BIOMASS PRODUCTION OF VETIVER (*Vetiveria zizanioides*)

USING VERMICOMPOST.

P. Lakshmanaperumalsamy, S. Jayashree and J. Rathinamala,

Department of Environmental Sciences,

Bharathiar University, Coimbatore, 641 046.

INDIA.

<u>Corresponding Author:</u>

Dr. P. Lakshmanaperumalsamy,
Professor and Head,
Department of Environmental Sciences,
Bharathiar University, Coimbatore – 641 046. INDIA
Tel: +91-422-2422222
Fax: +91-422-2422389

e-mail: plpsamy@rediffmail.com

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ABSTRACT

Vetiver (*Vetiveria zizanioides)* is a tall tufted, perennial, scented grass with long narrow leaves and an abundant network of roots. Generally it propagates by producing new shoots at the joints above the soil surface and by branching at the joints below soil surface that have inflorescence. Most spike lets are not subject to fertilization and the seeds that are very thin have a short dormancy period. Thus, there is limited opportunity to germinate and spread like a weed. Vetiver leaves will sprout from the bottom of the clump. Each blade is narrow, long and coarse. Vetiver leaves are used as animal fodder and for roof thatching and mulching. Roots are the most useful part of the plant. They are used for absorbing water, maintaining soil moisture, arresting soil erosion and also used for making sieves, blinds, hand fans, baskets, handbags, skin care substances, oil for making perfumes and aromatic ingredients in soaps and in insect repellents. In India as well as the world market, the demand for vetiver oil is increasing day by day. One more reason for increase in demand is that this oil can be substituted with reconstituted oil and cannot be made synthetically. The Indian consumption of oil at present is about 100 tons and more that 80% is met by import. In India, the wild plant roots are harvested for oil production and no attempt has been made to grow this plant under captivity. Hence, there is a need for enhancing the production of the roots because of its importance. Here we have attempted to find out the influence of various organic manure on the biomass production of vetiver.

In the present study, the vetiver is grown using soil, vermicompost, cow dung and coir pith at ratios of 1:1, 1:1:1, 1:1:1:1 respectively. Appropriate controls were maintained. At the end of the experimental period (120 days) morphological and physico – chemical parameters were analyzed. The 120th day results showed that the ideal combination for the growth of the vetiver is soil: vermicompost: cow dung: coir pith.

The morphological parameters showed that the maximum root and shoot length, fresh weight, dry weight, number of culms, number of leaves and total chlorophyll were 64 cm, 197 cm, 312 g, 1.83 g, 26 culms / plant, 198 leaves / plant and 40.57 mg/g. The physico – chemical parameters like pH, EC, NPK, OC and C/N ratio showed 7.3, 0.38, 0.78%, 99.63 ppm, 8.7 ppm, 15 % and C/N ratio 19.2. When compared to the control, the root length, shoot length, fresh weight of vetiver grown in the ratio of 1:1:1:1 was two times higher. The result showed that the bacterial (41 x 10^5 cfu/g), actinomycetes (14 x 10^4 cfu/g) and fungal (9 x 10^4 cfu/g) population were found to be high in the rhizosphere soil in the ratio of 1:1:1:1 than the non – rhizosphere soil, other ratios and the control. The antimicrobial activity of *Vetiveria zizanioides* leaves and roots by disc diffusion method showed the maximum zone of inhibition against various pathogenic microorganisms. The extracts effectively inhibited the growth of gram – negative microorganisms except *Shigella flexneri*. However, the root extract showed larger zone of inhibition than leaf extract. It was observed that the maximum zone of inhibition was found to be 18 mm against *Solmonella typhi* and *Staphylococcus aureus* and the minimum zone of inhibition was found to be 10 mm against *Pseudomonmas aeruginosa*. The results showed that the extracts of vetiver are pharmacologically important that may be tested for control of pests and other ailments of human beings.

INTRODUCTION:

Vetiveria zizanioides is a densely tufted, wiry, perennial grass that is considered sterile outside its natural habitat. It has no rhizomes or stolen and is propagated by root divisions. The plant grows in large clumps from a branched 'spongy' rootstock with erect culms. The leaf blades are relatively stiff, long and narrow-up to 75cm long and downward tough along the edges. Spike lets are narrow and acute. One spike let is sterile, hermaphrodite, with 3 stamens and two plumose stigmas. The other spike let is pedicelled and staminate. Some cultivated forms rarely flower. Both a xerophytes and a hydrophyte, *V. zizanioides* can withstand extreme drought condition. It has wide pH range, seems to be able to grow in any type of soil regardless of fertility and has been found to be unaffected by the temperature as low as –9 ºC to as high as 45 ºC. Further more it can bear repetitive mowing and does not encroach farmland. It is also a potential feed resource.

It holds the soil together and serves as an underground wall, which not only retards water flow but also allows it to seep into the soil. It helps to conserve water, land reclamation and hillside protection. The roots are also capable of absorbing mineral nutrients and other chemical substances like chemical fertilizers or pesticides before they flow into the water sources; thus protecting water form pollutants and maintaining the water quality. It has almost no enemies; snakes, rats and other pests dislike it. Vetiver is cultivated as a relay crop. The vetiver has high nutritive value for dairy cattle and beef fattening animals. The goat and sheepherders also use the fodder as popular forage. The animals with other balanced food and vetiver show a remarkable gain in body weight within a short period. Small-scale farmers benefit both socially and economically.

The leaves are used in basketry, mat weaving and also make an excellent roof thatching. The fragrant roots are woven into screens, fans and other household items.

The dried, chopped roots are steam-distilled and the oil known as vetiver oil is extracted. It is thick amber in colour and is extensively used as a fixative in perfumery. It is used in aromatherapy to help relieve stress and to promote relaxation, as an antiseptic, antispasmodic, circulatory stimulant, fixative and sedative. Vetiver oil calms the mind and reduces the tension. Chemical components of vetiver root are also very important because they possess fungicidal, herbicidal and insecticidal properties. Vetiver oil and one of its minor constituents, nootkatone, have been shown to inhibit germination and seed expansion in a variety of economically important weed species (Mao *et al*., 2004) and possess repellent properties useful against ants, cockroaches, bedbugs, head lice, flies, moths and termites (Henderson *et al*., 2005a,b; Ibrahim *et al*., 2004; Zhu *et al*., 2001a, 2001b, National Research Council, 1993). The main objective of this study is to enhance the production of the roots by using various organic manure and assess the antibacterial activity of leaf and root.

METHODOLOGY:

Selection of the plant species:

Vetiver (*Vetiveria zizanioides)* was selected for the study because of its economical importance and easily marketable, healthy and uniform size of vetiver culm was selected. Organic manures tested: Vermicompost (VC), Cow dung (CD) and Coir pith (CP).

Experiments were designed to study the impact of VC, CD and CP on the growth of vetiver. The Vetiver (*V. zizanioides)* was collected from the field in the University campus, Coimbatore, India. The culms of vertiver was planted with 5 cm shoot and 5 cm root length in the bags of 2 feet height and 1 feet containing garden soil, vermicompost, cow dung and coir pith at the ratio of 1:1, 1:1:1 and 1:1:1:1 respectively. Appropriate controls were maintained. Three replicates were maintained in all the ratios throughout the study. After plantation, proper irrigation was done once in two days. Weeding was carried out at regular intervals.

Trials:

CONTROL	Ratio		
	1:1	1:1:1	1:1:1:1
C1 - Soil	T1 – Soil: VC	T4 – Soil: VC: CD	T7–Soil: VC: CD: CP
C2. - VC	T2 – Soil: CD	T5 – Soil: VC: CP	
C3 - CD	T3 – Soil: CP	T6 – Soil: CD: CP	

C4 - CP

The initial and final pH, EC, nitrogen, phosphorus, potassium, organic carbon and C/N ratio of the different substrates and the controls were determined. The morphological parameters like root length, shoot length, fresh weight, dry weight, number of culms, number of leaves, total chlorophyll were analyzed on the final (120^{th}) day. The population of bacteria, actinomycetes and fungus in the rhizosphere and non-rhizosphere soil, vermicompost (VC), cow dung (CD), coir pith (CP) and samples from the ratios 1:1, 1:1:1 and 1:1:1:1 were enumerated.

ANTI BACTERIAL ACTIVITY:

The agar plate disc diffusion method was followed (Vincent and Vincent 1944) for testing antibacterial activity. The extracts of fresh matured roots and leaves of *V. zizanioides* were prepared using different solvents such as water, hexane, and carbon tetrachloride using Soxhlet apparatus. The 7 mm sterile discs were dipped in the root and leaf extracts. The bacterial strains used for this assay were *Shigella flexneri*, *Solmonella typhi*, *Pseudomonmas aeruginosa* and *Staphylococcus aureus*. Inoculum of each bacterial strain was suspended in nutrient broth and incubated for 18 hrs at 37º C. The bacteria were swabbed on the agar medium and the discs were carefully placed using a sterile forceps. The plates were then incubated at 37º C for 18 – 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicates and the mean of the diameter of the inhibition zones was calculated.

RESULTS

Physico-chemical parameters:

EC of substrate varied from 0.30 (ds/m) to 0.32 (ds/m) in 1:1 ratio and 0.31 to 0.36 in 1:1:1 ratio and in 1:1:1:1 ratio, it was 0.30 to 0.38. EC was lesser than the control. pH varied between 5.9 to 7.9 in the control sets and 7.4 to 8.1 in 1:1 ratio and 7.2 to 7.8 in 1:1:1 ratio and 7.1 in 1:1:1:1 ratio in experimental substrates. (Figures 1 and 2).

Figure -1 pH of the soil samples

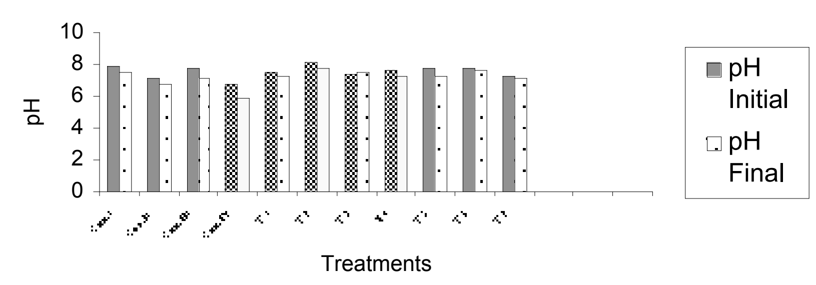
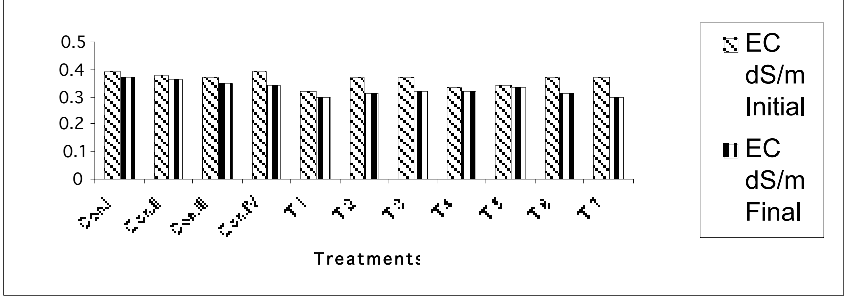


Figure - 2 EC of the soil samples



Chemical components:

Total nitrogen, phosphorus, potassium, organic carbon and C: N ratio were estimated in the soil, vermicompost, cow dung and coir pith and the experimental substrates the results are presented in Table 1 (Figures 3 to 7). Nitrogen varied between 0.31 and 0.9%. Similarly phosphorus, potassium and organic carbon varied widely in the 1:1:1:1 ratio. Phosphorus varied from 1.44 ppm to 99.63 ppm. It was less in the control sets. Potassium varied between 4.0 ppm and 16.02 ppm. Organic carbon was found to be high in T5 and least in T7. But in control set it was found to be more. C: N ratio varied from 19.23 to 79.00.

Figure - 3 Nitrogen content of the samples

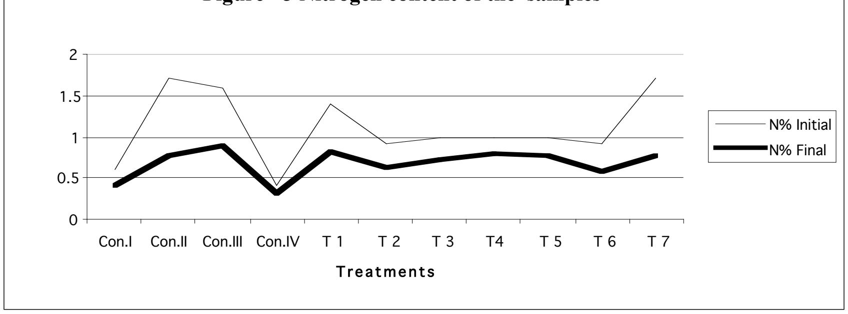


Figure - 4 Phosphorus content of the samples

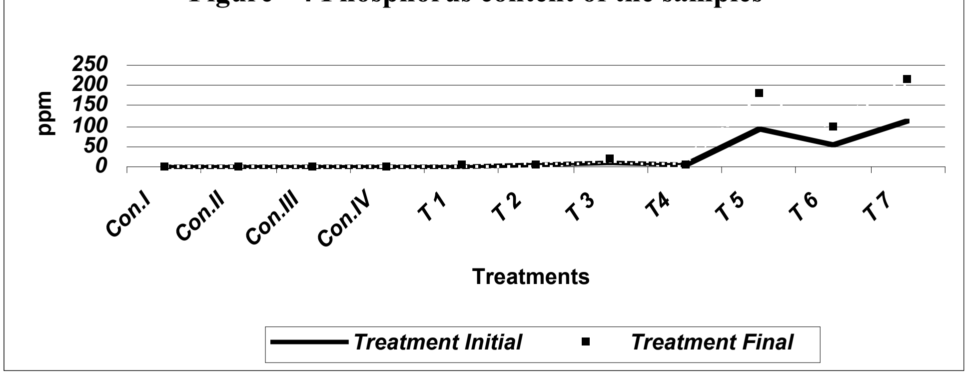


Figure - 5 Potassium content of the samples

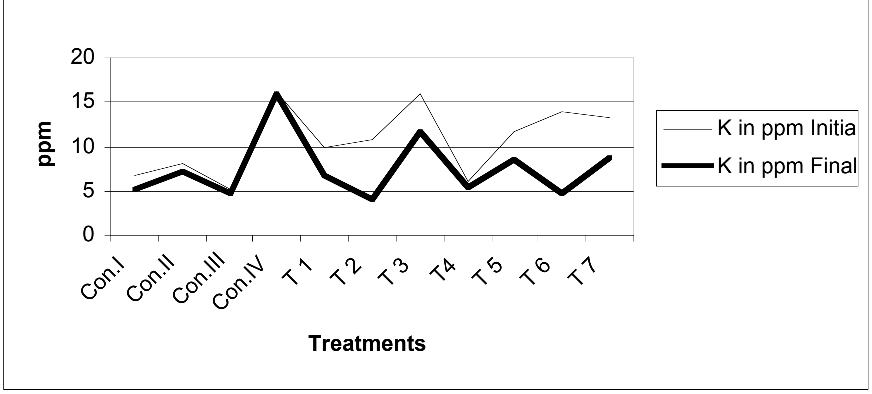


Figure - 6 Organic Carbon content of the samples

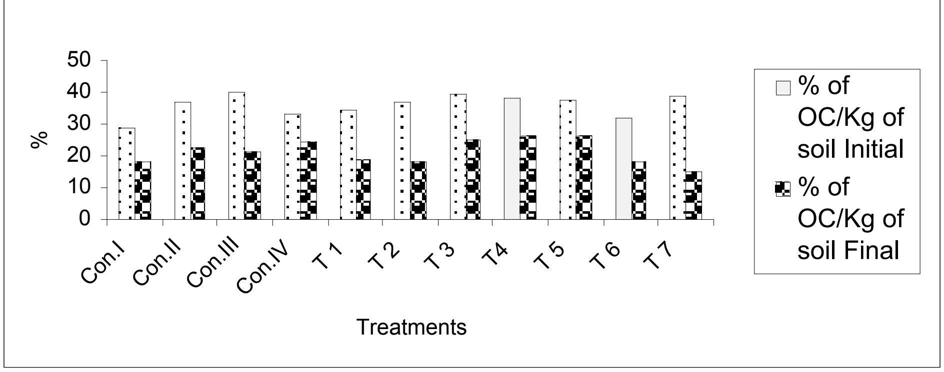
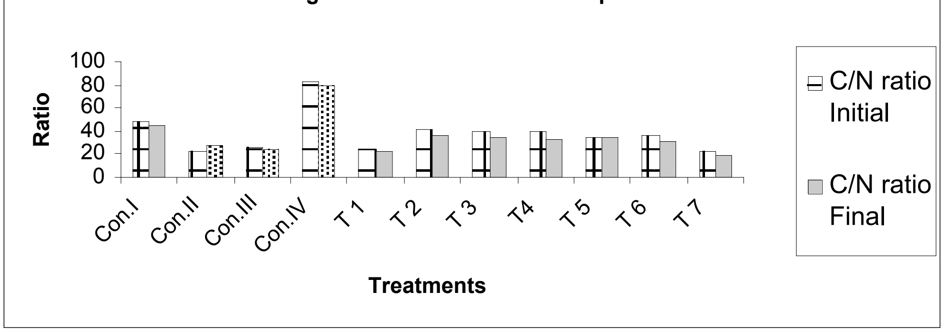


Figure - 7 C/N ratio of the samples



Microbial analysis:

Total heterotrophic bacteria, actinomycetes and fungi were enumerated from the rhizosphere and non – rhizosphere soil samples and the results are given in table 3. (Figure 8 to 10)

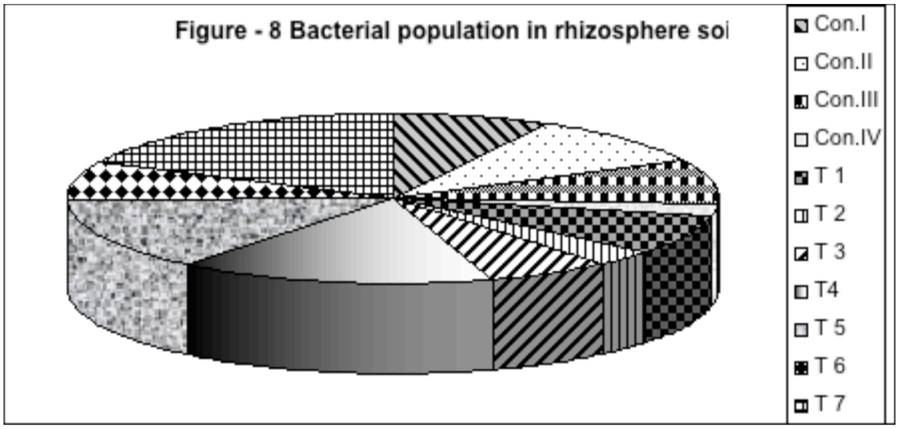


Figure - 8 Bacterial population in rhizosphere soi

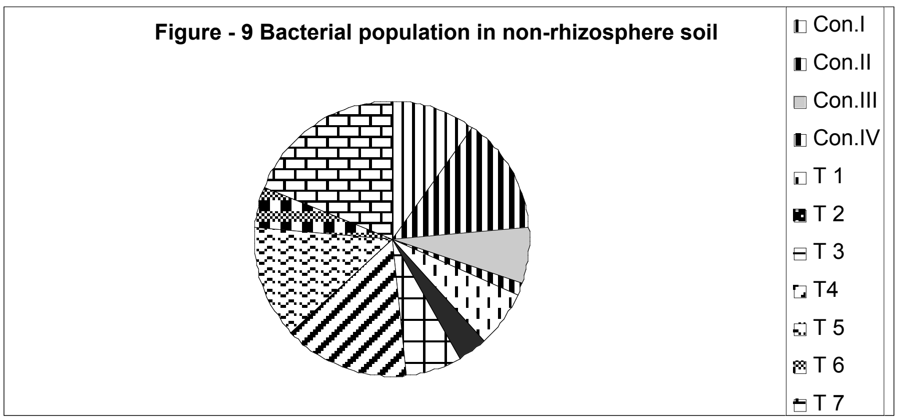


Figure - 9 Bacterial population in non-rhizosphere soil

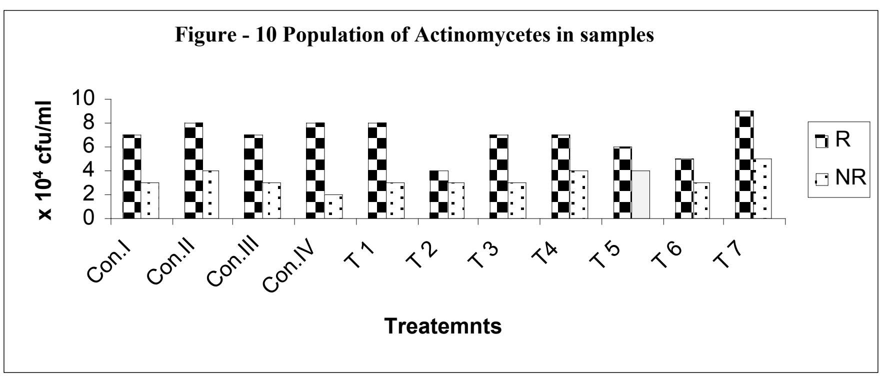
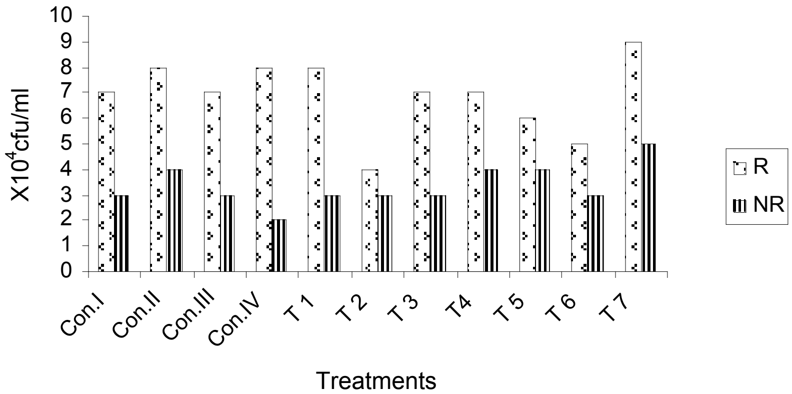


Figure - 10 Population of Actinomycetes in samples

Figure - 11 Population of fungus in the samples



Rhizosphere soil:

The total heterotrophic bacteria was found to be maximum of 41 x 10^5 cfu/g in T7 and minimum of 5 x 10^5 cfu/g in Control IV (CP). The maximum actinomycetes population was 14 x 10^4 cfu/g in T 7 and minimum was 5 x 10^4 cfu/g in Control I (S), Control IV (CP) and T 2 (S + CD). The maximum fungal population was 9 x 10^4 cfu/g in T 7 and minimum was 4 x 10^4 cfu/g in T 2 (S+CD).

Non – rhizosphere soil:

The maximum heterotrophic bacteria was 28 x 10^5 cfu/g in T 7 and minimum was found as 4 x 10^5 cfu/g in Control IV. The maximum actinomycetes population was found to be 6 x 10^4 cfu/g in T 7, and minimum was 2 x 10^4 cfu/g in T 5. The maximum fungal population was 5 x 10^4 cfu/g and minimum was found to be 2 x 10^4 cfu/g in Control IV.

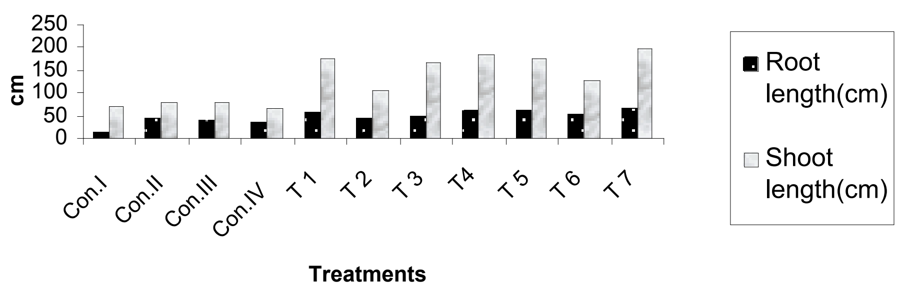
Growth parameters:

The results of growth parameters such as shoot length, root length, fresh weight, dry weight, chlorophyll content, number of leaves and number of culms in *V. zizanioides* on 120th day are given in figures 11 to 15.

The root length showed a gradual increase in the control sets and in all ratios. However the growth was at higher level in 1:1:1:1 than the other ratios. The maximum root length was 64 cm in T7 followed by T 5 with the root length of 63 cm where the root length in T 4 was 60 cm and minimum of 15 cm was found in control 1 (Soil). All the treatments showed better growth than the control sets.

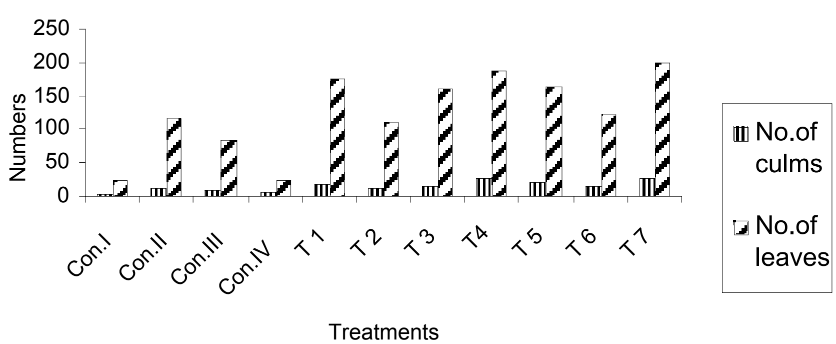
The shoot length was maximum (197 cm) in T7. T 4 showed next highest shoot length, with 188 cm, followed by T 5, T 1, T 3, T6 and T 2, where the shoot length were 176 cm. 174 cm, 165 cm, 127 cm and 107cm respectively (Figure 11).

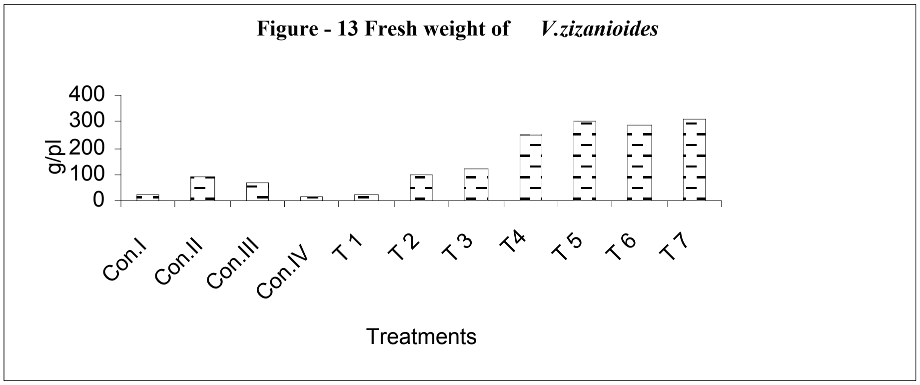
Figure - 11 Root length and shoot length of *V.zizanioides*



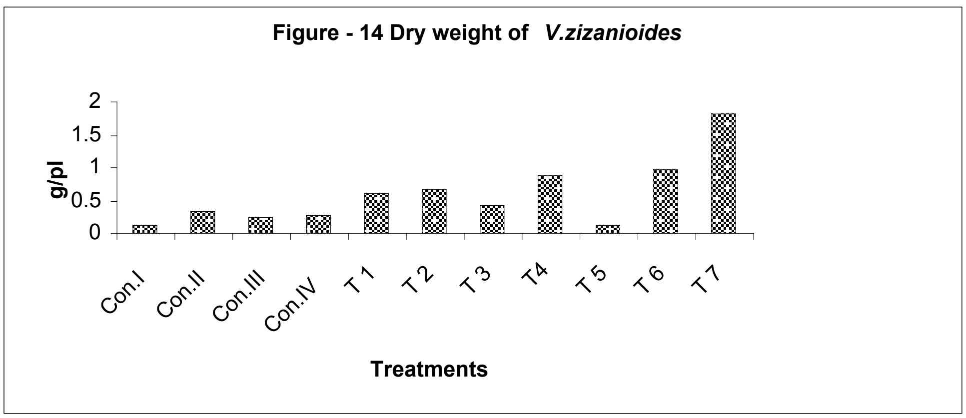
There was an increase in the number of culms and leaves in almost all treatments and also in control sets. Twenty-six culms were observed in T 4 (S+VC+CD) and T 7 (1:1:1:1 ratio). Twenty-two culms were observed in T5 (S+VC+CP), (Figure 12). The fresh weight of the plant in the treatment T7 was 312 g/p, which was highest among the treatments. In other treatments it showed higher results when compared with that of the control. The minimum fresh weight was 18 g/p seen in the control IV (Figure 13).

Figure - 12 Number of culms and leaves in *V.zizanioides*

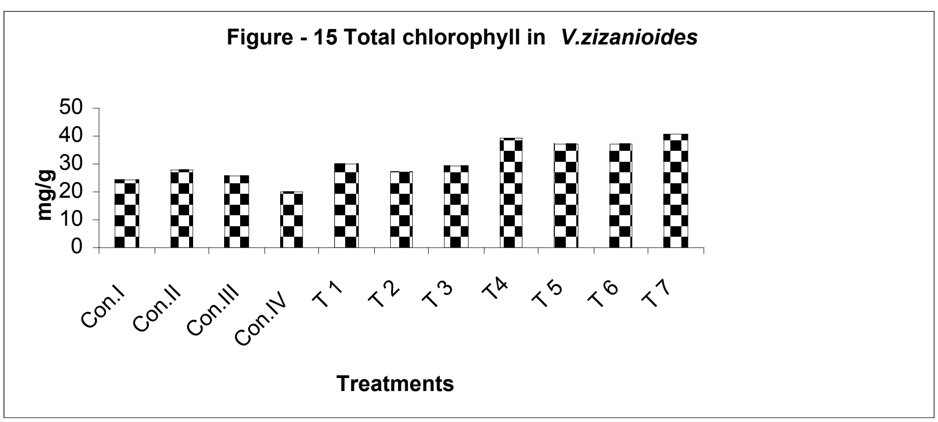




The maximum dry weight of *V. zizanioides* was 0.183 g/p in T 7. In T 6, the dry weight was found to be 0.96 g/p and the least of 0.12 g/p was noticed in the control I (Figure 14).



Maximum chlorophyll content of 40.87 mg/g was observed in *V. zizanioides* in the ratio of 1:1:1:1 (T 7) and minimum was 19.8 mg/g in Control IV (Figure 15).



Antibacterial activity:

The extracts of root and leaf effectively inhibited the growth of gram – negative microorganisms except *Shigella flexneri*. However, the root extract assay showed better zone of inhibition (18mm) against *Salmonella typhi* and *Staphylococcus aureus* and the minimum zone (10 mm) of inhibition was found against *Pseudomonas aeuginosa*.

DISCUSSION:

Vetiver (*Vetiveria zizanioides* (Linn) Nash) (2n=20) or Khus, of the family Poaceae is a densely tufted, perennial grass, found growing on various types of soils. In India, the plant is known as Khus-Khus. The commercial part of the plant is the root, which possesses a most agreeable aroma and is employed scent clothes, either by itself or in the form of sachets. From the time immemorial, vetiver roots have been employed to make baskets, hand –fans and mats which when sprinkled with water and hung like curtain in houses, cool the air and emanate a pleasant odour. Vetiver roots have become common stuffing item in ventilating panels used in electric desert coolers (Lavania, 2003). Also, compressed hard panels could be made from the roots of vetiver (Chomchalow and Chapman, 2003). Probably high tensile strength of vetiver roots and the inherent anti-micorbial property on account of its essential oil, make vetiver roots an ideal natural material for making compressed hard boards and panels. Decoction of roots is believed to dissolve kidney stones, and a past made from pounded fresh roots is considered as an abortifacient (Weiss, 1997). Medicinally vetiver oil is reported to be used as a carminative in flatulence and colic and as anthelminthic and possesses stimulant and refrigerant properties. It is locally applied to relieve pains on the body. Young leaves are used as fodder and are good bedding for horses and cattle. The leaves are also used for thatching purposes. At present, the vast majority of studies on vetiver grass focus on the functions of soil and water conservation, land reclamation and hillside protection etc. There are very few studies on the grass production of vetiver.

Biomass production of *V. zizanioides was* determined using organic manure in various combinations. The results of the organic manures individually and in combinations were analyzed.

In the present study, pH and EC decreased from the initial day to the final day (120th day). The same decreasing trend of pH and EC was observed by Mayalagu and Jawahar (2000) who reported a decrease in the soil pH indicating the acidifying effect of organic acid produced upon decomposition and EC as the compoundization of free ions present in the compost and organic soil.

The results of total nitrogen, phosphorus, potassium, organic carbon and C: N ratio showed a decrease with increase of time. According to Mba (1983), Lee (1985), Ramalingam (1997) and Karmegam (1997), the application of organic manures increased the NPK contents of the soil. Singh *et al.,* (1983), Yadhuvanshi *et al.,* (1985) and Bhat *et al.,* (1991) reported that the continuous application of organic manures and recycling of crop residues increase the available phosphorus content of soil. Singh (1978) and Sharma *et al.,* (1988) observed that farmyard manure registered highest organic carbon and available phosphorus content in the soil. All the above findings substantiate out present study very well.

The total heterotrophic bacteria, actinomycetes and fungi were found to be maximum in treatment T 7. The earthworm casts found to exhibit maximum microbial population (Teotia *et al.,* 1950, Ruschmann 1953, Kale *et al.,* 1991). The increasing trend of microbes from the initial day to the final day in the present study was attributed to the organic humus content of the soil, which is enriched heavily in the initial day due to the application of organic amendments. The effect of organic matter in increasing the microbial population has been reported (Gaur and Mukharjee,1980).

The ideal combination for the production of vetiver roots was noticed in treatment T 7. The various treatments applied to the plants started exhibiting its effects from 7th day to 120th day. The growth parameters recorded in various treatments differed according to the manure used. The best assay was observed in the treatment T7. Sharma and Bhalla (1995) reported that application of organic manures showed better response to the growth parameters.

The increase of growth parameters like root length, shoot length, number of culms, number of leaves, dry weight, fresh weight and chlorophyll content was noticed. Reddy (1998) also observed that the length and weight of the shoot and root system of *Vinca rosea* and *Oriza sativa* showed significant increase when they were applied with the casts of *Perionyx.* The application of vermicompost enhanced root initiation, root elongation, root biomass and rooting percentage. Srinivasan Reddy and Uma Mahesh (1995), Grappalli *et al.,* (1985) also observed a significant increase in dry matter and yield of green gram upon application of vermicompost and farmyard manure. Kale and Bano (1986) and Karmegam *et al.,* (1997) also observed that the application of vermicompost had greatly influenced the growth and yield of crop. Root system of the plant is the system by which water and nutrients are absorbed by the plants, so a plant with better root system obviously will give a better growth of the plant. The biomass of *V. zizanioides* was significantly greater under the treatment of domestic refuse and complex fertilizers (NPK) (Shu *et al.,* 2002a).

Antibacterial activity:

Staphylococcus aureus showed sensitivity to vetiver root extracts with an inhibition zone of 18 mm, which was equavalent to the inhibition zone exhibited by chloramphenicol disc with 30 μg concentration. Gangrade *et al.,* (1990); Hammer *et al.,* (1999), Leupin *et al*., (2000) also reported the susceptibility of *Staphylococcus aureus* to vetiver oil. Further inhibiting zone against *Pseudomonas aeruginosa*, *Solmonella typhi* showed that the vetiver extract may inhibit wide range of pathogenic bacteria.

Conclusion:

The trials reported in this paper indicate that the organic manure including vermicompost has increased vetiver biomass production. Hence it is recommended that the vermicompost either alone or supplemented with other organic manures be an excellent substrates for the maximum root production of *V. zizanioides*.

Acknowledgement:

The authors are thankful to the University for providing necessary facilities to carry out the work.

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