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MAIZE ROOT EXUDATES IN RELATION TO THE ACTIVITY OF SOME PATHOGENIC FUNGI AND *Azotobacter chroococum*

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ABSTRACT

The effect of maize root exudates of two maize cultivars (SC10 and Balady) on the growth of certain pathogenic fungi (*Cephalosporium maydis* Samra, Sabet and Hingorani and *Fusarium moniliforme* Sheldon), which caused maize root and stalk rot diseases and *Azotobacter chroococum* as well as component of these exudates were studied.

Root exudates of maize plants increased the dry weight of the pathogenic fungi and total count of *Azotobacter chroococum* cells. The exudates contained ten amino acids, reducing and non-reducing sugars, free and conjugated phenols as well as eight organic acids. The chemical component of the maize root exudates increased by plant age and maize variety SC10 was more active in its root exudates than Balady one.

INTRODUCTION

Root exudates play an important role of establishment and maintenance of the rhizosphere microorganisms. This effect came from stimulating or inhibition growth of microorganisms by root exudates of maize (El-Arosi *et al.*, 1971 and Samra *et al.*, 1971). The maize root exudates contained amino acids, reducing and non-reducing sugars, conjugated and free phenols as well as organic acids (Brown, 1975; Alexander, 1977; Czaplinska *et al.*, 1979; Mohamed *et al.*, 1981; Abd El-Ghany, Haifaa, 1987; El-Tony, 1987; Botros, 1988 and Ismail, 1994). The present investigation was planed to prepare the maize root exudates and study its effect on linear growth of both organisms, *Cephalosporium maydis* and *Fusarium moniliforme*, as well as on the total count of *Azotobacter chroococum* cells. Analysis of chemical composition of maize root exudates was also carried out to study the effect of root exudates of the two varieties under study on the activity of some pathogenic fungi and total count of *Azotobacter chroococcum* cells.

MATERIALS AND METHODS

Root-exudates of two maize cultivars (single cross SC 10 and Balady) were obtained after planting of seeds in acid-washed sterilized sand. Pots (40 cm in diameter) were filled with equal amounts of 4 kg acid washed sand. Irrigation of sand cultures were carried out when required by using sterilized distilled water. Roots exudates were obtained after 25 and 40 days by carefully removing the seedling with their root system as intact as possible. Sand cultivated was transferred to acid washed flask, vigorously shaken in sufficient amounts of ethanol (70%) sand washing was repeated twice, the exudates were strained through glass wool, and air dried in wineglass vessels. The dried film was dissolved in 5 ml isopropanol (50%), and kept

frozen in small vial, for testing their effect on both mycelial dry weights of the pathogenic fungi and total counts of *Azotobacter chroococum* (Al-Laithy, 1983) in relation to chemical constituents of such root exudates according to Georgey (1977).

Effect of root-exudates on the growth rate of the pathogenic fungi and/or total count on non-symbiotic nitrogen fixing bacteria (NSNFB) (*Azotobacter chroococum*):

To test the effect of root exudates of maize cultivars on the dry weight of the pathogenic fungi (*C. maydis* and *F. moniliforme*) either separately or mixed and on the total count of *Azotobacter chroococum* cells the following methods were carried out. Conical flasks (250 ml) each containing 200 ml of autoclaved Potato Dextrose Yeast Extract (PDYE) (Samra *et al.*, 1968) or Ashby's liquid medium (Abd El-Malek and Ishac, 1968) were used. Equal discs (5 mm in diameter) of *C. maydis* and *F. moniliforme* grown on PDA medium for two weeks at 27°C were used either separately or mixed to inoculate the PDYE liquid medium (one disc/flask). Also, 0.1 ml of *Azotobacter chroococum* suspension was grown on Ashby's liquid medium for one week at 30 °C was added to each flask of Ashby's liquid medium (0.1 ml/flask). For the combined treatment of the pathogenic fungi and NSNFB (*C. maydis* + *F. moniliforme* + *Az. chroococum*), 0.1 ml of the bacterial suspension was added to flasks containing PDYE medium and inoculated with *C. maydis* and *F. moniliforme*, as well as, *Az. chroococum*. Root exudates previously prepared from the plants of the two cultivars were sterilized by passing through autoclaved Seitz filter. Each of the inoculated flasks received 10 ml of sterilized root-exudates of either SC 10 or Balady cultivars. Flasks of the control treatment were treated similarly, but without root exudates. All flasks in four replicates were incubated at 30°C for one week, after which the mycelial dry weights of pathogenic fungi (*C. maydis* and *F. moniliforme*) were measured as usual. The total count of *Azotobacter chroococum* cells was determined according to MPN method as mentioned before (Hussain Allam, 1953).

Chemical composition of the root exudates:

Amino acids were determined by chromatography according to the method described by Smith (1958) as well as Ambe and Toppel (1961).

Sugar content was determined according to Trevelan *et al.* (1950) and Block *et al.* (1958). Total and free phenols in root exudates were determined as methods described by Snell and Snell (1954). Organic acids in root exudates were determined by using the methods described hy Buch *et al.* (1952) and Rieo (1959).

RESULTS AND DISCUSSION

The results presented in Table 1 revealed that different concentrations of root exudates of the two maize cultivar SC 10 (resistant) and Balady (susceptible) increased the mycelial dry weight of *F. moniliforme* and *C. maydis*. Also, root exudates of both cultivars were collected after 40 days from planting, were more effective on mycelial weight compared with that

were collected after 25 days from planting. Root exudates of resistant cultivar (SC 10) was less effective than that of susceptible one Balady. Stimulation in mycelial dry weight was obtained by increasing concentration of the root exudates.

Table 1. Effect of different concentrations of root-exudates (v/v) from two maize cultivar (SC 10 and Baldy) after 25 and 40 days from sowing on the mycelial dry weight of certain pathogenic fungi.

Strains	Concentration (v/v)	Mycelial dry weight (g) and tested cultivar			
		SC 10		Balady	
		25 *	40	25	40
F. moniliforme	0.0	0.466	0.466	0.425	0.480
	10.0	0.580	0.700	0.650	0.690
	20.0	0.600	0.720	0.675	0.700
	30.0	0.610	0.730	0.700	0.725
	40.0	0.650	0.750	0.720	0.750
	50.0	0.700	0.760	0.730	0.800
C. maydis	0.0	0.425	0.450	0.466	0.450
	10.0	0.450	0.470	0.520	0.560
	20.0	0.460	0.480	0.550	0.590
	30.0	0.475	0.530	0.600	0.620
	40.0	0.520	0.580	0.630	0.680
	50.0	0.580	0.630	0.700	0.720
F + C	0.0	0.475	0.490	0.480	0.480
	10.0	0.666	0.710	0.750	0.870
	20.0	0.670	0.710	0.900	0.920
	30.0	0.700	0.730	0.920	0.940
	40.0	0.720	0.750	0.930	0.950
	50.0	0.850	0.770	0.950	0.980
F + C + Az	0.0	0.470	0.480	0.490	0.495
	10.0	0.568	0.620	0.650	0.770
	20.0	0.571	0.620	0.800	0.820
	30.0	0.601	0.635	0.830	0.850
	40.0	0.620	0.650	0.830	0.860
	50.0	0.750	0.670	0.850	0.880

LSD at 5%			
	Tested organism	(A)	0.153
	Concentration	(B)	0.123
	Cultivars	(C)	0.202
	Tested planting period	(D)	NS
			0.233
	A x B		0.211
	A x D		0.355
	A x C		0.165
	B x D		0.453
	A x B x C		0.566
	A x B x C x D		

*: Plant-age by days from sowing.

With respect to the effect of root exudates on non-symbiotic nitrogen fixing bacteria (NSNFB), obtained data revealed that, all tested concentrations of root-exudates from both maize cultivars SC 10 and Balady after 25 and 409 days from planting, increased total count of *Azotobacter chroococum* cells. This effect was differed with the different cultivars. Root exudates of the resistant cultivar (SC 10) was less effective than the susceptible one Balady. These results are in agreement with the finding of Mohamed *et al.* (1981), Arafa *et al.* (1986), Botros (1988) and Ismail (1994).

Table 2. Effect of different concentrations of root-exudates (v/v) on the total count of *Az. chroococum* cells.

Concentration (v/v)	Total count of *Az. Chroococum* cells MPN (x 10^5 / 100 ml)			
	SC 10		Balady	
	25 days	40 days	25 days	40 days
0.0	7.00	10.00	12.00	11.00
10.0	15.00	12.00	16.00	17.00
20.0	17.00	14.00	28.00	20.00
30.0	22.00	20.00	30.00	27.00
40.0	27.00	25.00	33.00	39.00
50.0	30.00	28.00	38.00	40.00
LSD at 5%	5.10	4.80	5.90	6.10

These simulative effect on dry weight of both fungi and total count of bacteria might be due to that root exudates of maize plants contain more and/or fatty, amino acids, sugars, vitamins and certain growth regulators. Such substances encourage fungal multiplication and helped the dormant stages of the pathogenic fungi to germinate (Arafa *et al.*, 1986; Botros, 1988 and Ismail, 1994). Chemical analysis of root-exudates of SC 10 and Balady cultivars as shown in Table 3 and Fig. 1 revealed the presence of L-cystine, DL-methionine, DL-thrironine, L-leucine, Dl-alanine, DL-aspartic, DL-2-amino butyric acids, L-byroxy praline, L-lisine mono HCL and L-arginine mono HCL.

Table 3. Amino acid groups in root-exudates of two maize cultivars in sandy culure as mg/ml exudates.

Amino acids	Amino acids (mg/ml root exudates			
	SC 10		Balady	
	25 *-	40	25	40
Cystine	0.432	0.169	0.611	0.213
DL-Methionine	0.623	0.317	1.920	0.426
DL-Thrionine	1.311	--	0.812	0.490
L-Leucine	0.901	--	0.514	0.560
DL-Alanine	0.650	0.466	0.600	0.481
DL-Aspartic	0.322	0.290	0.930	0.330
DL-2-amino butyric acid	0.624	0.422	0.800	0.232
L-Hydroxy proline	0.721	0.507	0.790	0.612
L-Lysine mono HCL	0.790	0.125	0.911	0.264
L-Arginine mono HCL	0.500	0.33	0.800	0.611
Total amino acids	6.774	2.629	8.488	4.219

*: Plant-age by days from sowing.

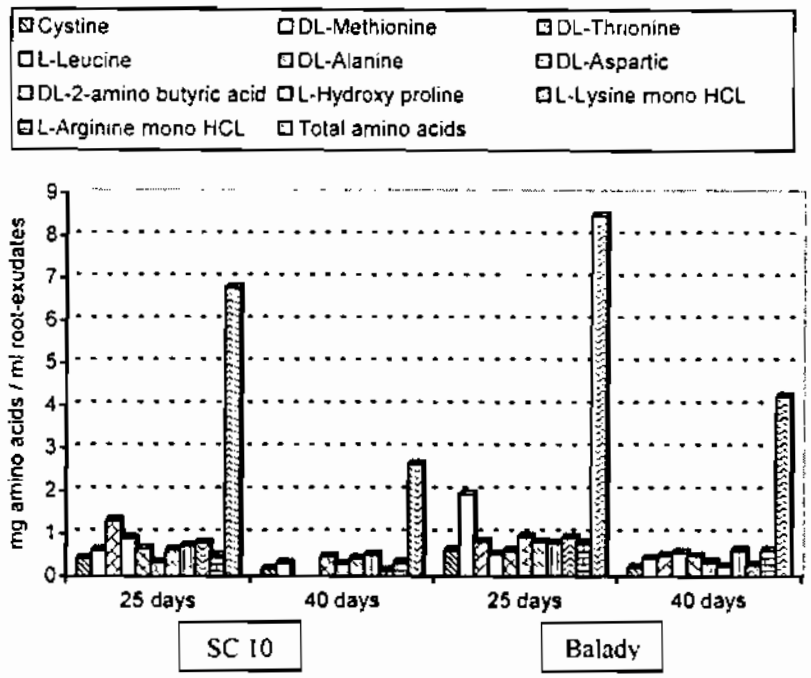

Fig.1. Sugar contents in root-exudates of two maize cultivars growing in sandy culture as mg glucose / ml root-exudates.

Table 4. Sugar contents in root-exudates of two maize cultivars as mg glucose / ml root-exudates.

Sugars	mg glucose / ml root-exudates of tested cultivars			
	SC 10		Balady	
	25 *	40	25	40
Reducing	284.12	612.90	153.17	322.80
Non-reducing	327.16	507.13	194.63	400.00
Total sugars	611.28	1120.03	347.80	722.80
LSD at 5%	Cultivars		(C)	266.3
	Sowing periods		(S)	260.4
	C x S			276.6

*: Plant-age by days from sowing.

The concentration of such amino acids were higher in root exudates of SC 10 cultivar compared with Balady cultivar. Also, the amount of amino acids was higher after 40 days from planting compared with 25 days from planting. These results are in harmony with those reported by Youssef and Youssef (1971), Botros (1988) and Ismail (1994).

Results in Table 4 and that diagrammatic in Fig. 2 also indicated that the root-exudates of the two maize cultivar (SC 10 and Balady) contain a very

high level of reducing and non-reducing sugars, but reducing and non-reducing sugars in case of SC 10 cultivar were higher than in Balady. Their concentrations at 40 days from planting were more than found after 25 days. These results are in agreement with the finding of Botros (1988) and Ismail (1994).

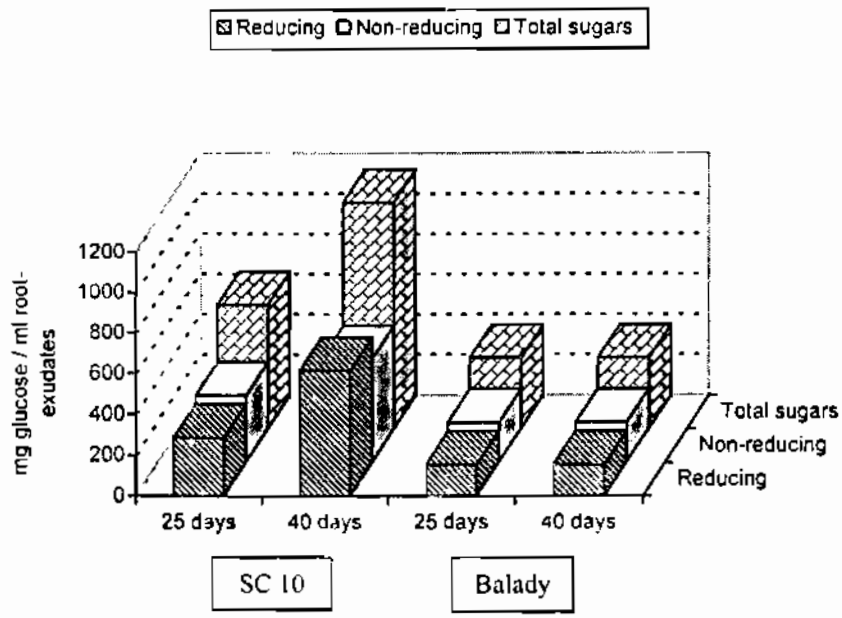

Fig.2. Sugar contents (reducing, non-reducing and total) in root-exudates of two maize cultivars (SC 10 and Baldy) collected after 25 and 40 days from sowing in sandy culture as mg glucose / ml root-exudates.

Table 5. Total, non-conjugated (free phenols) and conjugated phenols in root-exudates of two maize cultivars.

Sugars	Root exudates (mg catiol / ml)			
	SC 10		Balady	
	25 *	40	25	40
Free phenols	3.250	4.650	2.000	2.836
Conjugated phenols	0.515	1.090	0.325	0.753
Total phenols	3.765	5.740	2.325	3.609
LSD at 5%	Cultivars		(C)	1.180
	Sowing periods		(S)	0.750
	Interaction		(C x S)	0.950

***: Plant-age by days from sowing.**

As far as organic acids in root exudates are concerned, data in Table 5 showed that the kind and concentration of organic acid differed from one cultivar to another and at plant ages. These results are in harmony with those obtained by Sabet *et al.* (1972), Mohamed *et al.* (1981) and Ismail (1994).

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إفرازات جذور الذرة الشامية وعلاقتها بنشاط بعض الفطريات الممرضة للنبات وبكتريا الأزوتوباكتر كروكوكم

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تم دراسة تأثير إفرازات جذور الذرة الشامية على نمو بعض الفطريات الممرضة للنبات خصوصا (سيفالوسبوريوم مايدز ، فيوزاريوم مونيلفورم) وعلى أعداد بكتريا الأزوتوباكتر كروكوكم المتواجدة فى التربة الزراعية. وقد تم التحليل الكيماوى لهذه الإفرازات الجذرية وقد إحتوت على عشرة أحماض أمينية وعلى سكريات مختزلة وغير مختزلة وعلى فينولات حرة ومرتبطة وثمانية أحماض عضوية. وقد إتضح أن مفرزات الجذور تزداد بزيادة المدة بعد الزراعة وقد أدت هذه المفرزات إلى زيادة الوزن الجاف لميسليوم الفطريات الممرضة وإلى زيادة أعداد خلايا بكتريا الأزوتوباكتر كروكوكم المثبته للنتروجين. وأتضح أن الصنف هجين فردى ١٠ ينتج مفرزات جذرية أكثر من الصنف البلدى. وأن إفرازات الجذور من الصنف المقاوم (هجين فردى ١٠) كانت أقل تأثيرا من تلك المفرزة من الصنف البلدى من حيث تأثيرها على الوزن الجاف لميسيليوم الفطريات.