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Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L.

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Abstract

The application of triazole fungicides is a common practice in the cultivation of carrot (*Daucus carota* L.) plants. It is there for seems important to test the changes that are occurring in this food crop under triazoles, the non-traditional plant growth regulators, treatments in order to identify the extent to which it tolerate the fungicide application and thereby make it an economical food crop. A field experiment was conducted to find out the effects of two triazole fungicides (hexaconazole (HEX) and paclobutrazol (PBZ) at 20 mg l^{-1} $plant^{-1}$) on the biomass, yield, electrolyte leakage, lipid peroxidation and antioxidant potential of carrot. The treatments were given to plants on 15, 30 and 45 days after sowing (DAS). The plants were uprooted for analyses of growth and biochemical parameters on 60 DAS. It was found that both HEX and PBZ have significant effects on the growth and biochemical parameters of this plant. Among the triazoles used, PBZ performed best in terms of anthocyanin, protein, amino acid, proline, starch and sugar, contents whereas HEX enhanced carotenoids, fresh weight, dry weight and biomass. There was no significant variation in chlorophyll ('a' and 'b') contents between the two triazole treated plants, but HEX and PBZ proved best when compared to untreated control plants. HEX and PBZ increased α- and β-amylases enzymes activities to a significant level. Out of these two triazoles, PBZ performed best in increasing the starch hydrolyzing enzymes activities. The non-enzymatic antioxidant, reduced glutathione (GSH) and antioxidant enzyme ascorbate peroxidase (APX) were increased under fungicide applications. The data suggests that, the application of triazole fungicides may be a useful tool to increase the tuber quality as well as quantity in carrot plants, apart from their fungicidal properties.

Keywords: *Daucus carota*; Hexaconazole; Paclobutrazol; Pigments; Protein; Proline; Amino acid; Electrolyte leakage; Antioxidants

1. Introduction

Roots and tubers were critical components in the diet during the early evolution of mankind. They are the most important food crops of very ancient origin in the tropics and subtropics, associated with human existence, survival and their socio-economic history. Carrot (*Dacaus carrota* L.) is an important cool season root crop cultivated almost all over the world for its edible tubers. Carrot is a largely grown hilly root cum vegetable crop providing a rich source of carotene (provitamin A) [1]. However, its per unit area production is very low due to lack of improved varieties and non-adoption of improved cultivation practices.

Electrolyte leakage is a common indicator of membrane damage and this leakage is closely related to the loss of water potential [2]. Carotenoids are a large class of isoprenoid molecules that are synthesized *de novo* by all photosynthetic and many non-photosynthetic organisms. The colours provided by these pigments are of important agronomical value in horticultural crops [3]. Anthocyanin pigments are widespread in the plant kingdom and provide many of the orange, red, and blue colors found in fruits, vegetables, flowers, leaves, roots and other storage organs [4]. Starch and sugars are the dominant storage polysaccharides and it is present in all major organs of higher plants and certain tissues can accumulate it to a higher level in

Abbreviations: APX, ascorbate peroxidase; DAS, days after sowing; DW, dry weight; FW, fresh weight; GSH, reduced glutathione; HEX, hexaconazole; PBZ, paclobutrazol; PGR, plant growth regulators; TDM, triadimefon

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tubers [5]. The role of proline in plant is related to survival rather than to maintenance of growth [6].

Manipulation of crop production with chemicals is one of the most important advancement achieved in agriculture. The tuber yield and qualities of crop plants can be increased by application of biofertilizers and plant growth regulators (PGRs) [7,8]. Triazoles are a group of compounds, which have fungicidal as well as plant growth regulatory properties [9]. Triazole compounds induce a variety of morphological and biochemical responses in plants including retardation of shoot growth, stimulation of rooting, inhibition of gibberellin biosynthesis and increases cytokinin and abscisic acid [10]. These qualities make them ideal for use in edible root tuber cultivation.

Being triazole compounds, paclobutrazol (PBZ) and hexaconazole (HEX) have exhibited plant growth regulating properties and induced many morphological changes like reduction in shoot elongation, stimulation of rooting, inhibition of gibberellin synthesis, increased chlorophyll content, altered carbohydrate status, increased cytokinin synthesis and a transient raise in ABA content [7,11]. Triazole inhibit cytochrome P-450 mediated oxidative dimethylation reaction, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellin biosynthetic pathway [7].

Previous works carried out in our lab revealed the increased antioxidant potentials and an enhancement in alkaloid production under TDM application in *Catharanthus roseus* [9], induction of salt stress tolerance by paclobutrazol in *C. roseus* [12] and drought stress tolerance in *Vigna unguiculata* by propiconazole treatment [13]. The application of triazole fungicides is a common practice in the cultivation of this plant. It is there for seems important to test the changes that are occurring in this tuber crop under triazoles treatments in order to identify the extent to which it tolerate the fungicide application and thereby make it an economical food crop. To the best of our knowledge, there is little information available so far about the effect of triazoles on the tuber production and quality of carrot plants. Therefore, the present investigation was carried out with an objective of evaluating the effect of triazole compounds like PBZ and HEX on growth and yield (fresh weight, dry weight, biomass), photosynthetic pigments (chlorophyll 'a', chlorophyll 'b', carotenoids, anthocyanin), biochemical constituents (starch, sugar, protein, amino acid, proline), membrane integrity (electrolytic leakage, lipid peroxidation) and activities of carbohydrate metabolizing enzymes (α-amylase, β-amylase) and antioxidant potential (reduced glutathione, ascorbate peroxidase) of *D. carota* under field conditions.

2. Materials and methods

2.1. Seed collection, plant cultivation and fungicide applications

The seeds of *Daucus carota* L. var. New Kuroor were obtained from M/s. Indo American Seeds, Bangalore, India. The seeds were surface sterilized with 0.2% $HgCl_2$ solution for 2 min with frequent shaking then thoroughly washed with tap water to remove $HgCl_2$. The land was ploughed and ridges of 30 cm width were prepared at a spacing of 45 cm. The plot soil mixture containing red soil, sand and farmyard manure (1:1:1). The seeds were sown on ridges and thinned the seedlings on 10 days after sowing (DAS). The electrical conductivity of soil was 0.10 dS m^{-1} and pH was 6.8. The HEX and PBZ at 20 mg l^{-1} $plant^{-1}$ were applied by soil drenching on 15, 30 and 45 DAS. The plants were uprooted randomly on 60 DAS, washed and separated into leaves and tubers, then used for analysis of growth and biochemical constituents.

2.2. Growth and yield

Fresh weight (FW) and dry weight (DW) of leaves and tubers were taken by using electronic weighing device (Model-Citizen XK3190-A7M) and calculated the biomass (BM).

2.3. Photosynthetic pigments

Chlorophyll and carotenoid were extracted and estimated by the method of Arnon [14]. Carotenoid content was calculated by using the formula of Kirk and Allen [15]. Anthocyanin content was estimated according to the method of Beggs and Wellmann [16].

2.4. Biochemical constituents

Soluble protein [17], amino acid [18], proline [19], starch [20] and sugar [21] were extracted and estimated from fresh leaf and tuber tissues.

2.5. Membrane integrity

Electrolyte leakage was calculated by following the standard method of Pinhero and Fletcher [22]. Peroxidation of membrane lipids was assayed by the method of Heath and Packer [23].

2.6. Enzymes of carbohydrate metabolism

The α-amylase (1,4-D-glucan glucanohydrolase, EC: 3.2.1.1) and β-amylase (1,4-D-glucan maltohydrolase, EC: 3.2.1.2) activities were estimated by following the standard method of Tarrago and Nicolas [24]. The estimation of β-amylase was done in low pH and with 0.1 M EDTA to inactivate α-amylase. α- and β-amylase activities were expressed in terms of units (U). One U is defined as micrograms of maltose liberated per min per mg enzyme protein.

2.7. Antioxidant potential

The reduced glutathione (GSH) content was assayed as described by Griffith and Meister [25] and the expressed in μg g^{-1} fresh weight (FW). Ascorbate peroxidase (APX) (EC 1.11.1.1) activity was determined according to Asada and Takahashi [26]. The enzyme activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).