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REGULAR ARTICLE

EFFECT OF NITROGEN FIXING MICROORGANISMS AND ORGANIC MANURES ON THE BIOMASS PRODUCTION OF VETIVER (*VETIVERIA ZIZANIOIDES*)

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SUMMARY

Vetiver (*Vetiveria Zizaniodies*) is a tall tufted, perennial, scented grass with long narrow leaves and an abundant network of roots. Roots are more useful part of the plant. In the present study, the vetiver is grown using garden soil, cowdung, vermicompost with and without the N_2 fixing bacteria *Azospirillum* and *Azotobacter*. The 120th day results showed that the ideal combination for the growth of vetiver is vermicompost + *Azospirillum* + *Azotobacter*. The morphological parameters showed that the maximum root and shoot length, dry weight, number of culms, number of leaves and total chlorophyll were 99.75 cm, 231.00 cm, 2.45 g, 40 culms/plant, 172 leaves/plant and 41.00 mg/g respectively. The antibacterial activity of *V. Zizanioides* leaves and roots by disc diffusion method showed the maximum zone of inhibition against various pathogenic bacteria. 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 32 mm against *Staphylococuss aureus* and the minimum inhibition was found to be 22 mm against *Enterobacter faecalis*. These results showed that, the extracts of vetiver are pharmacologically important they may be tested for control of pests and other ailments of human beings.

Keywords: Vetiver, *Azotobacter, Azospirillum*, Antibacterial activity.

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1. Introduction

Vetiver (*Vetiveria Zizanioides*) is a tall tufted, perennial, scented grass with long narrow leaves and an abundant network of roots. Vetiver can grow up to 1.5 meters high and form clumps as wide. The stems are tall and the leaves are long, thin and rather rigid.

Vetiver roots grown down up to 2-4 meters in depth. Roots are the most useful part of the plant. The dried, chopped roots are steam distilled and the oil known as vetiver oil. This oil calms the mind and reduces the tension. Chemical components of vetiver roots are very important because they possess fungicidal, herbicidal and insecticidal properties [5,6].

In this present scenario, sustainable crop production needs the use of integrated nutrient management systems involving organic and inorganic sources of plant nutrients. The organic

sources are very rich in nutrients and contain more available form of nutrients to the crop. By the inoculation of organic amendments such as vermicompost, cowdung enhance the biomass of vetiver.

Biological nitrogen fixation (BNF) based systems would enhance the crop production in the longterm in both economically viable and socially acceptable ways [13]. Crop responses to inoculation with *Azospirillum* and *Azotobacter* were well demonstrated in many cereal and ferage crops [1]. The main objective of this study is to enhance the production of vetiver roots by using N_2 fixing microorganisms *Azospirillum* and *Azotobacter* with organic manures cowdung and vermicompost.

2. Materials and Methods

Pot culture studies were conducted at the Experimental farm, Department of Microbiology, Annamalai University, Tamil Nadu.

Experiments were designed to study the impact of N fixing bacteria *Azospirillum, Azotobacter* amended with organic manures such as cowdung and vermicompsot on the growth and biomass production of vetiver.

The vetiver was collected from the field in Villupuram District, Tamil Nadu, India. The culms of vetiver was planted with 5cm shoot and 5 cm root length in the pots containing garden soil, cowdung and vermicompost. After plantation, proper irrigation was done once in two days. Weeding was carried out at regular intervals. Control was maintained, five replicates were maintained throughout the study.

Trials

T1- Control (Soil)

T2-Cowdung

T3- Vermicompost

T4- Soil + *Azospirillum* + *Azotobacter*

T5- Cowdung + *Azospirillum* + *Azotobacter*

T6-Vermicompost+*Azospirillum* + *Azotobacter*

Biometric Observations

Five plants were chosen for each treatment for recording the observation. The biometric observations such as root length, shoot length, dry weight, number of culms, number of leaves and total chlorophyll content were recorded at 120th day.

Root length and shoot length

Five plants were selected at random from each treatment on 120th day, their average root length, and shoot length in cm was worked out.

Plant dry weight

Five plants were selected at random from each treatment, plants were pulled out without damaging the roots and dried in shade and further oven dried at 60°C till it attained a constant weight and dry weight of the plant in gm was recorded.

Number of leaves and culms

From each treatment, five plants were selected, their average number of leaves and culms were counted and recorded.

Total chlorophyll content

The total chlorophyll content of the plant samples were estimated [10].

Antibacterial activity

The agar plate disc diffusion method [15] was followed for testing antibacterial activity. The extract of fresh matured roots and leaves of *V. zizanioides* were prepared using solvent acetone using soxhlet apparatus. The 7 mm sterile discs were dipped in the root and leaf extracts. The Bacterial stains used for this assay were *E. coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Staphylococuss aureus* and *Enterobacter faecalis*. Inoculum of each bacterial

strain was suspended in nutrient broth and incubated for 18 hours at 37°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 37°C for 18-24 hrs. 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.

3. Results and Discussion

The results of growth parameters such as root length, shoot length, dry weight, number of leaves number of culms, chlorophyll content in *V. zazanioides* on 120th day are given in Table 1.

In general all the treatments recorded significantly increased root length and shoot length compared to control. The maximum root length of 92.50 cm was recorded in the treatment T6 (Vermicompost + *Azospirillum*+ *Azotobacter*) followed by T5 and T4. The least root length was recorded in T1 (70.15 cm). The ideal combination for the production of vetiver roots was noticed in T6. The various treatments applied to the plants started exhibiting its effect from 7th day to 120th day. The growth parameters recorded in various treatments differed according to various manures used. Application of organic manures with beneficial organisms showed better response to growth parameters [12].

There was an increase in the number of culms and leaves in almost all the treatments 38 culm/plant and 168 leaves /plant were observed in T6 which was found to be highest effect. The maximum dry weight and chlorophyll content observed in treatment T6 were 2.32g plant-1 and 41.00 mg g^{-1} respectively. The combined effect of strain inoculation and vermicompost exhibited pronounced effect than individual effect.

The increase of growth parameters like root length, shoot length, dry weight, number of culms, number of leaves, chlorophyll content was noticed. 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 [11,3]. The application of vermicompost enhanced the root initiation, root elongation root biomass and rooting percentage [14,3] the application of vermicompost had greatly influenced the growth and yielded in various crops [4,8]. The biomass production of vetiver is increased by the inoculation of N_2 fixing bacteria and organic amendments[16].

Table 1. Effect of inoculation of *Azospirillum* and *Azotobacter* on the growth parameters of vetiver (*Vetiveria Zanzanioides*) on 120th day

S. No	Treatments	Root length (cm)	Shoot length (cm)	Dryweight (g $plant^{-1}$)	Leaves(No. $plant^{-1}$)	Culms (No. $plant^{-1}$)	Chlorophyll (mg g^{-1})
1.	T1	70.15	200.50	2.00	140	29	32.28
2.	T2	80.20	212.88	2.10	150	30	35.06
3.	T3	85.50	225.95	2.18	154	32	37.23
4.	T4	86.25	226.80	2.20	156	35	38.92
5.	T5	92.50	229.18	2.32	168	38	40.10
6.	T6	99.75	231.00	2.45	172	40	41.00
		SED 2.45	4.81	0.08	2.51	1.50	1.25
		C.D (0.05) 4.75	8.50	1.15	5.10	3.00	2.52

Table 2. Antibacterial activity of the acetone extracts of vetiver (leaves & roots) inhibition zone diameter in mm)

Plant	*E. coli*	*Pseudomonas aeroginosa*	*Staphylococcus auerus*	*Klebsiella pneumoniae*	*Enterobacter facecalis*
Vetiver leaves	20	18	29	25	17
Vetiver roots	25	25	32	27	22

Antibacterial activity

The extracts of root and leaf effectively inhibited the growth of gram positive and gram negative microorganisms [Table 2]. However the root extract assay showed maximum zone of inhibition (32 mm) against Staphylococcus aureus and minimum zone (22mm) of inhibition was found against Enterobacter faecalis. Many reports showed susceptibility of Staphylococcus aureus to vetiver oil [4,9,2].

This study revealed that the N_2 fixing bacteria *Azospirillum* and *Azotobacter* when combined with the organic manure vermicompost has increased biomass production of vetiver extracts effectively inhibited the gram positive and gram negative bacteria. 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 32 mm against *Staphylococcus aureus* and the minimum inhibition was found to be 22 mm against *Enterobacter faecalis*. These results showed that, the extracts of vetiver are pharmacologically important that may be tested for control of pests and other ailments of human beings.

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