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Research Article

EFFECT OF ORGANIC FERTILIZERS ON THE BIOCHEMICAL PARAMETERS OF *ABELMOSCHUS ESCULENTUS* (L.) MOENCH AND *AMARANTHUSTRICOLOR* L

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A B S T R A C T

The use of organic sources enhances the absorption and release of macro as well as micronutrients and thus ensure their availability to the plant throughout its growing season. Through bio-fertilizers, fertilizer application can be reduced by 50%. In the present study, the biochemical parameters of *Abelmoschusesculentus* (L.) Moench and *Amaranthustricolor* L. was estimated and on the 30th and 60th day, the chlorophyll contents were found to be maximum in T_4. On the 45th day, chlorophyll b was higher in T_2 in *Abelmoschus*. In amaranth, the biochemical parameters were tested on the 30th and 45th day. On the 30th day, chlorophyll a and chlorophyll b was more in T_1 and total chlorophyll was higher in T_2. But, on the 45th day, combination of fertilizers showed better chlorophyll contents. In lady's finger, the protein content was maximum in plants treated with Phosphobacteria on all the days tested. Carbohydrate content was observed to be maximum in plants treated with *Azospirillum*. In amaranth, the protein content was higher in T_2 on 30th day and T_4 on 45th day. The carbohydrate content was observed to be maximum on both the days in plants treated with *Azospirillum*, VAM and Phosphobacteria.

INTRODUCTION

Vegetables play an important role in human nutrition. Most are low in fat and calories but are bulky and filling. They supply dietary fiber and are important sources of essential vitamins, minerals and trace elements. Particularly important are the antioxidant vitamins A, Cand E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease and other chronic ailments. Research has shown that, compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke (Vegetables & Fruits, 2015). Fruit and vegetables, particularly leafy vegetables, have been implicated in nearly half the gastrointestinal infections caused by norovirus in the United States. These foods are commonly eaten raw and may become contaminated during their preparation by an infected food handler. Hygiene is important when handling foods to be eaten raw and such products need to be properly cleaned, handled and stored to limit contamination (Centres for Disease Control & Prevention, 2013). Excessive use of agro-chemicals like pesticides and fertilizers has affected the soil health and lead to decrease in crop yields as well as quality of products.

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Hence, a natural balance has to be maintained to make the life and property exist. Organic farming is the best way for sustainable production of vegetable crops.

Bio-fertilizers such as *Azospirillum*, phosphorus solubilizing bacteria and mycorrhiza are capable of improving the mineral nutrients of plants and enhance the soil fertility. Phosphorus solubilising bacteria are capable of solubilising unavailable form of phosphorus into available form and make it available to plants (Veena *et al*., 2009; Vijendrakumar *et al*., 2014).The use of organic fertilizers could increase the yield of the vegetable crop as well as improve the fertility of the soil. The present study on the vegetable crop *Abelmoschusesculentus* (L.) Moench and green leafy vegetable (*Amaranthustricolor* L.) is an initiative to grow the plant under different organic fertilizers and estimate the biochemical constituents available in both the plants.

MATERIALS AND METHODS

The plant materials taken for the present study were *Abelmoschusesculentus* (L.) Moench and *Amaranthustricolor* (L.) belonging to the family Malvaceae and Amaranthaceae respectively. A pot study was carried out to estimate the biochemical parameters such as chlorophyll, protein and carbohydrate present at various stages of growth under different organic fertilizer treatments.

Collection of seeds

Seeds of both *Abelmoschusesculentus* (L.) Moench and *Amaranthustricolor* (L.) were obtained from Tamil Nadu Agricultural University, Coimbatore.

Collection of Fertilizers

The bio-fertilizers such as *Azospirillum*, VAM and Phosphobacteria were collected from TNAU, Coimbatore. The dosage used were as per the TNAU Agriportal.

Organic 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

Pot culture experiment was conducted with the two test plants. The experiment was carried out in the period from December 2017 to February 2018. The size of the experimental pot was 30 cm × 24 cm × 30 cm. Triplicates were maintained for each treatment.

The soil was cleaned by removing stones and other unwanted materials. The red soil and sand soil were mixed in the ratio of 1:1 and filled in pots of 7 kg capacity. A study was undertaken to assess the effect of different bio-fertilizers on thebiochemical parameters of both the plants.

The seeds were soaked in different bio-fertilizers for 12 hours. The bio-fertilizers used for the study were *Azospirillum,* Phosphobacteria and Vesicular Arbuscular Mycorrhizal (VAM) fungi. In the growing stages of the plants, the bio-fertilizers were sprayed on the plants and biochemical assays were carried out on 30th, 45th and 60th days of the plants for *Abelmoschusesculentus* (L.) Moench and on 30th and 45th days of the plants for *Amaranthustricolor* (L.).

The infection to the plants by various insects were controlled by spraying thulasi extract on the leaves of both the plants.

Treatments

T_0 – Control
T_1 – *Azospirillum*
T_2 – VAM
T_3 - Phosphobacteria
T_4 – *Azospirillum*+ VAM + Phosphobacteria

Biochemical Parameters

The biochemical parameters studied in the leaves of the two plants were chlorophyll, protein and carbohydrate. In *Abelmoschusesculentus* (L.) Moench the biochemical parameters were estimated on 30th, 45th and 60th day and in *Amaranthustricolor* (L.), it was estimated on 30th and 45th days.

METHODS

Estimation of Chlorophyll Content

Chlorophyll '*a*', '*b*' and total chlorophyll were analysed following the method of Arnon (1949).

Estimation of Protein

Protein was estimated following the method of Lowry *et al.,* 1951.

Estimation of Carbohydrate

Carbohydrate was estimated following the method of 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 analysed.

RESULTS AND DISCUSSION

The experiments conducted in *Abelmoschusesculentus* (L.) Moench and *Amaranthustricolor* (L.) using different organic fertilizers treatments showed the following results.

Biochemical parameters

The biochemical parameters such as chlorophyll '*a'*, chlorophyll '*b'*, total chlorophyll, protein and carbohydrate were analysed on 30th, 45th and 60th day for *Abelmoschusesculentus*(L.) Moenchand on 30th and 45th day for *Amaranthustricolor*(L.) and tabulated.

Abelmoschusesculentus (L.) Moench

Cholorophyll 'a', chlorophyll 'b' and total chlorophyll

Cholorophyll '*a',* chlorophyll '*b'* and total chlorophyll content was found to be higher on the 30th day in plants treated with combination of *Azospirillum*, VAM and Phosphobacteria and the values were 0.0760 ± 0.0135 mg/g and 0.1570 ± 0.0192mg/g (Table 1) respectively. But, the chlorophyll *'b'* content was observed to more in T_1 i.e., plants treated with *Azospirilllum* (0.1733 ± 0.2166 mg/g).

Similarly, on the 45th day of growth, the chlorophyll *'a'* and total chlorophyll content was observed to be more in T_4 (0.2313 ± 0.0798 mg/g and 0.3587 ± 0.0984 mg/g) respectively. But, the chlorophyll 'b' content was higher in T_2 (0.1230 ± 0.0460) mg/g (Table 2).

On the 60th day, all the three chlorophyll parameters i.e., chlorophyll '*a',* chlorophyll '*b'* and total chlorophyll was found to be higher in plants treated with combination of organic fertilizers (Table 3). The values were 0.2087 ± 0.0117 mg/g (chlorophyll '*a*'), 0.1850 ± 0.0078 mg/g (chlorophyll '*b*') and 0.4210 ± 0.0201 mg/g (total chlorophyll).

The studies carried out by Uma Maheswari and Elakkiya (2014) have shown that combined inoculation of liquid bio-fertilizers such as *Rhizobium*, *Azospirillum* and *Azotobacter*could enhance the growth parameters as well as the biochemical constituents.

Protein

The protein content was estimated in 0.1 ml and 0.2 ml of the leaf sample on 30th day, 45th day and 60th day. On all the days, the protein content was higher in plants treated with Phosphobacteria (Table 4). In 0.1 ml sample tested, the protein content was found to be 4.97±0.47, 6.13±1.21 and 8.40±0.17 on the 30th, 45th and 60th day respectively. Similarly, in 0.2 ml sample tested, the protein content was found to be 3.13±0.06, 4.07±0.23 and 4.73±0.15 on the 30th, 45th and 60th day respectively. It shows that the presence of phosphate solubilizing bacteria has an effect on the protein content of the vegetable crop.

Carbohydrarte

The carbohydrate content was estimated on the 30th day, 45thday and 60th day andit was more in T_1 (Table 5). The values were 3.20±0.17, 4.00±0.20 and 5.63±0.78 in 0.1ml of the sample tested on the 30th, 45th and 60th day respectively. In 0.2ml of the sample tested, the carbohydrate content was found to be 2.07±0.06, 2.53±0.47 and 3.87±0.91 on the 30th, 45th and 60th day respectively.

The beneficial effect of different organic fertilizers on total carbohydrate content has been reported by Hussein *et al.* (2012). They have reported that the benefit may be due to the role of macro and micro nutrients provided by the organic fertilizers which stimulate the metabolic processes and phytosynthetic apparatus resulting in more photosynthesis and carbohydrate synthesis.

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

Treatments	Chlorophyll '*a'*	Chlorophyll '*b'*	Total chlorophyll
T_0	0.0577 ± 0.0075	0.0373 ± 0.0185	0.0910 ± 0.0406
T_1	0.0607 ± 0.0172	0.1733 ± 0.2166	0.1067 ± 0.0350
T_2	0.0630 ±0.0087	0.0333 ± 0.0139	0.1017 ± 0.0067
T_3	0.0627 ± 0.0390	0.0473 ± 0.0093	0.1430 ± 0.0066
T_4	0.0760 ± 0.0135	0.0710 ± 0.0231	0.1570 ± 0.0192
SEd	0.0168	0.0801	0.0211
CD (P<0.05)	0.0375	0.1784	0.0469

Values are mean ± SD of three samples in each group

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

Treatments	Chlorophyll '*a'*	Chlorophyll '*b'*	Total chlorophyll
T_0	0.1007 ± 0.0774	0.0643 ± 0.0140	0.2303 ± 0.0150
T_1	0.1077 ± 0.0517	0.0853 ± 0.0249	0.2627 ± 0.0304
T_2	0.1760 ± 0.0452	0.1230 ± 0.0460	0.3227 ± 0.0969
T_3	0.1583 ± 0.0303	0.1047 ± 0.0391	0.2840 ± 0.0722
T_4	0.2313 ± 0.0798	0.0963 ± 0.0129	0.3587 ± 0.0984
SEd	0.0490	0.0248	0.0582
CD (P<0.05)	0.1091	0.0554	0.1297

Values are mean ± SD of three samples in each group

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

Treatments	Chlorophyll '*a'*	Chlorophyll '*b'*	Total chlorophyll
T_0	0.1590 ± 0.0030	0.1677 ± 0.0258	0.3410 ± 0.0406
T_1	0.1747 ± 0.0376	0.1793 ± 0.0312	0.3770 ± 0.0662
T_2	0.1937 ± 0.0263	0.1310 ± 0.0330	0.3503 ± 0.0572
T_3	0.1827 ± 0.0437	0.1687 ± 0.0195	0.3753 ± 0.0693
T_4	0.2087 ± 0.0117	0.1850 ± 0.0078	0.4210 ± 0.0201
SEd	0.0236	0.0206	0.0440
CD (P<0.05)	0.0525	0.0458	0.0980

Values are mean ± SD of three samples in each group

Table 4 Protein Content of *Abelmoschusesculentus* (L.) Moench on the 30thday, 45thdayand 60thday (mg/g f.wt.)

Treatments	30th day		45th day		60th day	
	0.1	0.2	0.1	0.2	0.1	0.2
T_0	3.10 ± 0.26	2.20 ± 0.17	5.07 ± 0.38	2.73 ± 0.25	5.77 ± 0.06	3.47 ± 0.42
T_1	3.67 ± 0.87	2.40 ± 0.53	5.03 ± 0.81	3.40 ± 0.30	6.53 ± 0.40	4.37 ± 0.25
T_2	3.77 ± 0.76	2.63 ± 0.55	5.53 ± 0.40	3.47 ± 0.45	6.20 ± 0.10	3.73 ± 0.40
T_3	4.97 ± 0.47	3.13 ± 0.06	6.13 ± 1.21	4.07 ± 0.23	8.40 ± 0.17	4.73 ± 0.15
T_4	4.63 ± 0.31	2.83 ± 0.49	6.03 ± 0.55	3.77 ± 0.45	7.40 ± 0.92	4.43 ± 0.38
SEd			0.33830			
CD(P<0.05)			0.67670			

Values are mean ± SD of three samples in each group

Table 5 Carbohydrates content of *Abelmoschusesculentus* (L.) Moenchon 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	2.37 ± 0.38	1.47 ±0.25	3.60 ±0.40	2.13 ± 0.23	4.23 ± 0.67	2.67 ± 0.55
T_1	3.20 ± 0.17	2.07 ±0.06	4.00 ±0.20	2.53 ± 0.47	5.63 ± 0.78	3.87 ± 0.91
T_2	2.90 ± 0.20	1.90 ±0.26	3.40 ±0.35	2.50 ± 0.20	5.43 ± 1.29	3.67 ± 0.57
T_3	2.53 ± 0.32	1.73 ±0.06	3.50 ±0.20	2.37 ± 0.38	4.57 ± 0.31	3.10 ± 0.46
T_4	2.63 ± 0.35	1.73 ±0.06	3.63 ±0.38	2.13 ± 0.25	4.77 ± 0.42	3.47 ± 0.61
SEd			0.38413			
CD(P<0.05)			0.76838			

Values are mean ± SD of three samples in each group

Amaranthustricolor (L.)

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll

In *Amaranthustricolor* (L.), the chlorophyll '*a'* and chlorophyll '*b'* content was found to be higher in T_1 (*Azospirillum* treated plants) on the 30th day (Table 6) and the values were 0.3723 ± 0.4050 mg/g and 0.3507 ± 0.4844 mg/g respectively. The total chlorophyll content was observed to be more (0.3680 ± 0.0960 mg/g) in T_2 (VAM treated plants).

On the 45th day, all the chlorophyll parameters namely chlorophyll '*a*', chlorophyll '*b'* and total chlorophyll contents were higher in T_4 i.e., plants treated with the combination of organic fertilizers (Table 7).

Chlorophyll is one of the important pigment content which is used as an index of plant production capacity. The pigment content is an indication of photosynthetic and metabolic activity. The chlorophyll is an integral part of plant pigments

and play an important role in the process of photosynthesis. The highest chlorophyll content recorded in *Arachis hypogea* L. in vermicompost and AM fungi applied plants (Lenin *et al.,* 2012) correlate with the result obtained in the present study on lady's finger and Amaranth.

The results of the study carried out by Nalawde and Bhalerao (2015) on the growth of *Vigna*sp showed a significant improvement in the growth parameters. The total chlorophyll content was also found to be significantly higher in treated plants than the control plants.

Protein

The protein content was estimated at two different concentrations on 30th day and 45th day and presented in (Table 8). The protein content was higher in T_2 on the 30th day and T_4 on the 45th day. The values were significant at 5 % level.

Carbohydrate

The carbohydrate content of *Amaranthustricolor* (L.) was estimated on the 30thday and 45th day at two different concentration levels, it was found to be higher in T_4 treatment i.e., plants treated with the combination of organic fertilizer. The values were found to be 2.83 ± 0.06 mg/g and 4.63 ± 0.75 mg/g at 0.1 ml concentration on the 30th day and 45th day. Similarly, the higher value of 1.73 ± 0.21 mg/g and 3.77 ± 0.78 mg/g was found in 0.2 ml concentration on the 30th day and 45th day (Table 9).

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

Treatments	Chlorophyll '*a'*	Chlorophyll '*b'*	Total chlorophyll
T_0	0.1000 ± 0.0151	0.1017 ± 0.0023	0.1910 ± 0.0217
T_1	**0.3723 ± 0.4050**	**0.3507 ± 0.4844**	0.2387 ± 0.0346
T_2	0.1640 ± 0.0480	0.1480 ± 0.0957	**0.3680 ± 0.0960**
T_3	0.1013 ± 0.0354	0.0417 ± 0.0271	0.1563 ± 0.0386
T_4	0.1490 ± 0.0530	0.1520 ± 0.0148	0.3247 ± 0.0561
SEd	0.1508	0.1807	0.0455
CD (P<0.05)	0.3361	0.4025	0.1014

Values are mean ± SD of three samples in each group

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

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_0	0.1533 ± 0.0155	0.1133 ± 0.0253	0.2867 ± 0.0206
T_1	0.2430 ± 0.0471	0.0787 ± 0.0200	0.3543 ± 0.0541
T_2	0.2023 ± 0.0310	0.1287 ± 0.0049	0.3543 ± 0.0345
T_3	0.1627 ± 0.0177	0.1240 ± 0.0508	0.3337 ± 0.0354
T_4	0.3063 ± 0.0127	0.1420 ± 0.0090	0.4883 ± 0.0095
SEd	0.0228	0.0223	0.0280
CD (P<0.05)	0.0508	0.0496	0.0624

Values are mean ± SD of three samples in each group

Table 8 Protein Content of *Amaranthustricolor*(L.) on the 30thday and 45th day (mg/g f.wt.)

Treatments	30thday		45th day	
	0.1	0.2	0.1	0.2
T_0	2.67 ± 0.21	1.67 ± 0.15	5.47 ± 0.31	2.27 ± 0.12
T_1	2.77 ± 0.15	1.53 ± 0.31	3.57 ± 0.40	3.40 ± 0.10
T_2	2.83 ± 0.68	1.97 ± 0.21	4.07 ± 0.31	3.00 ± 0.30
T_3	2.57 ± 0.21	1.80 ± 0.10	4.03 ± 0.68	3.67 ± 0.78
T_4	2.77 ± 0.21	1.77 ± 0.15	4.57 ± 0.85	3.77 ± 0.78
SEd	0.34960			
CD(P<0.05)	0.70659			

Values are mean ± SD of three samples in each group

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

Treatments	30thday		45thday	
	0.1	0.2	0.1	0.2
T_0	2.13 ± 0.47	1.47 ± 0.15	2.80 ± 0.10	1.73 ± 0.15
T_1	2.40 ± 0.10	1.57 ± 0.21	2.87 ± 0.06	1.83 ± 0.06
T_2	2.53 ± 0.21	1.70 ± 0.10	3.93 ± 0.42	2.63 ± 0.40
T_3	2.37 ± 0.15	1.57 ± 0.12	4.03 ± 0.68	3.67 ± 0.78
T_4	2.83 ± 0.06	1.73 ± 0.21	4.63 ± 0.75	3.77 ± 0.78
SEd	0.31868			
CD(P<0.05)	0.64409			

Values are mean ± SD of three samples in each group

Bio-fertilizers are used to hasten the biological activity of the plants to improve the availability of plant nutrient (Kumari *et al*., 2015). 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. *Amaranthus* is one of the plants that accumulate nitrates especially when soil fertility is very high (Alegbejo, 2013). Green leafy vegetables represent an excellent component of the habitual dot in the tropical and temperate countries (Ashok kumar *et al.*, 2013).

The higher content of protein and carbohydrate on the 45th day in plants treated with *Azospirillum,* VAM and phosphobacteria in combination indicates that the plants are able to mobilize the phosphorus content from the soil through Phosphobacteria and also grow well with the help of the other organic fertilizers.

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