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Research Article

A STUDY ON EFFECT OF VERMICOMPOST VAM FUNGI NITROGEN FIXING BACTERIA AND PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH PARAMETERS AND PHYSIOLOGY OF *TRIBULUS TERRESTRIS* L.

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ABSTRACT

Objective: To find out the effect of biofertilizers on the growth and yield of *Tribulus terrestris* by using vermicompost, vesicular mycorrhiza (VAM), phosphate solubilizing bacteria (PBS), nitrogen-fixing bacteria individually and in combination under garden house environment.

Materials and Methods: The plant seedlings were gathered at different intervals on the 30th, 60th, and 90th days later the transplantations. By the inoculation of vermicompost, VAM fungi, and phosphate solubilizing, nitrogen-fixing microorganisms shows in improvement of growth parameters as like plant shoot height, root length, a number of leaves, leaf area, fresh shoot biomass and fresh root biomass, dry shoot biomass and dry root biomass, chlorophyll content, reducing sugar and non-reducing sugar, starch, protein and lipid content in the root samples.

Results: The result highlights the significance of biofertilizer inoculations for the fast development of seedlings of a medicinal plant (*Tribulus terrestris*) grown in a garden house and shows the benefit of inoculating soil with fewer microbial inhabitants with aboriginal microbes.

Conclusion: Overall results revealed that the biofertilizers are the better supplements that can improve and show the maximum growth and yield with all inoculations than the individual.

Key words*:* Bacillus megatherium, Azotobacter chrocoocum, Biofertilizer.

INTRODUCTION

Tribulus terrestris is a wild folk medicinal plant. The leaves, fruits, and roots are the economic parts of this corp. The active compound has insinuated itself into the field of the cosmetics industry. Therapeutically they are used in the treatment of, diuretic, analgesic, astringent, hyperuricemia, arthritis, rheumatism and impotency. And also the existence of active compounds like tannins, alkaloids, saponins, cardiac glycosides, flavonoids, proteins, amino acids, phenols, steroids, and terpenoids, have been reported in the roots. Enrichment of soil by the use of organic compost like vermicompost, VAM fungi, and phosphate solubilizing, and nitrogen-fixing microorganisms has focussed on the increase soil health and quality. The use of bio-fertilizers has also confirmed that it has a helpful relationship with the consumption of nutrients that are there in the soil. Microbial symbiosis with herb or with their company leads to the development of plants. Inoculations of microbes have also been informed that they have expanded the beneficial effects on plants [1]. Application of biofertilizers, FYM along with neem and castor cake cane can produce the maximum growth and increased the availability of calcium and phosphorus from farmyard manure [2]. Biofertilizers as a better supplement can progress the growth, nutrient grade, fruit superiority, and yield [3]. The nutrient supplement on the supply of the organic amendment increased the co_2 production in the soil which results in improved enzymatic activity [4]. Biofertilizers are important for medicinal and aromatic plants to produce the best in both quantity and quality and it is also not dangerous for animals, humans, and the surroundings. The atmospheric conditions and geographical distribution may play a very important function in the growth and biochemical activities of plants. Recently countless studies have a statement that biofertilizers can encourage plant growth in the course of nitrogen fixation and potassium and phosphate solubilization [5, 6]. VAM fungi are most capable in improving the growth and yield of medicinal plants along with ferns, bryophytes, and flowering plants [7-9]. Fungal hypha expands into the soil which leads to the absorption of nutrient micronutrients and lightens the stress effects on plants and improves the soil fertility [10]. Biogas slurry as a vital functions in which equilibrium of C and N metabolisms by which improvement in amino acid and protein content can be seen [11]. Many studies were reported the valuable role of dissimilar bacterial species to encourage plant expansion and varying cereal plant species such as *Triticum* with *Pseudomonas*, *Anabaena*, *Azospirillum* have different nutrient absorbtion rate[12-14]; *Beta Vulgaris* and *Hordeum Vulgare* with *Rhodobacter, Paenibacillus, Pseudomonas, Bacillus* [15, 16]; saccharum officinarum with *Herbaspirillum* [17]; *Helianthus* with *Bacillus sps* [18]; *Zea mays* with *Bacillus* and *Pseudomonas*, *Herbaspirillum* and *Trichoderma*, ([19-20). In the current study, the *Tribulus terrestris* plant was chosen due to its elevated medicinal value and belongs to the family Zygophyllaceae which is broadly distributed around the earth. It is an annual and creeping plant that grows in dry climates, popularly known as Gokhru, caltrop, puncture vine and tack weed. The aim of the toil is to swot the effect of the application of vermicompost, VAM fungi, phosphate solubilising bacteria and nitrogen-fixing bacteria on yield, growth, and chemical composition of *Tribulus terrestris*.

MATERIALS AND METHODS

Collection of seeds

Seeds of uniform size of *Tribulus terrestris* were collected from the Sri Venkateswara University campus area in Tirupati, Andhra Pradesh and before planting the surface sterilized with 0.05% sodium hypo chloride and carefully cleansed with running tap water.

Collection of biofertilizers

Bacillus megatherium and *Azotobacter chrocoocum* cultures were collected from Regional Biofertilizer Development Centre Bangalore Division, India.

Experimental design

This experiment was conducted in polythene bags under conservatory conditions to distinguish the response of the *Tribulus terrestris* plant to vermicompost, VAM fungi, *Bacillus megatherium* and *Azotobacter chrocoocum* inoculation. *Tribulus terrestris* plants were grown up in polythene bags containing an uncontaminated fusion of sand, clay and slit. Each treatment had three experimental replications, and the bags were arranged in randomized pattern. For three months, the plants were grown under regular photoperiods and deionized water was supplied. Inoculums of 20ml *Azotobacter chrocoocum* culture and/or 20ml *Bacillus megatherium* culture were combined with soil and put in bags according to the treatments. The details of experiment which are treated were explained as below

T_1:Control (No inoculation)
T_2:Inoculation with Vermicompost
T_3:Inoculation with VAM fungi
T_4:Inoculation with *Bacillus megatherium* (PBS)
T_5:Inoculation with *Azotobacter chrocoocum* (nitrogen-fixing bacteria)
T_6 :Inoculation with All (vermicompost, VAM fungi, *B.megatherium, Azotobacter chrocoocum)*

Growth parameters

All of the treated plants growth parameters characteristics were measured at the 30th, 60th and 90th days including shoot length, root length, leaf number, leaf area, fresh shoot biomass and fresh root biomass, dry shoot biomass and dry root biomass.

Physiological Parameters

On the 30th, 60th and 90th, physiological parameters such as chlorophyll-a, chlorophyll-b content, reducing sugars and non-reducing sugars, lipid, starch and protein contents in root samples were investigated with and without inoculums treated plants. Estimation of chlorophyll content by Arnon, 1949, total lipid by Bligh and Dyer, 1959, starch by Mc Cready et al., 1950, carbohydrates by Highkin and Frankel, 1962 and total protein by Lowry et al., 1951 were estimated for every 30 days.

Statistical analysis

The data were tabulated in Microsoft Excel 2010 and statistically analysis carried out using R statistical package version 4.1.1 from R Core Team (2021); one-way analysis of variance (ANOVA) was utilised at a 5 % level of significance. In the finding the values were given in relation to each table.

RESULTS

Effect of bioinoculants on the growth of *Tribulus terrestris* Shoot length

When compare to control plants, all inoculated plants have shown an increased shoot length. The greatest shoot length was recorded in T_6 plants (49.06) on the 90th day of plant growth after transplantation (Table 1, fig 1). At a 5% level, there was a significant difference between all the treatments as well as the days.

Root length

On 90th days of plant growth, T_6 treated plants have the longest root length (11.26) and shortest root length in T_1 (5.56) treated plants (Table 2, fig 2). From the 30th days to 90th days, there was a significant increase root length at a 5% level in all treatments.

Fresh biomass

On the 30th, 60thand 90th day, in T_6 treated plants the greatest shoot biomass was reported (8.92, 10.66 and 19.30) followed by T_5 (6.58, 8.97 and 15.30), T_4 (4.79, 7.17 and 10.07), T_3 (3.42, 6.75 and 9.89), T_2 (3.18, 5.20 and 8.18). The lowest fresh biomass was recorded in T_1 (2.61, 3.60 and 5.57). Alike, the maximum root fresh biomass was observed in T_6 (3.80, 5.40 and 9.64) plants. The next best treatment was T_5 (2.07, 4.74 and 8.04), T_4 (1.56, 3.68 and 5.74), T_3 (0.91, 3.55 and 5.01), T_2 (0.58, 2.71 and 4.79). The minimum root fresh biomass was found in T_1 (0.42, 1.66 and 2.44) plants (Table 3, fig 3a and fig 3b). The total fresh biomass of shoot and root was shown to rise significantly from 30 days to 90 days in all the treatments at a 5% level.

Dry biomass

When compared to the control plants T_1 on the 90th day, T_6 treated plants had the highest shoot dry biomass (6.67) followed by T_5 (5.54), T_4 (3.95), T_3 (3.35), and T_2 (0.91) than T_1 (0.88). The root dry biomass was also found highest in T_6 (3.62) followed by T_5 (2.46), T_4 (2.11), T_3 (1.86), T_2 (1.35) than T_1 (0.88) (Table 4, fig 4a, and fig 4b). At a 5% level, the total dry biomass of shoot and root increased significantly from 30 days to 90 days in all the treatments.

Number of leaves

The number of leaves were maximum in the T_6 (354.6) treatment plants when compared to the control plants from the 30th day to 90th day, followed by T_5 (328.6), T_4 (285.6), T_3 (278.6) and T_2 (271.6). T_1 (252.6) had the smallest numbers of leaves (Table: 5, fig: 5).

Leaf area

At a 5% level all of the treatments had a significant different in leaf area. The maximum leaf area in T_6 plants (3.63) and whereas the minimum leaf area in T_1 (3.23) was recorded on the 30th day. On 60th and 90th days, all treatments performed in the uppermost leaf area were recorded in T_6 (4.53). The later treatment was T_1 (4.40) followed by T_3 and T_4 (4.36) and followed by T_5 (4.33). In T_2 plants (4.26), the leaf area was determined to be the least (Table 6, fig 6).

Table: 1, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on shoot length (cms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	7.76 (0.15)	15.26 (0.40)	32.06 (0.77)
T_2	8.46 (0.15)	16.73 (0.20)	34.93 (0.35)
T_3	8.43 (0.25)	18.43 (0.25)	36.76 (0.35)
T_4	9.56 (0.25)	19.06 (0.41)	37.33 (0.51)
T_5	10.13 (0.30)	20.46 (0.15)	43.83 (0.65)
T_6	12.93 (0.25)	22.56 (0.20)	49.06 (0.49)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on shoot length of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	188.1485	5	37.62969	6.247871	0.007015	3.325835
Columns	2723.765	2	1361.882	226.121	4.74E-09	4.102821
Error	60.22802	10	6.022802			
Total	2972.141	17				

Significant at a 5% level

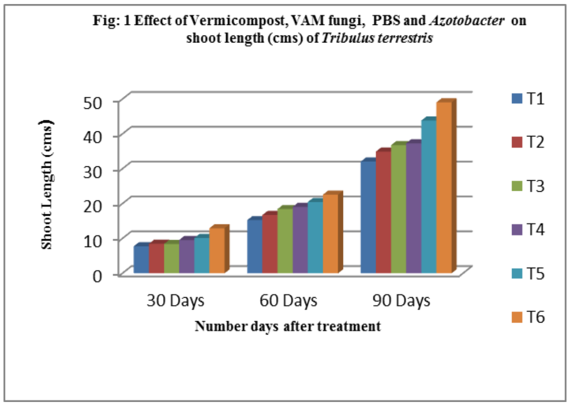

Table: 2, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on root length (cms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	2.93 (0.25)	4.53 (0.15)	5.56 (0.35)
T_2	3.06 (0.15)	5.73 (0.20)	6.46 (0.35)
T_3	3.43 (0.25)	6.13 (0.37)	6.53(0.32)
T_4	3.53 (0.25)	6.53(0.30)	8.73 (0.41)
T_5	3.56 (0.15)	5.56(0.30)	10.76 (0.37)
T_6	4.53 (0.20)	9.46 (0.30)	11.26 (0.41)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on root length of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	30.92698	5	6.185395	4.503483	0.020721	3.325835
Columns	67.4342	2	33.7171	24.54886	0.000139	4.102821
Error	13.73469	10	1.373469			
Total	112.0959	17				

Significant at a 5% level

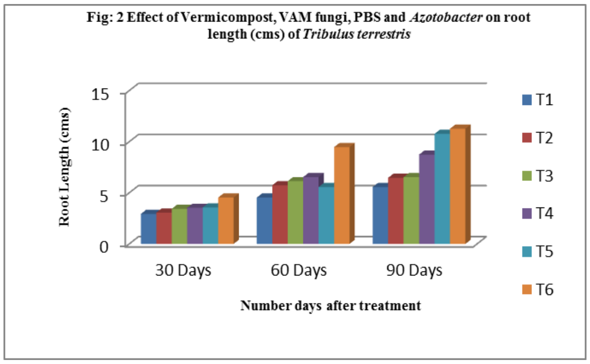

Table: 3, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on shoot fresh biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	2.61(0.28)	3.60 (0.17)	5.57 (0.21)
T_2	3.18 (0.27)	5.20 (0.26)	8.18 (0.32)
T_3	3.42(0.18)	6.75 (0.22)	9.89 (0.29)
T_4	4.79(0.21)	7.17 (0.24)	10.07 (0.30)
T_5	6.58 (0.28)	8.97 (0.28)	15.30 (0.58)
T_6	8.92 (0.28)	10.66 (0.27)	19.30 (0.61)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on shoot fresh biomass of ***Tribulus terrestris.***

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	163.2078	5	32.64155	13.45351	0.000359	3.325835
Columns	130.3785	2	65.18925	26.86834	9.51E-05	4.102821
Error	24.26248	10	2.426248			
Total	317.8487	17				

Significant at a 5% level

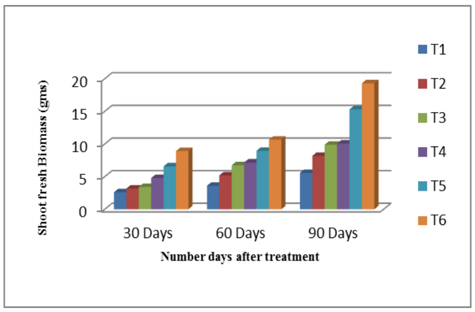

Fig: 3Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on shoot fresh biomass (gms) of *Tribulus terrestris*

Table: 3a, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on root fresh biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	0.42 (0.04)	1.66 (0.25)	2.44 (0.12)
T_2	0.58 (0.005)	2.71 (0.22)	4.79 (0.20)
T_3	0.91 (0.02)	3.55 (0.26)	5.01 (0.10)
T_4	1.56 (0.03)	3.68 (0.26)	5.74 (0.24)
T_5	2.07 (0.10)	4.74 (0.20)	8.04 (0.11)
T_6	3.80 (0.16)	5.40 (0.23)	9.64 (0.23)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on root fresh biomass of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	42.98036	5	8.596072	13.00178	0.000414	3.325835
Columns	57.7795	2	28.88975	43.69649	1.14E-05	4.102821
Error	6.611458	10	0.661146			
Total	107.3713	17				

Significant at a 5% level

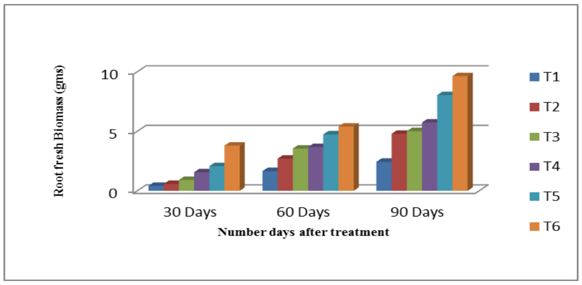

Fig: 3a Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on root fresh biomass (gms) of *Tribulus terrestris*

Table: 3b, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on total fresh biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	**60 days**	**90 days**
T_1	3.04 (0.27)	5.27 (0.26)	8.02 (0.15)
T_2	3.77 (0.28)	7.92 (0.21)	12.98 (0.15)
T_3	4.34 (0.20)	10.31 (0.38)	14.91 (0.34)
T_4	6.36 (0.18)	10.85 (0.08)	15.82 (0.43)
T_5	8.66 (0.38)	13.72 (0.24)	23.35 (0.55)
T_6	12.72 (0.28)	16.06 (0.29)	28.95 (0.73)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on total fresh biomass of ***Tribulus terrestri***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	373.0416	5	74.60833	13.66028	0.000337	3.325835
Columns	359.5607	2	179.7803	32.91657	3.99E-05	4.102821
Error	54.61697	10	5.461697			
Total	787.2192	17				

Significant at a 5% level

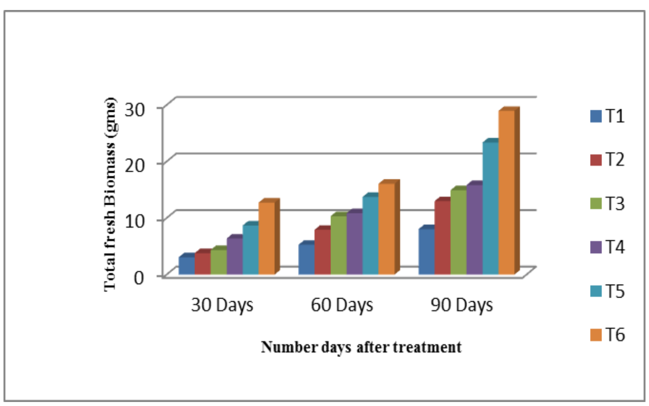

Fig: 3b Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on total fresh biomass of *Tribulus terrestris*

Table: 4, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on shoot dry biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	**60 days**	**90 days**
T_1	0.41(0.02)	0.68(0.04)	0.88(0.03)
T_2	0.68(0.04)	0.82(0.04)	0.91(0.02)
T_3	0.75(0.04)	2.14(0.05)	3.35(0.12)
T_4	1.01(0.09)	2.54(0.08)	3.95(0.07)
T_5	1.67(0.14)	3.36(0.11)	5.54(0.06)
T_6	2.79(0.14)	3.95(0.07)	6.67(0.21)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on shoot dry biomass of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	373.0416	5	74.60833	13.66028	0.000337	3.325835
Columns	359.5607	2	179.7803	32.91657	3.99E-05	4.102821
Error	54.61697	10	5.461697			
Total	787.2192	17				

Significant at a 5% level

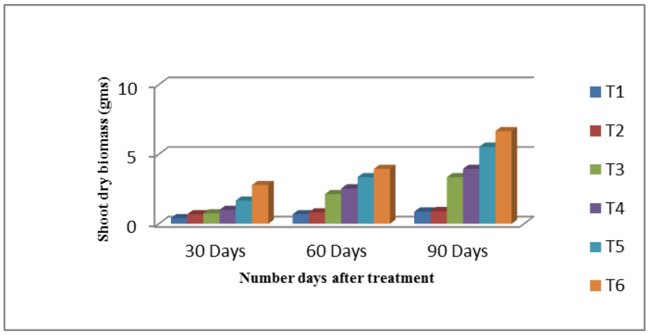

Fig: 4 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on shoot dry biomass (gms) of *Tribulus terrestris*

Table: 4a, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on root dry biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	0.16 (0.01)	0.17 (0.01)	0.88 (0.04)
T_2	0.27 (0.02)	0.91 (0.02)	1.35 (0.07)
T_3	0.35 (0.03)	1.19 (0.03)	1.86 (0.09)
T_4	0.46 (0.02)	1.84 (0.06)	2.11 (0.07)
T_5	0.67 (0.04)	2.10 (0.06)	2.46 (0.07)
T_6	1.72 (0.10)	2.75 (0.11)	3.62 (0.05)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on root dry biomass of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	9.544864	5	1.908973	21.15842	5.04E- 05	3.325835
Columns	6.351868	2	3.175934	35.201	2.98E-05	4.102821
Error	0.902228	10	0.090223			
Total	16.79896	17				

Significant at a 5% level

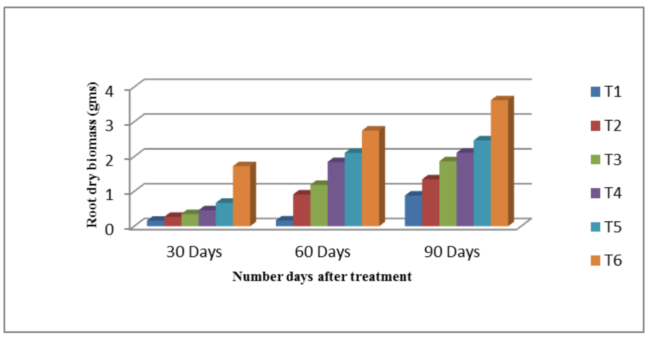

Fig: 4a Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on root dry biomass (gms) of *Tribulus terrestris*

Table: 4b, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on total dry biomass (gms) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	0.57 (0.005)	0.85 (0.04)	1.76 (0.07)
T_2	0.95 (0.05)	1.73 (0.03)	2.26 (0.08)
T_3	1.11 (0.01)	3.33 (0.04)	5.21 (0.06)
T_4	1.47 (0.11)	4.39 (0.15)	6.07 (0.07)
T_5	2.35 (0.10)	5.46 (0.17)	8.01 (0.12)
T_6	4.51 (0.18)	6.70 (0.15)	10.29 (0.21)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on total dry biomass of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	77.95066	5	15.59013	13.81267	0.000321	3.325835
Columns	42.7433	2	21.37165	18.93502	0.000398	4.102821
Error	11.28684	10	1.128684			
Total	131.9808	17				

Significant at a 5% level

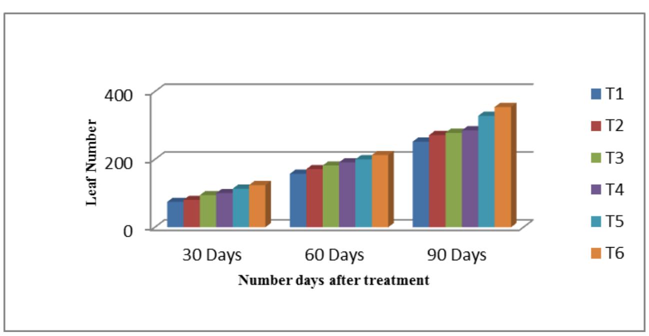

Fig: 5 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on leaf number of *Tribulus terrestris*

Table: 5 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on leaf number of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	60 days	90 days
T_1	74.66 (3.05)	157.66 (5.50)	252.66 (4.72)
T_2	80.66 (1.52)	171.33 (3.05)	271.66 (7.76)
T_3	94.66 (2.51)	182.00 (7.00)	278.66 (3.51)
T_4	100.66 (4.16)	191.33 (4.50)	285.66 (5.68)
T_5	113.66 (5.50)	200.33 (6.02)	328.66 (7.50)
T_6	124.33 (4.72)	212.66 (4.72)	354.66 (11.37)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on leaf number of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	9735.407	5	1947.081	13.62866	0.00034	3.325835
Columns	117159.3	2	58579.63	410.0301	2.54E-10	4.102821
Error	1428.667	10	142.8667			
Total	128323.3	17				

Significant at a 5% level

Table: 6, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on leaf area (sq mm) of *Tribulus terrestris*.

Treatments	Days after treatment		
	30 days	**60 days**	**90 days**
T_1	3.23 (0.05)	3.43 (0.15)	4.40 (0.26)
T_2	3.33 (0.11)	3.53 (0.25)	4.26 (0.20)
T_3	3.36 (0.15)	3.63 (0.20)	4.36 (0.20)
T_4	3.46 (0.15)	3.83 (0.25)	4.36 (0.20)
T_5	3.46 (0.15)	4.06 (0.15)	4.33 (0.11)
T_6	3.63 (0.15)	3.93 (0.25)	4.53 (0.25)

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on leaf area of ***Tribulus terrestris***.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.285185	5	0.057037	3.802469	0.034401	3.325835
Columns	2.871481	2	1.435741	95.71605	3.02E-07	4.102821
Error	0.15	10	0.015			
Total	3.306667	17				

Significant at a 5% level

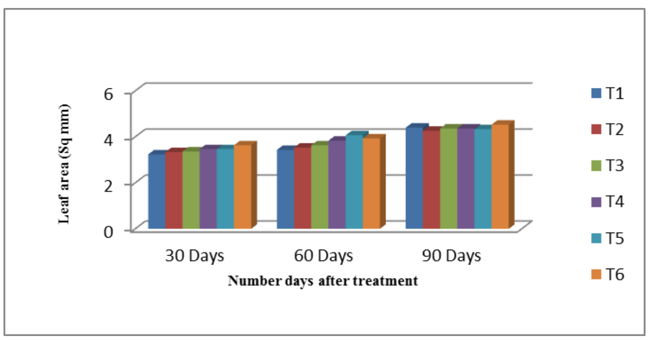

Fig: 6 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on leaf area (sq mm) of *Tribulus terrestris*

BIOCHEMICAL STUDIES

Total chlorophyll estimation

On the 30th, 60th and 90th day the total chlorophyll content was maximum in T_6 (13.672, 15.091, 18.167) and minimum in T_1 (9.850, 11.443, 12.478) plants. () treated plants followed by T_5 (12.667, 14.145, 15.682), T_4 (12.032, 13.646, 15.064), T_3 (11.185, 13.058, 15.624), T_2 (10.738, 12.441, 14.029) and minimum in T_1 (9.850, 11.443, 12.478) plants. On the 30th, 60th and 90th day, high amount of chlorophyll-a content was maximum in T_6 plants (6.350, 7.332, 8.476) followed by T_5 (6.367, 7.083, 8.050), T_4 (6.330, 6.858, 7.341), T_3 (5.922, 6.697, 7.256) T_2 (5.854, 6.667, 6.962) and minimum in T_1 (5.721, 6.017, 6.394) plants. The control plants exhibit a less level of chlorophyll on all days. The chlorophyll-b content was maximum in T_6 plants (7.321, 7.732, 9.690) followed by T_5 (6.297, 7.045, 7.631), T_4 (5.703, 6.666, 7.722), T_3 (5.262, 6.350, 8.367), T_2 (4.886, 5.803, 7.066) and minimum in T_1 (4.125, 5.439, 6.082) plants on 30th, 60th and 90th day sample. At a 5% level there was a significant difference in chlorophyll-a, chlorophyll-b and total chlorophyll content between the treatments and on various days (Table 7, fig 7a, fig 7b, fig 7c).

Table: 7 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Chlorophyll of *Tribulus terrestris*

Treatments	Chlorophyll 'a' (mg/g)			Chlorophyll 'b' (mg/g)			Total Chlorophyll (mg/g)		
	Days after treatment								
	30 days	**60 days**	**90 days**	**30 days**	**60 days**	**90 days**	**30 days**	**60 days**	**90 days**
T_1	5.721(0.0005)	6.017(0.003)	6.394(0.002)	4.125(0.004)	5.439(0.100)	6.082(0.002)	9.850(0.004)	11.443(0.100)	12.478(0.005)
T_2	5.854(0.002)	6.667(0.003)	6.962(0.003)	4.886(0.004)	5.803(0.063)	7.066(0.004)	10.738(0.004)	12.441(0.098)	14.029(0.005)
T_3	5.922(0.005)	6.697(0.042)	7.256(0.004)	5.262(0.002)	6.350(0.014)	8.367(0.05)	11.185(0.005)	13.058(0.007)	15.624(0.002)
T_4	6.330(0.004)	6.858(0.056)	7.341(0.005)	5.703(0.005)	6.666(0.113)	7.722(0.002)	12.032(0.005)	13.646(0.053)	15.064(0.005)
T_5	6.367(0.002)	7.083(0.007)	8.050(0.004)	6.297(0.005)	7.045(0.052)	7.631(0.004)	12.667(0.004)	14.145(0.058)	15.682(0.004)
T_6	6.350(0.005)	7.332(0.113)	8.476(0.002)	7.321(0.002)	7.732(0.096)	9.690(0.005)	13.672(0.002)	15.091(0.065)	18.167(0.002)

(Standard deviation and the mean of three replications are shown with in brackets).

Source of variation	Within Groups at every time interval								
	30 Days			60 Days			90 Days		
	Calculated	P-value	Table Value	F	P-value	F crit	F	P-value	F crit
Chlorophyll a	12569.04	7.1E-22	3.105875	202.0134	3.78E-11	3.105875	117934.6	1.04E-27	3.105875
Chlorophyll b	211231.2	3.15E-29	3.105875	321.0182	2.43E-12	3.105875	261545.1	8.75E-30	3.105875

Total Chlorophyll	283217.8	5.43E-30	3.105875	980.1282	3.1E-15	3.105875	537353.3	1.16E-31	3.105875

Significant at a 5% level

Source of variation	**Changes with time duration**								
	Chlorophyll 'a'			**Chlorophyll 'b'**			**Total Chlorophyll**		
	F	**P-value**	**F crit**	**F**	**P-value**	**F crit**	**F**	**P-value**	**F crit**
T_1	22684.74	2.31E-12	5.143253	884.0424	3.87E-08	5.143253	1569.265	6.95E-09	5.143253
T_2	116941.6	1.69E-14	5.143253	2650.202	1.45E-09	5.143253	2476.256	1.77E-09	5.143253
T_3	2144.868	2.72E-09	5.143253	93474.96	3.31E-14	5.143253	505653.6	2.09E-16	5.143253
T_4	715.2837	7.29E-08	5.143253	710.1567	7.44E-08	5.143253	7035.209	7.74E-11	5.143253
T_5	76110.37	6.12E-14	5.143253	1448.241	8.83E-09	5.143253	5890.966	1.32E-10	5.143253
T_6	792.9868	5.35E-08	5.143253	1552.947	7.17E-09	5.143253	11105.63	1.97E-11	5.143253

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on chlorophyll content of ***Tribulus terrestris.*** Significant at a 5% level.

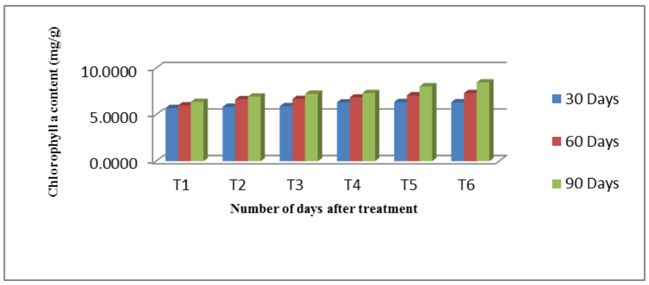

Fig: 7a Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on chlorophlly -'a' content of *Tribulus terrestris*

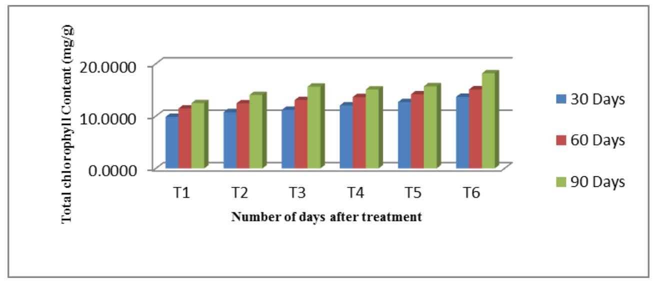

Fig: 7c Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on total chlorophyll content of *Tribulus terrestris*

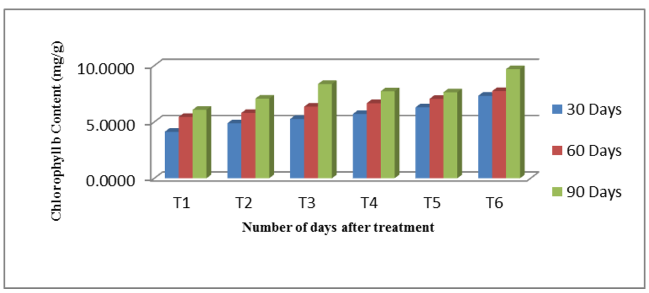

Fig: 7b Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on chlorophyll - 'b' content of *Tribulus terrestris*

Carbohydrate content

On the 30th, 60th and 90th days, the amount of reducing sugars (mg/g) of T_2 T_3 T_4 T_5 and T_6 plants were significantly higher when compared to T_1(2.50, 4.54, and 9.23). On the 30th, 60th and 90th days the maximum amount of reducing sugars was recorded in T_6 plants (5.09, 10.77, and 23.98) (Table 8, fig: 8).

When compare toT_1 (control) plants, T_2 T_3 T_4 T_5 T_6 plants demonstrated a significant increase in non-reducing sugars after 30, 60 and 90 days. When compare to T_1 plants (208.09, 234.94, and 267.54) the amount of non-reducing sugars in T_6 was at its greatest (313.08, 407.66 408.80) (Table 9, fig 9). There was a significant variation in the carbohydrate content of roots between treatments and on diverse days of the same treatment at a 5% level.

Table: 8, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Reducing sugar in roots of *Tribulus terrestris*

Samples	**Days after treatments**					
	30 Days	**60 Days**	**90 Days**	**Calculated**	**Table Value**	**P-value**
T_1	2.506 (0.595)	4.543 (0.595)	9.234 (0.595)	100.7849	2.42E-05	5.143253
T_2	3.123 (0.835)	5.901 (0.748)	11.271 (0.650)	91.89796	3.16E-05	5.143253
T_3	3.432 (0.466)	7.012 (0.650)	13.061 (1.019)	126.8844	1.23E-05	5.143253
T_4	4.543 (0.282)	8.123 (0.565	17.259 (1.335)	177.2356	4.61E-06	5.143253
T_5	4.666 (0.370)	9.913 (0.282)	20.407 (0.807)	665.4605	9.04E-08	5.143253
T_6	5.0987 (0.770)	10.777 (0.555)	23.987 (0.770)	564.4885	1.48E-07	5.143253
F	9.0032	49.6581	119.9363			
P-value	0.0009	1.33E-07	8.15E-10			
F crit	3.1058	3.1058	3.1058			

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on Reducing sugars content in roots of ***Tribulus*** terrestris. Significant at a 5% level.

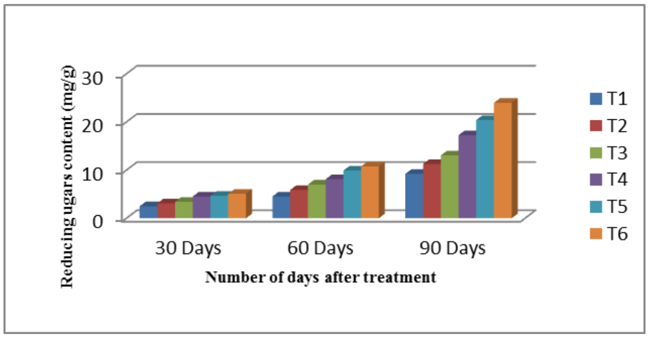

Fig: 8 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Reducing sugars content in roots of *Tribulus terrestris*

Table: 9, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Non-Reducing sugar content in roots of *Tribulus terrestris*

	Days after treatments					
Samples	**30 Days**	**60 Days**	**90 Days**	**Calculated**	**Table Value**	**P-value**
T_1	208.095 (0.762)	234.940 (1.045)	267.549 (29.651)	9.057317	0.015403	5.143253
T_2	217.347 (0.926)	249.399 (1.266)	258.519 (12.210)	27.76666	0.000927	5.143253
T_3	248.476 (0.871)	274.497 (2.288)	282.154 (2.295)	248.877	1.69E-06	5.143253
T_4	288.366 (0.569)	327.628 (0.914)	341.159 (2.000)	1292.074	1.24E-08	5.143253
T_5	294.084 (61.249)	366.660 (3.029)	389.579 (0.842)	5.907836	0.038199	5.143253
T_6	313.058 (0.668)	407.660 (3.767)	408.809 (1.305)	1661.978	5.85E-09	5.143253
F	9.0583	2643.949	72.73091			
P-value	0.00092	8.15E-18	1.49E-08			
F crit	3.105875	3.105875	3.105875			

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on Non-Reducing sugars content in roots of ***Tribulus terrestris.*** Significant at a 5% level.

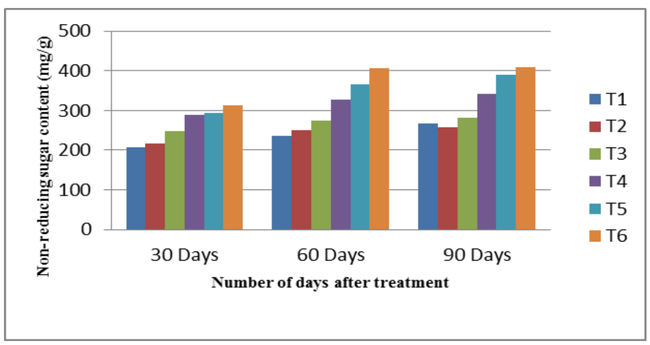

Fig: 9 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Non-Reducing sugars content in roots of *Tribulus terrestris*

Protein estimation

On the 30th 60th and 90th days the protein level in the roots of T_2 T_3 T_4 T_5 and T_6 plants was significantly higher than that of T_1 plants. T_6 (2.66) plants had the highest protein content, whereas T_1 (2.23) plants had the lowest. At a 5% level, there was a significant variation in the root protein content between the treatments and on different days of prearranged treatment (Table 10, fig 10)

Table: 10, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Protein content in roots of *Tribulus terrestris*

	Days after treatments					
Samples	**30 Days**	**60 Days**	**90 Days**	**Calculated**	**Table Value**	**P-value**
T_1	1.392 (0.018)	2.180 (0.030)	2.231 (0.022)	1124.047	1.89E-08	5.143253
T_2	1.501 (0.018)	2.225 (0.019)	2.281 (0.018)	1657.805	5.89E-09	5.143253
T_3	1.517 (0.022)	2.281 (0.018)	2.350 (0.066)	366.9482	5.33E-07	5.143253
T_4	1.686 (0.018)	2.384 (0.021)	2.440 (0.024)	1154.053	1.74E-08	5.143253
T_5	1.751 (0.024)	2.459 (0.035)	2.536 (0.030)	595.3875	1.26E-07	5.143253
T_6	1.805 (0.018)	2.538 (0.029)	2.663 (0.017)	1262.915	1.33E-08	5.143253
F	193.7324	82.70637	67.74742			
P-value	4.85E-11	7.11E-09	2.25E-08			
F crit	3.105875	3.105875	3.105875			

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on Protein content in roots of ***Tribulus terrestris.*** Significant at a 5% level.

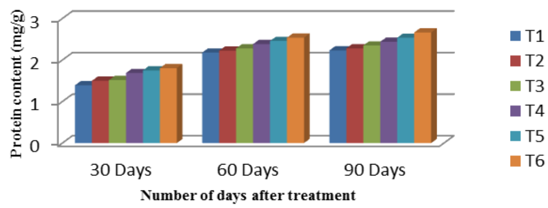

Fig: 10 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Protein content in roots of *Tribulus terrestris*

Starch estimation

On the 30th 60th and 90th days, the starch content in the roots of T_2 T_3 T_4 T_5 and T_6 plants were significantly higher than that of T_1 plants. T_6 (312.96) plants had highest starch content and the minimum in T_1 (203.33) plants. At a 5% level, **t**here was a significant variation in the starch content of the root between the treatments and on different days of prearranged treatment (Table 11, fig 11)

Table: 11, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Starch content in roots of *Tribulus terrestris*

	Days after treatments					
Samples	**30 Days**	**60 Days**	**90 Days**	**Calculated**	**Table Value**	**P-value**
T_1	123.703 (3.394)	182.592 (2.7962)	203.333 (6.186)	266.5786	1.38E-06	5.1432
T_2	127.407 (3.571)	189.629 (5.0102)	218.148 (11.349)	116.2691	1.59E-05	5.1432
T_3	141.481 (4.490)	223.333 (8.388)	241.481 (5.250)	216.2753	2.56E-06	5.1432
T_4	155.185 (8.190)	254.444 (3.333)	266.296 (10.791)	172.1438	5.03E-06	5.1432
T_5	158.518 (4.625)	268.888 (4.444)	285.925 (5.010)	649.8634	9.7E-08	5.1432
T_6	158.518 (5.592)	268.888 (7.286)	285.925 (4.490)	429.4606	3.34E-07	5.1432
F	9.003297	49.6581	87.41339			
P-value	3.105875	3.105875	3.105875			
F crit	0.000946	1.33E-07	5.16E-09			

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on Starch content in roots of ***Tribulus terrestris.*** Significant at a 5% level.

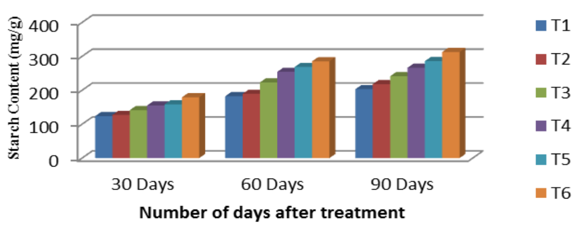

Fig: 11 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Starch content in roots of *Tribulus terrestris*

Lipid estimation

On the 30th 60th and 90th days, the lipid content in the roots of T_2 T_3 T_4 T_5 and T_6 plants were significantly higher than that of T_1 control plants. T_6 (19.40) plants had the maximum lipid content and whereas the minimum in T_1 (12.87) plants. At a 5% level, **t**here was a significant variation in the lipid content of the root between the treatments and on different days of prearranged treatment (Table 12, fig 12).

Table: 12, Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Lipid content in roots of *Tribulus terrestris*

	Days after treatments					
Samples	**30 Days**	**60 Days**	**90 Days**	**Calculated**	**Table Value**	**P-value**
T_1	7.616 (0.390)	9.073 (0.070)	12.876 (0.150)	370.8505	5.143253	5.17E-07
T_2	8.056 (0.096)	10.113 (0.370)	13.830 (0.246)	371.6234	5.143253	5.14E-07
T_3	8.146 (0.040)	10.786 (0.143)	14.126 (2.942)	9.312564	5.143253	0.014465
T_4	9.533 (0.090)	11.833 (0.180)	13.296 (2.377)	5.690324	5.143253	0.041139
T_5	9.676 (0.040)	12.256 (0.316)	17.976 (0.117)	1404.82	5.143253	9.68E-09
T_6	11.643 (0.070)	13.423 (0.436)	19.400 (0.393)	424.4398	5.143253	3.46E-07
F	225.719	91.5157	9.24276			
P-value	3.10588	3.10588	3.10588			
F crit	2E-11	4E-09	0.00084			

(Standard deviation and the mean of three replications are shown with in brackets).

One-way ANOVA for the effect of **Vermicompost, VAM fungi, PBS and *Azotobacter*** on Lipid content in roots of ***Tribulus terrestris.*** Significant at a 5% level.

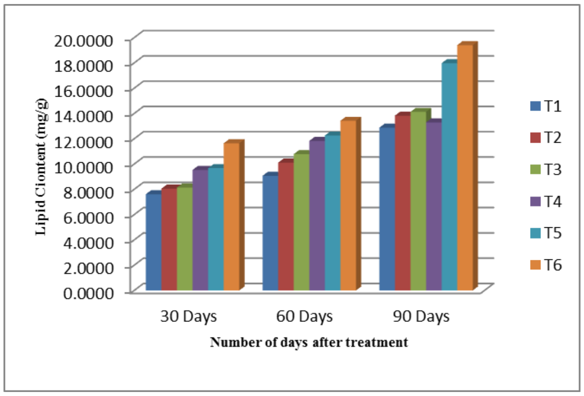

Fig: 12 Effect of Vermicompost, VAM fungi, PBS and *Azotobacter* on Lipid content in roots of *Tribulus terrestris*

DISCUSSION

Tribulus terrestris is one of the admired medicinal herb species around the world. The roots are used to cure appetizer, stomachic, carminative and diuretics and are also commonly used for rising testosterone levels in males. Biofertilizer is made up of living cells of effictive strains of microorganisms that helps that assist agriculture plants in nutrient uptake through their interaction in the rhizosphere when sprayed through the soil, contain the active strain of selective microorganisms like Vesicular arbuscular mycorrhizae, *Azotobacter*, *Bacillus* alone or in combination help the plant in growth in different mechanisms such as nitrogen fixation and solubilization of phosphate. Vermicompost is an organic matter derived from humus and other natural resources which increases the soil qualities. VAM is a beneficial fungus that improves soil nutrients as well as physical, chemical and biological properties. *Azotobacter chroococum* in crop production leads to improving plant nutrition and soil fertility by fixation of nitrogen from the atmosphere into ammonium ions and nitrates [21] and release of growth substances which causes high crop production [22]. *Bacillus* species are the most common bacteria that promote plant development by secreting a variety of compounds that stimulate plant growth while also preventing pathogen invasion. It using biofertilizer to soil can enhance the plant nutrient in rhizospheres and reprogram plant physiology changes to achieve biotic and abiotic stress tolerance. The findings of this investigation revealed that all inoculation of vermicompost, vesicular mycorrhiza (VAM), phosphate solubilizing bacteria (PBS), and nitrogen-fixing bacteria individually and in combination influence the growth and biomass of *Tribulus terrestris* seedlings. Among all the treatments in the inoculation of vermicompost + VAM fungi + phosphate solubilizing bacteria (PBS) + nitrogen-fixing bacteria produces excellent growth, biomass and biochemical parameters because of Vermicompost as organic matter, accumulation of nitrogen due to *Azotobacter* and phosphorus uptake by *Bacillus megtherium* and VAM fungi .

CONCLUSION

In this study, we conclude that by the treatment of bioinoculants mixture and in amalgamation with significantly superior the seedling growth parameters which include shoot height, root length, number of leaves, leaf area, fresh shoot biomass and fresh root biomass, dry shoot biomass and dry root biomass, chlorophyll content, starch, reducing and non-reducing sugar, protein and lipid contents in root samples in *Tribulus terrestris*.

CONFLICT OF INTEREST

We declare we have no conflict of interest.

ACKNOWLEDGMENT

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