Arab Gulf J. scient. Res. **2** (1), pp. 149-157 (1984)

Effect of Chromium Ions on the Growth of *Fusarium oxysporum* f.sp. *lycopersici* and *Cunninghamella echinulata*

M.I. Naguib, Nahed Z. Haikal and Souad Gouda

Botany Department, Faculty of Science, Cairo University, Cairo, Egypt

ABSTRACT. This investigation evaluates the role of chromium in the growth of a tomato phytopathogenic fungus (*Fusarium oxysporum* f.sp. *lycopersici*) and a tomato rhizospheric fungus (*Cunninghamella echinulata*).

Hexavalent chromium was much more toxic in the linear growth of the two experimental fungi than the trivalent ion, *Cunninghamella echinulata* being more tolerant to the large doses than *Fusarium oxysporum* f.sp. *lycopersici*. In the meantime, chromate and chromic had less effect on dry weight gain in *Fusarium oxysporum* than dichromate. Under all conditious, the inhibition of dry weight gain increased with the rise of concentration of any chromium ions but was partially overcome with age.

During the last decade, the role of trace amounts of heavy metals in the growth and metabolism of lower plants has attracted the attention of a number of investigators. Thus, Lamb and Tollefson (1978) reported that cupric ions were most toxic, followed by chromate and chromic to sludge microorganisms. Bieszkiewicz and Hoszowski (1978) proved that concentrations, of the above mentioned ions, above 0.8, 20 and 15 mg per litre, respectively, inhibited the purification efficiency of activated sludge microorganisms as indicated by their reduced intensity of respiration. Trivalent chromium has been listed as one of the most effective inhibitors of nitrification in soils (Laing and Tabatabai 1978); average inhibition being more than 50%. Similarly chromium reduced aryl sulphatase activity in soils (Al-Khafaji and Tabatabai 1979) to varying extents, dependent on type of soil.

In the meantime, Mangi *et al.* (1978) considered chromium (VI) and CrO^{2-} as moderately toxic to several reverine algae and to a lesser extent to duckweeds. Yongue *et al.* (1979) noticed that *Euglena gracilis* could tolerate 1 mg/L CrO_3 for 3 hr at room temperature. Petriya (1978) proved that growth and photosynthesis

of *Chlorella vulgaris* were stimulated at 10^{-4}-10^{-5}M chromium salts but larger doses were inhibitory.

Bacteria seem to be more sensitive to chromium than other microorganisms. According to Petrilli and de Flore (1977) all hexavlent chromium compounds caused complete inhibition of bacterial growth at doses of 400-500 μg per plate but the trivalent chromium compounds were neither toxic nor mutagenic to *Salmonella typhimurium*. Baldry *et al.* (1977) noticed that *Klebsiella aerogenes* was more tolerant to the trivalent than hexavalent ion.

Fungi seem to be more tolerant of heavy metals than other microorganisms. Schmidt and Ziemer (1976) were of the opinion that substrate, mass of inoculum and hyphal lesions were the primary influencing factors whereas adaptive or genetic phenomena did not participate in inducing high tolerance of fungi.

In this investigation, we tried to elucidate the effect of chromium on growth criteria of the pathogenic *Fusarium oxyporum* f.sp. *lycopersici* and the most common rhizospheric fungus, *Cunninghamella echinulata*, both of which were isolated from roots of the Money Maker tomato variety.

Material and Methods

The pathogenic fungus, *Fusarium oxysporum* f.sp. *lycopersici* was isoloted from wilted tomato plants, whereas the saprophytic fungus, *Cunninghamella echinulata*, was isolated from the rhizosphere of the same plant. Both fungi were purified and identified under the supervision of Prof. M.S. El-Abyad; Professor of Mycology, Faculty of Science, Cairo University, following the keys mentioned by Booth (1971) and Gilman 1957).

The procedures of Bollen (1972) were adopted for the measurement of the linear growth. 9 ml modified Dox agar (Naguib, 1967) were distributed in test tubes and autoclaved. 1 ml of cold-sterile, concentrated solution of the various chromiuum salts (Ammonium chromate, ammoniums dichromate and chromic sulplate) was added to the agar medium while still hot then asceptically poured in sterile 9-cm diameter Petri dishes. The final chromium concentrations would be 0.005, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 mM. 5 mm diameter disc from the perifery of 6-day old fungus mats was centrally inoculated in the agar media and daily measurements of the colony diameter were carried out after incubation at 28°C in the dark. This was performed on 5 replicate dishes for each chromium concentration, each chromium salt as well as controls.

Similar experiments were carried out using 100 ml aliquots of liquid modified Dox medium containing the same concentrations of the chromium salts in order to obtain the dry weight of the produced mycelial felts at 2-day (*Cunninghamella*) or 3-day (*Fusarium*) intervals. Here, the mycelial felts were separated from their

media by suction, thoroughly washed with distilled water and drained by suction. The mycelial pellets were then placed in a hot air drying oven at 110°C for one hour, then temperature was lowered to 80°C. The mycelia were left to dry till constant weight. Five replicate samples were used in each treatment including the controls. The modified Dox medium has the following constitution:

20 g sucrose, 4 g potassium nitrate, 2 g potassium dihydrogen phosphate, 1 g magnesium sulphate, 0.5 g sodium chloide, 0.1 ferrous sulphate, all dissolved in one litre solution.

Results

1. *Daily Linear Growth Rate*

Table 1 shows that during the first two days of the growth period, 0.005 and 0.01 mM Cr^{3+} did not prevent growth starting of *Cunninghamella*, whereas Cr^{6-} had little or no effect. With the lapse of time, chromic ions seemed to have a slight, if any, effect, whereas chromate ions lowered the daily growth rate of the fungus, particularly during the last two days and more prominently at higher concentrations. Further rise of cationic chromium concentration favoured a slower growth rate that was highly apparent during the early period of growth, but overcome with progress of age. On the other hand, raising the anionic chromium concentrations above 0.05 mM arrested growth of *Cunninghamella* for one or several days (dependent on concentration) followed by a comparatively low growth rate that was overcome only at 0.1 or 0.2 mM level. In most cases, dichromate was less effective than chromate in this respect. Under all conditions, growth of *Cunninghamella* was totally arrested above 0.8 mM concentration, even after 5 days incubation.

However, although chromic ions seemed to have similar effects on the daily growth rate of *Fusarium* and *Cunninghamella*, the inhibitory effect of the ion, particularly in larger doses, was not overcome even with passage of time. Hexavalent chromium lowered the daily growth rate of the fungus to a larger extent than the trivalent, the latter being more effective than chromate. The fungus could grow at 0.8 mM chromate but its growth was completely arrested above 0.2 mM dichromate.

2. *Dry Weight Gain*

Fig. 1 shows that during the first sample, 0.005 mM chromium salts resulted in a slight increase in dry weight gain in both fungi; more prominently chromic ion. This was followed by a gradual decline to the control level with time. Further increases in the chromium concentration gave a reduced rate of dry weight gain until complete cessation of growth (at 0.2 mM chromate) by both organisms during the first 2 or 3 days. Chromic, at the same level, had similar effects on *Cunninghamella echinulata*, whereas 0.2 mM dichromate arrested the growth of *Fusarlum*

Table 1. Effect of selected concentrations of various chromium ions on the daily linear growth rate of *Cunninghamella echinulata* and *Fusarium oxysporum lycopersici*. (Original diameter 5 mm. Mean of 5 replicates, in mm.)

Duration	Organism	Chromium ion	mM Chromium ion										
			0	0.005	0.01	0.05	0.1	0.2	0.4	0.6	0.8	1.0	2.0
1st Day	*C.echinulata*	Chromate	6.0 ± 0.22	6.0 ± 0.21	5.1 ± 0.18	4.3 ± 0.16	–	–	–	–	–	–	–
		Diochromate	6.0 ± 0.22	8.3 ± 0.29	7.1 ± 0.24	4.4 ± 0.17	–	–	–	–	–	–	–
		Chromic	6.0 ± 0.22	10.0 ± 0.33	8.3 ± 0.29	6.4 ± 0.22	5.6 ± 0.21	5.7 ± 0.20	4.1 ± 0.15	4.9 ± 0.18	3.3 ± 0.14	3.5 ± 0.15	3.1 ± 0.11
	F.oxysporum lycopersici	Chromate	5.6 ± 0.21	7.3 ± 0.26	6.7 ± 0.24	5.8 ± 0.21	3.6 ± 0.14	–	–	–	–	–	–
		Dichromate	5.6 ± 0.21	5.2 ± 0.20	4.7 ± 0.18	3.4 ± 0.14	–	–	–	–	–	–	–
		Chromic	5.6 ± 0.21	8.8 ± 0.29	7.5 ± 0.27	7.3 ± 0.27	6.9 ± 0.23	5.4 ± 0.20	4.3 ± 0.16	4.5 ± 0.18	3.2 ± 0.14	3.3 ± 0.15	2.7 ± 0.12
2nd Day	*C.echinulata*	Chromate	24.1 ± 0.76	19.2 ± 0.62	20.3 ± 0.64	11.7 ± 0.38	5.3 ± 0.19	–	–	–	–	–	–
		Dichromate	24.1 ± 0.76	24.1 ± 0.77	27.4 ± 0.86	23.2 ± 0.74	6.5 ± 0.23	–	–	–	–	–	–
		Chromic	24.1 ± 0.76	35.1 ± 0.16	27.7 ± 0.86	25.6 ± 0.79	20.8 ± 0.67	20.9 ± 0.66	21.5 ± 0.70	21.6 ± 0.70	12.3 ± 0.40	12.4 ± 0.41	12.2 ± 0.40
	F.oxysporum lycopersici	Chromate	11.2 ± 0.38	9.6 ± 0.33	8.3 ± 0.28	6.1 ± 0.21	5.4 ± 0.18	–	–	–	–	–	–
		Dichromate	11.2 ± 0.38	10.6 ± 0.36	6.4 ± 0.23	4.4 ± 0.16	5.7 ± 0.19	5.8 ± 0.21	–	–	–	–	–
		Chromic	11.2 ± 0.38	17.6 ± 0.57	8.8 ± 0.31	8.5 ± 0.29	8.7 ± 0.31	7.3 ± 0.26	7.4 ± 0.26	7.1 ± 0.25	6.6 ± 0.24	6.9 ± 0.25	5.4 ± 0.20
3rd Day	*C.echinulata*	Chromate	31.3 ± 1.05	30.6 ± 1.04	30.7 ± 0.97	21.1 ± 0.66	20.6 ± 0.65	5.5 ± 0.22	–	–	–	–	–
		Dichromate	31.3 ± 1.05	36.2 ± 1.20	30.6 ± 1.04	11.3 ± 0.37	10.7 ± 0.35	5.4 ± 0.20	–	–	–	–	–
		Chromic	31.3 ± 1.05	20.6 ± 0.67	21.2 ± 0.71	36.9 ± 1.52	31.2 ± 0.99	30.9 ± 0.98	25.4 ± 0.80	21.2 ± 0.67	30.7 ± 0.95	29.8 ± 0.95	26.6 ± 0.85
	F.oxysporum lycopersici	Chromate	13.3 ± 0.45	16.8 ± 0.55	12.7 ± 0.43	14.3 ± 0.48	15.6 ± 0.52	9.5 ± 0.33	5.2 ± 0.18	5.6 ± 0.20	4.0 ± 0.15	–	–
		Dichromate	13.3 ± 0.45	10.9 ± 0.37	14.2 ± 0.47	15.9 ± 0.52	14.3 ± 0.48	10.6 ± 0.37	–	–	–	–	–
		Chromic	13.3 ± 0.45	21.6 ± 0.70	11.4 ± 0.37	15.3 ± 0.51	16.4 ± 0.55	17.4 ± 0.57	15.6 ± 0.50	14.5 ± 0.47	15.8 ± 0.50	14.9 ± 0.50	10.7 ± 0.36
4th Day	*C.echinulata*	Chromate	12.5 ± 0.42	21.5 ± 0.69	20.8 ± 0.68	20.6 ± 0.66	15.8 ± 0.51	10.7 ± 0.36	5.6 ± 0.21	4.8 ± 0.16	–	–	–
		Dichromate	12.5 ± 0.42	17.4 ± 0.56	8.3 ± 0.28	18.9 ± 0.61	7.6 ± 0.28	12.8 ± 0.43	15.1 ± 0.50	4.3 ± 0.17	–	–	–
		Chromic	12.5 ± 0.42	20.4 ± 0.65	21.1 ± 0.68	23.2 ± 0.74	16.3 ± 0.53	15.6 ± 0.53	15.4 ± 0.49	20.1 ± 0.65	21.4 ± 0.70	20.7 ± 0.65	16.3 ± 0.53
	F.oxysporum lycopersici	Chromate	14.6 ± 0.46	15.9 ± 0.53	16.8 ± 0.53	8.6 ± 0.30	11.3 ± 0.36	15.1 ± 0.50	5.6 ± 0.20	5.1 ± 0.19	4.8 ± 0.19	–	–
		Dichromate	14.6 ± 0.46	21.3 ± 0.66	6.8 ± 0.25	7.9 ± 0.28	6.4 ± 0.21	11.2 ± 0.35	–	–	–	–	–
		Chromic	14.6 ± 0.46	18.4 ± 0.60	14.7 ± 0.47	16.2 ± 0.52	14.3 ± 0.45	13.8 ± 0.45	10.2 ± 0.35	6.4 ± 0.24	5.9 ± 0.21	7.3 ± 0.25	6.2 ± 0.24
5th Day	*C.echinulata*	Chromate	12.7 ± 0.44	20.9 ± 0.65	20.4 ± 0.65	21.6 ± 0.68	8.7 ± 0.30	5.3 ± 0.19	5.8 ± 0.20	5.3 ± 0.20	5.8 ± 0.22	–	–
		Dichromate	12.7 ± 0.44	7.8 ± 0.26	7.5 ± 0.25	16.6 ± 0.53	12.6 ± 0.44	3.3 ± 0.12	4.8 ± 0.18	3.1 ± 0.12	5.6 ± 0.21	–	–
		Chromic	12.7 ± 0.44	11.7 ± 0.40	19.3 ± 0.62	21.4 ± 0.65	24.6 ± 0.78	25.7 ± 0.80	30.9 ± 0.96	29.6 ± 0.95	28.8 ± 0.90	15.8 ± 0.50	9.7 ± 0.35
	F.oxysporum lycopersici	Chromate	27.7 ± 0.86	15.4 ± 0.50	17.1 ± 0.55	8.7 ± 0.30	7.9 ± 0.25	8.1 ± 0.27	5.8 ± 0.20	8.9 ± 0.30	10.2 ± 0.35	–	–
		Dichromate	27.7 ± 0.86	15.8 ± 0.51	11.2 ± 0.36	9.3 ± 0.32	8.6 ± 0.28	9.5 ± 0.33	–	–	–	–	–
		Chromic	27.7 ± 0.86	11.5 ± 0.37	8.6 ± 0.30	11.4 ± 0.37	9.6 ± 0.31	10.6 ± 0.39	9.3 ± 0.32	8.7 ± 0.31	6.8 ± 0.25	6.9 ± 0.25	2.6 ± 0.10

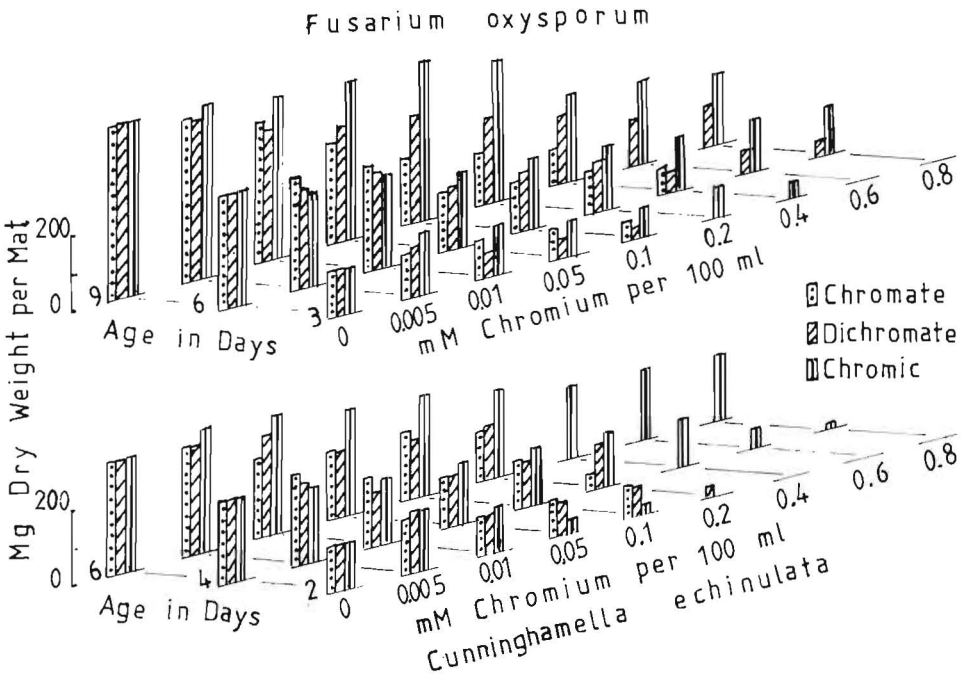

Fig. 1. Effect of selected concentrations of various chromium ions on the dry weight gain by mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* f.sp. *lycopersici* during 6 and 9 days growth period, respectively.

oxysporum f.sp. *lycopersici*. However, the *Fusarium* could grow on chromic media to 0.4 mM concentration. In time, both organisms grew on chromic or chromate up to 0.8 or 0.2 mM, respectively, where the *Fusarium* could continue growth on dichromate media to 0.8 mM level but *Cunninghamella* could tolorate only 0.2 mM of the ion.

Under all conditions, the inhibition of dry weight gain increased with the rise of concentration of any chromium ions but was partially overcome with age.

Figure 2 shows that, at the same age, chromate and dichromate ions caused almost the same percentage growth inhibition in both organisms, whereas the drop in dry weight gain, in chromic media, was far greater in the *Fusarium* than in the *Cunninghamella*.

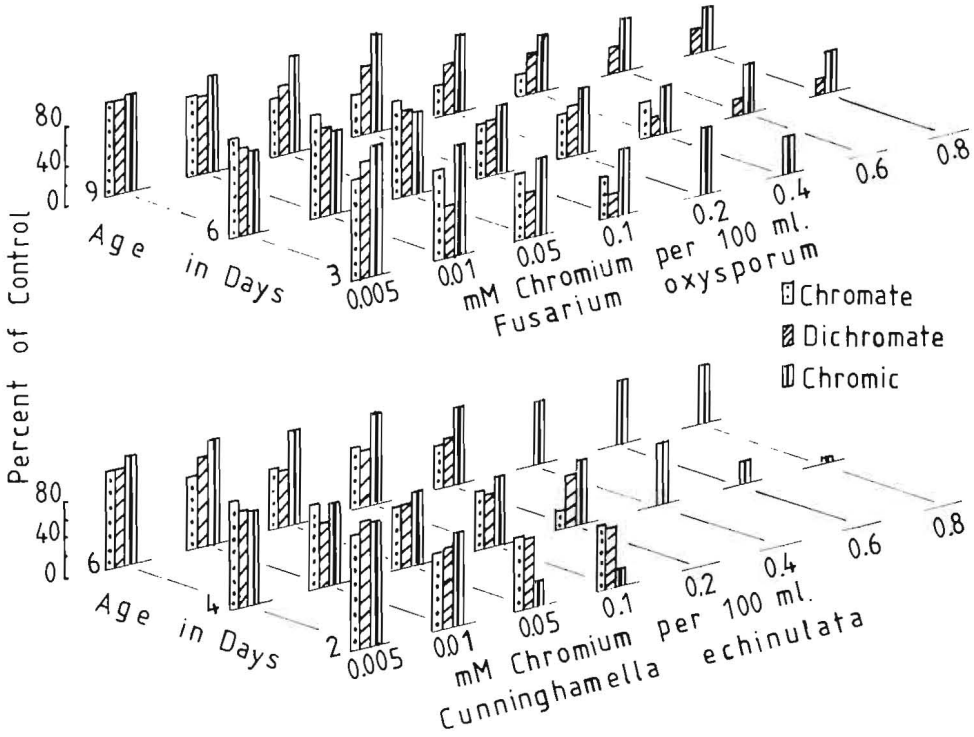

Fig. 2. Effect of selected concentrations of various chromium ions on relative gain in dry weight of mycelial felts of *Cunninghamella echinulata* and *Fusarium oxysporum* f.sp. *lycopersici* during 6 and 9 days growth period, respectively.

Discussion

This investigation demonstrates the higher toxicity, as evidenced by the daily rate of linear growth of the two experimental fungi, of hexavalent (anionic) as opposed to trivalent (cationic) chromium. Furthermore, *Cunninghamella echinulata* seemed to be more tolerant of high concentrations of chromic ions than *Fusarium oxysporum* f.sp. *lycopersici*. This is confirmed by the remarkable difference in growth rate of both fungi that was most apparent with progress of age. Furthermore, in spite of the fact that the *Fusarium* seemed to be more resistant to hexavalent chromium than the *Cunninghamella*, during the early periods of growth, yet with time, the *Cunninghamella* was also tolerant to the larger doses of such ion, in both of its forms, whereas tolerance of *Fusarium* to dichromate lagged behind that to chromate. At the same dose, chromate seemed less toxic than dichromate for both fungi. At low concentration, chromate stimulated the linear growth of *Cunninghamella* but inhibited that of the *Fusarium*, particularly towards

the end of the experimental period, whereas dichromate was mostly suppressive to growth of both fungi. Anionic chromium seemed to be more toxic than the cationic form, with dichromate being more effective than chromate.

These results are in accordance with the observations of Skeffington *et al.* (1976) and Fiussello and Novo (1977) that Cr^{6+} was more toxic than Cr^{3+} to crop plants including *Triticum vulgare, Phalaris canariensis, Pisum sativum, Hordium vulgare, Secala cereala, Linum usitatissimum, Phaseolus vulgaris, Raphanus raphanistrum, Lycopersicon esculentum* and *Zea mays*. Jan and Young (1978) reported that hexavalent chromium was the most toxic chemical species discharged to the sea through municipal waste water. It formed 75% of the chromium in uncontaminated coast sea waters of southern California.

These observations were confirmed by studies on the dry weight gain by both fungi, where parallel results were obtained although the fungi could then withstand to 0.8 mM and not 2 mM. Inoculum size and prolonged exposure to the ions are responsible for the apparent larger tolerance of *Fusarium* than *Cunninghamella* to the hexavalent forms. However, chromic seemed less toxic to the *Cunninghamella* than to the *Fusarium*. After 6 days incubation, the relative dry weight gain by the *Cunninghamella* was far greater than by the *Fusarium* in spite of the larger size of the latter's inoculum. At this stage of growth, the hexavalent ion exerted similar effects on both fungi.

Marsh *et al.* (1975) and Burzynska and Maciejska (1978) showed that 25 μg/ml tri- or hexavalent chromium did not influence the dry weight gain by various strains of *Aspergillus*. Similarly, Petrilli and de Flore (1977) concluded that 400-500 μg of hexavalent chromium per plate completely inhibited growth of *Salmonella typhimurium*. Similar observations were obtained by Baldry *et al.* (1977) working on *Klebsiella aerogenes*.

References

Al-Khafaji, A.A. and **Tabatabai, M.A.** (1979) Effects of trace elements on arylsulfatase activity in soils, *Sci.* **127:** 129-133.

Baldry, M.G.C., Hogarth, D.S. and **Dean, A.C.R.** (1977) Chromium and copper sensitivity and tolerance in *Klebsiella* (*Aerobacter*) *aerogenes, Microbios. Lett.* **4:** 7-16.

Bieszkiewicz, E. and **Hoszowski, A.** (1978) Effect of copper and tri- and hexavalent chromium on the work of an activated studge, *Acta Microbiol. Pol.* **27:** 147-154.

Bollen, G.J. (1972) A comparison of the vitro antifungal spectra of thiophantes and benomyl, *Neth. J. Plant. Pathol.* **78:** 55-64.

Booth, C. (1971) *Genus of Fusarium*, Common Wealth Mycological Institute, Kew, Surrey. England.

Burzynska, H. and **Maciejska, K.** (1978) The effect of some contaminants, occurring in food, on the growth of *Aspergillus flavus* and the production of aflatoxins, *Acta Aliment. Pol.* **4:** 119-128.

Fiusello, N. and **Novo, P.** (1977) The effect of chromium on plants, *Allionia* (*Turin*) **21**: 105-114.

Gilman, J.C. (1957) *A Manual of Soil Fungi*, Iowa State Univ. Press.

Jan, T. and **Young, D.R.** (1978) Chromium speciation in municipal waste water and seawater, *J. Water Pollut. Cont. Fed.* **50:** 2327-2336.

Laing, C.N. and **Tabatabai, M.A.** (1978) Effect of trace elements on nitrification in soils, *J. Ent. Qual.* **7:** 291-293.

Lamb, A. and **Tollefson, F.L.** (1978) Toxic effect of cupric chromate and chromic ions on biological oxidation, *Water Res.* **7:** 599-613.

Mangi, J., Schmidt, K., Pankow, J., Gaines, L. and **Turner, P.** (1978) Effects of chromium on some aquatic plants, *Environ. Pollut.* **16:** 285-292.

March, P.E., Simpson, M. and **Triuckesess, M.W.** (1975) Effect of trace metals on the production of aflatoxin, by *Aspergillus parasiticus, Microbiology* **30:** 52-57.

Naguib, M.I. (1967) Effect of colchicine on galactose absorption, carbon dioxide output and keto acid production by *Cunninghamella elegans, J. Bot. UAR* **10:** 19-24.

Petrilli, F.L. and **De Flore, S.A.** (1977) Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium, Environ. Microbiol.* **33:** 805-809.

Petriya, V. (1978) Influence of chromium salts from waste waters on physiological processes of the alga *Chlorella vulgaris, Rev. Roum. Biol.* **23:** 55-58.

Schmidt, O. and **Ziemer, B.** (1976) On preservative tolerance in fungi, *Mater Ore* (*Berlin*) **11:** 215-230.

Skeffington, R.A., Shervry, P.R. and **Peterson, P.J.** (1976) Chromium uptake and transport in barley seedlings, *Planta* (*Berlin*) **139:** 209-214.

Yongue, W.H. Jr., Berrent, B.L. and **Cairns, J. Jr.** (1979) Survival of *Euglena gracilis* exposed to sublethal temperature and hexavalent chromium, *J. Protozool.* **26:** 122-125.

(Received 30/10/1982;
in revised form 09/04/1983)

تأثير أيونات الكروم على نمو *الفيوزاريوم أكسيسبورم ليكوبرسيسي والكاننجها ميللا اكينولاتا*

محمد ابراهيم نجيب، ناهد زكريا هيكل، سعاد جوده
قسم النبات - كلية العلوم - جامعة القاهرة

تكشف هذه التجربة عن الدور الذى يلعبه عنصر الكروم فى نمو فطرة متطلفة على الطماطم (*فيوزاريوم اكسيسبورم ليكوبرسيسي*) وفطرة حول جذرية لنبات الطماطم (*كاننجها ميللا اكينولاتا*).

وقد تبين أن الكروم سداسى التكافؤ أكثر سميه من الكروم ثلاثى التكافؤ بالنسبة للنمو الطولى للفطرتين مع ملاحظة التحمل الأكبر لفطرة *الكاننجها ميللا* عن فطرة *الفيوزاريوم* للجرعات الكبيرة للمعدن.

وقد تبين أيضا أن أيون الكرومات وأيون الكروميك أقل تأثيرا عن أيون ثنائى الكرومات بالنسبة لزيادة الوزن الجاف لفطرة *الفيوزاريوم*. وفى جميع الحالات فقد ارتفع معدل تثبيط زيادة الوزن الجاف بزيادة تركيز أى من أيونات الكروم الثلاثة ولكن زيادة عمر الفطرة تقلل نوعا من هذا الأثر المثبط.