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### EFFECT OF VERMICOMPOST, NITROGEN FIXING BACTERIA, PSB ON PROTEIN AND LIPID CONTENTS IN SORGHUM

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#### ABSTRACT

**Issue:** The main aim of the present study is to elevate the protein and lipid content in *Sorghum vulgare* plants.

**Methodology:** To increase the protein and lipid content of *Sorghum*, plants were treated with vermicompost, nitrogen fixing bacteria (*A. chroococ-*



*cum*) and phosphate solubilising bacteria (*P. striata*) in combination and also in alone.

**Results:** The results of the present study clearly showed that plants treated with vermicompost, phosphate solubilising bacteria and nitrogen fixing bacteria showed increased protein and lipid content when compared

to other groups. This study will help farmers to gain increased productivity of *Sorghum vulgare* along with increased contents of protein and lipid.

**KEYWORDS :** Vermicompost, Nitrogen fixing Bacteria, Phosphate solubilising Bacteria.

#### INTRODUCTION

Sorghum is an annual cereal crop and it is the fourth most important world cereal following wheat, rice, and maize. Sorghum is one of the main staple food for the world's poorest and most food insecure people. About 26 percent of the Indian population is deficient in calories and 28 percent in protein (Chand et al. 2003). Sorghum is a cheap source of energy, protein, iron and zinc next only to pearl millet among all cereals and pulses (Rao et al. 2006). In addition to this it is a versatile crop which is grown not only for human consumption but it is a good quality for live stock and for some industrial products (Gomma et al. 1996). However, it is popularly grown for feed, fodder and more recently for bio-fuel purposes in the world (Kleih et al. 2000).

In India, sorghum is consumed and cultivated during both rainy (Kharif) and post-rainy (Rabi) seasons. However, the area under sorghum in India has declined drastically from 18.6 m ha in 1970 to 7.93 m ha in 2007-08. The total production also declined from 9.72 m t to 7.78 m t. Dependence on chemical fertilisers for future agricultural growth would mean further losses in soil quality solubilisation of water contamination and unsustainable burden on the fiscal system. More over these inputs have multiple beneficial impacts on the soil and can be relatively cheap and convenient for use. They offer an economically attractive and ecologically sound way for providing nutrient to the plant.

A great attention has recently directed towards the application of bio organic farming to avoid the heavy use of agrochemicals that result in erroneous environmental troubles (Abd El- Ghany, H.M., 2007). Bio fertilizer is a substance which contains living micro organisms which when applied to seed, plant surfaces, or soil colonizes the rhizosphere the interior of the plant and promotes growth by increasing the availability of primary nutrients to the host plant (Mazid et al., 2011).

Organic fertilizers contain organic compounds which directly or by their decay, increase soil fertility. Composting is considered to be one of the most suitable ways of converting organic wastes into products that are beneficial for plant growth (Stantford E.I, et al. 1987). Compost, Nitrogen fixing bacteria, PSB when inoculated can fix atmospheric nitrogen, solubilises phosphorous and stimulate plant growth respectively. The most commonly used bio inoculants to supply the nutritional need of the plants are Phosphorus Solubilising Bacteria (PSB), free living Nitrogen fixing bacteria (Azotobacter). They significantly contribute N, P, and K to plants and also provides resistance to drought. However very limited information is available regarding the effect of the biofertilizers on the growth and yield of crop plants. Vermicompost, PSB and Nitrogen fixing bacteria inoculums were prepared and its effect on plant growth was determined in terms of plant growth parameters and physiological parameters i.e total protein contents and lipid contents. PSB aid in improvement of plant uptake by phosphate solubilisation. PSB showed the potential to solubilise mineral phosphates and large unexploited resources of low grade rock phosphates which are available in many countries.

The present investigation was undertaken to study the physiological parameters like total protein content and lipid content in shoot and root of Sorghum.

## MATERIALS AND METHODS

### Collection of seeds:

The seeds of sorghum were obtained from Sri Venkateswara Agriculture Research Institute, Tirupati, Andhra Pradesh. The seeds of uniform size were separated and surface sterilized with 0.05% sodium hypo chloride after thorough washing with tap water before sowing.

### Vermicompost:

Fresh leafy vegetation was collected from different sites of Sri Venkateswara University campus at 10 -20 % flowering stage and chopped into the small pieces (2-3 cm). Equal amount of weed vegetation was used for each treatment. The material was uniformly spread into the pits to a height of about 5 cm and sprinkled with 10 percent cow dung slurry (1 kg dung in 10 liter water) and soil. Afterwards the remaining plant material was added and finally, the pits were sealed with dung slurry and fine clay to prevent loss of heat or exchange of gases. After partial decomposition (15 days), first turning was given for uniform decomposition and sufficient amount of water was sprinkled for maintaining 50 - 60 percent moisture. Then the earthworms of the species *Eisenia foetida* and *Eudrilus eugeniae* (50 - 60 individuals per pit) were released. Earthworms were identified with the help of monographs and other available literature on the subject Julka (1988). The vermicomposting process

was completed within 15 days and completely decomposed, fine, dark brown colored granular excreta was obtained for the field experiment.

#### **Azotobacter inoculum:**

Culture of *Azotobacter chroococcum* was obtained from Regional Biofertilizers Development Centre, Bangalore Division, India. From this mother culture carrier based inoculums was developed by growing it on nitrogen free nutrient broth as described by Ashby for 3 days at 25 °C temperatures. Then the culture was mixed with lignite powder and this carrier based inoculums was mixed with soil at the time of planting the test plants.

Composition of medium (Ashby's medium) used for growing *Azotobacter* mother culture (gm/lit): Mannitol - 20.0, K<sub>2</sub>HPO<sub>4</sub> 0.1, MgSO<sub>4</sub>·7H<sub>2</sub>O-0.2, NaCl-0.2, K<sub>2</sub>SO<sub>4</sub>-0.1, CaCO<sub>3</sub>-5.0, Distilled Water-1000ml with pH-7.2 at 25°C

#### **PSB inoculum:**

Culture of *Pseudomonas striata* was obtained from Regional Biofertilizers Development Centre, Bangalore Division, India. *P. striata* was inoculated in 500 ml sterilized Pikovskya broth and incubated at 30°C for 3 days in a BOD chamber. After obtaining the desirable growth (10<sup>7</sup>-10<sup>8</sup> cells/ml), the broth was mixed with wood charcoal by maintaining moisture content at 40 percent and pH 7.0. The slurry thus prepared was mixed with soil at the time of planting (Gaur, 1990).

#### **Composition of Pikovskaia's medium:**

The following constituents were mixed to prepare Pikovskya medium in gr/lit composition. Ca<sub>3</sub>PO<sub>4</sub>-5.0, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-0.5, Dextrose-10.0, KCl-0.2, MgSO<sub>4</sub>·7H<sub>2</sub>O-0.1, KCl-0.2, Yeast extract-0.5, MnSO<sub>4</sub>-0.0001, FeSO<sub>4</sub>-0.0001, Agar-15.0, Distilled water-1000 ml and pH-7.0.

#### **Location of experimental site:**

The plants were maintained under glass house conditions in the botanical garden of Botany department, S.V. University, Tirupati, Andhra Pradesh, India. The climate was warm and humid at the time of starting the experiment. The weekly average maximum and minimum temperatures ranged between 27.1°C to 36.2°C and 14.6°C to 23.7°C respectively during the experimental period.

#### **Experimental design:**

- T1 : Control (No inoculation)
- T2 : Inoculation with vermicompost
- T3 : Inoculation with nitrogen fixing bacteria (*A. chroococcum*)
- T4 : Inoculation with phosphate solubilizing bacteria (*P. striata*)
- T5 : Inoculation with vermicompost and *A. chroococcum*
- T6 : Inoculation with vermicompost and *P. striata*
- T7 : Inoculation with vermicompost, *A. chroococcum* and *P. striata*

#### **Seedlings:**

Sorghum plants were grown in plastic pots containing a sterilized mixture of soil and sand (1/1 w/w). Eight experimental replicates were prepared for each treatment. Seeds of sorghum were surface sterilized with 0.005% sodium hypochlorite for 45 min and rinsed twice with sterile water and then sown into a 5 cm depth in pots, grown in a greenhouse under natural photoperiods (23.5/18°C

day/night, 6000/4000 lux light intensity) for three months. Inoculums of vermicompost (20gm/kg soil), 20 ml of N<sub>2</sub> and PSB was laid around the seed.

#### Estimation of total proteins:

Protein content of the plants in different treatments was estimated on 30, 60 and 90 days of plant growth by the method of Lowry *et al.*, (1951) using Folin phenol reagent. Hundred mg of dry material was accurately weighed and macerated in a mortar with 10 % TCA (Trichloro acetic acid). The extract was centrifuged. The supernatant was discarded and the residue was washed with 5 % TCA to remove the interfering amino acids and phenols. The protein was then dissolved in 1 N NaOH and left for 2 hours at room temperature. Later this was made to a known volume.

To 0.1 ml of the protein sample (extract) 5 ml of Reagent 'A' (Alkaline copper reagent) was added and allowed to stand for 10 min. After 10 min, 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 colour was read at 660 nm in colorimeter. 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 concentrations.

#### REAGENTS:

##### Solutions

1.4 % Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH: 4 gm of sodium carbonate was dissolved in 100 ml of 0.1N NaOH solution.

2.0.5 % CuSO<sub>4</sub>.5H<sub>2</sub>O in 1 % Sodium potassium tartarate: 500 mg of copper sulphate was dissolved in 1 % sodium potassium tartarate (1 gm in 100 ml distilled water) solution.

##### Reagent 'A'

50 ml of solution (1) and 1 ml of solution (2) were mixed just before use.

##### Reagent 'B'

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

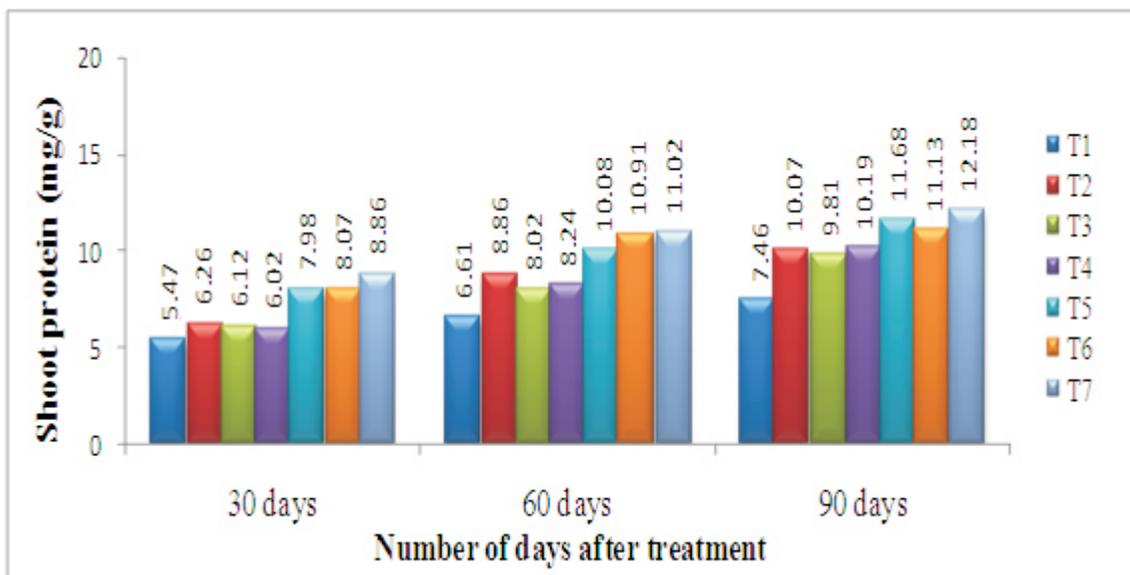
#### Estimation of total lipids:

Estimation of total lipids was carried out according to the method of Bligh and Dyer (1959). One gram dry plant powder was extracted with 14.4 ml of boiling mixture of chloroform: methanol: water (1:2:0.6 v/v/v). The supernatant was transferred into stopper tube and residue was re-extracted with 8.0 ml of hot methanol and again transferred to a stopper tube. To this, 12 ml of chloroform was added. Now the final ratio of chloroform, methanol and water was 2:2:0.6 (v/v/v). After keeping the samples overnight at 0 °C in the deep freeze, 11.2 ml of water was added, shaken well and centrifuged for phase separation. The final ratio of chloroform, methanol and water was 2:2:1.8 (v/v/v). The aqueous phase was removed with suction. The lipid phase was washed thrice with methanol: water (2:1.8 v/v) mixture. The contents were centrifuged in refrigerated centrifuge at 6000 rpm for 10min in order to remove the water soluble compounds. The lipid extract was evaporated to dryness.

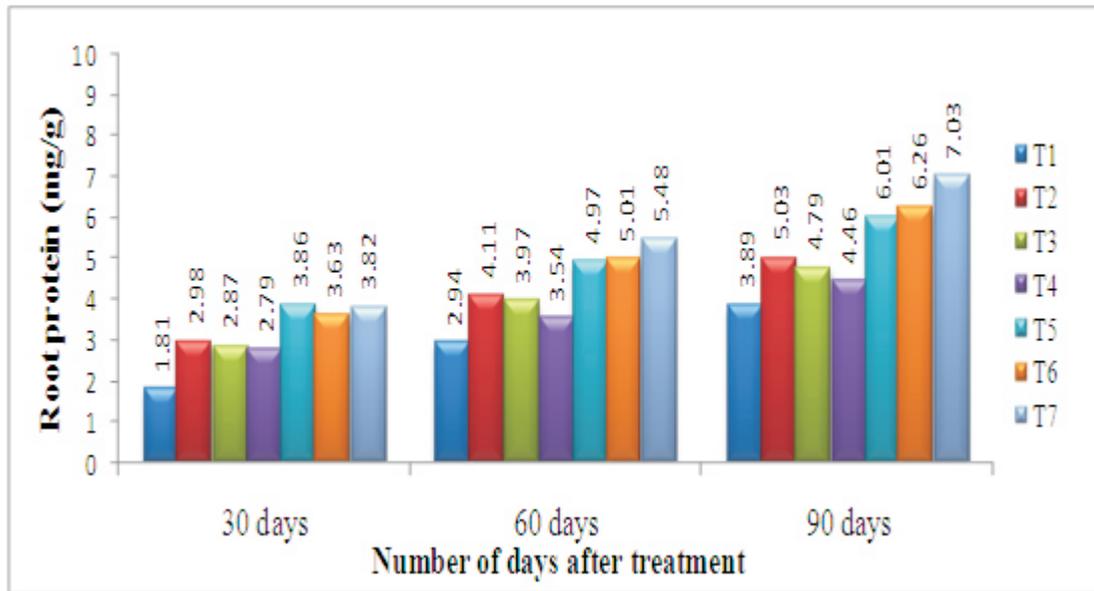
**Table 1: Effect of Vermicompost, Azotobacter and PSB on total protein content of Sorghum.**

Treat-ments	Shoot protein content (mg/g)			Root protein content (mg/g)			Leaf protein content (mg/g)		
	Days after treatment								
	30 days	60 days	90 days	30 days	60 days	90 days	30 days	60 days	90 days
T <sub>1</sub>	5.47	6.61	7.46	1.81	2.94	3.89	2.02	2.09	3.55
	(0.88)	(0.92)	(0.17)	(0.04)	(0.09)	(0.11)	(0.05)	(0.03)	(0.07)
T <sub>2</sub>	6.26	8.86	10.07	2.98	4.11	5.03	3.06	3.91	4.68
	(0.12)	(0.05)	(0.25)	(0.09)	(0.11)	(0.14)	(0.06)	(0.08)	(0.11)
T <sub>3</sub>	6.12	8.02	9.81	2.87	3.97	4.79	2.95	3.65	4.28
	(0.14)	(0.11)	(0.10)	(0.06)	(0.04)	(0.11)	(0.03)	(0.08)	(0.07)
T <sub>4</sub>	6.02	8.24	10.19	2.79	3.54	4.46	3.01	3.95	4.51
	(0.13)	(0.22)	(0.09)	(0.06)	(0.07)	(0.08)	(0.10)	(0.05)	(0.06)
T <sub>5</sub>	7.98	10.08	11.68	3.86	4.97	6.01	3.86	5.11	6.01
	(0.28)	(0.18)	(0.23)	(0.05)	(0.06)	(0.13)	(0.09)	(0.12)	(0.11)
T <sub>6</sub>	8.07	10.91	11.13	3.63	5.01	6.26	3.96	5.02	6.24
	(0.16)	(0.11)	(0.05)	(0.08)	(0.16)	(0.12)	(0.06)	(0.14)	(0.09)
T <sub>7</sub>	8.86	11.02	12.18	3.82	5.48	7.03	4.11	5.86	6.91
	(0.06)	(0.17)	(0.13)	(0.16)	(0.08)	(0.06)	(0.06)	(0.05)	(0.10)
CD	0.284	0.652	0.434	0.189	0.343	0.287	0.194	0.564	0.398
SEM	0.089	0.104	0.064	0.100	0.043	0.074	0.032	0.110	0.098

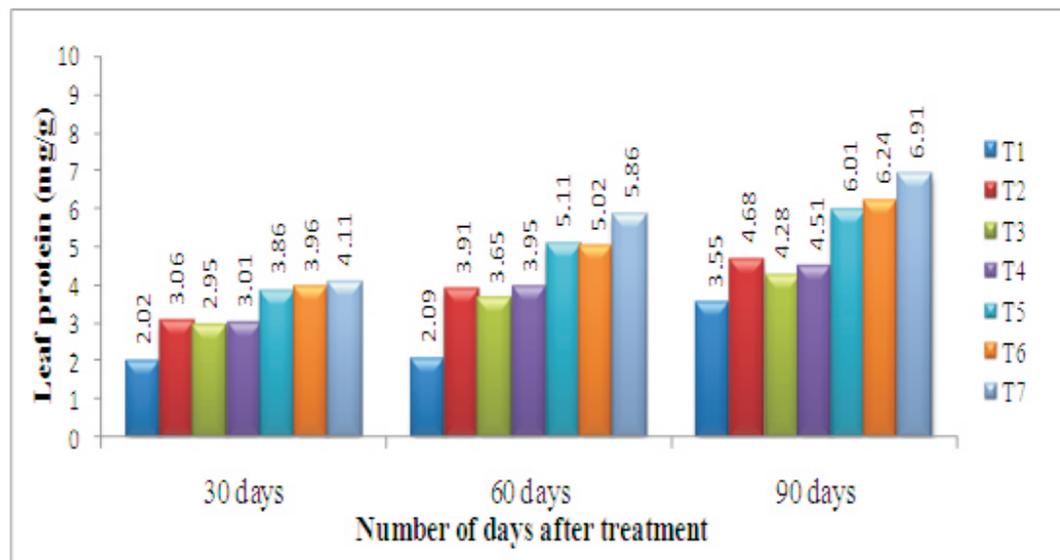
Values within the brackets indicate standard deviation.  
Each value represents mean of eight replications.

**Fig 1: Effect of Vermicompost, Azotobacter and PSB on shoot protein content of Sorghum.**

**Fig 2: Effect of Vermicompost, Azotobacter and PSB on root protein content of *Sorghum*.**



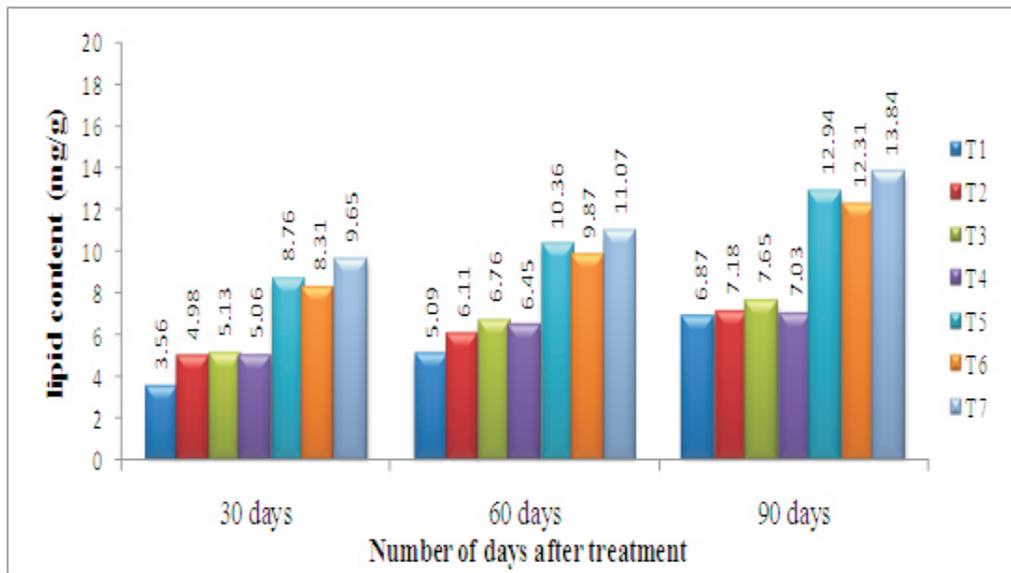
**Fig 3: Effect of Vermicompost, Azotobacter and PSB on leaf protein content of *Sorghum*.**

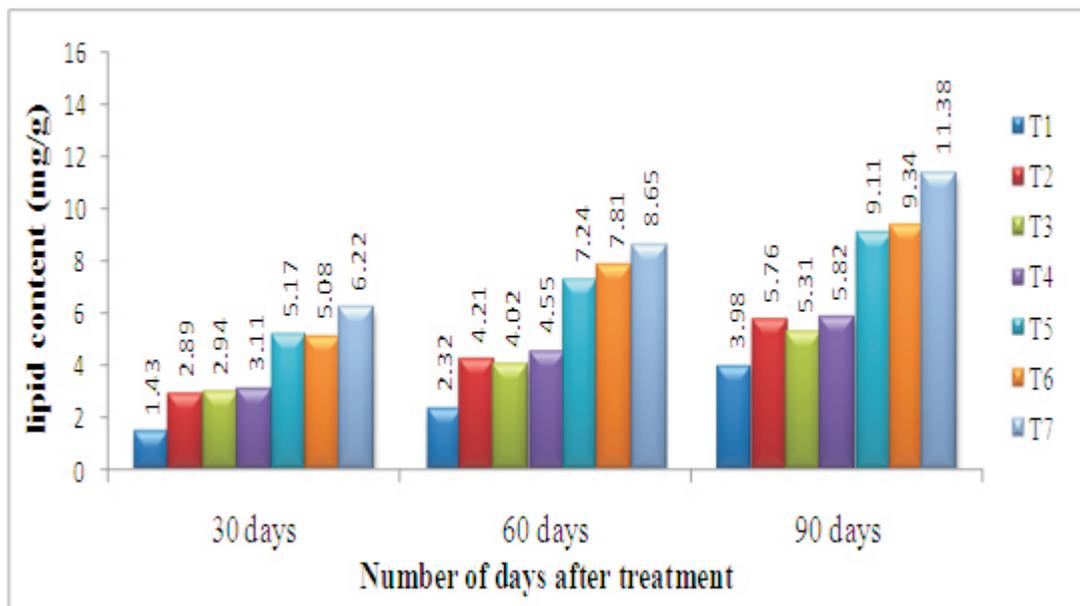


**Table 2: Effect of Vermicompost, Azotobacter and PSB on shoot and root lipid content of *Sorghum*.**

Treatments	Shoot lipid content			Root lipid content		
	Days after treatment					
	30 days	60 days	90 days	30 days	60 days	90 days
T <sub>1</sub>	3.56	5.09	6.87	1.43	2.32	3.98
	(0.04)	(0.03)	(0.08)	(0.02)	(0.05)	(0.04)
T <sub>2</sub>	4.98	6.11	7.18	2.89	4.21	5.76
	(0.06)	(0.04)	(0.03)	(0.02)	(0.06)	(0.05)
T <sub>3</sub>	5.13	6.76	7.65	2.94	4.02	5.31
	(0.09)	(0.14)	(0.06)	(0.04)	(0.05)	(0.07)
T <sub>4</sub>	5.06	6.45	7.03	3.11	4.55	5.82
	(0.05)	(0.08)	(0.23)	(0.10)	(0.09)	(0.07)
T <sub>5</sub>	8.76	10.36	12.94	5.17	7.24	9.11
	(0.14)	(0.11)	(0.08)	(0.05)	(0.08)	(0.12)
T <sub>6</sub>	8.31	9.87	12.31	5.08	7.81	9.34
	(0.17)	(0.32)	(0.21)	(0.09)	(0.14)	(0.08)
T <sub>7</sub>	9.65	11.07	13.84	6.22	8.65	11.38
	(0.18)	(0.14)	(0.23)	(0.09)	(0.10)	(0.12)
CD	0.346	0.262	0.337	0.406	0.283	0.486
SEM	0.096	0.084	0.183	0.040	0.053	0.084

Values within the brackets indicate standard deviation.  
Each value represents mean of eight replications

**Fig 4: Effect of Vermicompost, Azotobacter and PSB on shoot lipid content of *Sorghum*.**

**Fig 5: Effect of Vermicompost, Azotobacter and PSB on root lipid content of Sorghum**

## DISCUSSION

Significant increase is found in the protein content and lipid content of shoot root and leaf tissue of inoculated plants compared to uninoculated control plants. Maximum protein content in triple inoculated plants. This is in agreement with that reported by Kumutha (2005) and Lawje et al. (2005). In case of nutrient uptake, combined inoculation treatment (T7) with 2.01, 0.91 and 1.60 mg per plant recorded significantly highest N, P and K uptake, respectively compared to all other treatments. However, the N, P and K, uptake was lowest in uninoculated control with 1.52, 0.42 and 1.17 mg per plant, respectively. The increase in N, P and K uptake due to single inoculation of N<sub>2</sub>-fixers and phosphate solubilizers are reported by (Sharma and Namdeo, 1999; Parashar *et al.*, 1999; Defreitas *et al.*, 1997). The increase in N, P and K, uptake due to combined inoculation of vermicompost and microbial inoculants has been documented by several workers (Ramanjaneyulu *et al.*, 2010; Ghulam *et al.*, 2007; Devananda, 2000).

## CONCLUSION

Results revealed that inoculation of vermicompost, Azotobacter and PSB enhanced the Protein and lipid contents in shoot and root. This study will help farmers to gain increased productivity of *Sorghum vulgare* along with increased contents of protein and lipid.

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