ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 01(C), pp. 30321-30325, January, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFECT OF DIFFERENT BIO-FERTILIZERS ON THE BIOCHEMICAL PARAMETERS OF *SOLANUM NIGRUM* L. AND *AMARANTHUS VIRIDIS* L.

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1001.3034

ARTICLE INFO

Article History:

Received 4th October, 2018
Received in revised form 25th October, 2018
Accepted 18th December, 2018
Published online 28th January, 2019

Key Words:

Azospirillum, carbohydrate, chlorophyll, phosphobacteria, protein, VAM fungi

ABSTRACT

The bio-fertilizers play a major role in organic farming. In the present study, the various biochemical parameters such as chlorophyll, protein and carbohydrate were estimated at different stages of growth of the two test plants by the application of biofertilizers such as Azospirillum, Phosphobacteria and VAM fungi. The chlorophyll *a* total chlorophyll content was found to be higher in tomato plants treated with Azospirillum on 30th day and 45th day, but on the 60th day, the chlorophyll a content was found to be higher in plants treated with VAM fungi. In the case of chlorophyll *b*, it was found that on the 30th day, the content was higher in VAM treated plants and on the 45th day and 60th day, it was observed to be more in *Azospirillum* treated and combination of fertilizers respectively. In *Amaranthusviridis* L., the chlorophyll *a* was found to be higher in plants treated with *Azospirillum* on the 30th day. Chlorophyll *b* was found to be higher in plants treated with VAM fungi and total chlorophyll in plants treated with combination of fertilizers. On the 45th day, the chlorophyll *a* was found to be higher in $T_4$and chlorophyll *b* and total chlorophyll was higher in plants treated with Phosphobacteria. The protein content in tomato plant was higher in T_2 on the 30th day, T_4 on the 45th day and T_3 on the 60th day. The carbohydrate content was found to be more in T_2 on the 30th day, T_1 on the 45th day and T_4 on the 60th day. In *Amaranthusviridis* L., the protein content was observed to be higher in T_2 on the 30th day and T_4 on the 45th day. Similarly, the carbohydrate content was also found to be more in T_2 on the 30th day and T3 on the 45th day.

INTRODUCTION

India is an agriculture based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. Bio-fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agriculture science. Bio-fertilizers are applied in the agriculture field as a replacement of our conventional fertilizers consisting of compost, household wastes and green manure. One of the benefits from bio-fertilizers is a contribution from population of microorganism available. Bio-fertilizers such as *Rhizobium, Azospirillum* and Phosphobacteria provide nitrogen and phosphorus nutrients to crop plants through nitrogen fixation and phosphorous solubilization processes. These Bio-fertilizers could be effectively utilized for rice, pulses, millets, cotton, sugarcane, ladies finger, tomato, vegetable and other horticulture crops.Saeed *et al*. (2015) have reported a significant increase in fruit yield of tomato when treated with differentlevels of phosphate and bio-fertilizer.

A significant improvement in growth and biochemical parameters of *Vigna ungiculata*has been reported by Badar*et al*., (2015). They studied the influence of organic, inorganic and bio-fertilizes on physical and biochemical parameters of *Vigna ungiculata.*Kavitha *et al*. (2013) investigated different combinations of bio-fertilizer (*Azosprillum*) on the growth of a green leafy vegetable *Amaranthus tristis* and found a significant change in biometric parameters and increase in biochemical constituents.Earlier studies on the nutritional value of *Amaranthus* spp. (Schonfeltdt and Pretorius, 2011) have proved that raw leaves of *A.tricolor*, *Cleome gynandra* L. and *Corchorus olitorius* L. contain higher concentration of iron, zinc as well as phosphorus and calcium.

Nutrition

A tomato contains 95% water, 4% carbohydrates and less than 1% each of fatand protein. In a 100gram amount, raw tomatoes supply 18 calories and are a moderate source of vitamin C (17% of the Daily Value), but otherwise are absent of significant nutrient content.

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*Amaranthus viridis*L. is often harvested from the wild as a source of food and medicine for local use. It is sometimes cultivated in the Tropics for its edible leaves. *Amaranthus viridis* is a cosmopolitan species in the botanical family Amaranthaceae and is commonly known as slender amaranth or green amaranth.

The main objective of the current research is to study the efficiency of three different bio-fertilizers namely *Azosprillum,* Phosphobacteria and Vesicular Arbuscular Mycorrhizal fungi on the biochemical aspect of *Solanum lycopersicum* L. and *Amaranthus viridis* L. in pot culture study.

MATERIALS AND METHODS

The plants taken for the present study were *Solanumlyopersicum* L. belonging to the family Solanaceae and *Amaranthus viridis* L. belonging to the family Amaranthaceae. Biochemical studies were carried out under different treatments of biofertilizers namely *Azospirillum*, Phosphobacteria andVesicular Arbuscular Mycorhiza during different stages of growth of the plants.

Collection of the seeds

Seeds of *Solanum lycopersicum* L. and *Amaranthusviridis* L. were obtained from Tamil Nadu Agricultural University Coimbatore.

Collection of biofertilizers

The bio-fertilizers such as *Azosprillum*, VAM and Phosphobacteriawere collected from TNAU, Coimbatore.

Bio-Fertilizers

Azospirillum

They are called as associative endosymbiont on roots of grasses and similar types of plants. They are known to fix atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. *Azospirillum* is considered to be more efficient and it has been reported that *Azospirillum* inoculation increases the growth, nitrogen uptake and yield in number of crops (Mallikarjuna Rao *et al.,* 2014).

Vesicular Arbuscular Mycorrhiza (VAM)

Mycorrhiza is a mutualistic association between plant roots and fungal mycelia. Many graminaceous plants, legumes and horticultural crops are highly susceptible to VAM colonization. The transfer of nutrients mainly phosphorus from the soil to the cells of the root cortex is mediated by intracellular obligate fungal endosymbiont of the genera *Glomus,Gigaspora, Endosone*, etc. which possess vesicles for storage of nutrients and arbuscules for funneling these nutrients into the root system.

The mycorrhizal fungi mobilize phosphates and other micronutrients like zinc, boron and molybdenum from adjacent soil to the root system through hyphal network (Mallikarjuna Rao *et al.*, 2014)

Phosphobacteria

Microorganisms are also involved in the availability of phosphorus, the second most important nutrient required by crop plants. The phosphate solubilizing bacteria (PSB) solubilize the insoluble phosphates and make them available for crop plants in the rhizosphere region (Mallikarjura Rao *et al*., 2014)

METHODS

Pot Culture Experiment

The seeds obtained from TNAU,Coimbatore were soaked in different organic fertilizers overnight. Later,the seeds were sown in pots (30cm×24cm×30cm sized pots) containing red soil and sandy soil in the ratio 1:1. The treated pots were maintained in triplicates. The effect of different organic fertilizers on the growth, biochemical and yield parameters of *Solanum lycopersicum* L. and *Amaranthus viridis* L.were assessed. The biochemical parameters at different stages of growth of the plants were analyzed. Thulasi extract was sprayed at intervals to control the growth of insects. The different organic fertilizer treatments given were:

T_0-Control
T_1-*Azosprillum*
T_2-Vesicular Arbuscular mycorrhiza
T_3-Phosphobacteria
T_4-Azosprillum + VAM + Phosphobacteria

Biochemical Parameters

The estimation of Chlorophyll, Protein and Carbohydrate were done using standard procedures
Chlorophyll (Arnon, 1949)
Protein (Lowry *et al.,* 1951)
Carbohydrate (Hedge and Hofreiter, 1962)

Statistical Analysis

The data obtained from various biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978). The significance and critical differences of various treatments were analyzed.

RESULTS AND DISCUSSION

The experiments conducted in ***Solanum lycopersicum* L.**and *Amaranthus viridis* (L.) using different organic fertilizers treatments showed the following results.

Biochemical Parameters

***Solanum lycopersicum* L.**

Chlorophyll a, b and Total chlorophyll

In *Solanum lycopersicum*L. grown under different bio-fertilizer treatments, the chlorophyll contents were measured on the 30th day, 45th day and 60th day and tabulated (Table 1, 2 and 3).

Chlorophyll *a* was found to be higher in T_1 (0.166 ± 0.034 mg/g) on 30th day, T_1 on 45th day (0.242 ± 0.076 mg/g) and T_2 (0.386 ± 0.049) on the 60th day. This shows that *Azosprillum* increases the chlorophyll pigments initially, but at later stage of growth, presence of VAM increases the chlorophyll *a* pigment.

The chlorophyll *b* content was found to be higher in T_2 (0.131 ± 0.020 mg/g) on 30th day, T_1 (0.429 ± 0.051 mg/g) on 45th day and T_4 (0.498 ±0.204mg/g) on the 60th day. This result shows that the chlorophyll *b* pigment gradually increases when there is combination of bio-fertilizers and on the 60th day higher chlorophyll *b* content is observed in T_4 (Table 3).

Similarly, the total chlorophyll content was found to be higher in T_1 (Table 1) on 30th day and 45th day (Table 2). The values were 0.280 ± 0.023 mg/g and 0.682 ± 0.098 mg/g respectively. On the 60th day, total chlorophyll content was higher in T_4 and the value was 0.724 ± 0.204 mg/g. (Table 3).

Table 1 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Solanumlycopersicum* L. on the 30th day (mg/g f.wt.)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_0	0.121 ± 0.010	0.080 ± 0.005	0.201 ± 0.012
T_1	0.166 ± 0.034	0.115 ± 0.014	0.280 ± 0.023
T_2	0.151 ± 0.092	0.131 ± 0.020	0.197 ± 0.146
T_3	0.122 ± 0.013	0.087 ± 0.005	0.209 ± 0.016
T_4	0.131 ± 0.004	0.093 ± 0.009	0.225 ± 0.012
SEd	0.0364	0.0098	0.0547
CD(P<0.05)	0.0810	0.0219	0.1219

Values are mean ± SD of three samples in each group

Table 2 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Solanumlycopersicum* L. on the 45th day (mg/g f.wt.)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_0	0.175 ± 0.041	0.227 ± 0.054	0.406 ± 0.071
T_1	0.185 ± 0.047	0.429 ± 0.051	0.682 ± 0.098
T_2	0.226 ±0.036	0.319 ± 0.098	0.544 ± 0.126
T_3	0.242 ± 0.076	0.321 ± 0.072	0.564 ± 0.069
T_4	0.202 ± 0.064	0.334 ± 0.051	0.550 ± 0.016
SEd	0.0448	0.0553	0.0689
CD(P<0.05)	0.0999	0.1231	0.1535

Values are mean ± SD of three samples in each group

Table 3 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Solanumlycopersicum* L. on the 60th day (mg/g f.wt.)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_0	0.326 ± 0.024	0.355 ± 0.079	0.680 ± 0.095
T_1	0.278 ± 0.031	0.360 ± 0.077	0.569 ± 0.182
T_2	0.386 ± 0.049	0.368 ± 0.022	0.701 ± 0.099
T_3	0.253 ± 0.049	0.444 ± 0.071	0.714 ± 0.123
T_4	0.227 ± 0.013	0.498 ± 0.204	0.724 ± 0.204
SEd	0.0295	0.0887	0.1205
CD(P<0.05)	0.0656	0.1976	0.2685

Values are mean ± SD of three samples in each group

Earlier, similar results were reported by Selvakumar and Thamizhiniyan (2011) in chilli and Selvakumar *et al*. (2012) in black gram. Higher chlorophyll and protein content have been reported due to the application of *Azotobacter* and *Azosprillum*.

Protein

The protein content of *Solanum lycopersicum* L. was estimated on the 30th day, 45th day and 60th day and tabulated (Table 4). Two different concentrations 0.1 ml and 0.2 ml of the sample were used for estimation.

On the 30th day, the protein content was found to be 3.30±0.62mg/g in T_2 in 0.1ml concentration. On the 45th day, the protein was estimated to be higher in T_4 (4.77±0.06mg/g) in 0.1ml concentration. On the 60th day, the protein content was higher in T_3 (6.13±0.45mg/g).

Similarly, in 0.2ml concentration of the sample, the protein was higher in T_2 on the 30th day (2.07±0.40mg/g), T_4 on the 45th day (2.93±0.15mg/g) and T_4 on the 60th day (4.37±0.35mg/g). As the concentration varies, there is drastic variation in the protein content also under different bio fertilizer treatments (Table 4).

Earlier studies by Javed and Panwar (2013) on the effect of biofertilizers, vermicompost and chemical fertilizer on different biochemical parameters of *Glycine max* and *Vigna mungo* have shown increased protein, carbohydrate and phenol content

Carbohydrate

The carbohydrate content was estimated for *Solanum lycopersicum* L. under different bio fertilizer treatment on 30th day, 45th day and 60th day and tabulated (Table 5).

The carbohydrate content was found to be higher in T_2 on 30th day in both the concentrations and the values were 3.47±0.35mg/g and 2.03±0.15mg/g (Table 5).

On the 45th day of the plant growth, the carbohydrate content was estimated to be higher in T_1 in both the concentrations and the values were 4.97±0.32mg/g and 3.63±0.50mg/g (Table 5).
On the 60th day, the carbohydrate content was found to be more in T_4 in both the concentrations and the readings obtained were 6.47±0.38mg/g and 5.50±0.82mg/g (Table 5).

Table 4 Protein Content of *Solanumlycopersicum*L. on the 30th Day, 45th Day and 60th Day (mg/g f.wt.)

Treatments	30thDay (mg/g)		45thDay(mg/g)		60thDay(mg/g)	
	0.1	0.2	0.1	0.2	0.1	0.2
T_0	2.07 ± 0.25	1.60 ± 0.52	3.63 ± 0.21	2.33 ± 0.12	5.12 ± 0.40	3.03 ± 0.12
T_1	2.80 ± 0.30	2.00 ± 0.36	3.57 ± 0.25	2.40 ± 0.10	4.67 ± 0.15	3.57 ± 0.15
T_2	3.30 ± 0.62	2.07 ± 0.40	3.47 ± 0.38	2.37 ± 0.21	4.87 ± 0.47	3.97 ± 0.31
T_3	2.87 ± 0.25	1.97 ± 0.12	3.87 ± 0.40	2.63 ± 0.12	6.13 ± 0.45	4.27 ± 0.49
T_4	2.93 ± 0.72	2.03 ± 0.21	4.77 ± 0.06	2.93 ± 0.15	5.37 ± 0.38	4.37 ± 0.35
SEd			0.40925			
CD(P<0.05)			0.81961			

Values are mean ± SD of three samples in each group

Table 5 Carbohydrate content of *Solanumlycopersicum* L. on the 30th day, 45th day and 60th day (mg/g f.wt.)

Treatments	30thDay		45thDay		60thDay	
	0.1	0.2	0.1	0.2	0.1	0.2
T_0	3.23 ± 0.40	1.83 ± 0.06	4.00 ± 0.10	2.27 ± 0.23	4.63 ± 0.15	2.33 ± 0.32
T_1	2.90 ± 0.26	1.80 ± 0.44	4.97 ± 0.32	3.63 ± 0.50	5.90 ± 0.66	3.80 ± 0.10
T_2	3.47 ± 0.35	2.03 ± 0.15	3.70 ± 0.36	2.20 ± 0.35	6.10 ± 0.36	3.70 ± 0.46
T_3	3.10 ± 0.62	1.83 ± 0.40	4.70 ± 0.10	3.07 ± 0.15	5.83 ± 0.67	3.63 ± 0.50
T_4	3.00 ± 0.17	1.87 ± 0.32	4.70 ± 0.85	3.00 ± 0.20	6.47 ± 0.38	5.50 ± 0.82
SEd			0.28674			
CD(P<0.05)			0.57358			

Values are mean ± SD of three samples in each group

Amaranthus viridis L.

Chlorophyll a, b and total chlorophyll

The chlorophyll pigments present in the green leafy vegetables were calculated on the 30th day and 45th day and tabulated (Table 6 &7).

The chlorophyll *a* content was higher in T_1 (0.133±0.012mg/g) on the 30th day. Chlorophyll *b* was observed to be more in T_2 (0.225±0.129mg/g) on the 30th day and the total chlorophyll content on the 30th day was more in T_1 (0.173±0.015mg/g).
On the 45th day, there was variation in chlorophyll pigments. The chlorophyll *a* was higher in T_4 (0.300±0.045mg/g). The chlorophyll *b* and total chlorophyll was found to be higher in T_3 and the values were estimated to be 0.450±0.141mg/g and 0.678±0.147mg/g respectively (Table 7).

Protein

The protein content of *Amaranthus viridis* L. was estimated on the 30th day and 45th day (Table 8). On the 30th day, the protein content was found to be higher in T_2 (2.80±1.10mg/g) at 0.1ml concentration. On the 45th day, the protein content was found to be higher in T_4 (5.97±0.81mg/g).

Carbohydrate

The carbohydrate content of *Amaranthus viridis L.* was estimated on the 30th day and 45th day and tabulated (Table 9). Higher carbohydrate content was observed in T_2 on the 30th day and the value was 2.83±0.83mg/g. On the 45th day, the carbohydrate content was found to be higher in T_3 (Phosphobacteria treated plants) and the value was 5.97±0.12mg/g (Table 9).The statistical analysis of various biochemical parameters showed significance at 5% level.

In *Amaranthus dubius*,Manoharan *et al*. (2011) showed an increase in the amount of protein and carbohydrate in plant treated with cyanospray compared to other treatments.

Bio-fertilizers are used to hasten the biological activity of the plants to improve the availability of plant nutrient. The work on the growth and establishment of cashew grafts under greenhouse condition by Shankarappa *et al.* (2017) have shown that the bio fertilizers used increased the growth and nutrient uptake of the cultivar.

Table 6 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Amaranthusviridis* L. on the 30th day (mg/g f.wt.)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_0	0.131 ± 0.013	0.101 ± 0.129	0.159 ± 0.010
T_1	0.133 ± 0.012	0.026 ± 0.012	0.173 ± 0.015
T_2	0.118 ± 0.016	0.225 ± 0.189	0.153 ± 0.031
T_3	0.132 ± 0.022	0.032 ± 0.021	0.143 ± 0.010
T_4	0.090 ± 0.011	0.018 ± 0.002	0.094 ± 0.036
SEd	0.0124	0.0842	0.0190
CD(P<0.05)	0.0276	0.1875	0.0424

Values are mean ± SD of three samples in each group

Table 7 Chlorophyll *a*, Chlorophyll *b* and total chlorophyll content of the *Amaranthusviridis* L. on the 45th day (mg/g f.wt.)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T_0	0.237 ± 0.042	0.184 ± 0.097	0.479 ± 0.070
T_1	0.244 ± 0.080	0.244 ± 0.167	0.506 ± 0.141
T_2	0.250 ± 0.053	0.386 ± 0.091	0.637 ± 0.133
T_3	0.229 ± 0.027	0.450 ± 0.141	0.678 ± 0.147
T_4	0.300 ± 0.045	0.266 ± 0.307	0.577 ± 0.291
SEd	0.0429	0.1460	0.1407
CD(P<0.05)	0.0955	0.3253	0.3136

Values are mean ± SD of three samples in each group

Table 8 Protein Content of *Amaranthus viridis* L. on the 30th Day, and 45th day (mg/g f.wt.)

Treatments	30th day		45th day	
	0.1	0.2	0.1	0.2
T_0	2.20 ± 0.10	1.23 ± 0.15	3.87 ± 0.57	3.77 ± 2.20
T_1	2.30 ± 0.44	1.57 ± 0.32	5.07 ± 1.38	2.90 ± 0.70
T_2	2.80 ± 0.10	1.33 ± 0.32	3.63 ± 0.46	2.67 ± 0.70
T_3	2.60 ± 0.46	1.67 ± 0.49	4.87 ± 1.00	3.30 ± 0.95
T_4	2.43 ± 0.58	1.37 ± 0.21	5.97 ± 0.81	3.77 ± 1.27
SEd	0.67594			
CD(P<0.05)	1.36615			

Values are mean ± SD of three samples in each group

Table 9 Carbohydrate Content of *Amaranthus viridis* L. on the 30th day and 45th day (mg/g f.wt.)

Treatments	30th day		45th day	
	0.1	0.2	0.1	0.2
T_0	1.37 ± 0.32	1.07 ± 0.31	3.33 ± 0.15	2.13 ± 0.23
T_1	2.63 ± 0.25	1.47 ± 0.06	3.63 ± 0.12	2.03 ± 0.15
T_2	2.83 ± 0.83	1.73 ± 0.23	3.77 ± 0.15	2.20 ± 0.10
T_3	2.47 ± 0.40	1.83 ± 0.15	5.97 ± 0.12	3.43 ± 0.42
T_4	2.47 ± 0.06	1.80 ± 0.10	3.50 ± 0.26	2.97 ± 0.38
SEd	0.20303			
CD(P<0.05)	0.41035			

Values are mean ± SD of three samples in each group

Studies on the effect of PSB, *Azosprillum* and *Azotobacter* by Choudhary *et al.* (2017) have indicated that the application of bio-fertilizers not only improves the quality of Knol-Khol, but also gives a maximum monitory benefit. Bio-fertilizers are natural fertilizers containing microorganisms that enhance crop productivity through nitrogen fixation, solublizing of plant nutrients and produce plant growth regulators.

Microbial flora of soil plays an important role in soil health. The microbes present in the environment around the roots influence the plant growth and crop yield.The microorganisms in Bio-fertilizers restore the soils natural nutrient cycle and build soil organic matter.The increased amount of chlorophyll content in leaves treated with organic fertilizers indicates the photosynthetic efficiency of the plants.

Bio-fertilizers are carried-based preparations containing beneficial microorganisms in viable state intended for seed or soil application. In recent years, they have emerged as a promising component of integrated nutrient supply system. They are likely to assume greater significance as a complements or supplements to the chemical fertilizers because of high nutrient turnover, exorbitant cost of fertilizers, soil and environmental protection. Bio-fertilizers are less expensive, eco-friendly, providing plant hormones and help in sustainable crop production through maintenance of soil productivity.

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How to cite this article:

Gayathri, V and Malathi R.2019, Effect of Different bio-Fertilizers on the Biochemical Parameters of Solanum Nigrum l. and Amaranthus Viridis l.. *Int J Recent Sci Res.* 10(01), pp. 30321-30325. DOI: http://dx.doi.org/10.24327/ijrsr.2019.1001.3034

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