the help of epigenic group of earthworms. Special types of earthworms like *Eisenia foetida*, *Eudrilus* sp. *etc.* are used for the production of vermicompost. (Chaudhuri *et al.*, 2003). The intestine of earthworms have been rich occurrence of different microorganisms, enzymes *etc.* which get mixed with the digested food and help the essential minerals to decompose rapidly to form vermicompost (Kaushik and Garg, 2003; Venter and Reinecke, 1988).

Vermicompost has been emerging as an important source in supplementing chemical fertilizers in agriculture in view of sustainable development after Rio Conference. Vermicompost is a biofertilizer enriched with all beneficial soil microbes and also contains all the essential plant nutrients like N, P and K. Vermicompost that is prepared through conventional method has standard values of total nitrogen: 1.94%, phosphorus: 0.47% and potassium:0.70% it is also enriched with various micronutrients such as Mg (0.46%), Fe (7563 ppm), Zn (278 ppm), Mn (475 ppm), Bo (34 ppm), Cu (27 ppm) (Gupta, 2003). Thus eliminate usage of any further artificial chemical inputs. Further, nutrients in vermicompost are often much higher than traditional garden compost (Alam *et al.*, 2007). It is non toxic, utilize low energy input for composting and recycled bio-organic product. Due to absence of toxic enzymes it is also ecofriendly and has beneficial effect on the biochemical activities of the soil (Ali and Jahan, 2001). It also increases the quality, fertility and mineral content of the soil structure. It enhances soil aeration, texture and jilt thereby reducing soil compaction. It also build up water retention capacity of soil because of its high organic matter content and promotes better root growth and nutrient absorption (Nourbakhsh, 2007). Vermicomposting is a bio-oxidation and stabilization process of organic material that involves the joint action of earthworms and microorganisms. The earthworms are the agents of turning, fragmentation and aeration. It also increase N_2 fixation by both nodular and free living N_2 fixing bacteria and thus enhance plant growth. Vermicompost has been proved as cheapest source of nitrogen and other essential elements for better nodulation and yield particularly in legumes. Such plants can meet their N needs through both biological nitrogen fixation (symbiosis) and native nitrogen in the soil (Parthasarthi and Ranganathan, 2002).

In this article the effects of different types of fertilizers such as DAP, FYM (Farm Yard Manure) along with Vermicompost (VC) from *Eisenia foetida* produced in the college Vermicomposting unit. Experimental data accounted on the different morphological parameters such as root length, shoot length, number of root branches, number of stem branches, number of leaves, number flowers and number of pods of two leguminous plants (Family: Leguminosae); *Pisum sp.* and *Cicer sp.* A comparison was also

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made between the effect of fertilizers on the *in vitro* growth of a number of soil inhabiting N_2 fixing free living bacterial colonies and colony diversity.

Materials and Methods

Experimental plot preparation and treatment: Experimental field in the college campus was divided into two groups of four plots making a total of eight plots. The field experiment was laid out in a randomized block design (RBD) with four treatments replicated thrice. In the first group, *Cicer arietinum, var* B-124 (Marked as 'C') and in the other *Pisum sativum var. arvense* (Marked as 'P') was cultivated. The seeds were bought from an agricultural outlet in Asansol. It should be mentioned that the soil type is loamy in nature. The average meteorological data's during the time of the experiments (January- '08 - March-'08) are temperature (max-29°C and min 15.6°C) and rainfall (1.42 cm). Each group of plot consists of 4 plots (1.2 × 0.6 m). In each plot, 36 plantlets are sown. The plots were marked as C1 and P1, C2 and P2, C3 and P3 and C4 and P4 and are pre-treated with none (untreated control), FYM, DAP and VC for *Cicer* sp. and *Pisum* sp. respectively. The rate of application of the fertilizers are148, 1453, 2.7 tons ha^{-1} of VC, FYM and DAP respectively (dose estimated on the basis of nitrogen content of the fertilizers) during the time of plot preparation. Though the recommended and standard dose of vermicompost application is about 5-10 ton ha^{-1} (Nagavallemma *et. al*., 2004) but in this work much higher dose of vermicompost had to be applied, this is because the soil in experimental field is of undulating lateritic in nature having underlying sometimes exposed Gondowana rocks and belongs to a fragile nutrient deficient ecosystem, further the soil was also not conditioned.

Collection of soil sample: Land was prepared on 10th December 2005, fertilizers were applied after five days, 4 samplings were made during Jan-March, 2006 with an average interval of fifteen days. Soils were collected in U.V. irradiated sterile packets from the soil sub-surface *i.e.* 2-3 inches below the soil surface of the experimental field where *Pisum sp.* and *Cicer sp.* were grown and pre-treated with DAP, FYM and Vermicompost (VC). Different soil samples were collected and brought to the laboratory for further study. Soil samples collected from eight different plots, were serially diluted and diluted samples were plated in N_2 free glucose medium (Thompson and Skerman, 1979). In the medium, molybdenum was included to activate nitrogenase enzyme for N_2 fixation. The pH was adjusted to 6.8-7 with NaOH prior to sterilization. The medium was solidified by adding 20 g of Bacto-agar per 1000 ml unless otherwise mentioned, media was sterilized at 15 psi for 15 minutes.

Plates were incubated at 37°C for five days. The free living N_2 fixing bacteria were counted in terms of their colony forming units (CFU). The average number of N_2 fixing free living bacteria present in 1 g of soil was calculated in eight different samples for a regular period of three months.

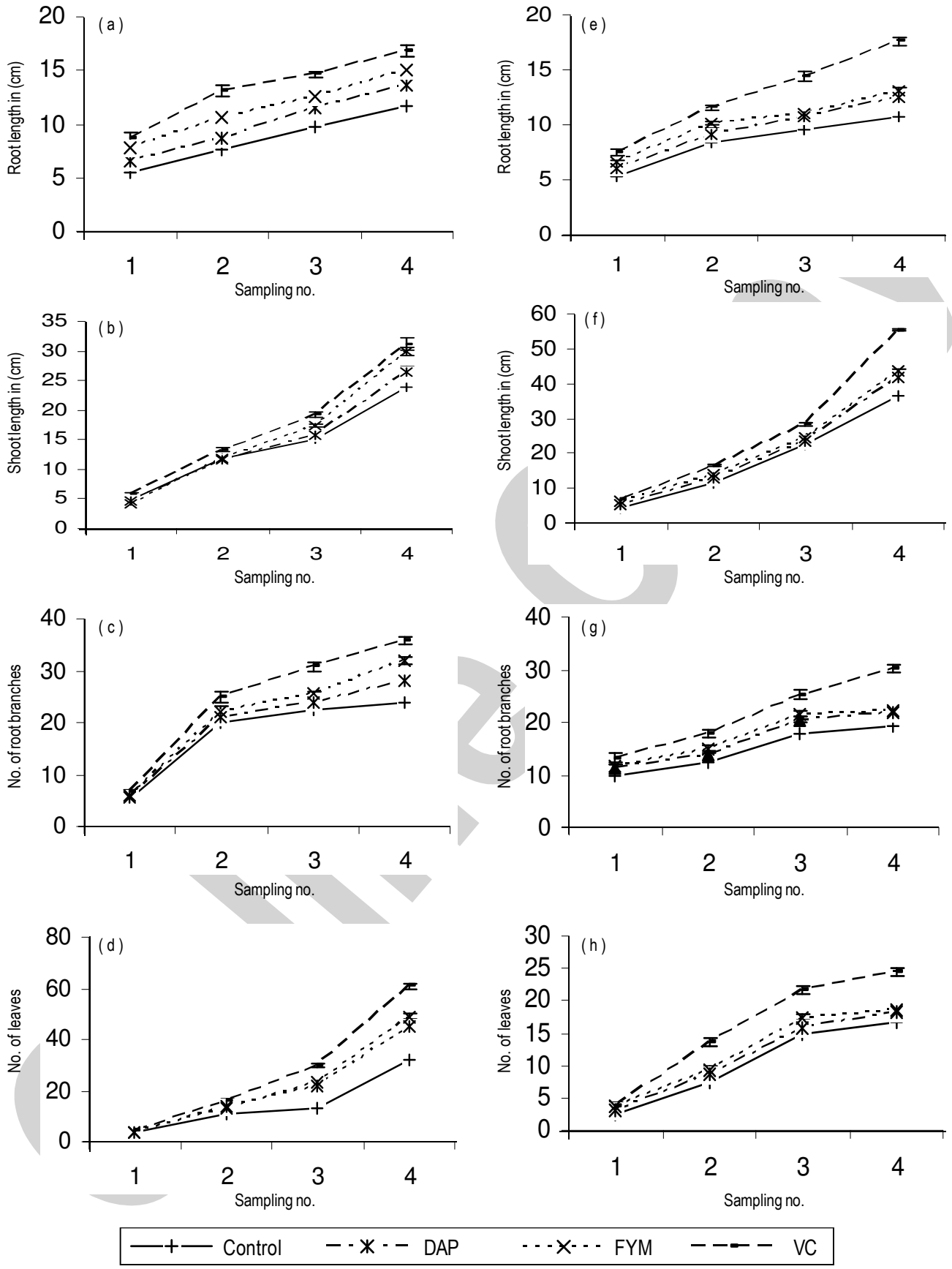
Isolation of free-living nitrogen fixing bacteria: For isolation of bacteria, 1 g of soil sample was serially diluted in steps of 10 with sterile distilled water up to a dilution of 10^{-4}. Molten autoclaved agar was poured to each of the sterile petri-plate. Aliquots of 0.2 ml of soil suspension from 10^{-4} dilution tube was transferred to sterile agar plates in triplicate and then spread over solidified agar medium with the help of spreader. Number of colony forming unit (CFU) was assessed in different time points (1st, 2nd and 3rd month) after proper indexing, maintaining in nitrogen free slants and sub-cultured at regular intervals.

Ecological diversity indices: Ecological Diversity Indices *i.e.* Shannon Weaver Index ($\bar{H}$) and Evenness Index (e) of different colonies of bacteria were also calculated using specified formulas (Odum, 1971).

Statistical analysis: In the Randomized block design (Kothari, 2004) for our experiment there are 3 variables first the Treatment factor (T) including various levels *viz.* untreated control, DAP treated, FYM treated and Vermicompost treated, second the Blocking factor (B) including various levels *viz.* 1st, 2nd, 3rd and 4th sampling and third the corresponding effect. Hypothesis is constructed in the following manner: Treatments : Null Hypothesis (H_0): $\mu_1=\mu_2=\mu_3=\mu_4$, where μ_1,μ_2,μ_3 and μ_4 are the means of morphological parameters for different treatments *viz.* untreated control, DAP treated, FYM treated and Vermicompost treated; Alternative Hypothesis (H_α): At least two of the means (μ) differ. If H_0 is rejected (for p-value<0.5=α; significance level), then various treatment and untreated control were affecting different morphological parameters such as root length, shoot length, number of root branches, number of stem branches, number of leaves, number of flowers, number of pods and number of root nodules in two and a half months of sampling. One way ANOVA has been performed to assess the difference in means of morphometric parameters and bacterial population counts using MS-Excel software.

Results and Discussion

Analysis of morphological parameters: Effect of the application of various fertilizers on different morphological parameters such as root length, shoot length, number of root branches, number of stem branches, number of leaves, number of flower, number of pods and number of root nodules were determined at regular intervals. The results of 4 samplings has been exhibited in Fig. 1(a to o). All the morphological parameters increased significantly for the plants treated with vermicompost belonging to *Cicer sp. and Pisum sp.* At the end of the final sampling *i.e.* after 2½ months the root length increased 24.6, 5.5 and 43.7%; shoot length increased 21.6, 26.4 and 48.6%; number of root branches increased 37.5, 53.84 and 49.1%; number of stem branches increased 0, 4.16 and 77.7% ; number of leaves increased 29.9, 63.1 and 82.9%; number of flowers increased 36.1, 63.88 and 148.4%; number of pods increased 40, 40 and 160% after FYM, DAP and VC treatment respectively in *Cicer sp.* Whereas in case of *Pisum sp*. the root length increased 17.64, 14.7 and 63.2%; shoot length increased 15.21, 22.82 and 24.4% number of root branches increased 17.3,


(a)
Root length in (cm)
Sampling no.
(b)
Shoot length in (cm)
Sampling no.
(c)
No. of root branches
Sampling no.
(d)
No. of leaves
Sampling no.
(e)
Root length in (cm)
Sampling no.
(f)
Shoot length in (cm)
Sampling no.
(g)
No. of root branches
Sampling no.
(h)
No. of leaves
Sampling no.
Control
DAP
FYM
VC

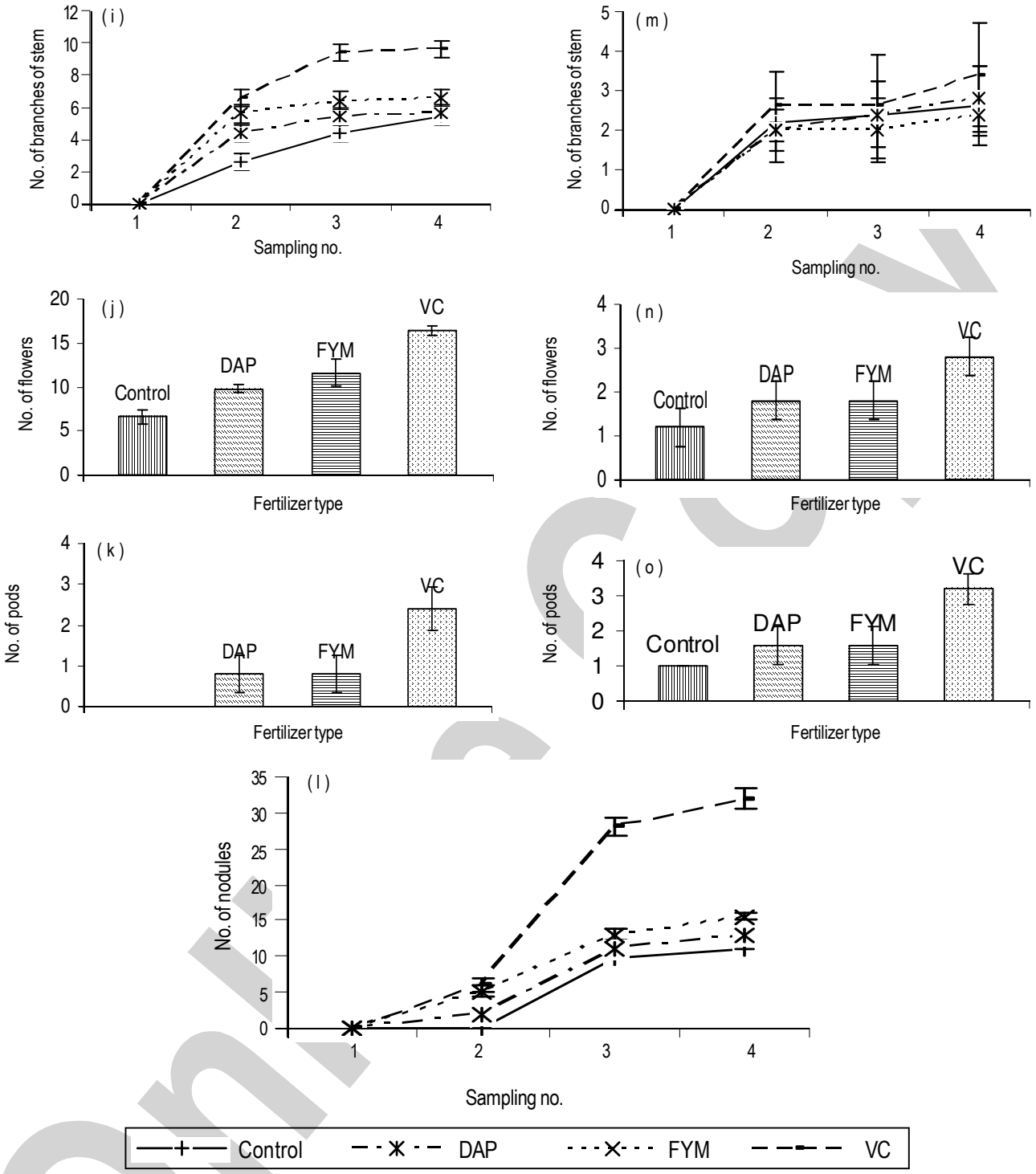


Fig. 1(a-o): Effect of various fertilizers on different morphological parameters of *Cicer* sp. (a-d and i-k) and *Pisum* sp. (e-h and l-o)

20.4 and 57.29%; number of stem branches increased 2.8, 5.7 and 53.3%; number of leaves increased 5.88, 12.94 and 46.9%; number of flowers increased 33, 50 and 133.3%; number of pods increased 60, 120 and 220% after FYM, DAP and VC treatment respectively. Percent increase of the morphological parameters after various treatments was calculated against the untreated control.

Thus there is an excellent increase of different morphological parameters in both the plants when treated with VC especially in case of the reproductive parts *i.e.* the flowers and the pods. The result is consistent with work of Alam *et al.*, 2007, carried on red amaranth. Similar work was also done on the growth and yield of wheat (*Triticum aestivum)* by Agrawal *et al.*, 2003. Further, considerable increase in the number of root nodules was observed

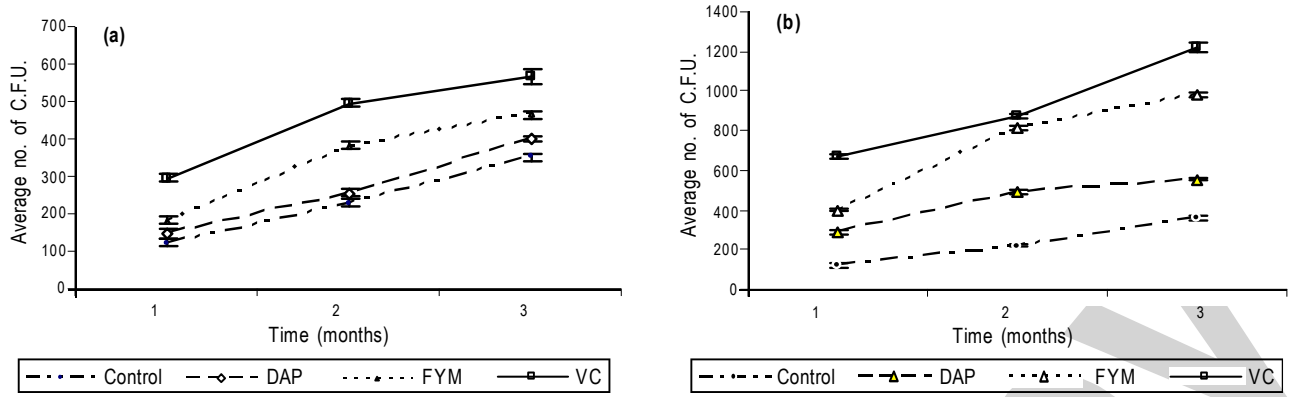


Fig. 2: Effect of various treatments on the number of free living nitrogen fixing bacteria in a) *Cicer* sp. and b) *Pisum sp.*

Table - 1: Shannon Weaver Index (H) and Evenness Index (e) of bacterial species (colonies) of soil of *Cicer sp*. and *Pisum sp.*

Diversity Index		Control	DAP	FYM	VC
$\overline{H}$	*Cicer* sp.	0.818	0.972	1.4	1.6
	Pisum sp.	0.98	1	1.3	2
e	*Cicer* sp.	0.59	0.88	0.95	0.99
	Pisum sp.	0.89	0.9	0.92	0.99

Table - 2: RBD related blocked ANOVA for assessing the effect of various treatments on morphological parameters

Morphological parameters	F (Treatment)	Significance *p* (Treatment)	Morphological parameters	F (Treatment)	Significance *p* (Treatment)
Cicer-root-length	4.251	0.040	*Pisum*-root-length	24.529	0.000
Cicer-shoot-length	4.351	0.037	*Pisum*-shoot-length	4.006	0.046
Cicer-no. of root branches	5.501	0.020	*Pisum*-no. of root branches	6.852	0.011
Cicer-no. of leaves	3.943	0.048	*Pisum*-no. of leaves	4.824	0.029
Cicer-no. of stem branches	7.029	0.010	*Pisum*-no. of stem branches	5.193	0.024
			Pisum-no. of root nodules	4.523	0.034

Note for morphological parameters *viz*. *Cicer*- no. of flower, *Cicer*-no. of pods, *Pisum*-no. of flower, *Pisum*-no. of pods results are not available in all the samplings and respective treatments so are no feasible for blocked ANOVA calculation

in *Pisum* sp. due to VC treatment compared to other fertilizers. This is in conformity with results from the vermicompost treatment on *Phaseolus aureus* (Karemegam *et al*., 1999). The plant growth may be due to directly stimulation through nitrogen fixation (Han *et al*., 2005). The results are also in agreement with findings of Parthasarthi and Ranganathan, 2002, who stated that vermicompost, enhances growth and yield in leguminous crops such as *Vigna mungo* and *Arachis hypogaea*.

Effect of Vermicompost on the growth of free living N_2 fixing bacterial colonies: Effect of vermicompost on the growth of free living N_2 fixing bacterial colonies was observed as a reflection in the increment of number of bacterial colonies in soil. A comparison of the effect of treatment with VC to other fertilizers like DAP, FYM and untreated soil on the growth of number of bacterial colonies was made.

Soil from the plot in which vermicompost was added (*i.e.* plot C4 and P4) showed highest number of bacterial colonies per gram of soil compared to other samples. The other soil samples from C2, P2 and C3, P3 in which FYM and DAP were added respectively showed greater number of bacterial colonies compared to untreated control (*i.e.* plot C1 and P1) but lesser then VC treated soil samples (Fig. 2).

The increment of free living nitrogen fixing bacteria was due to the fact that vermicoposting earthworms specially *Eisenia* sp. encourages the formation in the organic substrate of conditions favorable for the nitrogen fixing bacteria, changes in the structure of the microbial community of the substrate in support of non-spore forms of bacteria, and suppression of the growth of saprophyte bacilli, the main competitors of nitrogen fixing bacteria for carbon nourishment sources (Tereshchenko and Naplekova, 2002).

Effect of Vermicompost on microbial population diversity: Assessments were made on bacterial population diversity as reflected through colony morphology. Results showed that VC treated soil had the highest diversity index ($\overline{H}$ and e) compared to other fertilizers such as DAP and FYM pre-treated and untreated control (Table 1). Vermicompost increases the microbial diversity and populations (Barakan *et al*., 1995).

$F_{calculated}$ (Table 2) values derived from randomised block ANOVA revealed that means (μ_1, μ_2, μ_3 and μ_4) of different morphological parameters for various treatments for both *Cicer* and *Pisum* varies significantly from $F_{Tabulated\ values}$ (since $p \leq 0.05 = \alpha$; significance level), thus the H_0 is rejected.

Hence growth as reflected through various morphological parameters of two economically important leguminous plant *viz.*, *Pisum* and *Cicer* increases through the treatment of different fertilizers. But highest growth was observed in cases where soils are pre-treated with Vermicompost in comparison to FYM or DAP. Addition of vermicompost to soil increased nutrient contents in the substrate and gave higher concentrations of P, Ca, Mg, Cu, Mn and Zn in shoot tissues of red clover and cucumber (Sainz *et al.*, 1998). Further Kumari and Ushakumari, 2002 reported that enriched vermicompost was a better treatment for enhancing uptake of N, P, K, Ca and Mg by cowpea. Thus nutrient uptake enhancement aided through vermicompost, increases the growth of plants.Vermicomposts are comprised of large amounts of humic substances, some of the effects of which on plant growth are similar to those of soil-applied plant growth regulators (Muscolo *et al.*, 1999). Aracnon *et al*. (2006) reported that enhanced availability of plant growth influencing substances produced by microorganisms in vermicomposts were factors considered to have contributed to increased fruit yields in peppers. These findings support our observations that vermicompost significantly enhanced the growth of the plants and also increases the microbial diversity of vermicompost applied soil.

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