IMPACT OF EXOGENOUS ASCORBATE ON GROWTH AND PHYSIOLOGY OF PEANUT SEEDLINGS TREATED BY NORFLURAZON

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Abstract - Norflurazon is an herbici de which inhibits the phytoene desaturase implicated in the carotenoids biosynthesis. Norflurazon 100 µM alone or in combination with ascorbate (50 mM) was applied in pre-emergence to peanut seedlings (Arachis hypogaea L.). Norflurazon treatment allowed to photobleached plants which were noticeably smaller than the control. Norflurazon impaired the photosynthetic activity by decreasing photosynthetic pigments (carotenoids and chlorophylls). The determination of malondialdehyde (MDA) a marker of lipid peroxidation showed that its contents was higher in treated plants in relation with enhanced of reactive molecules species by the herbicide and decreased of endogenous α-tocopherol. The addition of exogenous ascorbate, minimize the damage done by the herbicide on the growth (shoot length, leaf area), at the membrane (malondialdehyde content) and increase the pigments content. Furthermore, the norflurazon decreased the glutathione S-transferase activity in the leaves of seedlings of peanut, while it increased the level of reduced glutathione. This activity decreased even more with the application of exogenous ascorbate in combination with the herbicide. The herbicide alone or in association with the antioxidant increased ascorbic acid content. The supplementation of ascorbate partially alleviate the toxicity of norflurazon.

Index terms - Arachis hypogaea, Ascorbate, Carotenoids Glutathione S-transferase, Norflurazon, Oxidative stress, Peroxidation.

I. INTRODUCTION

Norflurazonis a model of bleaching herbicide used in many laboratories of research; the target of norflurazonis the phytoene desaturase the key enzyme of carotenoid biosynthesis [1]. Carotenoid deficiency causes oxidative stress due to significantly increasing the content of reactive oxygen species (ROS) [2]. The reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism. They damage photosynthetic components, inactivate proteins, destroy cell membrane structure and permeability by causing lipid peroxidation [3]. Norflurazon also inhibits the unsaturation of chloroplast lipids [4].

Plants possess various antioxidants systems to cope with the oxidative stress. These include non enzymatic antioxidants as tocopherols, carotenoids, ascorbic acid, glutathione, phenolic compounds and enzymatic antioxidants (superoxide dismutase, catalase, and enzymes of the ascorbate/glutathione cycle) [5]. Ascorbate (or vitamin C) is a water soluble antioxidant; it has a central role in the process of cellular detoxification involved in scavenging of ROS induced by environmental stresses [6]. Indeed, it quickly reacts with superoxide anion and the oxygen Singlet, or even with hydrogen peroxide; this last reaction is catalyzed by ascorbate peroxidase (APX). Ascorbate is also essential by its ability to reduce other antioxidants oxidized such as vitamin E and carotenoids [7].
The objectives of this study were to evaluate the effects of norflurazon on peanut seedlings, and the role of ascorbate antioxidant in the reversibility of the damage caused by the application of the herbicide norflurazon.

II. MATERIALS AND METHODS

Norflurazon 100µM alone or in combination with ascorbate (50 mM) was applied in pre-emergence to peanut seedlings Arachis hypogaea L. The analyzes were performed after 4 weeks of growth.
The contents of pigments (chlorophylls and carotenoids), malonyldialdehyde (MDA) and antioxidants (ascorbate and glutathion) were determined by spectrophotometry. The α-tocopherol was determined by HPLC. Glutathion s transferase activity was also measured.

III. RESULTS

3.1. Peanut plant growth on soil soaked with norflurazon and ascorbate

We measured plant growth over 30 days and compared control plants with plants growing in norflurazon-polluted soils (Fig. 1A). Control plants reached an average 10.65 (±1.05) cm.
Norflurazon at concentration 100 µM had a severe effect on plant growth; It induced a decrease in size (67%) (Fig. 1A) due probably to a perturbation in the energetic metabolism.
Norflurazon reduces leaf area; in control seedlings leaf area is 22.85 ± 6.36 cm^2, it decreased in treated seedlings very significantly (52%) compared to the control (Fig. 1B).
The association of ascorbate with norflurazon meliorate the growth of peanut seedlings.

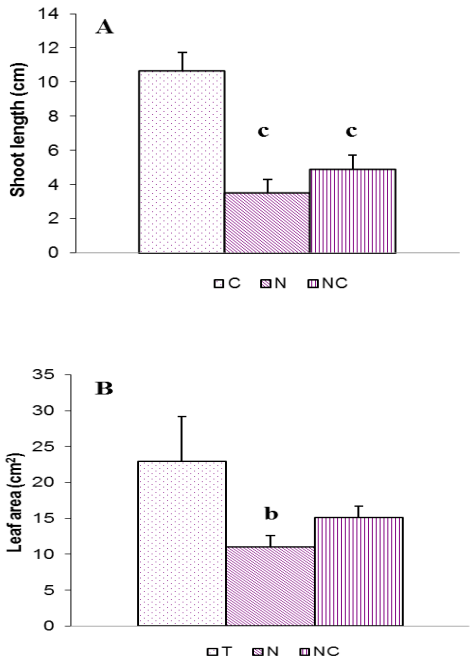
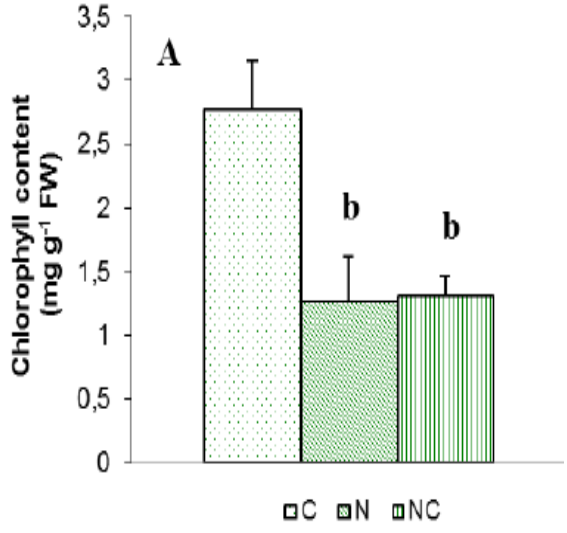


Figure 1.Effects of norflurazon on peanut growth (C= control;N= 100 µMnorflurazon; NC= 100 µM norflurazon + 50mMascorbate).

3.2. Content of chlorophyll and carotenoids in plants exposed to norflurazon and ascorbate

Thirty days after treatment, we quantified photosynthetic pigments, which were lowered. From 2.77 mg g^{-1} FW chlorophyll in control plants, chlorophyll content was 54 and 52% less in plants grown on norflurazon alone or in association with ascorbate, respectively (Fig. 2A). Concurrently, we found that plants grown in presence of norflurazon alone or with the ascorbate accumulated less carotenoids to that of control plants (202.03 and 226.09 µg g^{-1} FW compared to 423.57 µg g^{-1} FW in control plants) (Fig. 2B).



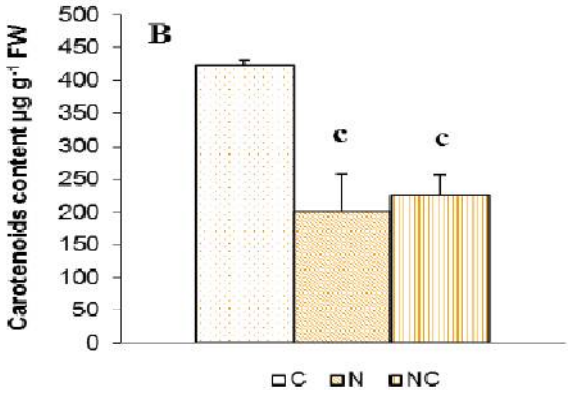


Figure 2.Effects of norflurazon on the content of total chlorophyll (A) and carotenoids (B) in peanut plants.

3.3. Norflurazon induced oxidative damage in lipids

The basal level of MDA which is 0.09 nmol g^{-1} in control seedlings, increased to 64% with 100 µM norflurazon; the presence of ascorbate lowered the magnitude of increasing of MDA to become 10% (Fig. 3).

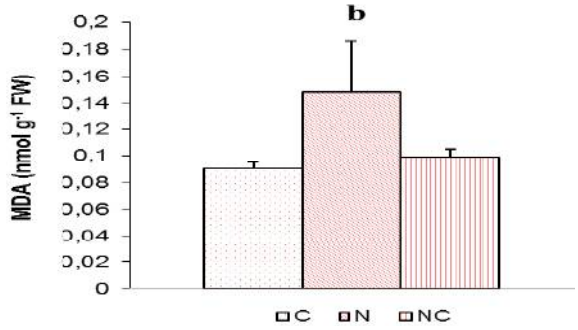


Figure 3.Effects of norflurazonon the content of TBARS in peanut plants.

3.4. Norflurazon modified non-enzymatic antioxidant level

Alpha tocopherol content in the control plants is 0.43 ± 0.01 mg g^{-1}FW, this content decreased in presence of norflurazon treated plants to 0.18 ± 0.08 mg g^{-1}FW and to 0.13± 0.01 mg g^{-1}FW in seedlings treated with norflurazon associated to ascorbate, corresponding respectively to 56% and 69% of reduction (Fig. 4A).
The content of of ascorbic acid in control seedlings was 1.90 µmol g^{-1} FW. Norflurazon treatment resulted in non significant accumulation of ascorbic acid contents compared to controls (Fig. 4B). The presence of ascorbate associated with norflurazon elevated the level of ascorbic acid (2.86µmol g^{-1} FW) (Fig. 4B).

GSH content in leaves increase for norflurazon and ascorbate treatment (Fig. 4C). The leaves GSH content in plants growing in presence of 100 µM norflurazon was 98% higher relative to controls and in presence of norflurazon associated with ascorbate it was 19% relative to controls.

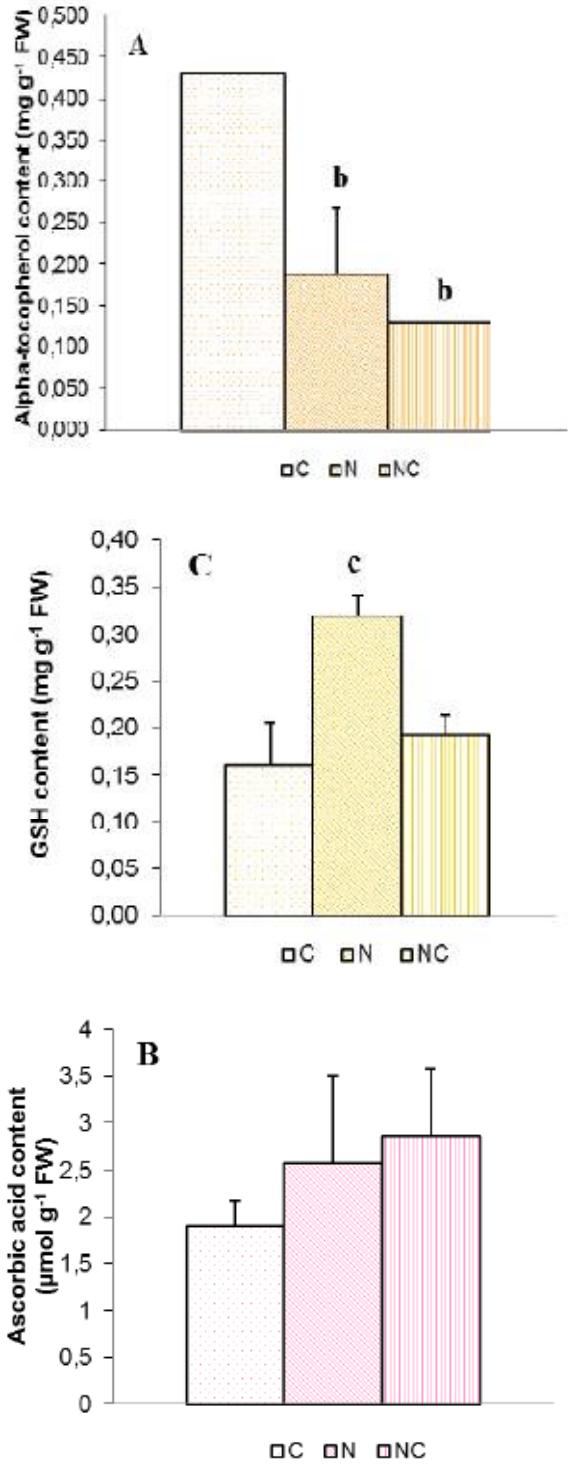


Figure 4.Effects of norflurazon on the content of alpha tocopherol (A), ascorbic acid (B) and glutathione (GSH) (C) in peanut plants.

3.5. Norflurazon induced changes in glutathione-S-transferase (GST) activity

The herbicide application resulted in decreasing of GST activity in leaves which was lower up to 47%. In the presence of Norflurazon associated with ascorbate, the GST activity is also reduced compared to the control (a reduction of 91%) (Fig. 5).

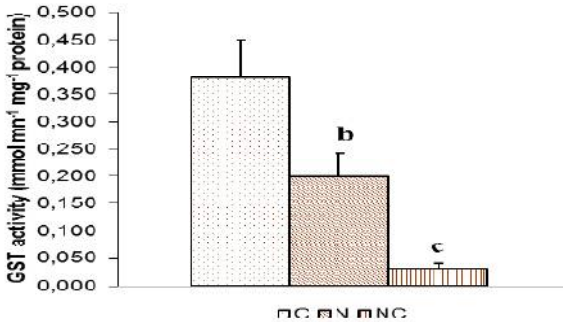


Figure 5.Effects of norflurazon on the activities of GST in leaves in peanut plants.

DISCUSSION AND CONCLUSION

Our results demonstrate that norflurazon by inhibiting the biosynthesis of carotenoids, induces a lethal physiological state because the most important role of these pigments is to protect against photo-oxidation damage of the photosynthetic apparatus [8]. The absence of carotenoids leads to the formation of active oxygen molecules [2] so, both growth and biochemical parameters are profoundly affected.
The decrease in the activity of glutathione Stransferase in seedlings treated with norflurazon indicate that a part of this herbicide is not conjugated in the leaves of treated plants.

The exogenous contribution of vitamin C appears to attenuate the inhibitory effects of norfluarzon on the growth of seedlings of peanut. Furthermore, although the level of MDA decreases in the leaves of seedlings subjected to the norflurazon associated with an addition of vitamin C, vitamin E content decreases in these same seedlings which suggests that the latter is not recycled by the Vitamin C.

Our results are in agreement with those of [9], which show that an artificial supply of ascorbate does not improve the recycling of alpha tocopherol in corn seedlings under cold stress. Carotenoids, tocopherols, ascorbate and glutathione are interdependent in the control of ROS levels in plants under stress and thus in limiting damage due to oxidative stress induced. The increased glutathione level in the norflurazon treatment peanut plants could be interpreted in such a way that it may have triggered alterations in the ROS signaling networks, allowing these plants to achieve a new homeostasis to cope with abiotic stress. Glutathione was previously proposed as a candidate transmitter of intracellular ROS signals [10].

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