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Triadimefon and Hexaconazole Enhances the Photosynthetic Pigment Composition of Tapioca, an Important Tuber Crop

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Abstract: An investigation was carried out to study the effect of Triadimefon and Hexaconazole on the photosynthetic pigment characteristics of tapioca (*Manihot esculenta* Crantz) during the growth and maturation period. One litre of 20 mg L-1 triadimefon and 15mg L-1 hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and 100 DAP by soil drenching. Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem, leaf, root and tuber were used for determining photosynthetic pigment characteristics. From the results it is clear that triadimefon and hexaconzole enhanced the Chlorophyll, carotenoid, Xanthophyll and Anthocyanin contents of tapioca.

Key words: Chlorophyll, Carotenoid, Xanthophyll, Anthocyanin, Tapioca, Triazole

INTRODUCTION

The traditional means of increasing crop productivity are reaching their limits and the increasing demand for plant production necessitates new strategies for future improvements in crop yields [1-3]. The identification of the methods responsible for manifestation of growth and yield have now become one of the most important tasks to provide food security for the future generation [4,5].

It has been widely accepted that plant growth and development is controlled by the plant hormones, which are endogenously produced by the plants [6]. However, synthetic plant growth regulators are also increasingly used to modify growth, development, stress behaviour, the qualitative and quantitative yield of crop plants. Plant growth regulators open new opportunities to improve agricultural crop yield by circumventing several barriers imposed by genetics and environment [7]. Manipulating the crop morphology by using plant growth regulators also increases the utilization of solar radiation and alter photo assimilate distribution in favour of yield increment [8].

Tapioca (*Manihot esculenta* Crantz) also known as Cassava, Mandioca and Yucca is a bushy shrub belongs to the family Euphorbiaceae is an important food crop grown throughout the tropics for its enlarged tuberous roots. The tubers are used for sago industry for starch extraction and grown in rainfed areas where a number of sago and starch mills exist. In addition the boiled tubers are consumed as staple food. The tuber crops become the most important food crop after cereals and legumes. They form a rich source of energy for people living near sustenance level in Tropical East and West Africa, East and South pacific islands and part of South America and India [4].

Plant growth regulators have been successfully used to increase the yield in many tuber crops [9-11]. Gibberellins, Cytokinins are broadly used to induce bulb formation and increase the yield [6]. The enhancement of yield in tuber crops will be beneficial to the farmers. The main objectives of this study are to asses the effect of Triadimefon and Hexaconazole on Pigment composition of Manihot esculenta Crantz during the growth and maturation period.

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MATERIALS AND METHODS

The land was prepared by ploughing thoroughly five times to a depth of 35 cm and the soil was sandy loam without any stones and pebbles. The Farm yard manure (FYM) was applied at the rate of 10 tonnes per hectare. The stem cuttings of *Manihot esculenta* Crantz. (Tapioca) CV-H-226 were were used for planting. The stem cuttings were dipped for 10 minutes in 1% Bavestin before planting to avoid fungal infections. Each stem cuttings was planted in a plot of 1.5 × 1.5 to a depth of 5 cm inside the soil and Completely Randomized Block Design (CRBD) was used for this experiment.

No inorganic fertilizer was used throughout the experiment and no systemic pesticide or fungicide was used during the experiment. Only ground water was used for irrigation. In preliminary experiments, 5, 10, 15, 20, 25 and 30 mgL^{-1} triadimefon and hexaconazole were used for treatment to determine the optimum concentration of triadimefon and hexaconazole.

Among these treatments, 20mg L^{-1} triadimefon and 15mg L^{-1} hexaconazole concentrations were found to increase the dry weight significantly and in higher concentrations they slightly decreased the growth and dry weight. Hence 20 mg L^{-1} triadimefon and 15 mgL^{-1} hexaconazole concentrations were used to determine the effect of these chemicals on the growth and metabolism of tapioca. One litre of 20 mg L^{-1} triadimefon and 15mg L^{-1} hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and 100 DAP by soil drenching. The EC of the soil was 0.21 dSm^{-1} and pH was 6.8 after the treatment. The average temperature was 32/26°C (maximum and minimum) and relative humidity (RH) varied between 60-75 percent during the experimental period.

Triadimefon [1- (4- chlorophenoxy) –3, 3- dimethyl –1- (1H-1, 2, 4- triazole –1 –Y1) –2 butanone] [$C_{14}H_{16}ClN_3O_2$] M.W. 293.75 has been obtained from Bayer India Ltd., Mumbai and Hexaconazole (2- (2, 4- dichlorophenyl)-1- (2 H-1, 2, 4- triazole-1-Y1) hexan –2-01) [$C_{14}H_{17}Cl_2N_3O$] M. W. 314.2 has been obtained from Rallis India Ltd., Mumbai used for this study. Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem, leaf, root and tuber was used for determining photosynthetic parameters.

Pigments

Chlorophyll and Carotenoid Content: Chlorophyll and carotenoids were extracted from the leaves and estimated according to the method of Arnon [12].

Extraction: Leaf discs (0.8 cm diameter) were taken from the third leaf on either side of the mid rib at the intra venal region for the determination of chlorophyll and carotenoid contents. Five hundred milligrams of fresh leaf discs were ground with 10 ml of 80 percent acetone at 4°C in a pestle and mortar and centrifuged at 2,500 g for 10 minutes, at 4°C. The residue was re-extracted with 80 percent acetone until the green colour disappears in the residue and the extracts were pooled and transferred to graduated tube and made upto 20 ml with 80 per cent acetone and assayed immediately.

Estimation: Three ml of extract was transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm in a spectrophotometer against 80 percent acetone as blank. Chlorophyll content was calculated using formula of Arnon [12].

$$\text{Total Chlorophyll (mg/ml)} = (0.0202)\times(A.\ 645)+(0.00802)\times(A.\ 663)$$
$$\text{Chlorophyll "a" (mg/ml)} = (0.0127)\times(A.\ 663)-(0.00269)\times(A.\ 645)$$
$$\text{Chlorophyll "b" (mg/ml)} = (0.0229)\times(A.\ 645)-(0.00468)\times(A.\ 663)$$

Carotenoid content was calculated using the formula of Kirk and Allen [13].

$$\text{Carotenoid (mg/ml)} = A.\ 480+(0.114\times A.\ 663)-(0.638\times A.\ 645)$$

Xanthophyll: Xanthophyll contents were estimated by the method of Neogy *et al*. [14].

Five hundred mg of fresh weight of tissues taken from the 3rd leaf and periphery of the tuber were used for the assay. The tissue was ground with 10ml of 80 percent acetone at 4°C in a pestle and mortar and centrifuged at 1000 g for 15 minutes. The residue was re-extracted with 80 percent acetone until the colour completely disappeared in the residue. The aqueous acetone extract was shaken thrice with an equal volume of hexane in a separating funnel and the combined hexane fractions were washed with equal volumes of water. To separate xanthophylls from carotenes the hexane fraction containing carotenoid was extracted repeatedly with 90 percent methanol. The methanol fraction containing xanthophylls was measured for absorbance at 450 nm in a spectrophotometer. The results were expressed in absorbance per gram fresh weight.

Anthocyanin: Anthocyanin was extracted and estimated by the method of Kim *et al*. [15].

In a pestle and mortar, five hundred mg of fresh tissue taken from the third leaf and from the periphery of the tuber tissue (0.5 cm from the epidermis and 1 cm from head of the root tuber) was ground in liquid nitrogen and extracted with 20 ml of 50 percent acetic acid overnight. The homogenate was centrifuged at 19,000 g for 15 minutes. The resultant supernatant was made upto 20ml and 80ml of McIlvaine's buffer (pH 3.0). The absorption measured at 530 nm in spectrophotometer. The anthocyanin contents were expressed in colour value cv = 0.1 × A530/g fw.

Statistical Analysis: The data was analysed using the analysis of variance (ANOVA) as described by the method outlined by Ridgman [16]. Means were compared between treatments from the error mean square by LSD (Least Significant Difference) at the P = 0.05 and P = 0.01 confidence level using Tuckey's [17] test.

RESULTS

Pigments

Total Chlorophyll: The total chlorophyll content of the leaves increased with the age in the control and triazole treated plants (Table 1). Triazole treatments significantly increased the total chlorophyll content to a larger extent when compared to control. Triadimefon and hexaconazole treatments increased the total chlorophyll content to 134.91 and 132.14 percent over the control on 240 DAP.

Chlorophyll "a" and " b ": Chlorophyll "a" (Fig. 1) and "b" (Fig. 2) content in the tapioca leaves increased with the age in the control and treated plants. Triazole treatments increased the chlorophyll "a" and "b" content to a higher level. There was no significant variation between the chlorophyll "a" and "b" content within the trizole treated plants.

Carotenoid (Fig. 3): Triazole treatments significantly increased the carotenoid content of the leaves of tapioca when compared to control. Among the triazole treatments triadimefon and hexaconazole increased the carotenoid content to 133.09 and 131.97 percent over the control on 240 DAP.

Table 1: Triazole induced changes in the total chlorophyll content in the leaves of Tapioca (values are the mean of three replicates expressed in mg/g1 fresh weight)

DAP	CON	TDM	HEX	F- ratio	LSD
40	0.129	0.161	0.135	**	0.016
80	0.145	0.184	0.162	**	0.012
120	0.152	0.195	0.175	**	0.017
160	0.168	0.212	0.194	**	0.022
200	0.196	0.248	0.231	**	0.021
240	0.335	0.426	0.418	**	0.034

NS-Non significant; *-Significant at 0.05 level; **-Significant at 0.01 level

Xanthophyll: The Xanthophyll content in the tapioca increased with the triazole treatments (Fig. 4). Among the triazoles, triadimefon treatment increased it to a higher level than hexaconazole and it was 130.15 and 124.44 percent over the control on 240 DAP.

Anthocyanin

Leaf (Fig. 5): Anthocyanin content in the tapioca leaves increased with triazole treatments. Triadimefon and hexaconazole treatments increased the anthocyanin content in the leaves to 131.85 and 128.77 percent over the control respectively on 240 DAP.

Tuber: Anthocyanin pigment in the tubers of tapioca, triazole treatments significantly increased the anthocyanin in the outer cortical region when compared to control (Fig. 6). Treatment with triadimefon and hexaconazole increased it to 130.06 and 120.45 percent over the control respectively on 240 DAP.

DISCUSSION

Triadimefon and hexaconazole treatments increased the total chlorophyll and chlorophyll "a" and "b" content in the leaves of tapioca. Increased chlorophyll content with triadimefon treatment was observed in *Catharanthus roseus* [18,19], *Solenostemon rotundifolius* [20]. Triazole treated plants typically appear dark greener and this has been correlated with increased chlorophyll content [21]. The increased chlorophyll content was also attributed to more densely packed chloroplasts in a small leaf area as reported in maize [22]. Increased chlorophyll synthesis in *Zea mays* seedlings was observed with paclobutrazol treatment [23]. Similar results were observed with uniconazole, LAB-150978, BAS-110W treated *Cucumis* cotyledons [24] paclobutrazol treated tomato [25].

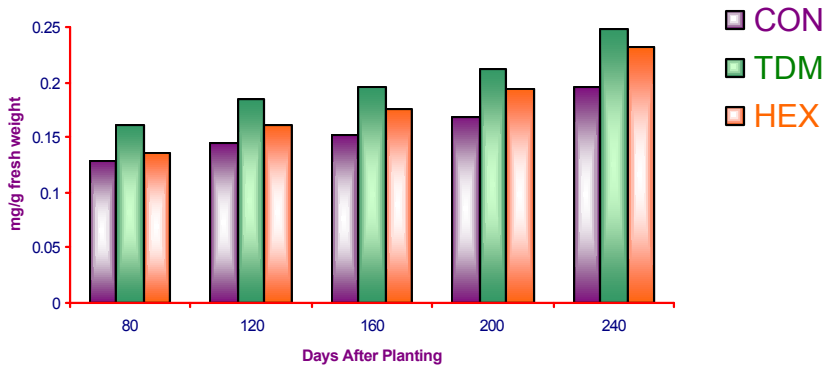

Fig. 1: Triazole induced changes in the chlorophyll 'a' content in the leaves of Tapioca

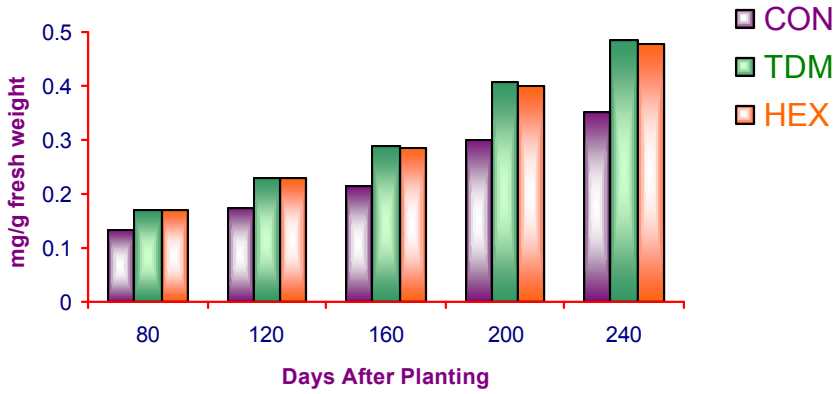

Fig. 2: Triazole induced changes in the chlorophyll 'b' content in the leaves of Tapioca

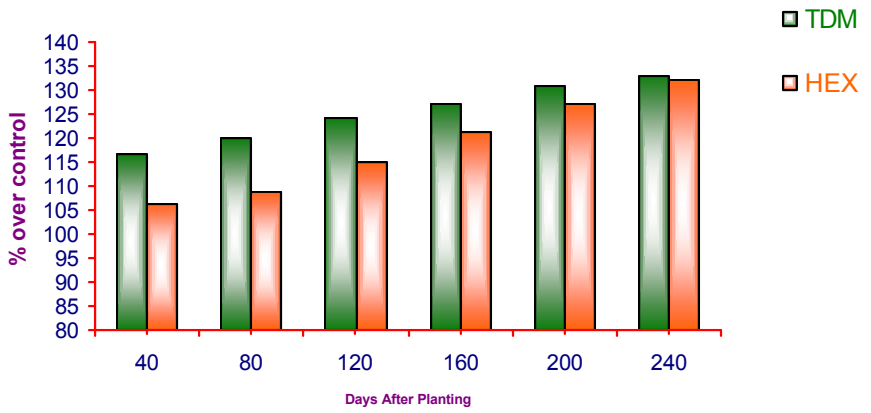

Fig. 3: Triazoles induced changes in the carotenoid content in the leaves of Tapioca

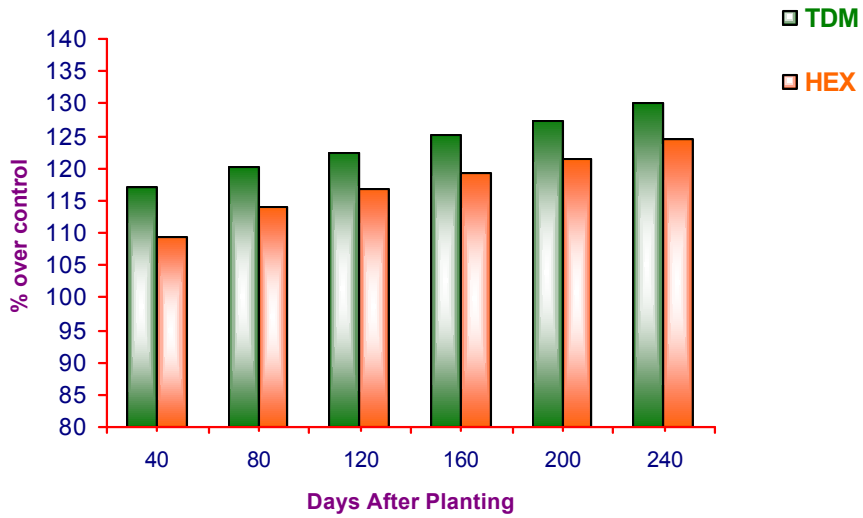

Fig. 4: Triazoles induced changes in the xanthophyll content in the leaves of Tapioca

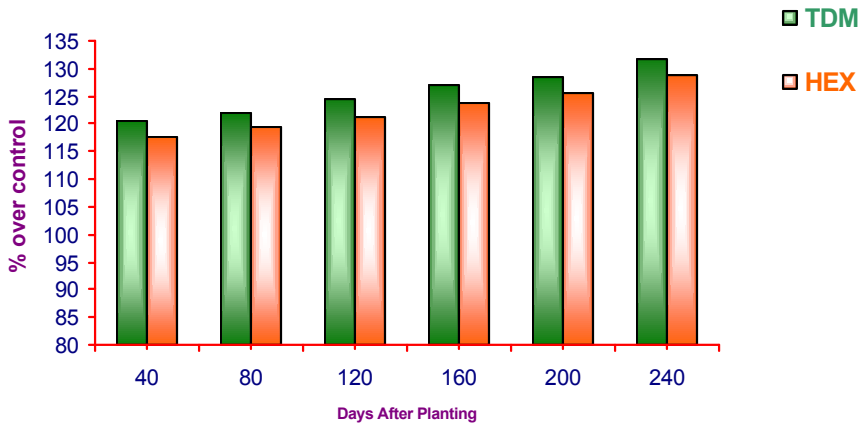

Fig. 5: Triazoles induced changes in the anthocyanin content in the leaves of Tapioca

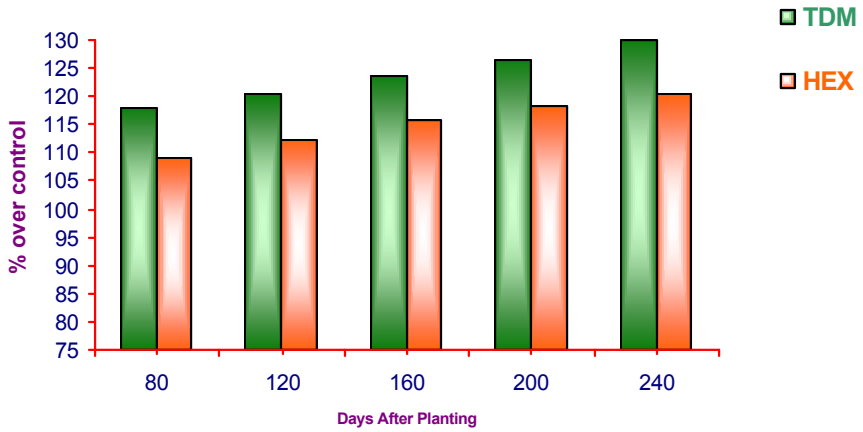

Fig. 6: Triazoles induced changes in the anthocyanin content in the Tuber of Tapioca

Triadimefon and hexaconazole treatment increased the carotenoid content in tapioca leaves. Triadimefon treatment induced higher level of carotenoid content in cow pea [26]. Similar results were observed in ketoconazole [27] and paclobutrazol [28] in *Catharanthus roseus* treatment.

Increased level of cytokinin particularly transzeatin and its riboside has been reported in sunflower cell suspension, rice, soybean and rape seedlings after uniconazole treatment and thus increased zeatin might be responsible for the increased synthesis of carotenoid in the plants [29].

Triazole treated tapioca leaves showed increased xanthophyll content at all stages of growth. Xanthophyll participates in light harvesting photosynthetic membranes and protects the photosynthetic apparatus from excessive light energy by quenching chlorophylls and singlet oxygen [30]. Triazole treatment increased the chlorophyll, carotenoid and xanthophyll content in the leaves of *Catharanthus roseus* [27,28].

Triazoles increased the anthocyanin content to a larger extent in the leaves and tubers of tapioca. Triadimefon increased the chlorophyll and anthocyanin content in *Catharanthus roseus* and its effects can be compared to that produced by cytokinin [18,21]. Treatment with ABA increased anthocyanin accumulation in *Ocimum sanctum* [22]. Triazoles induced a transient raise in abscissic acid content in *Catharanthus roseus* [31]. This increased ABA content induced by triazole might be the cause for the increased anthocyanin content.

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