Volume 5, Issue1, August 2012

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Sudan Journal of Science (SJS)

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Effect of potassium chlorate on nitrate uptake and reduction in *Zea mays L.* seedlings.

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Abstract

Foliar application of potassium chlorate (5 ppm and 15 ppm) on maize seedlings caused distinct lowering of nitrate content and nitrate reductase in the leaves. After 5 days of spraying diminishment of nitrate amounted to 16% and 45% with 5 ppm and 15 ppm respectively. More decline was observed after 10 and 15 days after spraying.

Nitrate reductase behaved similarly and decreased progressively with time. After 5 days of spraying the enzyme declined by 20% and 31% with the lower and higher doses respectively.

Decline in the two parameters is probably provoked by reduction in carbon assimilation as a result of chlorate treatment, and that nitrate uptake, translocation and ultimately reduction to nitrate reductase are known to be high energy expending processes, and factors which negatively affect energy availability reduce nitrate uptake and its reduction to nitrate reductase. Nitrate reductase is an inducible enzyme depending on de novo synthesis to nitrate availability.

Introduction

Chlorates are highly oxidizing substances that cause toxic effects to plants (Hofstra, 1977). Among the major sources of chlorate input to the environment, are its extensive use as a defoliant and weed control in agriculture (Agaev et al 1986). This herbicide is phototoxic to all green parts of plants, and absorption of chlorates may occur through both roots and leaves. When foliary applied, the chlorate ions penetrate the cuticle, and when absorbed by plant roots, the ions are translocated through the xylem to the leaves. Borges et al (2004) showed that chlorate treatments provoked severe damage to rice leaves. They demonstrated that the electron microscopy of the chloroplast revealed severe damage in the organelle organization, in which the integrity of outer membrane and the internal network of thylakoid membranes were almost completely lost. They verified that the photochemical efficiency of photosynthesis was drastically affected and severe damages to the exodermis, endodermis, cortex calls and root hairs were observed. Eltahir (2010) showed that potassium chlorate spraying on seedlings of cotton, caused significant reduction of nitrate uptake and reduction in the roots and leaves.

The primary objective of this research was to examine the effect of potassium chlorate on uptake and reduction of nitrates to nitrate reductase at early growth stages of maize.

Materials and Methods

Plant culture:

Grains of *Zea mays* (maize), variety Ohio 43 obtained from a seed shop in New Jersey, U.S.A. were used in this study. The grains were surface sterilized with 1% hydrogen peroxide, rinsed several times with distilled water and germinated in deep plastic trays containing sand and clay (1:1) and watered every other day. Seedlings of comparable size, aged three weeks were used in this study. Potassium chlorate (5 ppm and 15 ppm) was foliary applied with suitable sprayers, and great care was taken to prevent the herbicide from coming onto direct contact with the soil. Nitrate reductase and nitrates were assayed in leaves after 5,10 and 15 days of spraying with the herbicide.

Assay of nitrate reductase:

In vivo nitrate reductase in leaves was assayed as outlined by Radin (1978). One g of fresh material was thoroughly washed and incubated for 1h in 10 ml potassium phosphate buffer (pH 7.5) containing few drops of 1% 1-propanol as a wetting agent. Prior to assay, the buffer solution was purged with N_2 gas for 30 min to remove oxygen. Nitrite was quantitatively released into the medium, and it was determined by combining 1 ml dilute sample with 1 ml sulfanilamide (1% w/v in 1.5 M HCl), and 1 ml naphthylethlene diamine hydrochloride (0.02% w/v). After 15 min, absorbance was read at 540 nm in a spectrophotometer and nitrite concentrations (representing nitrate reductase activity) were calculated from a standard curve.

Nitrate assay:

Dried samples were ground, and the powder further dried at 70°C. One hundred mg of powder were mixed with 10 ml distilled water for 1 h at 45°C and then centrifuged (5000 rpm) for 5 min. The supernatant was used for nitrate determination by the salicylic acid method (Cataldo et al., 1975). To 0.2 ml of the extract 0.8 ml of the salicylic acid reagent (5% in conc. H_2 SO_4) were added and the mixture left to cool. The nitrate content was measured spectrophotometrically at 410 nm and concentrations were derived from a standard curve.

Results and Discussion

Results presented in Fig. 1 show that the nitrate content was drastically diminished compared to controls. After 5 days of spraying, the nitrate content declined by 16% and 45% with 5 ppm and 15 ppm respectively. Reduction of the ion continued with prolongation of the experiment, and decline after 15 days amounted to 27% and 39% with the lower and higher dose of the herbicide respectively.

On the other hand, nitrate reductase activity followed exactly a similar pattern with decline of the enzyme throughout the run of the experiment (Fig. 2). It was observed that changes in nitrate content and nitrate reductase followed a parallel trend with reduction of both parameters suggesting induction of nitrate reductase by nitrate availability. This corroborates the findings of other workers (Erlrich and Hageman 1973; Thiobodeaux and Jaworski 1975) that nitrate reductase is a limiting enzyme and is substrate dependent.

It was observed that nitrate reductase activity and nitrate content declined towards the termination of the experiment (i.e. with age). This result is consistent with that obtained by Harper and Hageman (1972). Who showed that seedling age affects both the steady state level of the enzyme and the inducible levels. The steady state level of the enzyme is normally low in the tissues of young seedlings and then declines in the senescent ones. Eltahir (2010) showed that 5 herbicides including potassium chlorate tended o decrease the chlorophyll content, stomatal conductance and total soluble sugars in cotton seedlings. An important consequence of the depressive effects of herbicides on the above mentioned parameters is lowering carbon assimilation and production of glucose as the final product of photosynthesis which is extremely vital in liberating energy (ATP) via the respiratory metabolic pathway. Nitrate reductase is an inducible enzyme depending on nitrate availability, and nitrates are known to consume great energy in a triple process starting form absorption, translocation and ultimately reduction to nitrate reductase.

The depressive effects of chlorates on the two studied parameters could also be attributed to their reduction to chlorites by nitrate reductase (nitrates and chlorates are known to be competitive inhibitors of each other), and chlorites cause drastic effects and irreparable damage at the reduction site (Mackown et al 1996). Although the toxicity of chlorates on plants is evident, the levels of damage caused by the stressor on the physiology and biochemistry of plants are still poorly characterized (Borges et al 2004.

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تأثير كلوريت البوتاسيوم على امتصاص النترات واختزالها في بادرات الذرة الشامية.

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الخلاصة:

رش كلوريت البوتاسيوم بتركيزي 5 و 15 جزء في المليون على بادرات الذرة الشامية أحدث انخفاضاً واضحاً في محتوي النترات وإنزيم النترات ردكتيز في الأوراق. فمثلاً بعد 5 أيام من الرش انخفض محتوي النترات بـــ 16% و45% بتركيزي 5 و 15 جزء في المليون بالتتالي. إنزيم النترات ردكتيز انخفض بصورة مماثلة تماماً لمحتوي النترات فبعد 5 أيام من المعاملة انخفض تركيز الإنزيم بـــ 20% و 31% بتركيزي 5 و15 جزء في المليون بالتتالي.

الانخفاض في المتغيرين يمكن أن يعزي إلى انخفاض في تمثيل الكربون نتيجة المعاملة بالكلوريت، فامتصاص النترات واختزالها من العمليات التي تحتاج إلى طاقة عالية على هيئة ثلاثي ادنتوسين الفوسفات، فالعوامل التي توثر سلباً على توفر الطاقة تؤدي إلى تخفيض امتصاص النترات واختزالها إلى إنزيم النترات ردكتيز، فتخليق الإنزيم يعتمد على توفر النترات والتي بدورها تحاج لطاقة عالية لامتصاصها وانتقالها واختزالها.

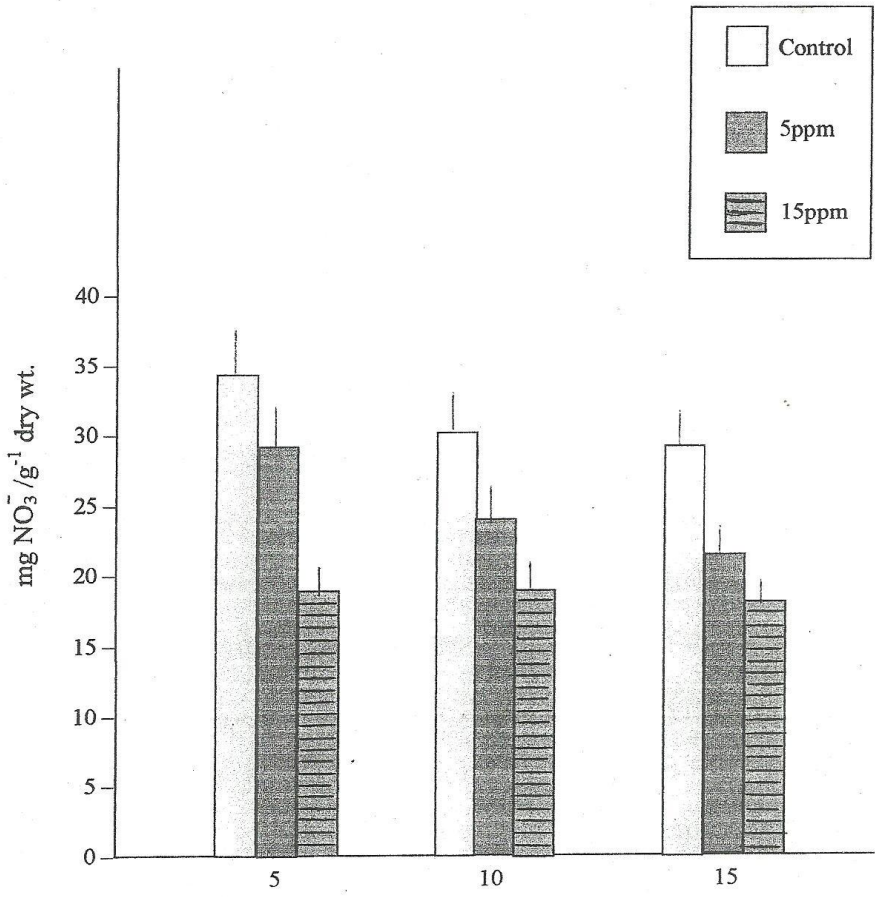

Fig. 1. Effect of foliar application of two concentrations of $KClO_3$on nitrate content in the leaves of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).

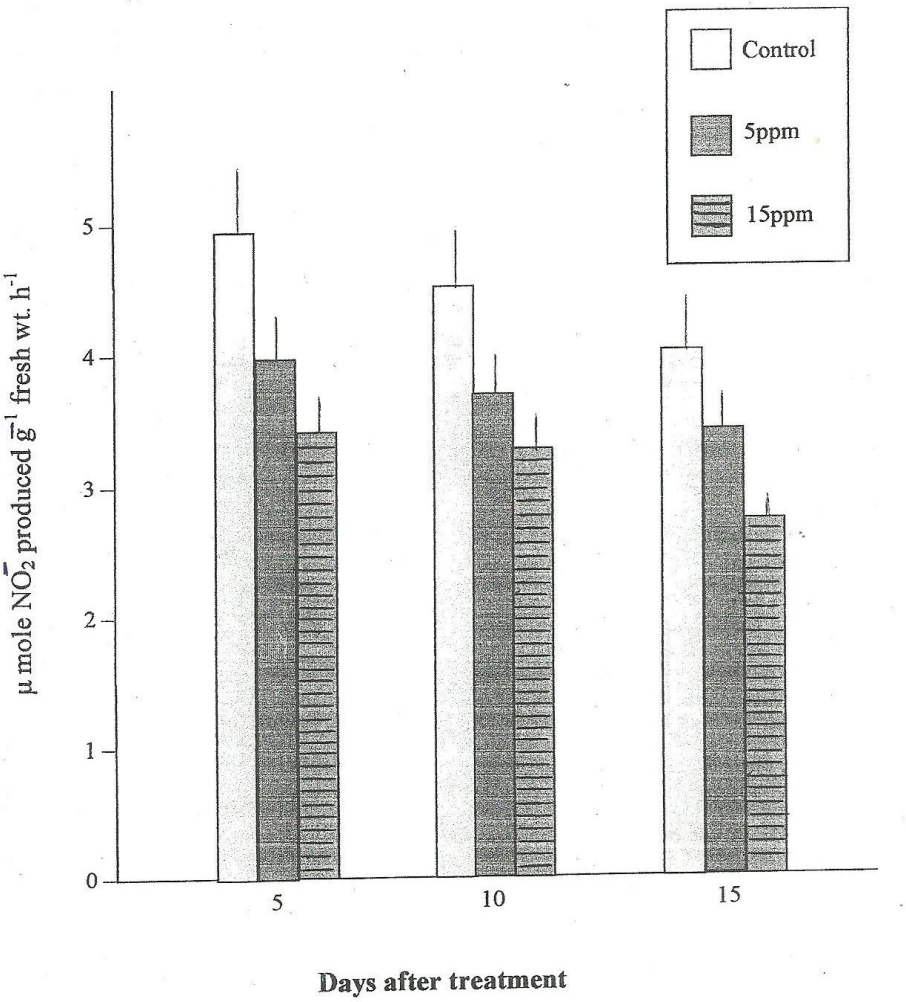

Fig. 2. Effect of foliar application of two concentrations of $KClO_3$ on nitrate reductase activity in the leaves of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).