JKAU: Sci., vol. 9, pp. 17-27 (1417 A.H. / 1997 A.D.)

Study of the Single and Combined Genotoxic Effects of the Insecticide Furadan and Cadmium Nitrate in *Aspergillus terreus*

JAMAL S.M. SABIR
Biology Dept., Faculty of Science,
King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. In attempt to investigate the genotoxicity of the insecticide Furadan and the heavy metal cadmium in the form of cadmium nitrate in *Aspergillus terreus*, two main treatments were involved:

1. Single treatment, which consists of: **a** - Treating the conidia of the fungus with three different concentrations of Furadan (0.3g/10ml, 0.35g/10ml and 0.4g/10ml conidial suspension.) for 0, 15, 30, 45, 60 minutes of exposure. **b** - Treating the conidia with three different concentration of cadmium nitrate (0.0038g/10ml, 0.038g/10ml and 0.38g/10ml) for 0, 15, 30, 45, 60 minutes of exposure.

2. Combined treatment, which also consists of: **a.** Treating the conidia with the optimal dose of Furadan followed by the optimal dose of cadmium nitrate, and vise versa in **b** -. In **c** - the conidia were treated by a mixture of the optimal doses of the two substances.

As a results, it was observed in both of the two single treatments that, the increase of Furadan or cadmium nitrate concentration and exposure time, was always met by a decrease in survivals and an increase up to a certain peak in mutation frequency.Whereas, an antagonistic effect on both lethals and mutation induction was obtained in the first two combined treatments, and a synergistic effect on induced mutation occurred in the final combined treatment.

Introduction

It has been reported by Pusztai[1], Sharma and Singh[2], Sabir and Baeshin[3] and Baeshin *et al.*[4] in a different studies that, the commonly-used agricultural insecticide Furadan, is as potent genotoxic agent as the well known chemical mutagen (N.T.G.), when studied either in a single treatment or combined with some other genotoxic substances such as heavy metals.

The heavy metal cadmium, tended to have similar genotoxic effects in some organisms. Mitra and Bernstein[5], found that, cadmium chloride caused a DNA breakage in

E. coli. Ochi *et al.*[6] and Ochi and Ohsawa[7], stated the ability of cadmium in induction of mutation in Chinese hamster. Similar result were obtained by Mandel and Ryser[8], following the treatment of Salmonella by cadmium. In this study, an attempt was made to elucidate the genotoxic effects of the two substances individually and simultaneously.

Materials and Methods

1. Strain

The wild type strain of *A. terreus* was used throughout this study. It was obtained from the Department of Biology, Faculty of Science, K.A.U., Jeddah, where it had been maintained for several years. It was isolated from Makkah road by El-Sharqawi *et al.*[9]. It has been identified by the Commonwealth Mycological Institute, Kew, Surrey, England.

2. Chemicals

a. The insecticide (2,3-dihydro-2,3-dimethyl-7-benzofuranyl methyl carbamate), commercially known as Furadan was supplied by the distributor in Saudi Arabia, Al-Selouly Agricultural Est., Jeddah.

b. Cadmium nitrate was supplied by BDH Reagents Laboratories, England.

3. Media

a. Synthetic Media

Difco Czapek dox agar medium (Dox) was used as a minimal medium. The composition (gram/lit) of this medium as indicated by the supplier (Difco Laboratories, Detroit, Michigan, U.S.A.) is as follows: 2.0g sodium nitrate ($NaNO_3$); 0.5g potassium chloride (KCl); 0.5g magnesium glycerophosphate; 0.1g ferrous sulfate ($FeSO_4$); 0.35g potassium sulphate (K_2SO_4); 30.0g sucrose; 12.0g oxoid agar.

b. Non-synthetic Media

Modified Prune-Extract agar medium (PE) was employed as the complete medium. It was prepared as reported by Talboys[10] according to the formula: prune 5.0g, yeast extract 1.0g, oxoid agar 15.0g, distilled water 1000ml.

Induction and Isolation of Mutants

1. *The single treatment:* Method of mutagenesis was followed after Baeshin and Sabir[11]. A dense conidial suspension was made and the number of conidia/ml was estimated using a hemocytometer. Furadan or cadmium nitrate solution, made by dissolving the agent in 5ml water, was immediately added to the conidial suspension (5ml) and a 1ml sample of this mixture was immediately diluted in 9ml sterile water to serve as untreated control. Subsequent samples were taken at regular intervals and serially diluted in sterile distilled water to halt the mutagenic treatment. Samples of the final dilutions containing about 100 conidia were spreaded on PE plates and incubated. These procedures were repeated by using three different concentrations of Furadan, 03g/10ml,

0.35g/10ml and 0.4g/10ml or three different concentrations of cadmium nitrate, 0.0038g/10ml, 0.038g/10ml and 0.38/10ml respectively.

2. *The combined treatment:* Three experiments were attempted to investigate the combined mutagenic effects of Furadan and cadmium nitrate. In the first one, conidia of *A. terreus* were treated with Furadan (optimal concentration of 0.35g/10ml) for 45min. exposure, as explained before. After 45min. of exposure, the Furadan solution was discarded by centrifugation at 2,000rpm for 10min. and the conidia were washed three times with sterile distilled water. The conidia then were resuspended in 5ml sterile distilled water and cadmium nitrate was added to final concentration of 0.038g/10ml (optimal dose) for 60min. exposure. Subsequent samples were taken and serially diluted in sterile distilled water to halt the mutagenic treatment. Samples of the final dilution's containing about 100 conidia were spreading on PE plates and incubated.

In the second experiment, the conidia were first treated with 0.038g/10ml of cadmium nitrate for 60min. exposure, followed by 0.35g/10ml of Furadan for 45min. exposure as previously described.

In the third experiment, conidia were treated with mixture of Furadan 0.35g/10ml and cadmium nitrate, 0.038g/10ml, simultaneously for 60 min. exposure.

Total isolation method described by Fincham *et al.*[12] was followed for isolating mutants. At each of the previously mentioned doses and exposure intervals a single conidium was inoculated in each of 26 loci/plate containing PE which served as a template. The template was in turn replicated on Dox medium to detect auxotrophic mutants.

Statistical analysis was used to score linear regression to ensure the linear relationship between the period of exposure to the mutagen and survival percentage and concentration of mutagen and percentage of mutation, with the aid of Microsoft Excel Version 5.

All replicates were incubated for 24hr at 24°C. Auxotrophic mutants are those which fail to grow on the minimal medium after incubation.

The equation of Sharma and Grover[13] was applied to determine whether the combined effects of the two substances is synergestic or antagonistic as follows:

$$\text{Combined effect} = AB / A + B$$

where AB = percentage of mutation resulted from combined treatment (cumulative effect).

A = Percentage of mutation resulted by Furadan alone.

B = Percentage of mutation resulted by cadmium nitrate alone.

A + B = Additive effect.

If AB > A + B then there is synergism since AB/A + B would be more than 1

If AB < A + B then there is antagonism since AB/A +B would be less than 1.

Results

1. The Single Treatment

The survival percentage and recovery of auxotrophic mutants among survivals are summarized in Tables 1-3 for Furadan and in Tables 4-6 for cadmium nitrate. Variation in survival and mutant percentage is noticed in these tables.

TABLE 1. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Furadan (0.3g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	900	100	390	0	0
15	873	97	390	0	0
30	846	94	390	3	0.77
45	828	92	390	5	1.3
60	702	78	390	5	1.3
Total	4149		1950	11	0.56

TABLE 2. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Furadan (0.35g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	800	100	390	0	0
15	672	84	390	0	0
30	624	78	390	3	0.77
45	658	71	390	9	2.3
60	560	70	390	8	2.05
Total	3192		1950	20	1.02

TABLE 3. Survival and recovery of autotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Furadan (0.4g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	750	100	390	0	0
15	615	82	390	0	0
30	555	74	390	5	1.29
45	555	74	390	7	1.8
60	526	70	390	7	1.8
Total	3031		1950	19	0.97

TABLE 4. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by cadmium nitrate (0.0038g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	800	100	390	0	0
15	720	90	390	1	0.256
30	656	82	390	2	0.512
45	640	80	390	2	0.512
60	632	79	390	3	0.769
Total	3448		1950	8	0.41

TABLE 5. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by cadmium nitrate (0.038g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	500	100	390	0	0
15	410	82	390	1	0.256
30	375	75	390	2	0.512
45	350	70	390	2	0.512
60	340	68	390	4	1.025
Total	1975		1950	9	0.46

TABLE 6. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by cadmium nitrate (0.38g/10ml).

Treatment (min.)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No.)	(%)		(No.)	(%)
0	700	100	390	0	0
15	525	75	390	3	0.769
30	469	67	390	3	0.769
45	448	64	390	1	0.256
60	413	59	390	2	0.512
Total	2555		1950	9	0.46

The effect of Furadan and cadmium nitrate dose and exposure time on survival percentage is shown in Fig. 1 and 2. In both cases it can be inferred that an increase in mutagen dose and time of exposure causes a decrease in survival percentage as confirmed by linear regression calculation. The relation between the mutagen dose, exposure time and the percentage of mutants is presented in Fig. 3, 4 which show that an increase in dose and exposure time, to a certain limit, leads to an increase in mutation percentage.

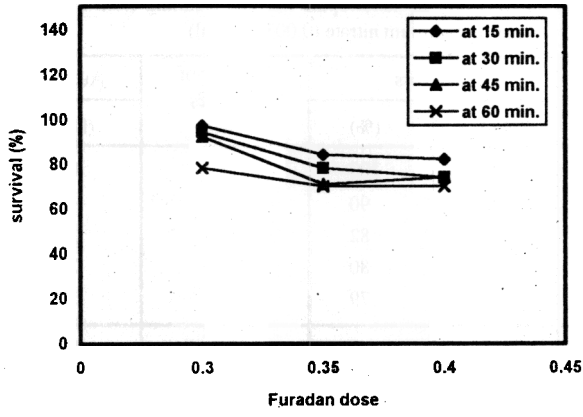

FIG. 1. Effect of Furadan dose and exposure time on survival percentage.

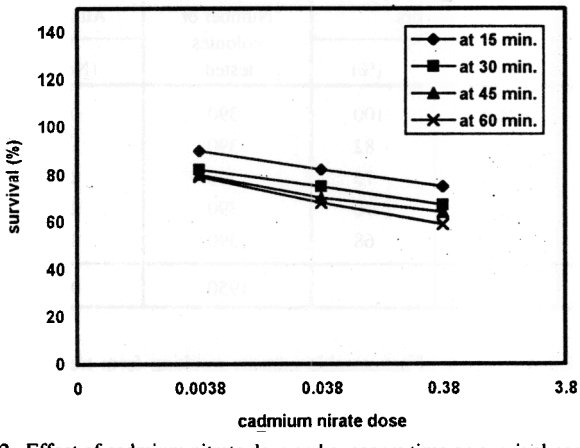

FIG. 2. Effect of cadmium nitrate dose and exposure time on survival percentage.

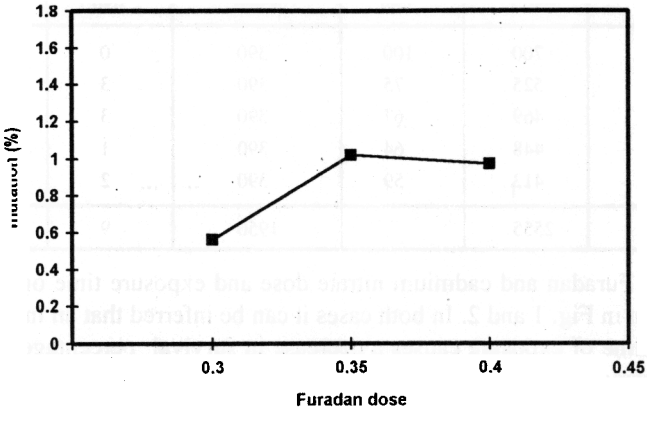

FIG. 3. Effect of Furadan dose on mutation percentage.

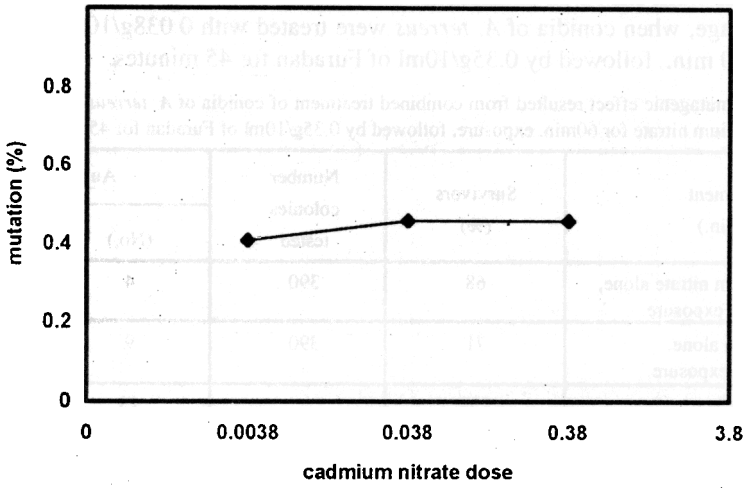

FIG. 4. Effect of cadmium nitrate dose on mutation percentage.

The highest possible percentage of mutation was achieved with the dose 0.35g/10ml of Furadan for 45min. of exposure and 0.038g/10ml of cadmium nitrate for 60min. exposure. Therefore, these doses would be the optimal doses for induction of mutation in this fungus with these substances.

2. *The Combined Treatment*

Table 7, shows a cumulative mutagenic effects when conidia of *A. terreus* were treated with 0.35g/10ml of Furadan for 45min., followed by 0.038g/10ml of cadmium nitrate for 60min., since less survivals and auxotrophs were obtained, it means that there was an antagonistic effect for this combined treatment on both of the percentage of mutants and survival percentage.

TABLE 7. The mutagenic effect resulted from combined treatment of conidia of *A. terreus* by 0.35g/10ml of Furadan for 45min. exposure, followed by 0.038g/10ml cadmium nitrate for 60min. exposure.

Treatment (min.)	Survivors (%)	Number colonies tested	Auxotrophic	
			(No.)	(%)
By Furadan alone, for 45min. exposure.	71	390	9	2.3 (A)
By cadmium nitrate alone, for 60min. exposure.	68	390	4	1.025 (B)
Additive effect	139		13	3.325 (A + B)
Combined treatment for 105min. exposure. (Cumulative effect)	64	312	5	1.6 (AB)
Combined effect	0.46			0.48 AB / A + B

Table 8, as well, shows an antagonistic effect on both survival percentage and mutation percentage, when conidia of *A. terreus* were treated with 0.038g/10ml of cadmium nitrate for 60 min., followed by 0.35g/10ml of Furadan for 45 minutes.

TABLE 8. The mutagenic effect resulted from combined treatment of conidia of *A. terreus* by 0.038g/10ml of cadmium nitrate for 60min. exposure, followed by 0.35g/10ml of Furadan for 45min. exposure.

Treatment (min.)	Survivors (%)	Number colonies tested	Auxotrophic	
			(No.)	(%)
By cadmium nitrate alone, for 60 min. exposure.	68	390	4	1.025 (A)
By Furadan alone, for 45min. exposure.	71	390	9	2.3 (B)
Additive effect	139		13	3.325 (A + B)
Combined treatment for 105min. exposure. (Cumulative effect)	69	312	6	1.9 (AB)
Combined effect	0.49			0.571 AB / A + B

Finally, it is quite obvious that Table 9 shows a synergistic effects for both substances on the mutation percentage, but an antagonistic effects as far as the survival percentage is concerned when conidia were treated by a mixture of the optimal doses of the two substances simultaneously.

TABLE 9. The mutagenic effect resulted from combined treatment of conidia of *A. terreus* by a mixture of 0.038g/10ml of cadmium nitrate and 0.35g/10ml of Furadan for 105min. exposure.

Treatment (min.)	Survivors (%)	Number colonies tested	Auxotrophic	
			(No.)	(%)
By cadmium nitrate alone, for 60min. exposure	68	390	4	1.025 (A)
By Furadan alone, for 45min. exposure.	71	390	9	2.3 (B)
Additive effect	139		13	3.325 (A + B)
Combined treatment for 105min. exposure (Cumulative effect)	57	350	12	3.428 (AB)
Combined effect	0.41			1.03 AB / A + B

Discussion

It was found that the mutagen dose of both substances is inversely proportionate to survival percentage, which means that an increase in the dose is met by a decrease in

survival percentage. It was also found that the survival percentage is inversely proportionate to the mutagen dose and exposure time, whereas the survival percentage decreases with an increase of dose and exposure. Thus, mutation percentage was found to be increased to a certain limit as dose of both substances and exposure time increased.

All these results are in general agreement with the rule mentioned by Fincham *et al.*[12], who stated that by using chemical mutagens there was a constant relation between the dose and mutation percentage which increases to a certain limit with the increase in dose.

The present study confirmed that Furadan is a potent mutagenic substance as compared with the potent chemical mutagenic agent N.T.G. In a study by Baeshin and Sabir[11]. It was found that N.T.G. gave a percentage of 3.8% of auxotrophs with the optimal dose of 0.0075g/10ml at 70 min. of exposure, whereas Furadan gave a percentage of 2.3% of auxotrophic mutants with the dose of 0.35g/10ml at 45min. of exposure as shown in the present study. Compared with both of Furadan and N.T.G. cadmium nitrate has a less mutagenic effects since the highest auxotrophic mutants percentage obtained by the present study was 1.025% compared with 1.7% auxotrophs obtained by cadmium chloride (data in publication processes), by the optimal dose of 0.038g/10ml at 60 min. of exposure. This is supported by the study of Kanematsu *et al.*[14], who reported that cadmium chloride was more effective in inducing genetic changes than cadmium nitrate in *Bacillus subtillus.* Reddy and Vaidyanath[15], found that cadmium chloride was of less mutagenic effect than γ-rays in rice. Another study of Reddy and Vaidyanath[16] in rice as well, demonstrated that combined treatment with both γ-rays and cadmium chloride brought about significantly more mutagenic effects than single treatments with either of the two of them. Would this apply to *A. terreus* or not deserves further study with this fungus.

The alternative treatment with cadmium nitrate first, followed with Furadan, seems to be enhanced the effect of Furadan in inducing genetic changes by the capability of cadmium in abolishing the DNA repair mechanism as stated by Klein *et al.*[17] and Zasukhina and Sinelschikova[18].

The absence of synergism on mutation and survival percentage in both of the alternative treatments, may due to that, Furadan dose not interfere with DNA repair system. On the other hand, the difference in the modes of induction of mutation between Furadan treatment and other treatments could be attributed to the nature of the mutagen, although, more investigations are needed before reaching a firm conclusion on this matter.

The synergistic effect on mutation percentage resulted from the simultaneous combined treatment, can be interpreted as the two substances stimulate each other to increase genotoxicity specially when they are applied for a long period. Since cadmium tends to have cytotoxic effect much more than genotoxic effect as demonstrated by Glass[19], an antagonistic effect on survival percentage has been obtained in this treatment. The result of the present study support the few data reported on the induction of mutation by combined treatments in organisms other than *A. terreus* (Mandel and Ryser[8], Degraeve[20] and Ruposhev and Garina[21]).

References

[1] **Pusztai, T.,** Chromosomal aberrations and chlorophyll mutations induced by some pesticides in barley.. *Acta-botanica-Hungaria,* (1983) **5:** 55-56.

[2] **Sharma, A.** and **Singh, R.M.,** Mutagenic effects of Carbofuran on *Pisum* and *Vicia. India. Geobios (Jodhpur),* (1990) **17**(5/6): 273-276.

[3] **Sabir, J.** and **Baeshin, N.,** Study of single and combined mutagenic effects of the pesticide Furadan and cadmium chloride in *Aspergillus terreus.* Accepted for publication in *The Proceedings of the Third International Symposium on New Genetical Approaches.* Tandojam, Pakistan (1997).

[4] **Baeshin, N., El-Seoudy, A.** and **Al-Ahmedi, M.,** Single and combined genotoxic effect of Furadan and Sequestrene 138 Fe 100 SG in root meristem of *Allium cepa.* Accepted for publication in *The Proceedings of the Third International Symposium on New Genetical Approaches.* Tandojam, Pakistan (1997).

[5] **Mitra, R.** and **Bernstein, I.,** Nature of repair process associated with the recovery of *Escherechia coli* after exposure to Cd^{2+}. *Biochem. Biophys. Res. Commun.,* (1977) **74:** 1450-1455.

[6] **Ochi, T., Mogi, M., Watanabe, M.** and **Ohsawa, M.,** Induction of chromosomal aberrations by the short-term treatment with cadmium chloride in cultured Chinese hamster cells. *Mutat. Res.,* (1984) **137:** 103-109.

[7] **Ochi, T.** and **Ohsawa, M.,** Participation of active oxygen species in the induction of chromosomal aberrations by cadmium chloride in cultured Chinese hamster cells. *Mutat. Res.,* (1985) **143:** 137-142.

[8] **Mandel, R.** and **Ryser, I.,** Mutagenicity of cadmium in *Salmonella typhimurium* and its synergism with two nitrosamines. *Mutat. Res.,* (1984) **138:** 9-16.

[9] **El-Sharqawi, H.M., Malibari, A., El-Shaieb, M.K.Z.** and **Tawfik, K.,** Some edapho-ecological factors controlling the distribution of soil fungi in the western region of Saudi Arabia. *Bull. Fac. Sci., K.A.U., Jeddah,* (1981) **5:** 103-115.

[10] **Talboys, P.,** A culture medium adding the identification of *Verticilium albo atrum* and *V. dahliae. Plant Pathol.,* (1960) **9:** 57-58.

[11] **Baeshin, N.** and **Sabir, J.,** Some genetic studies on *Aspergillus terreus* Thom., *Res. Sci., K.A.U.,* (1987) 215-224.

[12] **Fincham, J.R.S., Day, P.R.** and **Radford, A.,** *Fungal Genetics,* University of California Press, Berkeley and Los Angeles (1979).

[13] **Sharma, A.** and **Grover, P.,** Interaction of mutational lesions induced by EMS and gamma-rays in *Drosophila melanogaster., Mutat. Res.,* (1970) **10:** 221-226.

[14] **Kanematsu, N., Hara, M.** and **Kada, T.,** Rec assay and mutagenicity studies on metal compounds. *Mutat. Res.,* (1980) **77:** 109-116.

[15] **Reddy, T.** and **Vaidyanath, K.,** Mutagenic potentiating and antimutagenic activity of certain metallic ions in the rice genetic system. *Current Sci.,* (1978) **47:** 513-515.

[16] **Reddy, T.** and **Vaidyanath, K.,** Synergistic interaction of gamma rays and some metallic salts in the induction of chlorophyll mutations in rice. *Mutat. Res.,* (1978) **52:** 361-365.

[17] **Klein, W., Kocsis, F.** and **Wottawa, A.,** DNA repair and environmental contaminants. *Ber Oster. Studienges. Atom Energ.,* (1976) 2613, 89.

[18] **Zasukhina, G.** and **Sinelschikova, T.,** Mechanism of the action of certain mutagens on the DNA on human cells. *Dokl. Acad. Nauk SSSR,* (1976) **230:** 719-721.

[19] **Glass, E.,** Die verteilung von Fragmentationen und a chromatischen stellen auf den chromosomen von *Vicia faba* nach Behandlung mit Schermetallsamzen, *Chromosoma.,* (1956) **8:** 260-284.

[20] **Degraeve, N.,** Modification des effects du methane sulfonate d ethyl au niveau chromosomique, I. Les ions metalliques, *Rev. Cytol. Biol. Veget.,* (1971) **34:** 233-244.

[21] **Ruposhev, A.** and **Garina, K.,** Modification of mutagenic effects of ethyleneimine by cadmium in *Crepis capilloris. Genetika,* (1977) **13:** 32-36.

دراسة التأثير الوراثي السام المفرد والمشترك للمبيد الحشري الفيورادان ونترات الكادميوم في فطر *أسبرجلس ترّس*

جمال صابر

قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز

جـــــدة - المملكة العربية السعودية

المستخلص . في محاولة لمعرفة التأثير الوراثي السام المفرد والمشترك للمبيد الحشري الفيورادان والمعدن الثقيل الكادميوم (في صورة نترات الكادميوم) ، تم تعريض الجراثيم الكونيدية لفطر *أسبرجلس ترّس* لهاتين المادتين في المعاملات التالية :

١ - المعاملة المفردة : والتي تتألف من أ - معاملة الجراثيم الكونيدية بثلاث تركيزات مختلفة من الفيورادان (من بينها التركيز الحقلي) وهي ٠,٣ جم ، ٠,٣٥ جم و ٠,٤ جم/ ١٠مل معلق جرثومي ولفترات تعريض مختلفة . ب - معاملة الجراثيم الكونيدية بثلاث تركيزات مختلفة من نترات الكادميوم وهي ٠,٠٣٨ جم ، ٠,٠٣٨ جم و ٠,٠٣٨ جم/ ١٠مل معلق جرثومي ولفترات تعريض مختلفة .

٢ - المعاملة المشتركة : والتي تتألف بدورها على أ - معاملة الجراثيم الكونيدية بالتركيز الأمثل من الفيورادان أولاً يعقبه المعاملة بالتركيز الأمثل لنترات الكادميوم . ب - معاملة الجراثيم الكونيدية بالتركيز الأمثل لنترات الكادميوم أولاً يعقبه المعاملة بالتركيز الأمثل للفيورادان . ج - معاملة الجراثيم الكونيدية بخليط المادتين معًا .

ووجد في جميع المعاملات المفردة أن الزيادة في تركيز الفيورادان ونترات الكادميوم وفترات التعريض تؤدي دائمًا إلى انخفاض النسبة المئوية للبقاء والزيادة إلى حد معين في النسبة المئوية للطفرات . بينما كان هناك تأثير مشترك تضادي لكل من المعاملتين المتعاقبتين على النسبة المئوية للبقاء والنسبة المئوية للطفرات . أما المعاملة المشتركة الأخيرة فقد أدت إلى ظهور تأثير تضافري لكلا المادتين على النسبة المئوية للطفرات .