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Effect of Different Fertilizers on Biochemical Characteristics of Leaf of Groundnut (*Arachis hypogaea* L.).

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ABSTRACT

The study was conducted to observe the comparative effect of different organic fertilizers (cowdung, vermicompost), inorganic fertilizer (DAP) and biofertilizer on the biochemical characteristics of leaf of groundnut legume plant. The study was conducted at University of Rajasthan Jaipur. Five types of treatments were conducted to analyse Arachis hypogaea L. plant. T1- Control plant (Without any fertilizer), T2- DAP treated plant, T3- Cow-dung treated plant, T4-Vermicompost treated plant and T5-Biofertilizer treated plant. Biochemical parameters of leaf analyzed were viz. total soluble sugar, starch, protein, total lipid, total phenol, ascorbic acid, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and activity of antioxidant enzymes like peroxidase and catalase. Results of experiment revealed that chlorophyll a (0.99±0.01 mg/gm), chlorophyll b (0.37±0.02 mg/gm), total chlorophyll (1.36±0.03 mg/gm), protein (240.67±7.57 mg/gm), total soluble sugar (6.56±0.18 mg/gm), starch (8.971±0.32 mg/gm), total phenol (1.66±0.06 mg/gm), antioxidant enzyme catalase activity (1.80±0.26 µml/l/g/fw) and total lipid content (15.33±0.15 mg/gm) were significantly influenced with vermicompost application (T4) as compared to other treatments and control. Carotenoids (0.52±0.00 mg/gm) with DAP treatment (T2), ascorbic acid (14.25±0.19) with cowdung treatment (T3) and peroxidase activity (2.63±0.45 µml/l/g/fw) with rhizobium biofertilizer treatment (T5) were influenced significantly as compared to other treatments and control. The work concluded that quality and quantity of food for growing world population requires sustainable management of fertilizers and new technology.

Keywords: Biochemical parameters, cowdung, vermicompost, DAP and rhizobium biofertilizer.

INTRODUCTION

Groundnut is one of the important oil-crops of India. In Rajasthan groundnut is cultivated in .56 million hectare with a total production of 1.14 million tonnes. Rajasthan shares 15.08% groundnut and ranked second in India. Bikaner is highest groundnut producing district in Rajasthan (Rajasthan agriculture statistics at a glance 2017-18).

Peanut or groundnut is a native of South America. Botanical name of groundnut is *Arach0is hypogaea* L. and it belongs to fabaceae family. Peanut is high in functional compounds like oil, proteins, vitamin B complex, fiber, polyphenol, antioxidants and minerals. Cholesterol adsorption

blocking compounds like resveratrol, flavonoides, phenolic acid and phytosterol are found in groundnut. It is also a good source of co-enzyme Q10 and consist all the 20 amino acids with highest amount of arginine. Boiling and roasting methods increase bioactive compounds (Arya *et* al. 2016). Oil is obtained from seeds and is used as cooking oil and in processing margarine, soap, and lubricants.

To increase agricultural production use of fertilizers is essential factor, but long term use of chemical fertilizers has detrimental effect on soil health which in turn causes decrease in productivity. Nutrient management in cropping system is a crucial step among the agronomic practices which is responsible for the sustainable production (Kulkarni et.al.2018). Proper utilization of growth resources depends on effective use of NPK fertilizers. Nitrogen plays crucial role for better growth and development of groundnut. Nitrogen determines effective utilization of phosphorus (Parameshwarareddy et. al. 2019). Excessive use of chemical fertilizers cause serious issues to human health, soil and environment. Recent studies showed that organic farming production system aims at promoting agro ecosystem health, soil biological activities, biodiversity and biological cycles (Mathivanan and Jayaraman, 2019).

MATERIALS AND METHODS

This experiment was laid out at Department of Botany, University of Rajasthan, Jaipur which is located at latitude 26° 53′ 22.9″ N and longitude 75° 49′ 02.3″E. Jaipur is included in semi arid eastern plain agro climatic zone of Rajasthan. The experiment was undertaken in complete randomize design (CRD) with five treatments and four replicates. During the kharif season (July 2018) seeds were sown in pots. During the season the highest temperature, lowest temperature, average temperature and average humidity were 42°C, 24°C, 30°C and 71% respectively. Chemical properties of soil observed were water holding capacity 46.5%, pH 8.5, conductivity .15 ds/m, organic carbon percentage .22%, phosphate 23 kg/ha, potash 242 kg/ha, Zn 1.02 ppm, Fe 4.48 ppm, Cu .24 ppm and Mn 2.28 ppm.

In order to investigate the effect of different fertilizers viz. DAP, cowdung, vermicompost and rhizobium biofertilizer on biochemical parameters of groundnut leaf, five treatments T1, T2, T3, T4 and T5 with four replicates were taken to pots plants. There were T1-control (without any fertilizer), T2-NP 15:60 kg ha⁻¹ (.59 gm DAP in 10 kg soil), T3-cow-dung 10 t ha⁻¹ (45.50 gm in 10 kg soil), T4-vermicompost 10 t ha⁻¹ (45.50 gm in 10 kg soil) and T5- biofertilizer (5 ml Rhizobium biofertilizer in 10 kg soil and seed treatment). Recommended doses of vermicompost and cowdung were applied 15 and 30 days before sowing of seeds respectively. DAP (Diammonium phosphate) was applied in two splits first: before sowing and second: during flowering. Rhizobium biofertilzer was applied to pots by soil application and seed inoculation method before sowing of seeds. Pots were irrigated twice in a week with tap water.

Total soluble sugar, starch, total phenol, total lipid, ascorbic acid and protein content were measured by shade dried leaves powder collected after harvesting the plant (120 DAS). Quantitative estimation of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and activity of antioxidant enzymes catalase and peroxidase were done by using fresh, fully expanded, homogenous fresh leaves collected before harvesting the plants at the age of 4 months. Estimation of chlorophyll a, chlorophyll b, carotenoids and total chlorophyll was done by Arnon's (1949) method. Total phenol

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was measured by Bray and Thorpe (1954) method. Total soluble sugar was quantified by phenol-sulphuric acid (Dubois *et al.* 1951) method. Starch content estimated by method developed by Mc Cready *et al.* (1950). Protein content was evaluated by Lowry et al. (1951) method. Peroxidase was quantified by Putter (1974) method. Catalase was measured by Aebi (1974) method. Evaluation of total lipid was done by Barnes and Blackstock (1973) method. Ascorbic acid was measured by Omaye *et al.* (1979) method.

One Way ANOVA, T test and Post Hoc Dunnett's test were done by using MS excel, Analystat and OPSTAT software. The One Way ANOVA is used to test statistically significant difference among the means of two or more groups. T test is run to determine whether two groups are different from one another. When finding of ANOVA test is significant it doesn't reveal which groups of means are different. So a Post hoc Dunnett test is run after One Way ANOVA to determine treatments against a control and identify the pairs with significant different.

RESULTS AND DISCUSSION

Application of organic, inorganic fertilizer viz. DAP, cow-dung, vermincompost and rhizobium biofertilizer in groundnut plant had great influences on biochemical characteristics. Biochemical parameters studied in this experiment were like chlorophyll-a, chlorophyll b, total chlorophyll, carotenoids, total phenol, total lipid, protein content, total soluble sugar, starch, and ascorbic acid and activity of antioxidant enzymes catalase and peroxidise.

Chlorophyll a was measured 0.75 ± 0.02 , 0.80 ± 0.01 , 0.88 ± 0.02 , 0.99 ± 0.01 and 0.97 ± 0.01 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 1). Chlorophyll b was analyzed 0.35±0.01, 0.36±0.01, 0.30±0.01, 0.37±0.02 and 0.31±0.01 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 1). Total chlorophyll was calculated 1.11±0.02, 1.17±0.01, 1.13±0.19, 1.36±0.03 and 1.25±0.00 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 1). Carotenoids were measured 0.34±0.02, 0.52±0.00, 0.33±0.01, 0.49±0.01 and 0.45±0.00 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 1). Chlorophyll a and total chlorophyll content was increased significantly with vermicompost treatment (T4) followed by biofertilizer treatment (T5) (Figure 4). Chlorophyll b was observed maximum in vermicompost application (T4) followed by DAP treatment (T2) as compared to treatments and control but difference between groups is not significant (Figure 4). Carotenoids were significantly influenced with DAP treatment (T2) followed by vermicompost treatment (T4) (Figure 4). Influence in chlorophyll a, chlorophyll b and total chlorophyll with vermicompost and phosphorous treated plants compared with other organic and inorganic fertilizer in Vigna radiata seedling was reported by Vaithiyanathan and Sundaramoorthy (2016). Increase in chlorophyll a with rhizobium biofertilizer as compared to control in Black Gram (Vigna mungo) was reported by Tiwari et al. (2017). The enhancement of chlorophyll a, chlorophyll b and total chlorophyll content in vermicompost treated plants might be increased due to easily and eco-friendly availability of nutrients. The similar outcomes were found by Manikandan and Thamizhiniyan (2016). They observed that chlorophyll a, chlorophyll b and total chlorophyll were recorded higher with compost treatment compare than other treatments. In compost treatment the availability of N, P and K was higher than other treatments which cause speedy decomposition and mineralization in compost as reported by Tensing and Muthulakshmi (2017).

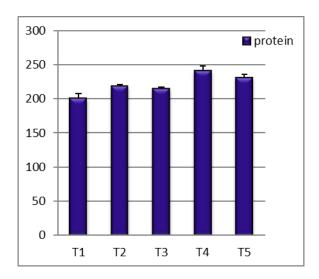


Figure 1. Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer on protein content of groundnut leaf

Amount of catalase enzyme was measured 1.56 ± 0.28 , 1.57 ± 0.53 , 1.64 ± 0.24 , 1.80 ± 0.26 and 1.57 ± 0.53 µml/l/g/fw in T1, T2, T3, T4 and T5 respectively (Table 2). Activity of peroxidase enzyme in was measured 1.85 ± 0.43 , 2.04 ± 0.20 , 1.99 ± 0.52 , 2.30 ± 0.34 , 2.63 ± 0.45 µml/l/g/fw in T1, T2, T3, T4 and T5 respectively (Table 2). Antioxidant enzyme catalase activity in leaves was reported higher with vermicompost treatment (T4) but not statistically different (Figure 2). Peroxidase activity in leaves was recorded maximum biofertilizer treatment (T5) but not statistically different from other treatments (Figure 2). Rachel and Sirisha (2016) were reported maximum amount of peroxidase in biofertilizer treated plant.

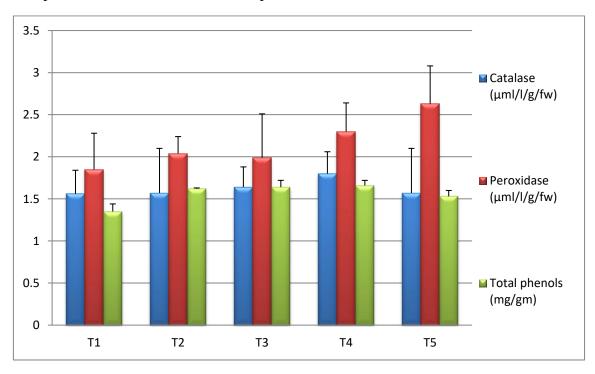


Figure 2. Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer on total phenol and antioxidant enzyme catalase and peroxidase of groundnut leaf.



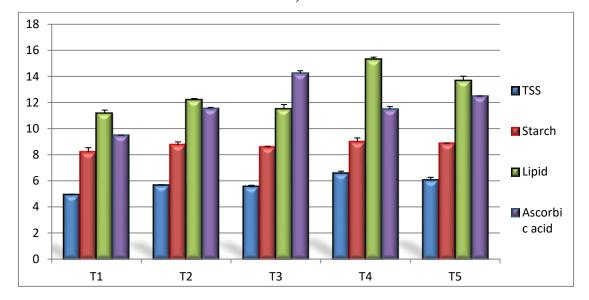


Figure 3.Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer total soluble sugar, starch, lipid and ascorbic acid of groundnut leaf.

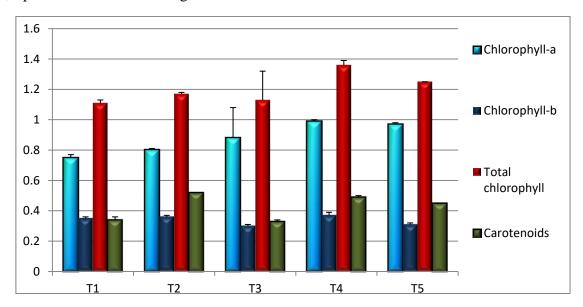


Figure 4. Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of groundnut leaf.

Table 1. Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and protein content of groundnut leaf.

Treatment	Chlorophyll-a (mg/gm)	Chlorophyll-b (mg/gm)	Total chlorophyll (mg/gm)	Carotenoids (mg/gm)	Protein (mg/gm)
T1	0.75 ± 0.02	0.35 ± 0.01	1.11±0.02	0.34 ± 0.02	200.33±7.09
T2	0.80±0.01 **	$0.36{\pm}0.01~^{\mathrm{NS}}$	$1.17{\pm}0.01^{\text{ NS}}$	0.52±0.00 ***	218.5±2.29 *
Т3	.88±0.02 ***	0.30±0.01 **	1.13±0.19 NS	$0.33\pm0.01^{\text{ NS}}$	214.5±3.12 NS

T4	0.99±0.01 ***	0.37±0.02 NS	1.36±0.03 *	0.49±0.01 ***	240.67±7.57 ****
T5	0.97±0.01 ***	0.31±0.01 **	1.25±0.00 NS	0.45±0.00 ***	230.67±5.50 ***
SE(m)	.007	.007	.049	.007	3.194
CD (0.05)	.023	.022	0.157	.021	10.194

^{***=}p 0.001, **=p 0.01, *=p 0.05, NS=Non significant

Table 2. Effect of DAP, cowdung, vermicompost and rhizobium biofertilizer total soluble sugar, starch, total phenol, lipid, ascorbic acid activity of catalase and peroxidase in groundnut leaf.

Treatm ent	TSS (mg/gm)	Starch (mg/gm)	Total phenols (mg/gm)	Lipid (mg/gm)	Ascorbic acid	Catalase (µml/l/g/f w)	Peroxidas e (µml/l/g/f w)
T1	4.92±0.05	8.202±0.34	1.35±0.09	11.20±0.2 2	9.50±0.01	1.56±0.28 NS	1.85±0.43 NS
T2	5.65±0.08	8.755±0.23	1.62±0.01	12.22±0.0	11.55±0.08	1.57±0.53	2.04±0.20
	***	NS	*	9**	***	NS	NS
Т3	5.55±0.11	8.571±0.08	1.64±0.08	11.53±0.3	14.25±0.19	1.64±0.24	1.99±0.52
	**	NS	*	2 ^{NS}	***	NS	NS
T4	6.56±0.18	8.971±0.32	1.66±0.06	15.33±0.1	11.50±0.19	1.80±0.26	2.30±0.34
	***	*	**	5***	***	NS	NS
T5	6.04±0.23	8.846±0.06	1.53±0.07	13.70±0.4	12.50±0.01	1.57±0.53	2.63±0.45
	***	*	NS	5***	***	NS	NS
SE(m)	0.083	0.137	0.047	0.161	0.077	0.216	0.232
CD (0.05)	0.266	0.437	0.152	0.513	0.245	NS	NS

^{***=}p 0.001, **=p 0.01, *=p 0.05, NS=Non significant

Protein content was analyzed 200.33±7.09, 218.5±2.29, 214.5±3.12, 240.67±7.57 and 230.67±5.50 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 1). Protein content in leaves was significantly increased with vermicompost (T4) followed by biofertilizer treatment (T5) as compared to other treatments and control (Figure 1). Higher protein content was reported by Tensing and Muthulakshmi (2017) in vermicompost + biofertilizer, vermicompost treatment than control plants in okra (*Abelmoschus Esculentus* (L.) moench). Biofertilizer provides macronutrients and micronutrients which are assimilated by plants and used for various metabolic activities. They reported that protein content was increased with Nitrogen availability. Choudhary *et* al. (2017) reported higher protein content in vermicompost and farmyard manure treated plants than control. Densilin *et* al. (2010) in in chilli (Ns-1701) reported higher amount of protein in vermicompost, inorganic fertilizer and biofertilizer treated plants. Results are in agreement with all above findings.

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Ascorbic acid was observed 9.50±0.01, 11.55±0.08, 14.25±0.19, 11.50±0.19 and 12.50±0.01 in T1, T2, T3, T4 and T5 respectively (Table 2). Ascorbic acid in leaves was significantly influenced with cowdung treatment (T3) followed by biofertiliaer treatment (T5) as compared to other treatments and control (Figure 3). Khan *et* al. 2017 reported higher amount of ascorbic acid with compost/ biocompost (*Trichoderma*-enriched biofertilizer) treatment than NPK fertilizer and control in tomato.

Total lipids were recorded 11.20±0.22, 12.22±0.09, 11.53±0.32, 15.33±0.15 and 13.70±0.45 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 2). Total lipid content in leaves was increased significantly with vermicompost (T4) followed by biofertilizer treatment (T5) compare than other treatments and control (Figure 3). The Nitrogen stimulates synthesis of photosynthetic pigments through increased amount of stromal and thylakoid protein in leaves which leads to increase in lipid content of leaves (Singh *et* al. 2016). Bekele *et* al. (2019) also reported higher seed oil in groundnut with vermimcompost than nitrogen and phosphorous fertilizer.

Total soluble sugar was measured 4.92 ± 0.05 , 5.65 ± 0.08 , 5.55 ± 0.11 , 6.56 ± 0.18 and 6.04 ± 0.23 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 2). TSS content in leaves was significantly influenced with vermicompost (T4) followed by biofertilizer treatment as compared to other treatments and control (Figure 3). Antonious *et* al. (2019) also reported higher soluble sugar content with vermicompost treatment than other inorganic and organic fertilizer in tomato.

Starch content was analyzed 8.202±0.34, 8.755±0.23, 8.571±0.08, 8.971±0.32 and 8.846±0.06 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 2). Starch content in leaves was significantly influenced with vermicompst treatment (T4) followed by biofertilizer treatment (T5) as compared to other treatments and control (Figure 3). Sanjivkumar (2014) reported higher amount of starch content in *Zea mays* with different fertilizer treated plants like cowdung, compost and vermicompost compare than control plant. Starch was recorded maximum in Triple 17 complex + vermicompost followed by different other fertilizers and lowest was found in control plant (Densilin *et al.* 2011). Their study revealed that the biochemical parameters of the chili fruits increased in the different treatments. They found that inorganic fertilizers with vermicompost or different organic manures enhance the nutrient composition of chili fruit.

Total phenol content was measured 1.35 ± 0.09 , 1.62 ± 0.01 , 1.64 ± 0.08 , 1.66 ± 0.06 and 1.53 ± 0.07 mg/gm in T1, T2, T3, T4 and T5 respectively (Table 2). Total phenol contents in leaves were significantly influenced with vermicompost treatment (T4) followed by cowdung treatment (T3) as compared to other treatments and control (Figure 2). Similar results were also reported by Antonious et al. (2019) in tomato with vermicompost treatment as compared to organic and inorganic fertilizer. Influence in total phenol content was also reported with vermicompost as compared to control in fenugreek (Alaghemand et al. 2017).

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CONCLUSION

It can be concluded that under hot semi arid climatic conditions of Rajasthan synthesis of chlorophyll

a, total chlorophyll, protein, total soluble sugar, starch and lipid content can be promoted by application of vermicompost followed by rhizobium biofertilizer as compared to than other treatments and control. Total phenol and catalase activity was found influenced with vermicompost followed by cowdung application as compared to treatments and control.

Influence in ascorbic acid with cowdung treatment, peroxidase activity with rhizobium biofertilizer, chlorophyll b with vermicompost and caroteinoids with chemical fertilizer treatment were reported as compared to other treatments and control.

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