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A COMPARATIVE STUDY ON THE IMPACT OF FERTILIZERS ON PIGMENTS IN *AMARANTHUS CAUDATUS* AND *AMARANTHUS TRICOLOR*

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ABSTRACT

The primary function of pigments in plants is photosynthesis, which uses the green pigment chlorophyll along with several red and yellow pigments that help to capture as much light energy as possible. The concentration of the pigments present in plants decides the photosynthetic efficiency of the same. The present study aims at understanding the effect of organic and inorganic fertilizers on the concentration of pigments in *A.caudatus* and *A.tricolor*. The work puts light into the photosynthetic efficiency of the species and suggests which type of fertilizer could improve the pigment concentration of the species.

Key words: *Amaranthus*, NPK fertilizer, Chromatography, Pigments.

INTRODUCTION

Plant pigments include a variety of different kinds of molecule, including porphyrins, carotenoids, anthocyanins and betalains. All biological pigments selectively absorb certain wavelengths of light while reflecting others. These pigments decide the photosynthetic efficiency of the plants (Grotewold, 2006). Chlorophyll is the primary pigment in plants; it is a chlorin that absorbs yellow and blue wavelengths of light while reflecting green. It is the presence and relative abundance of chlorophyll that gives plants their green color. Carotenoids are red, orange, or yellow tetraterpenoids. During the process of photosynthesis, they have functions in light-harvesting (as accessory pigments), in photoprotection (energy dissipation via non-photochemical quenching as well as singlet oxygen scavenging for prevention of photooxidative damage), and also serve as protein structural elements. In higher plants, they also serve as precursors to the plant hormone abscisic acid (Lee, 2007). There are 6 plant pigments obtained from *Amaranthus* when separated by TLC using petroleum ether acetone chloroform in 3:1:1 ratio. The R_f value of the different pigments are carotene -0.98, chlorophyll a - 0.59, chlorophyll b – 0.42, pheophytin 0.81, xanthophyll1 - 0.28, xanthophyll2 – 0.15. The pigment colour for carotenes are orange, chlorophyll a is blue green, chlorophyll b are yellow green, xanthophyll are yellow and phaeophytin are olive green (Resis Carol, 1994).

MATERIALS AND METHODS

Seeds of *Amaranthus caudatus* and *Amaranthus tricolor* (cultivated varieties) were collected from outlets of Kerala Agricultural University. The seeds were raised in nursery beds containing potting mixture. 20 polythene bags need to be prepared. A sufficient quantity of soil to fill 20 bags were collected and mixed thoroughly to ensure uniform composition. The experimental plot was then divided into two isolated regions- Subplot A and Subplot B, each with 10 polythene bags. Both the regions had identical humidity, temperature and sunlight availability. Seedlings were transplanted into the prepared polythene bags containing the experimental soil at one week after their growth on nursery beds. 10 seedlings of *A. caudatus* and 10 seedlings of *A. tricolor*, were transplanted, with each region of the experimental plot having 5 each of both species. The bags were then labeled.

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Fertilizer treatment

Fertilizers for treatment come under two classes- Organic (Cow dung, Ash and bone meal in the ratio 2:1:1) and inorganic (NPK fertilizer). 5 days after transplantation, fertilizer treatment was performed- organic fertilizer in Subplot A and inorganic fertilizer in Subplot B. The fertilizers were applied by ring method, 5 cm radius and about 2 cm deep around the *Amaranthus* plant at the rate of 100 kg N/ha (Adeoluwa and Adeogun 2010). In each region, 8 plants (sample) were treated with respective fertilizer and 2 from each species were left as control in each region. Temperature and humidity at the experimental plot was recorded every day and the plants were watered evenly two times a day. At the third week, fertilizer treatment was performed again.

Harvesting of the experimental plants: The harvesting of plants was done on the sixth week after planting. Freshly harvested plants were cleaned to remove adhering dust and impurities. The plants from each subplot were classified based on species and fertilizer treatment. The following groups of plants were obtained. The samples were subjected to further pigmental analysis. Thin Layer Chromatography as used to quantify the various pigments and column chromatography using silica gel as used to quantify the various pigments present in samples.

Thin layer chromatography (TLC)

Thin layer chromatography was carried out to identify the different pigments present 4. Precoated silica plates were used as the soild phase and solvent phase was petroleum ether and acetone in the ratio 3:1. 0.1 g of sample was extracted using acetone and was spotted at a distance of 1 cm from the bottom of slica plate. After spotting the silica plate is placed in slanding position in the solvent inside a beaker. The plate was left under disturbed for 15minutes and the distance travelled by the solvent and the pigment is noted. The various pigments are identified on the basis of their R_f value using the formulae R_f value = Distance travelled by pigment / distance travelled by pigment.

Column Chromatography

The method was followed as stated in the Pharmacia fine chemical handbook-97, 1974.Column chromatography was carried out to estimate the volume and concentration of chlorophyll a chlorophyll b and carotenoids. Silica gel for column chromatography was taken and mixed with benzene. It was used to set bed inside the column without forming lumps. The bed was left undisturbed for 10 minutes. The sample extracted in hexane was (0.1g in 10 ml hexane) poured into the column without disturbing the silica bed. The pigments on the basis of their affinity extracted out and is collected and measured in a measuring cylinder. The pigments were identified and read at 645 and 663 nm for quantifying chlorophyll a and chlorophyll b and was calculated using the formula: (12.7 * A 663) – (2.69 * A645) for chlorophyll a and (22.9 * A645) – 4.68 * A663) for chlorophyll b For quantification of carotenoids the silica for preparing column was mixed with acetone and hexane in the ratio 2:3 the sample was also extracted using the same in the ratio 1:9.The extract was measured at 436nm.

TLC (Thin layer Chromatography)

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. The compounds separate on the basis of their affinity in the mobile and stationary phase. Following are the R_f values, that is the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase of the pigments identified from *A.caudatus* and *A. tricolor* treated with the two classes of fertilizers. .*A. caudatus* treated with organic fertilizers showed the presence of orange, blue green, yellow green, yellow and olive green which corresponds to carotene, chlorophyll a, chlorophyll b, xanthophyll and pheophytin. The Rf values of the pigments obtained are given in the Table 2.

Table 1. R_f values of the various pigments identified using Thin Layer Chromatography in *A. caudatus* treated with organic fertilizers

Colour of pigment	Distance travelled by pigment	Distance travelled by solvent	Rf value of pigment	Name of pigment
Orange	2.5	2.7	0.925	Carotene
Blue green	1.6	2.7	0.59	Chlorophyll a
Yellow green	1.1	2.7	0.40	Chlorophyll b
Yellow	0.8	2.7	0.29	Xanthophyll
Olive green	2.4	2.7	0.88	Pheophytin

Table 2. R_f values of the various pigments identified using Thin Layer Chromatography in *A. caudatus* treated with inorganic fertilizers

Colour of pigment	Distance travelled by pigment	Distance travelled by solvent	Rf value of pigment	Name of pigment
Orange	2.4	2.6	0.923	Carotene
Blue green	1.4	2.6	0.53	Chlorophyll a
Yellow green	1.1	2.6	0.42	Chlorophyll b
Yellow	0.6	2.6	0.23	Xanthophyll

Table 3. Rf values of the various pigments identified using Thin Layer Chromatography in *A. tricolor* treated with organic fertilizers

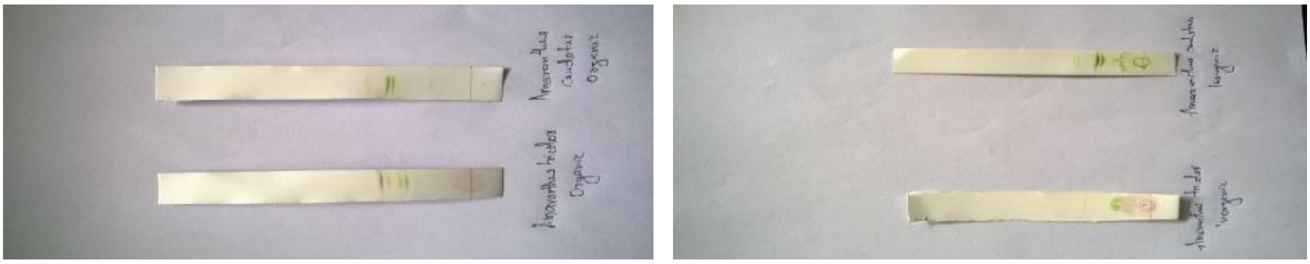
Colour of pigment	Distance travelled by pigment	Distance travelled by solvent	Rf value of pigment	Name of pigment
Blue green	1.6	2.9	0.55	Chlorophyll a
Yellow green	1.3	2.9	0.44	Chlorophyll b
Yellow	0.8	2.9	0.27	Xanthophyll
Olive green	2.6	2.9	0.89	Pheophytin

Table 4. R_f values of the various pigments identified using Thin Layer Chromatography in *A. tricolor* treated with in organic fertilizers

Colour of pigment	Distance travelled by pigment	Distance travelled by solvent	Rf value of pigment	Name of pigment
Orange	2.3	2.4	0.95	Carotene
Blue green	1.2	2.4	0.51	Chlorophyll a
Yellow green	1.1	2.4	0.45	Chlorophyll b

Table 5. Volumes (in mg) of the various pigments isolated using column chromatography determined spectrophotometrically

Species	Treatment given	Concentration of chlorophyll a	Concentration of chlorophyll b	Concentration of carotenoid
A.caudatus	Organic	0.916	0.861	0.198
	Inorganic	0.783	0.654	0.097
A.tricolor	Organic	0.867	0.768	0.765
	Inorganic	0.689	0.561	0.541



(a) (b)

Figure 1. (a) Paper chromatography of *A.tricolor* and *A.caudatus* treated with organic fertilizer, (b) Paper chromatography of *A.tricolor* and *A.caudatus* treated with inorganic fertilizer

(a) (b) (c)

Figure 2. (a) & (b) Column chromatography of *A.tricolor* treated with organic and inorganic fertilizers. (c) column chromatography of *A.caudatus* treated with organic and inorganic fertilizers

Thin Layer Chromatography performed in *A. caudatus* treated with inorganic fertilizers, showed the presence of only four pigments. Orange, blue-green, yellow green, yellow bands were obtained which corresponded for carotene chlorophyll a, chlorophyll b and xanthophyll. The Rf values of the pigments are given in Table 3. Thin Layer Chromatography in *A. tricolor* treated with organic fertilizers showed the presence of four different bands Blue green, Yellow green, Yellow and Olive green which corresponds to Chlorophyll a, chlorophyll b, xanthophyll and pheophytin. The Rf values of the pigmens are given in Table 4. Thin Layer Chromatography in *A. tricolor* treated with inorganic fertilizers showed the presence of three different bands Blue green, Yellow green, and orange which corresponds to Chlorophyll a, chlorophyll b, and carotene. The R_f values of the pigments are given in Table 5 The TLC results show that in *A. tricolor* and *A. caudatus* given organic treatment has all the five pigments, viz. carotene, chlorophyll a, chlorophyll b, xanthophyll and pheophytin .*A. caudatus* given inorganic treatment lacks pheophytin and *A. tricolor* given inorganic treatment lacks xanthophyll and pheophytin. The results obtained by Thin layer chromatography is similar to the work done by Resis & Carol in1994.

He reported that there are 6 plant pigments obtained from *Amaranthus* when separated by TLC using petroleum ether acetone chloroform in 3:1:1 ratio. The Rf value of the different pigments were carotene -0.98, chlorophyll a - 0.59, chlorophyll b – 0.42, pheophytin 0.81, xanthophyll1 -0.28, xanthophyll2 – 0.15, but in the present study only 5 bands were obtained which corresponds to various pigments (Table 2,3,4 &5). In the samples which were treated with inorganic fertilizers the presence of certain pigments was absent which implies that the intake of nutrients from the soil influences the pigment composition in *Amaranthus.*

Column chromatography

It was carried out and concentration of pigments present in each sample was found out. Column chromatography was carried out to find the concentration of the pigments. Chlorophyll a was highest in *A.caudatus* treated with organic fertilizer. While the least content of it was shown by *A.tricolor* treated with inorganic fertilizer. The value differed by 0.227 (Table 6). Chlorophyll b content was also highest in *A.caudatus* treated with organic fertilizer and lowest value was shown by *A .tricolor* treated with inorganic fertilizer. The concentration differed by 0.3 (Table 6). Carotene content was higher in *A. tricolor* treated with organic fertilizer and least value was shown by *A.caudatus* treated with inorganic fertilizer. The difference in the concentration in *A. tricolor* was 0.224. *A.caudatus* had only minimum amount of carotenoid and the variations in concentration were shown by sample subjected to different treatment. The concentration of carotenoid in *A.caudatus* treated with organic fertilizer to that of the one treated with inorganic fertilizer was 0.101.

Conclusion

The pigment composition studied using column chromatography showed a marginal higher value of photosynthetic pigments in plants treated with organic fertilizers. The lower concentration of photosynthetic pigments could cause lesser photosynthetic efficiency and that could account for the lesser nutrient content in plants treated with inorganic fertilizers as exhibited by the studies carried out in this work. In the light of this experiment it could be concluded that usage of organic fertilizers has the ability to increase the concentration of different pigments and thereby influences the photosynthetic ability of plants.

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