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EFFECT OF FOLIAR APPLICATION ON THE HERBICIDE STOMP ON NITRATE REDUCTASE IN THE LEAVES OF *Gossypium hirsutum* L.

Wasfi, M. A. and Ekhlas Eltahir
Dept. of Botany, U. of K. P.O. Box 321. Khartoum – Sudan

ABSTRACT

Foliar application of stomp resulted in drastic diminishment of three parameters in leaves of young plants of cotton. The higher dose of the herbicide (15ppm) provoked reduction in nitrate reductase activity by 46% , 57% after 5, 10 and 15 days respectively. The same dose lowered the total soluble sugars by 40% , 64% and 73% after 5, 10 and 15 days respectively. The nitrate content showed similar pattern and decreased progressively with increasing the dose of the herbicide throughout the run of the investigation.

The negative effect of the herbicide on these parameters could be attributed to reduction in carbon assimilation. Inadequate supply of sugars reduces nitrate uptake and reduction and consequently nitrate reductase being a substrate inducible enzyme is negatively affected.

Keywords: Herbicide , stomp, cotton, nitrate reductase , total soluble sugars, nitrates.

INTRODUCTION

The voluminous and previous research on herbicides was mainly focused on elimination of invasive weeds and other plants competing with crops for nutrients and water. The effects of the herbicides on physiological and biochemical parameters on non target crops received little attention, and publications concerning these parameters are meager and scanty. The herbicide "stomp" contains pendimethalin, a selective diniltroalanine that effectively controls a wide spectrum of annual grasses and broad-leaved weeds in many important horticultural practices. Stomp alone , and in combination with other herbicides is registered for use in over 60 countries in 70 crops. Weed control by stomp in cereals , maize , cotton, vegetable and other agronomic and horticultural crops has been outstanding. Ahmed (2003) evaluated the efficiency of three preemergence herbicides (stomp, diuron and goal) in controlling weeds affecting growth and yield of banana. Amel (2003) investigated the chemical weed control in garlic by stomp and obtained positive results.

The objective of this study was to foliary apply stomp on young plants of cotton and analyse the effect on nitrate reductase, total soluble sugars and nitrate content of the leaves.

MATERIALS AND METHODS

Plant culture:

Seeds of *Gossypium hirsutum* , medium stable cotton, variety Barakat 90 , were obtained from the Gezera Scheme (Sudan). The seeds were surface sterilized for 10 min. 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 (5ppm and 15 ppm) was foliary applied using suitable sprayers, and great care was taken to prevent the herbicide from coming into direct contact with the soilk.

Nitrate reductase activity, total soluble sugars and nitrates were assayed in the leaves after 5, 10 and 15 days of treatments with the herbicide.

Assay of nitrate reductase:

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

Assay of total soluble sugars:

Total soluble sugars were extracted by methanol: chloroform: water (12:5:3 v/v/v) mixture as described by Dickson and Larson (1975) from fresh leaves. Three extractions were done followed by centrifugation (2000 g for 15 min) . The upper (lighter) fraction, dried (evaporated under pressure at 30^{o}C) and redissolved in 10 ml water was used for analysis.

The total soluble sugars were assayed using the anthrone method as described by Halhoul and Kleinberger (1972). Six ml of anthrone reagent (0.15% in conc. H_2 SO_4) were added to 0.4 ml distilled water and 0.1 ml of the sugar extract in a test tube placed in a boiling water bath for exactly 6 min. Afterwards, the tube was transferred to cold water to stop the reaction. After thorough shaking, absorbance was read at 630nm in a spectrophotometer and calculations were made from a standard curve.

Nitrate assay:

Dried samples were ground, and the powder further dried at 70^{o}C. One hundred mg of powder were mixed with 10 ml distilled water for 1 h at 45^{o}C and then centrifuged (5000 ppm) for 5min. 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 reagent (5% in conc. H_2 SO_4) were added and the mixture left to cool. The nitrate content was measured spectrophotometrically at 410nm 410nm and concentrations were derived from a standard curve.

RESULTS

The results presented in Fig.1 show the negative effect of the two doses of the herbicide on nitrate reductase activity throughout the run of the experiment. Drastic reductions in the activity of the enzyme were caused by the herbicide especially with the higher dose (15ppm) where 46% , 57% and 78% were recorded after 5,10 and 15 days respectively in reference to controls. It should be observed that enzyme activity progressively declined with age of the plants.

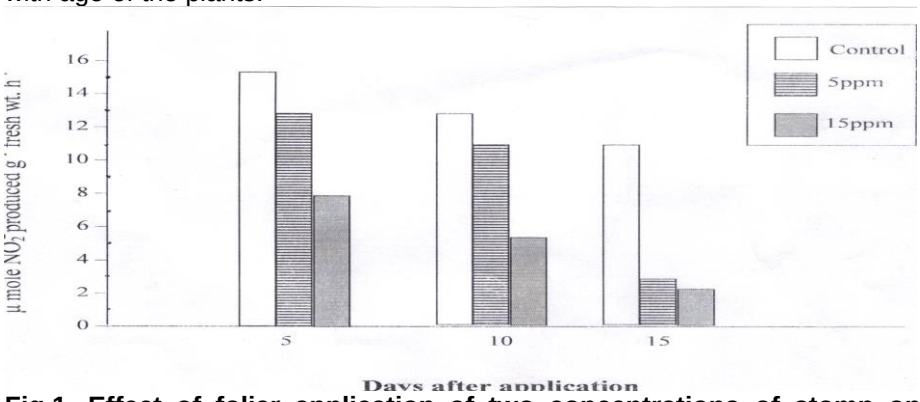

Fig.1. Effect of foliar application of two concentrations of stomp on nitrate reductase activity in the leaves of cotton. Vertical bars indicate ± SD, (n=3).

The total soluble sugars were also significantly reduced by the herbicide. Again, the higher dose of the herbicide resulted in greater lowering of the total soluble sugars compared to control value. Decrements amounted to 40% , 64% and 73% after 5,10 and 15 days respectively. Also, as in the enzyme activity , the total soluble sugars decreased progressively with age and with both concentrations of the herbicide.

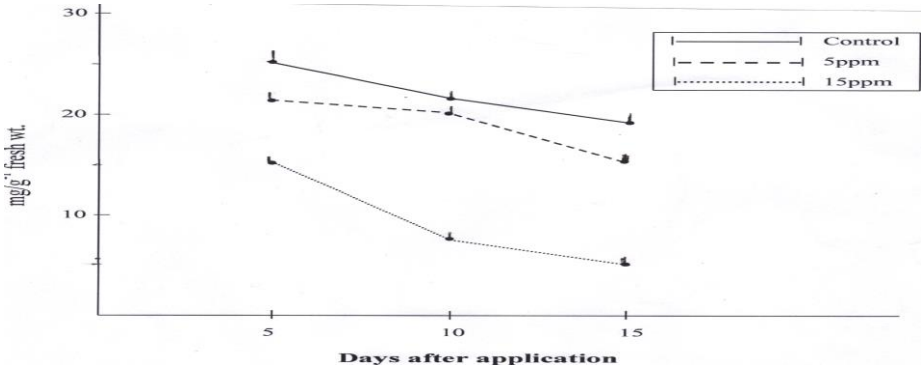

Fig.2. effect of foliar application of two concentration of stomp on the total soluble sugars in the leaves of cotton. Vertical bars indicate ± SD, (n=3).

Obvious reductions in the content caused by the herbicide were presented in Table 1, and a more or less similar pattern as in the previous two parameters was observed.

Table 1. Effect of foliar application of two concentrations of stomp on nitrate content in the leaves of cotton. Values shown are means of three estimations ± SD , (n=3) expressed as mg/g^{-1} dry wt.

Treatments	Days after treatment		
	5	10	15
Control	9.2 ± 1.3	8.6 ± 1.1	7.2 ± 1.1
5 ppm	7.6 ± 1.0	6.9 ± 0.9	5.9 ± 0.85
15 ppm	6.5 ± 0.80	5.9 ± 0.75	5.0 ± 1.60

DISCUSSION

Stomp herbicide is registered to alter some chemical processes in higher plants including changes in sugar content, amino acids, malic acid as well as inhibition of photosynthesis (Robert, 1982 and Vouzouis, 1995). It was observed that changes in nitrate reductase activity and nitrate content in this study followed the same trend suggesting induction of nitrate reductase by nitrate availability nitrate reductase is an inducible enzyme depending on nitrate supplies, and nitrates are known to consume great energy in a triple process starting from absorption, translocation and ultimately reduction. Earl *et al.,* (2004) and Bigot *et al*., (2007) have shown that herbicides, in general, cause reductions in carbon assimilation, stomatal conductance and water use. Therefore, it is suggested that, stomp treatment in this study reduced the photosynthetic rate and hence, inadequate supplies of sugars resulted in lowered nitrate uptake and reduction.

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تأثير رش المبيد العشبي ستومب على إنزيم النترات ردكتيز في أوراق نبات القطن.

ميرغنى عبد الرحمن وصفى و إخلاص الطاهر
قسم النبات- كلية العلوم – جامعة الخرطوم- جمهورية السودان

رش المبيد على المجموع الخضري لنبات القطن تسبب في نقص واضح لثلاثة متغيرات في الأوراق. التركيز الأعلى للمبيد (١٥ جزء في المليون) أحدث انخفاضا في إنزيم النترات ردكتيز بـــ ٤٦% و ٥٧% و ٧٨% بعد ٥و ١٠ و ١٥ يوم على التوالي مقارنة بالنباتات غير المعاملة. نفس تركيز المبيد تسبب في نقص السكريات المذابة بـــ ٤٠% و ٦٤% و ٧٣% بعد ٥و ١٠و ١٥ يوم على التوالي مقارنة بالنباتات غير المعاملة. كذلك انخفض محتوى الأوراق من النترات بطريقة مماثلة.

التأثير السلبي للمبيد على هذه المتغيرات يمكن أن يعزى إلى نقص تثبيت الكربون في عملية التمثيل الضوئي ، إذ أن انخفاض تخليق السكر يتسبب في نقص امتصاص النترات اللازمة لتحفيز تخليق إنزيم النترات ردكتيز.

قام بتحكيم البحث

أ.د / على على عبد الهادى	**كلية الزراعة – جامعة المنصورة**
أ.د / محمود ابراهيم حسن	**كلية الزراعة – جامعة المنوفيه**