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EFFECT OF THE HERBICIDE OXYFLUORFEN ON NITRATE REDUCTASE AND NITRATE CONTENT IN *Zea mays* L. SEEDLINGS

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ABSTRACT

Oxyfluorfen spraying resulted in distinct lowering of nitrate reductase with both doses of the herbicide (5 ppm and 15 ppm) and in both roots and leaves of maize seedlings throughout the run of the experiments. After 5 days of spraying, diminishment of the enzyme amounted to 37% and 56% with the lowest dose of the herbicide in roots and leaves respectively. A similar pattern was observed after 10 and 15 days of spraying.

On the other hand, the nitrate content behaved similarly indicating induction of nitrate reductase by nitrate availability. Lowering of nitrate reductase and nitrate content by oxyfluorfen treatment could be attributed to decline in carbon assimilation by some herbicides as verified by several workers, and nitrate uptake and reduction to nitrate reductase are energy expending processes.

INTRODUCTION

Oxyfluorfen (Trade name: Goal and molecular formula (C_{15} H_{11} Cl F_3 NO_4) is a selective pre- and post-emergent herbicide used to control certain annual broadleaf and grassy weeds in vegetables, fruits, cotton and ornamentals. This herbicide is reported to cause necrosis and sometimes chlorosis when applied to foliage, while pre-emergence application inhibits seed germination, early seedling growth and meristem activity (Robert, 1982 and Mohamed, 1995). Gilham and Dodge (1982) have shown that oxyfluorfen induces light independent bleaching and lipid peroxidation; the level of damage being greater at higher light intensities. It is activated by a light dependent process which required photosynthetic electron transport and affects non-cyclic electron flow or promotes superoxide formation.

In Sudan, the effects of different herbicides on many species and different varieties of main crop plants and weeds were investigated. The different species and varieties greatly varied in their response to the herbicides used. Ahmed (2003) evaluated the efficiency of three pre-emergence herbicides (oxyfluorfen included) in controlling weeds affecting growth and yield of banana and obtained good results. Two pre-emergence herbicides (oxyfluorfen and pendimethalin) used for the control of annual weeds in garlic-crop, gave selective control of most annual broad-leaved weeds, and a significant increase in garlic yield (Amel, 2003).

The objective of this study was to foliary apply oxyfluorfen (5 ppm and 15 ppm) on seedlings of maize and analyze the effect on nitrate reductase and nitrate content in the roots and leaves.

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. The herbicide (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 roots and leaves after 5, 10 and 15 days of spraying with the herbicide.

Assay of nitrate reductase:

In vivo nitrate reductase in roots and 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 sufanilarnide (1% w/v in 1.5 M MCl), 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_2SO_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

Oxyfluorfen spraying provoked distinct diminishment of nitrate reductase activity in the roots (Fig. 1) and leaves (Fig 2).

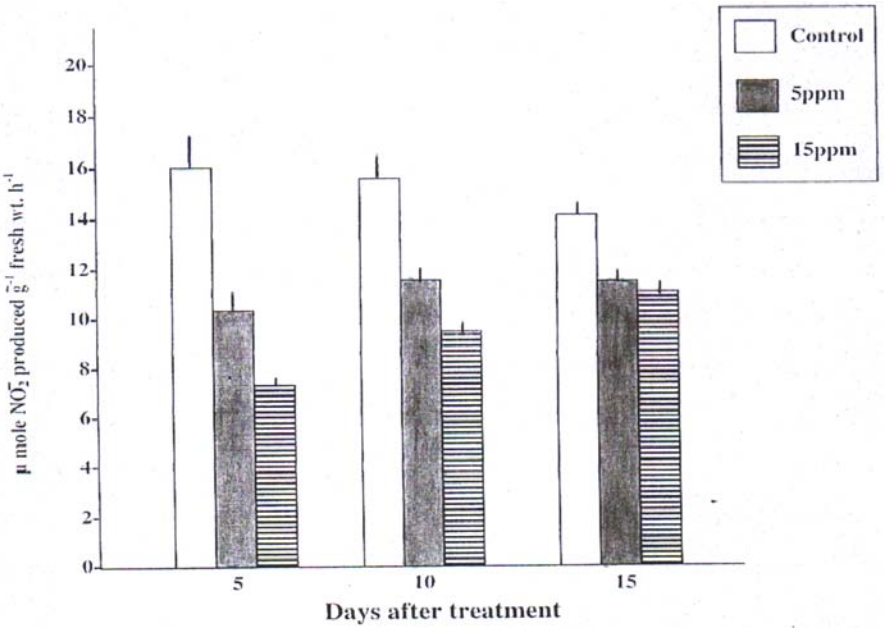

Fig. 1: Effect of foliar application of two concentrations *of* oxyfluorfen on nitrate reductase activity in the roots of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).

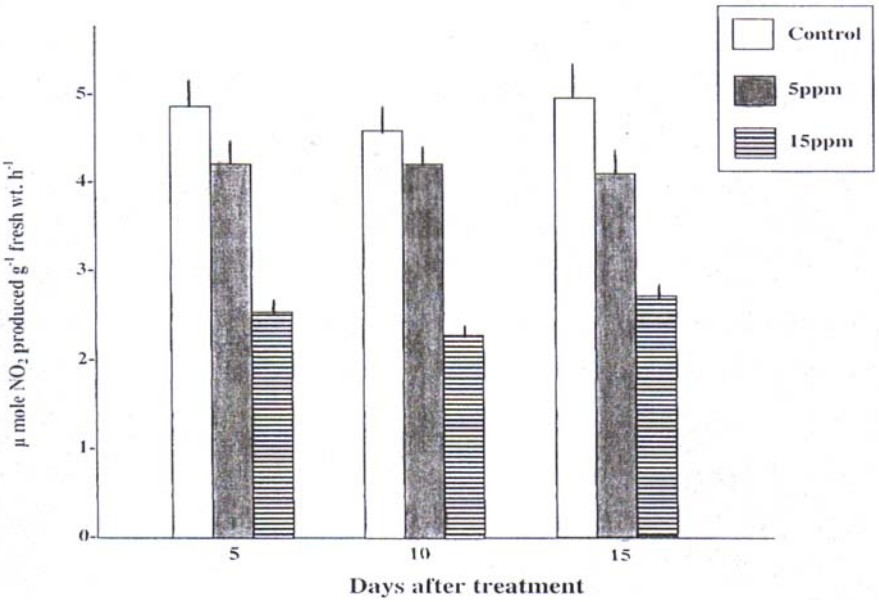

Fig. 2: Effect of foliar application of two concentrations *of* oxyfluorfen on nitrate reductase activity in the leaves of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).

It is noteworthy that the degree of decline increased with increasing the dose of the herbicide. Herbicides are known to influence various aspects of nitrogen metabolism on which seed protein production and yield depend. Several herbicides have been reported to influence leaf nitrate concentration (Kees and Krumery, 1978) and nitrate reductase activity (Esmama and Juliano, 1976). Knowledge of the factors which affects nitrate reductase activity is vital, as nitrate reductase is considered to be a limiting factor for growth, development and production in plants.

Information with regard to herbicide application on physiological and biochemical changes on treated crops is extremely meager. Very little information referred to these parameters, and the major information on the influence of herbicides in Sudan and even elsewhere was focused on the eradication of unwanted weeds completing with crops. It is of great concern to understand the effect of herbicides on non-targeted crops they are meant to protect (Xia *et al*., 2006). Omokaro and Ajakaiye (1989) demonstrated that four herbicides applied on two cultivars of cowpea decreased their nitrate reductase activity. El Tahir (2010), also showed that five herbicides (including oxyfluorfen) lowered nitrate reductase activity in cotton seedlings.

A distinct decline of nitrate content in roots and leaves was observed following spraying with the two doses of the herbicide (Figs. 3 and 4 respectively).

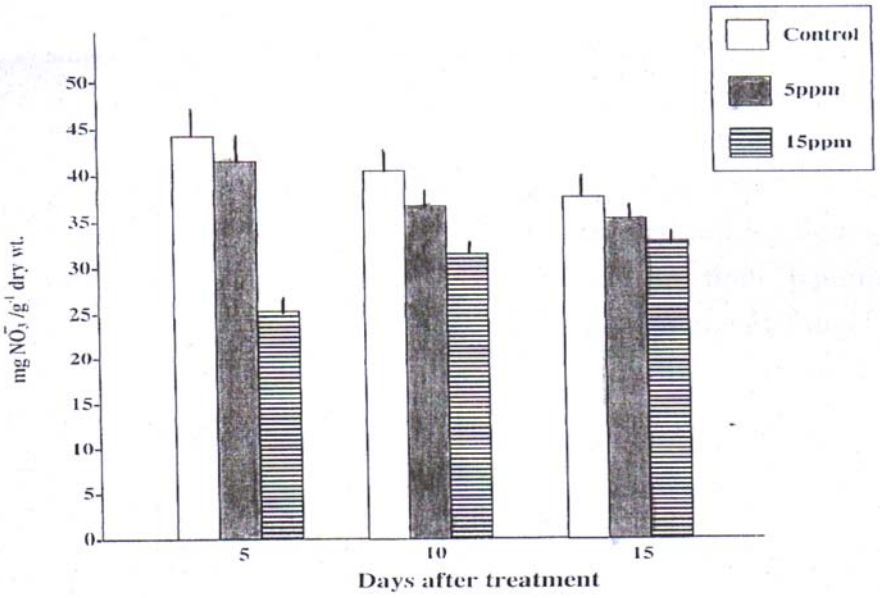

Fig. 3: Effect of foliar application of two concentrations *of* oxyfluorfen on nitrate content in the roots of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).

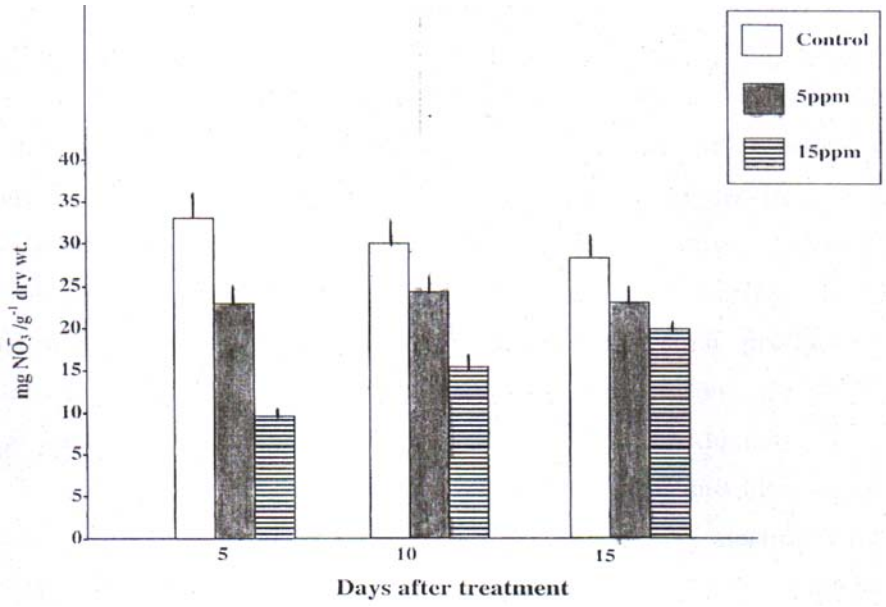

Fig. 4: Effect of foliar application of two concentrations *of* oxyfluorfen on nitrate content in the leaves of three weeks old maize seedlings. Vertical bars indicate ± SD, (n=3).

Decline in the nitrate content was more conspicuous after 5 days of treatments especially with the higher dose of the herbicide. It was observed that changes in nitrate reductase activity and nitrate content followed a parallel pattern with reduction in 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. El Tahir (2010) showed that 5 herbicides including oxyfluorfen tended to 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 from absorption, translocation and ultimately reduction to nitrate reductase.

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تأثير رش المبيد العشبى أوكسيفلورفين على إنزيم النترات ردكتيز ومحتوى النترات فى بادرات الذرة الشامية

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رش المبيد العشبى أوكسيفلورفين بتركيزى 5 و 15 جزء فى المليون تسبب فى نقص واضح لإنزيم النترات ردكتيز فى جذور وأوراق بادرات الذرة الشامية طيلة فترة التجربة. بعد 5 أيام من الرش بالتركيز المتدنى إنخفض إنزيم النترات ردكتيز بـ 37 % و 56 % فى الجذور والأوراق وبالتالي – تكرر هذا التأثير السلبى بعد 10 و 15 يوم من الرش بالمبيد.

وفى المقابل ، فإن محتوى النترات إنخفض بصورة مماثلة للإنزيم وفى ذلك إشارة لأهمية وجود النترات لتحفيز إنزيم النترات ردكتيز.

إنخفاض الإنزيم والنترات بمعاملة المبيد العشبي يمكن تفسيره بنقص في تمثيل الكربون ببعض المبيدات العشبية والذي أكدته الكثير من أعمال سابقة وكما هو مثبت حالياً فإن إمتصاص النترات وإختزالها تحتاج إلى طاقة عالية تمد بواسطة عملية البناء الضوئي.

قام بتحكيم البحث

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