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INFLUENCE OF SELENIUM ON THE EFFICIENCY OF A FUNGICIDE ON CERTAIN FUNGI

A. A. Razak, H. EL-Tantawy, H. H. El-Sheikh and M. M. Gharieb**

Department of Botany, Faculty of Science, Al-Azhar Univ. and El-Menoufia Univ.* , EGYPT.

ABSTRACT

Aspergillus funiculosus was isolated from rotted banana fruits, while Alternaria tenuis and Fusarium sp. were isolated from rotted tomato fruits. The isolated fungi tolerated relatively ghigh levels of the fungicide; Dithane up to 2560 ppm. on solid medium, but grew well at 40 ppm. when supplemented with liquid medium. They are able to tolerate selenite up to 2% (w/v) sodium selenite. A. funiculosus showed no growth in the presence of mixture of 2.5 ppm selenium and 20 pmm Dithane, while Fusarium sp. failed to grow at 2.5 ppm selenium and 10 ppm Dithane or at 10 ppm of each. Neverthless, Alternaria tenuis is more tolerable, it showed growth in the presence of relatively high levels of selenium and Dithane; up to 10 ppm. selenium and 40 ppm. Dithane, however, its growth was inhibited by the presence of a mixture of both. The results suggested new form of highly active fungicides. Selenium as an essential nutirent at such very low concentrations, as well as the application of very low concentrations of the fungicide would certainly reduce the hazardous effect of such pollutant in the environment.

INTRODUCTION

Several metal ions are known to have a fungicidal action. The fungitoxic activities of several heavy metals; especially silver, mercury, copper, sulphur, cadmium, chromium, nickel, lead, zinc, arsenic and calcium attracted the attentions of several investigators since the nineteen's century (Rassow *et al.*, 1961, Martin, 1969). However, mostly inorganic compounds have contributed little to the control of fungal pathogens, the main reason for that was given by Martin (1969) that inorganic compounds with biological activity are nonspecific, possessing a general toxicity to both pathogen and host.

Although, sulphur is a good broad-specrum fungicide which controls a

multitude of diseases (Sharvella, 1961; Tweedy, 1969) a very serious problem that arises when sulphr is used as a fungicide is its phytotoxic effect on secveral plants (Tweedy, 1969).

On the other hand, organometallic compounds are more active fungicides than the metal ions, e.g. scarcely any biological effect is known for different forms of tin. However, trialkyltin compounds are classified with the most active fungicides (Kaars Sijpesteijn *et al.*, 1969).

Although, the volume of fungicides produced peaked in 1960 and fluctuated between 1961 and 1976 at levels somewhat below peak production (Sheets, 1980), several derivatives have been introduced recently. Dithane is a widely distributed fungicide; the active ingredients are a complex of zinc ions, 2% ; manganese, 16% and ethylene bisdithiocarbamate, 62%. Its marketed in the form of an 80% wettable powder. Generally, the applications of such high concentrations is a source of environmental pollution that have had advrese effect on plants and animals including human.

The present communication is a trial aiming at investigating the influence of certain heavy metals ; selenium, on the efficiency of a widely distributed sulphur containing fungicides ; Dithane, in order to minimize the application of high levels of such fungicides by increasing efficiency with such heavy metal.

MATERIALS AND METHODS

Fungicide : Manozeb (Mazeb, Dithane M 45) was purchased commercially as produced by Rohm and Hoss, U.S.A.. Its common name is Dithane.

Fungi : A seleno- as well as a fungicide-tolerant fungi were isolated from rotted fruits ; banana and tomato fruits on Dox medium supplemented with different concentrations of sodium slelenite; 0. 0.05, 0.1, 0.5, 1, 2 and 2.5% (w/v). The banana pathogen was identified as *Aspergillus funiculosus* according to Raper and Fennel (1977). While the tomato pathogens were identified as *Alternariatenuis and Fusarium* sp. according to kendrick *et al.* (1980). Ellis (1971) as well as Domsch *et al.*, (1980).

Determination of fungal mycelial dry weight :

0.1 ml. fungal spores suspension was inoculated into 100 ml. conical flasks capacity, containing 25 ml. Dox liquid medium supplemented with either different selenite concentrations, different Dithane concentrations or a mixutre of both. They were sterilized with diethylether for 24 hr. then supplied to the sterilized medium. The flasks were incubated at 28 °C, unless otherwise stated, for 7 days. The cultures were filtered and the produced mycelia were washed thoroughly with dist. water several times and dired at 60 °C for 3 days. Mean of triplicate set dry were taken as criterion for dry weight determinations.

Radial growth was measured by subculturing discs of 6 mm. diameter from the margin area of a young growing colony on correspondence Dox solid medium and incubated at 28 °C for 8 days.

Counting conidia :

Six millimeter disc was taken from the margin of a standardized growing colony as shown previously. The disc was transferred into a sterlie test tube with 2 ml. sterile distilled water, shacked for 5 minutes. Count was carried out in a known volume using Hemocytometer.

Protein determination :

Protein was determined according to the method of Lowry *et al.* (1951), using bovine serum albumin as a standard protein.

Carbohydrate determination :

Carbohydrates were determined according to the method uf Umbriet *et al.* (1959), using sucrose as a standrard carbohydrate.

Lipid determination :

Lipids content were determined by the phosphovanilline method according to Barnes and Blackstock (1973), cholesterol was used as a standard lipid.

RESULTS

The isolated fungi seems able to tolerate high levels of selenite, mostly, up to 2.5 % (w / v). The growth of the fungi were inhibited with increasing selenium concentrations in the environment (Table 1). The number of conidia were markedly inhibited as well. *A. funiculosus* were able to form conidia up to 2.5 % (w / v), while no conidia were detected at 1.5 and 2 % by *Fusarium* sp. and *A. tenuis* respectively.

On the other hand, the isolated fungi succeeded to grow up to 2560 ppm / ml in the presence of the fungicide Dithane in solid medium, but only up to 40 ppm / ml when provided with liquid medium (untabulated results). The *Fusarium* sp. was less tolerable than the other two fungi (Table 2). Generally, the gross growth as well as conidial formation were inhibited with increasing the fungicide in the environment.

Interestingly, the addition of selenite into the fungicide inhibited the fungal growth completely at very low concentrations. Generally, the radial growth of the fungal colonies were inhibited in the presence of a mixture of both selenium and Dithane.

At a mixture of 2.5 ppm selenium and 20 ppm Dithane was the lethal dose for *A. funiculosus*. Contrary to that, *Alternaria tenuis* was more tolerable, it grew up to 10 ppm selenium and 40 ppm Dithane (Table 3). Nevertheless, when the fungi were cultured on selenium and Dithane containing liquid medium, very low concentration of that mixture ceased the fungal growth (Table 4). At a mixture of 2.5 ppm selenium and 20 ppm Dithane, *A. funiculosus* showed poor growth. While it failed completely to grow at a mixture of 10 ppm of selenium and 20 ppm Dithane. Moreover, its growth in the presence of a mixture of 10 ppm of both selenium and Dithane was very poor, the obtained mycelial dry weight was unconsiderable. While the *Fusarium* sp. was less tolerable. In the presence of 2.5 ppm selenium and 5 ppm Dithane, it failed completely to grow. Even at 0.5 ppm selenium and 5 ppm Dithane, the jobtained mycelial dry weight was unconsiderable; approximately 95 % inhibition was obtained.

Table 1: Effect of different concentrations of selenite on the growth of the isolated fungi, cultivated on Dox media. Data are expressed as mean diameter of radial fungal growth (mm) as well as number of conidia.

Selenite conc. (% w/v)	*Aspergillus funiculosus* colony radius (mm)	*Aspergillus funiculosus* No. of conidia (x 4 x 10^4)	*Fusarium* sp. colony radius (mm)	*Fusarium* sp. No. of conidia (x 4 x 10^4)	*Alternaria* sp. colony radius (mm)	*Alternaria* sp. No. of conidia (x 4 x 10^4)
0.00	57.1	251.6	77.3	24.0	73.83	21.66
0.05	52.0	189.3	74.1	58.3	66.16	15.33
0.10	27.3	120.0	60.0	116.3	63.66	11.66
0.50	26.6	71.6	20.8	118.3	40.66	7.66
1.00	24.6	40.6	16.3	120.3	19.50	8.33
1.50	21.1	20.3	12.6	000.0	14.16	6.33
2.00	19.8	17.6	11.5	000.0	10.66	00.00
2.50	17.1	16.6	9.1	000.0	00.00	00.00

Table 2: Mycelial dry weight (mgm), mean diameter of radial growth (mm) and number of conidia / cm^2 (x 4 x 10^4) per of the isolated fungi as influenced by different concentrations of Dithane, supplemented to Dox medium.

Dithane conc. (ppm)	*Aspergillus funiculosus* Dry weight	*Aspergillus funiculosus* Mean diameter	*Aspergillus funiculosus* No. of spores	*Fusarium* sp. Dry weight	*Fusarium* sp. Mean diameter	*Fusarium* sp. No. of spores	*Alternaria* sp. Dry weight	*Alternaria* sp. Mean diameter	*Alternaria* sp. No. of spores
00.0	363.3	00.0	77.7	204	88.8	71.0	376.0	85.0	28.0
5.0	356.7	00.0	85.0	209	84.8	23.3	375.7	50.8	18.0
10.0	358.0	73.5	82.7	141	76.8	14.0	351.7	43.8	10.0
20.0	317.7	64.2	81.0	120	71.0	6.0	249.7	31.5	11.0
40.0	38.0	56.5	71.0	000	19.6	2.3	23.7	9.0	9.7

Table 3: Effect of mixtures of selenite and Dithane on the radial growth (mm) of the isolated fungi; *A. Funiculosus* (AF), *Fusarium* sp. (F) and *Alternaria tenuis* (AT), cultivated on Dox medium.

Dithane conc. (ppm)	Selenite concentrations (ppm).														
	0.0			0.1			0.5			2.5			10		
	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT
00.0	57.2	89.9	57.5	60.0	89.1	76.2	80.5	87.3	79.0	72.5	88.5	78.2	57.7	85.1	57.0
5.0	80.2	78.5	65.0	72.8	77.7	64.8	71.3	67.7	64.5	65.3	61.0	64.8	49.8	49.2	59.0
10.0	65.0	62.7	58.5	62.8	41.3	57.7	60.8	19.2	55.5	58.8	00.0	56.7	55.5	00.0	47.5
20.0	5.3	16.8	54.2	49.2	00.0	52.8	41.5	00.0	49.2	00.0	00.0	44.3	00.0	00.0	40.5
40.0	00.0	00.0	45.6	00.0	00.0	40.2	00.0	00.0	32.5	00.0	00.0	34.7	00.0	00.0	10.5

Table 4: Effect of different concentrations of selenium and Dithane mixtures on the mycelial dry weights of the isolated fungi; *A. Funiculosus* (AF), *Fusarium* sp. (F) and *Alternaria tenuis* (AT), cultivated on Dox medium.

Dithane conc. (ppm)	Selenite concentrations (ppm).														
	0.0			0.1			0.5			2.5			10		
	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT
00.0	120.3	223.3	46.7	125.0	281.0	74.3	177.9	288.0	67	134.3	240.7	59.0	126.7	247.3	43.3
5.0	144.0	259.6	45.0	133.0	95.0	46.0	157.0	43.3	45	118.0	000.0	70.0	111.3	000.0	66.0
10.0	156.7	226.0	62.3	170.7	32.0	117.3	125.7	000.0	102	108.3	000.0	44.7	12.0	000.0	41.7
20.0	118.0	36.0	118.3	114.7	17.3	83.3	84.0	000.0	41	23.3	000.0	19.3	000.0	000.0	00.0
40.0	69.7	000.0	43.3	51.7	000.0	40.0	14.7	000.0	000	000.0	000.0	00.0	000.0	000.0	00.0

On the other hand , the *Alternaria* sp. was more tolerable than the other two fungi; it grew up to a mixture of 0.5 ppm selenium and 40 ppm Dithane as well as 2.5 ppm selenium and 20 ppm Dithane and 10 ppm of both selenium and Dithane. Moreover, the exerted inhibition on its mycelial formation at different concentrations was insignificant. Its growth in the presence of different concentrations of either selenium or Dithane was nearly similar, the obtained mycelial dry weights were fluctuated within a narrow range.

It worth to mention that the same treatments were carried out on banana and tomato natural media, the results were similar to that on Dox media.

Data in Table (5) show that the conidiogenesis in the studied fungi were drastically inhibited in the epresence of both selenium and Dithane. *Aspergillus funiculosus* was alble to conidiate up to 40 ppm Dithane and 10 ppm selenium or even more when supplemented separately. However, over 2.5 ppm selenium and 10 ppm Dithane, no conidia were observed. Since, the pathogenicity stored by invasion of fungal spores. It seems so crucial to investigate the influence of selenium and Dithane on sporogenesis (Table 5). The result shows that sporogenesis in *A. funiculosus* and the *Fusarium* sp. were drastically affected, while, *Alternaria tenuis* seems not hardly affected. Number of conidia were decreased sharply by the treatment with a mixture of both selenium and Dithane. At a mixture of 2.5 ppm selenium and 20 ppm, Dithane, the *Aspergillus* isolate failed to form any conidia, while the *Fusarium* sp. failed to form any conidia at 2.5 ppm selenium and 10 Dithane. At lower concentrations of their mixture, the number of conidia were reduced considerably. Contrary to that, the conidia formation by *Alternaria tenius* fluctuated irregularly in the presence of different concentrations of selenium and Dithane. The obtained results in this case are amazing and no conclusive action could be derived. Nevertheless, data in Table (6) show that the presence of selenium in the growth environment increased the *Aspergillus* cellular contents of carbohydrates, proteins and lipids while the presence of Dithane decreased the cellular contents of lipids and carbohydrates, but increased the protein contents. Nevertheless, a mixture of 0.5 ppm selenium and 20 ppm. Dithane increased the cellular contents of carbohydrates, proteins and mostly decreased the lipid contents.

Table 5: Effect of different concentrations of selenium and Dithane mixtures on tconidiogenesis of the Fungal isolates; *A. Funiculosus* (AF), *Fusarium* sp. (F) and *Alternaria tenuis* (AT), cultivated on Dox medium. Data are expressed as number of conidia ($x\ 4 \times 10^4$) / cm^2 of the fungal colony.

Dithane conc. (ppm)	Selenite concentrations (ppm).														
	0.0			0.1			0.5			2.5			10		
	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT	AF	F	AT
00.0	194.3	57.3	77.0	183.7	60.3	7.0	177.3	59.0	4.3	159.0	70.7	5.0	83.7	73	10.7
5.0	68.0	4.0	8.0	69.3	4.3	2.3	70.0	10.0	6.0	69.0	12.0	5.3	68.0	13	3.7
10.0	48.3	6.7	8.3	50.3	9.6	6.0	52.0	20.3	3.0	69.3	00.0	3.0	59.0	00	4.7
20.0	44.0	13.0	4.7	45.0	00.0	4.7	51.1	00.0	6.7	000.0	00.0	6.3	00.0	00	7.3

Table 6: Influence of mixtures of dithane and selenium on the fungal cellular contents carbohydrates, proteins and lipids.

Dithane conc. (ppm)		*Aspergillus funiculosus*		*Fusarium* sp.		*Alternaria teuias.*	
		Selenium concentrations (ppm).					
		Control	0.5	Control	0.5	Control	0.5
Control	Carbohydrates	9542.17	10112.68	3680.92	3602.45	21792.49	18752.4
	Proteins	3971.29	6762.49	1497.88	1352.87	6242.23	6023.8
	Lipids	6983.40	9427.60	1518.94	1916.74	5421.59	2851.9
20	Carbohydrates	8078.77	11362.5	1309.91	4829.17	9600.59	18195.7
	Proteins	4998.83	10029.92	2031.37	2719.78	6375.69	7192.86
	Lipids	1593.84	3067.60	383.80	1156.00	3613.95	795.49

The picture of the *Fusarium* sp. is somewhat different. The presence of selenium has no marked influence on the carbohydrates and protein contents, while increased the lipids content. On the other hand, the presence of Dithane decreased the cellular contents of both carbohydrates and lipids while increased the protein content.

A mixture of 0.5 ppm slelenium and 20 ppm Dithane increased the protein and to some extent carbohydrate content, while, the lipids content was relatively decreased, presumably, due to the presence of selenium.

In the *Alternaria* sp., the presence of selenium slightly decreased the carbohydrates and the protein contents but markedly decreased the lipids content.

Alternatively, the presence of Dithane decreased carbohydrates and lipids, but has unconsiderable action on the protein content. Similar pattern was obtained by the presence of a mixture of 0.5 ppm selenium and 20 ppm Dithane.

DISCUSSION

Environmental pollution generally results from industrialization particularly with the chemical revolution. Plenty of foreign chemical complexes have become an integral part of the biosphere. Unfortunately, the contaminants are extensively introduced by man. We must confess that, as the problem has been produced by human and their activities, so it must be solved by human as well, each in his field.

Extensive flow of newly introduced fungicides are in progress enormously. Fungicides are catregorized as pollutants. However, the survival of mankind depends upon the economical and abundant production of foods, this wouldn't be ensured otherwise causative agents, e.g. fungi, are destroyed. Consequently, fungicides are to be used crucially.

Nevertheless, fungicides or pesticides in general can be transformed microbiologically to new toxicants that act on their host. Some fungicides may be converted to human-carcinogens (Stirling, 1980).

Fortunately, the presented results showed the increase of the efficiency of the fungicide, Dithane, by adding very low concentrations of selenium. Interestingly,

the tested fungi were completely inhibited by the applications of very low concentrations of selenium and the fungicide Dithane. Such finding would certainly open new era in the aplicable uses of fungicides. Serving in two ways; firstly, the use of low concentrations of fungicides will indeed avoid the disruption of the environment by minimizing the distribution of pollutants. Secondly, the addition of selenium in such very low concentrations will serve as nutrient for the host plant or even to the feeder animal including man. It was reported that selenium is an essential nutrient, as well as integral part of certain enzymes and may have a role in other biologically active compounds (Frost, 1972 ; Schroder and Mitchner, 1972). Neverthless, methods used to provide nutritionally required selenium include parenteral injection (1 mg. 50 kg), addition to compelete feeds (0.1 - 0.2 µg / g) and application of small amounts to the soil or foliage (Cary and Alloway, 1973).

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الملخص العربى

تأثير السيلينيوم على فاعلية مبيد الفطريات على فطريات معينة

تحملت فطريات الأسبرجلس فينيكلوزيس المعزولة من ثمار الموز والألترناريا تنيس والفيوزاريوم المعزولين من ثمار الطماطم تركيزات مرتفعة نسبيا من دايثان (حتى تركيز ٢٥٦٠ جزء فى المليون وذلك عندما نميت على وسط غذائى صلب ولكن لم يتجاوز تحملها تركيز ٤٠ جزء فى المليون عند الأنبات على الوسط الغذائى الصلب .

كما نمت هذه الفطريات متحملة تركيزات مختلفة من عنصر السيلنيوم ٢٪ (وزن /جم)

وقد أورت الفطريات المذكورة تبانيا فى درجة التحمل والنمو على التركيزات المختلفة من خليط كل من السيلنيوم والدايثان .

وقد أثبتت الدراسة أن قطرة الأسبرجلس فينيكلوزيس انعدم نموها عند تركيز ٢ر٥ جزء فى المليون من السيلينيوم +٢٠جزء فى المليون من الدايثان وقد انعدم ايضا نمو فطرة الفيوزاريم عند تركيز ٢ر٥ جزءفى المليون سيلينيوم + ١٠ أجزاء فى المليون من الدايثان.

وجد أن فطرة الالترناريا التى استطاعت النمو وتحمل مستوياً مرتفعة من تركيز السلينيوم (١٠جزء فى المليون) وكذلك تركيزات مرتفعة من المبيد (٤٠جزء فى المليون) يثبط نموها عندما عوملت بخليط من كل من السيلنيوم والمبيد عند نفس مستوى التركيزات .

ويخلص البحث إلى إقتراح تصور جديد فعال لمبيد فطرى يحوى تركيز منخفض من السيلينيوم وأيضا تركيز منخفض من المبيد الفطرى تكون دون مستوى التلوث مما من شأنه إختزل تلوث البيئة .