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Effect of biofertilizers and mangampet barytes mine waste on the growth of *vitex negundo*

Ganta Nalini and Vijaya Tartte

Abstract
Over the last few years, focus on medicinal plants research has increased all over the world to identify and validate plant derived substances to protect human health from various diseases. In the next few decades human population raises a big threat to diseases. Therefore it is essential to enhance the productivity of medicinal plants. Too much dependence on chemical fertilizers for more crop production damages environment and human health. Exploitation of microbes like Bacteria, Fungi and Cyanobacteria as biofertilizers is an alternative method to enhance the productivity of medicinal plants. Biofertilizers along with mine waste increases the growth of medicinal plants. This present article elucidates that Biofertilizers and mine waste from Mangampet Barytes (Located in Andhra Pradesh, Kadapa district) promotes growth of *Vitex Negundo* a medicinal plant and was observed by studying various physiological parameters an experimental approach and also protects against various plant pathogens.

Keywords: Biofertilizers, mine waste, *Vitex negundo*, physiological parameters

1. Introduction
Mangampet deposit is one of the largest barytes in the world located in Kadapa district of Andhra Pradesh which reserves about 74 million tonnes accounts for 98% of known Indian reserves and 25% of known world reserves. It is used mainly in oil well drilling industry as drilling mud, small quantities are used in the manufacture of Barium chemicals. My research work mainly focuses on these mine waste that has organic matter which can be utilised for the growth of medicinal plants. In recent years, microbial inoculants as a source of biofertilizers are utilised for the growth of plants as plants have number of relationships with fungi, bacteria and algae especially Mycorrhiza which delivers alot of benefits for the growth of plants like plant nutrition, disease resistance and tolerance to adverse soil and climatic conditions. Vesicular Arbuscular Mycorrhizae helps plants in uptaking of nutrients, hormones from soil and also protects it from root pathogens (Bhagyaraj *et al.*, 1992) [12].
Vitex negundo is widely cultivated plant in India as a valuable medicinal herb has wide application in most of the drug formulation. The plant is antipyretic, anti-inflammatory, expectorant, depurative, sudorific, anti helmenthic, dizertive and stomachi. (Chawla *et al.*, 1992) [11]. It is also useful in hyperdipsia, wounds, ulcers, chronic fever, malarial, information, cough, bronchitis, skin diseases, leprosy, intestinal worm's flatulence, diarrhoea, colic dysentery and haemorrhoids. Because of its importance in Pharmaceutical industries *Vitex negundo* has been selected for the present study. (Chandramu *et al.*, 2003, Tandon *et al.*, 2005) [4, 10].

2. Materials and Methods
The present research work was undertaken to study and examine the effect of biofertilizers and mangampet barytes mine waste on the growth and biochemical aspects of *Vitex negundo* an important medicinal plant.

2.1 Preparation of Biofertilizers
2.1.1 Preparation of Vesicular Arbuscular Mycorrhizal (VAM) inoculums
The inoculums of VAM fungi was developed in pots on the roots of maize in sterilized soil. (Amani *et al.*, 1986) [7]. The pots were maintained in the glass house by watering to field capacity. After four months of growth, the mycorrhizal roots were air dried, homogenised by mixing the soil and cutting the roots into pieces less than 1cm in length. (Hymaru *et al.*, 1983) [9].

2.1.2. Preparation of vermicompost

Vermicomposting is the process of composting waste organic materials and earthworms to get high yield in crop varieties. Vermicomposting unit should be cool, moist and shady site. In the form of bed cow dung and chopped dried leafy materials are mixed in 3:1 and decomposed for 2-3 weeks. (Divya *et al.* 2001) [8]. Earthworms are released on the upper layer of the bed water should be sprinkled on the bed daily by covering with gunny bags or polyethylene bags. Bed should be turned for every week in order to maintain aeration and proper decomposition. After 45days vermicompost can be utilized for plant growth. (Kale *et al.,* 1982) [5].

2.2. Collection of Mangampet barytes mine waste

Mine waste was collected from Mangampet barytes located in Kadapa district of Andhra Pradesh. The collected material was crushed and sieved.

2.3. Collection of plant

The medicinal plant *Vitex Negundo* was selected based on economical and pharmaceutical importance for detailed study on enhancement of biomass and biochemical contents through the microbial applications. The plant parts are being used in pharmaceutical industry for the preparation of various drugs either solely or in combination with other medicinal plant extracts. Stem cuttings of *Vitex Negundo* were collected from Biotrim, Tirupati. Stems were cut into small nods and placed in Bavistin solution for 10min and then in different concentrations of Indole butaric acid at 3000 rpm, 4000 rpm, 5000 rpm and the stem cuttings were planted and kept in shade house for 30days. Finally planted in the botanical garden, Department of Botany, SV University, Tirupati, Andhra Pradesh.

2.4. Experimental design

Medicinal plants were maintained at 14.6 ^{0}C to 35 ^{0}C temperature in the botanical garden, Department of Botany, SV University, Tirupati, Andhra Pradesh. The pot culture experiment was carried out under green house conditions to know the response of *Vitex Negundo* to VAM fungi, Vermi compost and Mangampet barytes mine waste. The treatment details of the pot experiment were generalized as follows:

T_1 : Vesicular Arbuscular Mycorrhiza (VAM)
T_2 : Vermicompost
T_3 : Mangampet barytes mine waste
T_4 : VAM + Vermicompost
T_5 : VAM+ Vermicompost + Mangampet barytes mine waste
T_6 : Vermicompost + Mangampet barytes mine waste
T_7 : Control

2.5. Physiological parameters

2.5.1. Estimation of Chlorophyll content

The chlorophyll content of the plants was estimated in all the treatments on 30th, 60 th and 90 th days according to the method of *Arnon et al.,* 1994. Small pieces of plant material was taken and washed with distilled water, blotted dry and homogenized in pre-chilled mortar with 80% acetone by adding a pinch of $CaCo_3$ to facilitate easy grinding and centrifuged at 5000rpm for 15min. Supernatant was filtered by using watmann no.1 filter paper. The absorbance was measured at 645nm and 663nm for the determination of chlorophyll a, chlorophyll b and total chlorophyll using 80% acetone as blank in Shimadzu (UV240) double beam spectrophotometer.

Chlorophyll a and chlorophyll b concentrations can be measured by the following equations.

Chlorophyll a = (0.0127XOD at 663nm) - (0.000269XOD at 645nm)

Chlorophyll b = (0.0229XOD at 645nm) - (0.00408XOD at 663nm)

2.5.2. Estimation of total proteins

100mg of dry material was accurately weighed and macerated with mortar and pestle by adding 10% TCA (Tri Chloro Acetic acid). The extract was centrifuged at 3500rpm for 20min. The supernatant was discarded and the residue was washed with 5% TCA (Tri Chloro Acetic acid) to remove the interfering of amino acids and phenols. The protein was dissolved in 1N NaOH and left for 2hrs at room temperature.

Protein content in medicinal plants can be estimated by following various treatments. Lowry method is one of the standard methods which was selected for the estimation of protein in *Vitex Negundo* at 30th, 60th and 90th day of plant growth (Lowry *et al*., 1951) [2].

Solution 1: 4gms of Sodium Carbonate was dissolved in 100ml of 0.1N NAOH.

Solution 2: 500mg of Copper Sulphate was dissolved in 1% Sodium potassium tartarate.

Reagent A: 50 ml of Solution 1+ 1ml of Solution 2 were mixed just before use.

Reagent B: 1ml of Folin-Ciocaltan reagent was added to 1ml of distilled water to give a solution of 1N in acid.

Protein content of the plants in different treatments was estimated on 30th, 60th, 90th days of the plant growth by Lowry's method using Folin phenol reagent. To 0.1 ml of protein sample (extract) 5ml of reagent 'A' (Alkaline copper reagent) was added and allowed to stand for 10 min. After 10 minutes 0.5 ml of Reagent 'B' (Folin- Phenol Reagent was added with instantaneous and vigorous shaking. The sample was incubated for 30 minutes at room temperature. The color was read at 660nm in calorimeter. A blank was prepared in a similar manner without protein. The total amount of protein was calculated from the standard curve prepared using Bovine Serum Albumin (BSA) of different concentration.

3. Results and Discussion

An experiment was carried out to study the effect of VAM fungi, Vermicompost and Mangampet barytes mine waste on the growth and biochemical aspects of the medicinal plant *Vitex negundo.*

3.1. Chlorophyll estimation (mg/g)

On 30th day the total chlorophyll content was maximum in T_5 (2.41) and minimum T_2 (1.23) plants on 30th, 60th and 90th day also the total chlorophyll content was maximum T_5 (2.41, 3.33, 3.83) treated plants followed by T_4 (2.34, 1.12, 3.46) and T_6 (1.98, 1.53, 2.84) and T_1 (1.27, 1.75, 2.65) and T_3 (1.62, 1.37, 3.63) plants minimum amount of chlorophyll was found in T_7 (Plants 1.92, 2.51,2.70) on these days varied difference in chlorophyll a content was observed among the treatments on 30th, 60th and 90th day samples. On 30th, 60th and 90th day more amount of Chlorophyll 'a 'was found in T_5 (1.80, 1.85, 2.50), T_1 (6.8, 1.23, 1.95) and T_3 (1.67, 1.03, 1.68) plants the chlorophyll 'b' was also observed maximum in T_5 (1.80, 1.85, 2.5) and Minimum T_1 (1.68, 1.23, 1.95) plants in 30th, 60th and 90th day sample.

Table 1: Effect of VAM fungi Vermicompost and MBMW chlorophyll 'a' content of *Vitex negundo*

Treatments	Days of Treatment		
	30 Days	60 Days	90 Days
T_1	1.27±0.02	1.75±0.08	2.65±0.09
T_2	1.23±0.06	2.16±0.12	3.83±0.08
T_3	1.62±0.57	1.37±0.03	3.63±0.09
T_4	2.34±0.05	1.12±0.11	3.46±0.24
T_5	2.41±0.01	3.3±0.28	4.10±0.21
T_6	1.98±0.16	1.53±0.07	2.84±0.06
T_7	1.92±0.66	2.51±0.44	2.70±0.07

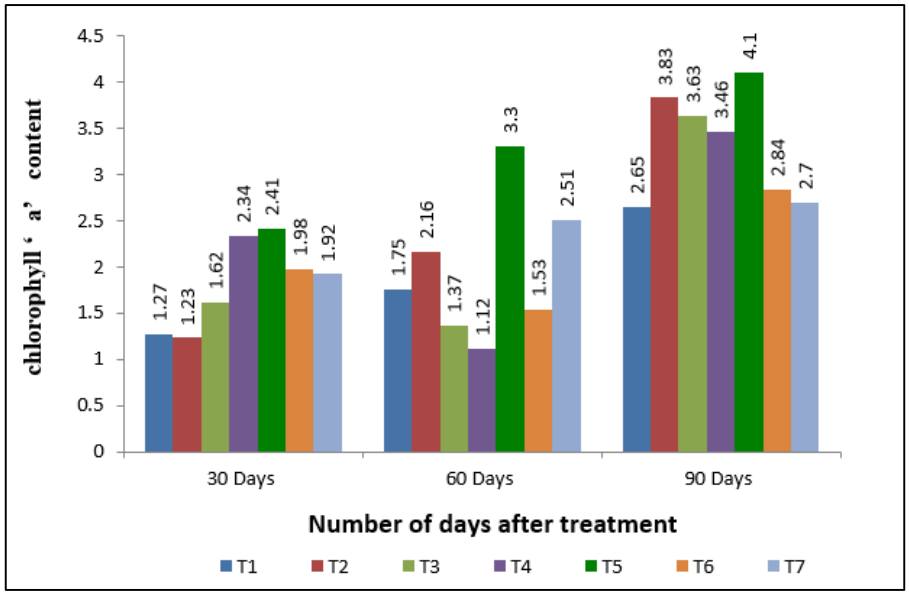

Fig 1: Effect of VAM fungi, Vermicompost and MBMW on chlorophyll 'a' content of *Vitex nigundo*

Table 2: Effect of VAM fungi Vermicompost and MBMW chlorophyll" b" content of *Vitex negundo*

Treatments	Days of Treatment		
	30 Days	60 Days	90 Days
T_1	1.68±0.30	1.23±0.11	1.95±0.03
T_2	1.88±0.08	1.47±0.06	2.50±0.02
T_3	1.67±0.40	1.03±0.03	1.76±0.06
T_4	1.74±0.11	0.76±0.12	1.60±0.09
T_5	1.80±0.01	1.85±0.06	2.51±0.15
T_6	2.47±0.29	0.92±0.03	1.84±0.07
T_7	1.19±0.05	1.63±0.07	1.68±0.12

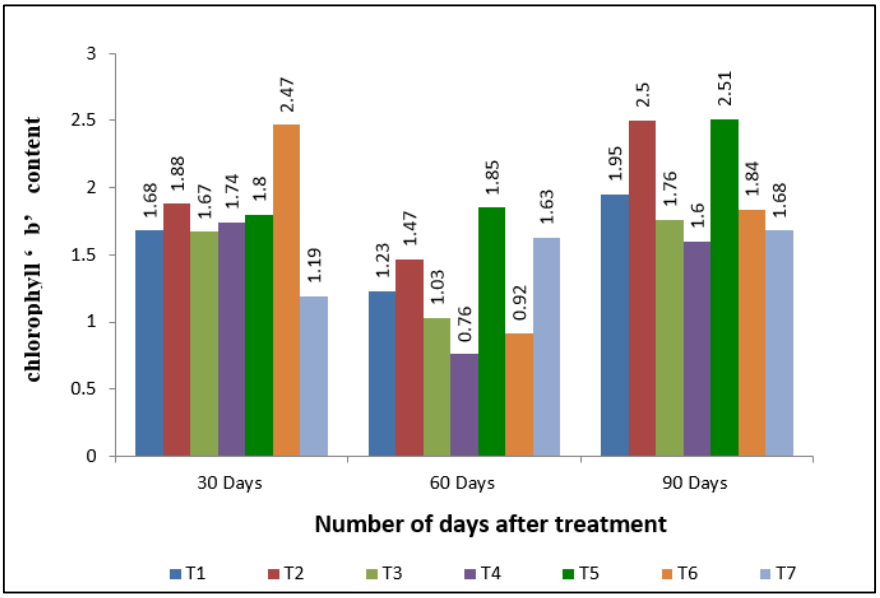

Fig 2: Effect of VAM fungi, Vermicompost and MBMW on chlorophyll 'b' content of *Vitex nigundo*

3.2. Carbohydrate estimation

The amount of reducing sugars (mg/g) of T_1, T_2, T_3, T_4, T_5, T_6 plants on 30th, 60th and 90th days were significantly high when compared to T_7 plants. Maximum amount of reducing sugar's were recorded in the T_5 plants on 30th, 60th and 90th day (14.16, 15.71, 16.32) in T_7 plants the amount of reducing sugars was found minimum T_2 (7.53, 8.39, 10.39)

There was a significant increase in the amount of non-reducing sugars (mg/g) in T_1, T_2, T_3, T_4, T_5, T_6, over T_7 control plants on 30th, 60th and 90th days. The amount of non-reducing sugars being maximum *T_5 plants on* 30th day 60th day and 90th days were (14.16, 15.71, 16.32) followed by T_1, T_5 and T_4, T_6, T_1, T_3, T_2 the plants the amount was 7.53, 8.39, 10.53 respectively.

Table 3: Effect of VAM fungi Vermicompost and MBMW on carbohydrate (Reducing sugars) content of *Vitex negundo*

Treatments	Days of Treatment		
	30 Days	60 Days	90 Days
T_1	8.34±0.51	9.27±0.24	10.68±0.24
T_2	7.53±0.19	8.39±0.03	10.53±0.11
T_3	10.48±0.24	12.33±0.43	10.39±0.24
T_4	12.65±0.15	13.89±0.11	14.21±0.13
T_5	14.16±0.13	15.71±0.18	16.32±0.30
T_6	11.41±0.34	10.52±0.26	11.35±0.38
T_7	5.64±0.11	11.15±0.03	10.20±0.12

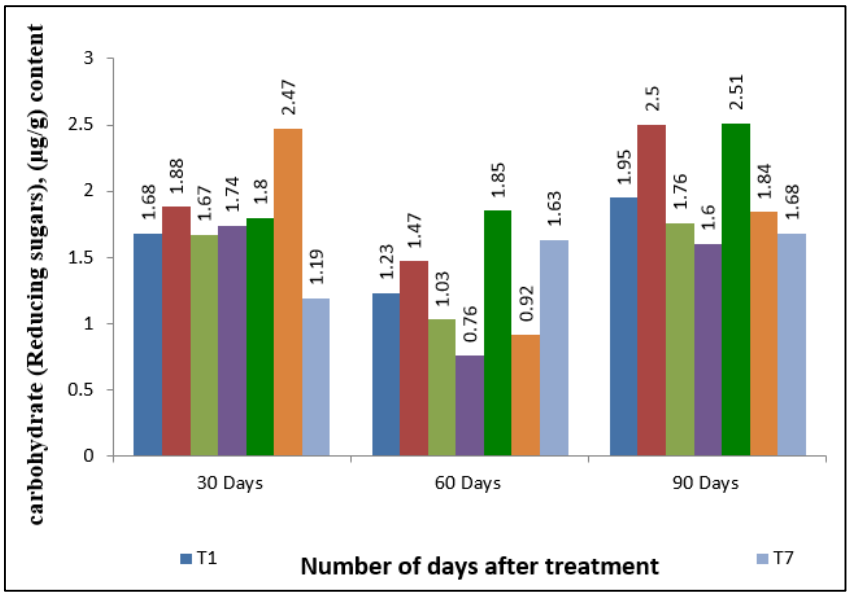

Fig 3: Effect of VAM fungi, Vermicompost and MBMW on carbohydrate (Reducing sugars), (µg/g) content of *Vitex nigundo*

Table 4: Effect of VAM fungi Vermicompost and MBMW on carbohydrate (Non Reducing sugars) content of *Vitex negundo*

Treatments	Days of Treatment		
	30 Days	60 Days	90 Days
T_1	7.63±0.27	10.26±0.11	13.26±0.27
T_2	5.91±0.10	8.86±0.06	10.63±0.10
T_3	8.57±0.16	12.15±0.14	9.75±0.12
T_4	10.76±0.07	12.82±0.04	10.91±0.06
T_5	12.33±0.26	13.64±0.11	15.6±0.20
T_6	10.63±0.06	8.32±0.04	10.34±0.36
T_7	6.13±0.14	12.14±0.16	12.35±0.06

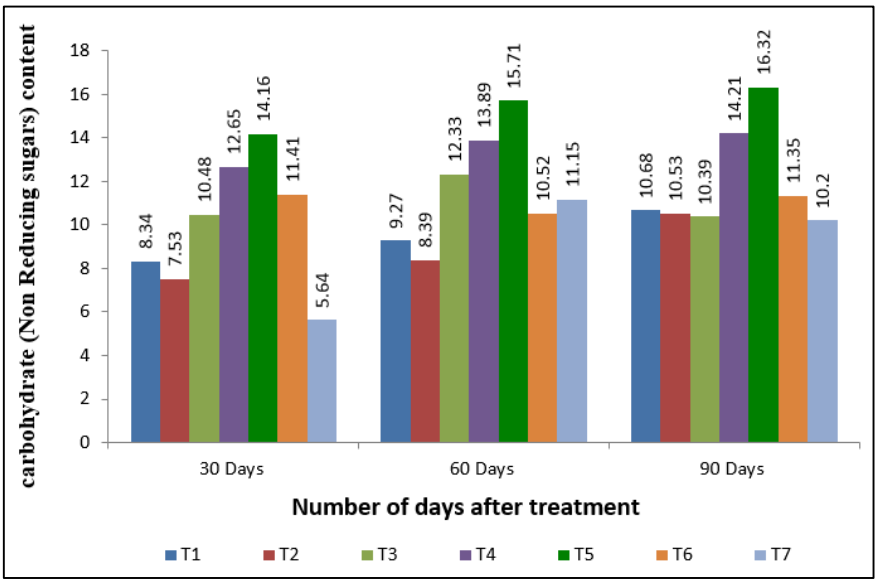

Fig 4: Effect of VAM fungi, Vermicompost and MBMW on carbohydrate (Non Reducing sugars) content of *Vitex nigundo*

4. Conclusion

During the last fifty years due to the high use of synthetic fertilizers not only the overall soil fertility is decreased but also the biological systems adversely affected, visible in the decreasing trend of production even after using higher doses of fertilizers. Besides, the use of synthetic fertilizers is causing several environmental problems- Experiencing the adverse effects of synthetic in put dependent agriculture the concept of organic / sustainable / natural fanning in gaining momentum. The usage of organic fertilizer is well popularized in agriculture and horticulture. But the impact of organic fertilizer on the growth of medical plants is less known. Hence in the present study an attempt has been made to study the effect of VAM fungi Vermicompost and MBMW on the growth and yield of *Vitex negundo.*

The results revealed that inoculation of VAM alone enhanced the growth of plant, chlorophyll content and carbohydrates Plants responded more positively to triple inoculation which attributes to the existence of synergistic interaction between vermicompost and VAM fungi. The maximum growth and biomass increase was found in Treated plants. Based on the results obtained in the present study it was observed that the combination of VAM and vermicompost is favourable for obtaining maximum growth and yield. However all the treatments have positively responded and showed significantly high growth over the control-Based on the results obtained in the present study the amendment of combination of VAM fungi Vermicompost is recommended for the cultivation of *Vitex negundo* to meet the pharmaceutical demand.

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