Genotoxicity of Two Organophosphorous Pesticides: Dimecron and Diazinon in *Aspergillus terreus*

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ABSTRACT. In attempt to investigate the genotoxicity and the mode of action of the organophosphorous pesticides; Dimecron (2-chloro-N,N-diethyl-3-hydroxycrotonamide dimethyl phosphate) and Dizinon [o,o-diethyl o-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate], the conidial spores of *Aspergillus terreus* were treated by four different concentrations (including the field concentration) of each pesticide individually as follows:

Conidia were treated with 0.1 ml/10 ml, 0.15 ml/10 ml, 0.2 ml/10 ml and 0.25 ml/10 ml conidial suspension of the pesticide Dimecron, and survival and mutation frequencies were calculated to find out the optimal dose for induction of mutation (by scoring the auxotrophs if possible). In the same way, conidia were treated with 0.075 ml/10 ml, 0.1 ml/10 ml, 0.15 ml/10 ml and 0.2 ml/10 ml-conidial suspension of the pesticide Diazinon, and the same procedure above mentioned was followed.

As a results, it was found in both of the two experiments that, with increasing of Dimecron or Diazinon concentration and exposure time, a decrease in survival percentage and an increase up to a certain limit in mutation frequency were always observed. The optimal dose for inducing mutation by Dimecron was found to be 0.2 ml/10 ml-conidial suspension for 30 min. and 0.15 ml/10 ml conidial suspension of Diazinon for 30 min. too.

Introduction

Many organophosphorous-derived chemicals form a class of pesticides that are widely used in agriculture. Some of these pesticides are esters or amide esters of phosphoric acid, therefore, are named organophosphates (Wild[1]). Because most of this group have alkylating properties (Mohn[2]), many work has been done on their genotoxic activity. Malathion, for instance, is a widely used organophosphorous pesticide that has been found to induce several types of genetic damage (Nicholas *et al.*[3], Dulout *et al.*[4],[5]). Other organophosporous pesticide, has also been found positive for inducing chromosome aberrations in bone marrow cells (Kurinny[6]) or for sister-chromatid exchange (SCE) in mammalian cells (Tezuka *et al.*[7], Chen *et al.*[8]).

The organophosphorous pesticide Dimecron was reported to be positive for inducing a high ratio of sex-linked recessive lethal mutations to *Drosophila melanogaster* by Tripathy *et al.*[9], chromosome aberrations in *Allium sativum* by Padmaja *et al.*[10] and in rat by Adinkari and Grover[11]. The other organophosphorous pesticides Diazinon was not fully studied as far as genotoxicity is concerned, except that Kaur and Grover[12] found that Diazinon causes a chromosome aberrations in barley.

In this work, the mutagenic activity of Dimecron and Diazinon which belong to the organophosphorous pesticides group, were studied in the conidial spores of *Aspergillus terreus*, and found the induction of mutations by both agents.

Materials and Methods

1. Strain

The wild type 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.*[13]. It has been identified by the Commonwealth Mycological Institute, Kew, Surrey, England.

2. Chemicals

The organophosphorous pesticides; Dimecron (2-chloro-N, N-diethyl-3-hydroxycrotamide dimethyl phosphate) and Dizinon [o,o-diethyl o-[2-isopropyl-4-methyl-6-pyrimidyl] phosphorothioate], were supplied by the distributor in Saudi Arabia, Al-Selouly Agricultural Est., Jeddah.

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: 2.0 g sodium nitrate ($NaNO_3$); 0.5 g potassium chloride (KCl); 0.5 g magnesium glycerophosphate; 0.1 g ferrous sulphate ($FeSO_4$); 0.35 g potassium sulphate (K_2SO_4); 30.0 g sucrose; 12.0 g 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[14] according to SS formula: prune 5.0 g, yeast extract 1.0 g, oxoid agar 15.0 g, distilled water 1000 ml.

Induction and Isolation of Mutants

The method of experimental mutagenesis was followed after Baeshin and Sabir[15]. A dense conidial suspension was prepared and the number of conidia/ml was estimated using a hemocytometer. Dimecron and Diazinon solution, made by dissolving the agent in 5 ml water, was immediately added to the conidial suspension (5 ml) and a 1 ml sample of this mixture was immediately diluted in 9 ml sterile water to serve as control. Subsequent samples were taken at regular intervals and serially diluted in sterile distilled water to halt the mutagenic treatment. Samples of the final dilution's containing about

100 conidia were spread on PE plates and incubated. These procedures were repeated by using four different concentrations of Dimecron , 0.1 ml/10 ml, 0.15 ml/10 ml, 0.2 ml/10 ml and 0.25 ml/10 ml conidial suspension and four different concentrations of Diazinon, 0.075 ml/10 ml, 0.1 ml/10 ml, 0.15 ml/10 ml and 0.2 ml/10ml conidial suspension, respectively.

Total isolation method described by Fincham *et al.*[16] 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 24 hr at 24ºC. Auxotrophic mutants are those which fail to grow on the minimal medium after incubation.

Results

The survival percentage and recovery of auxotrophic mutants are summarised in Tables 1-4 for Dimecron and in Tables 5-8 for Diazinon. Variation in survival and mutant percentage was observed in these tables.

TABLE 1. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Dimecron (0.1 ml/10 ml).

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	500	100	320	0	0
15	490	98	320	0	0
30	385	77	320	0	0
45	355	71	320	0	0
60	330	66	320	0	0
Total	2060		1600	0	0

TABLE 2. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Dimecron (0.15 ml/10 ml).

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	500	100	350	0	0
15	450	90	350	0	0
30	370	74	350	0	0
45	325	65	300	0	0
60	285	57	280	1	0.36
Total	1930		1630	1	0.06

TABLE 3. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Dimecron (0.2 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	350	0	0
15	462	84	350	1	0.29
30	374	68	350	3	0.86
45	303	55	300	0	0
60	253	46	250	0	0
Total	1942		1600	4	0.25

TABLE 4. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Dimecron (0.25 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	350	0	0
15	375	75	350	2	0.57
30	310	62	310	1	0.32
45	230	46	230	0	0
60	190	38	190	0	0
Total	1605		1430	3	0.21

TABLE 5. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Diazinon (0.075 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	390	0	0
15	523	95	390	0	0
30	506	92	390	0	0
45	440	80	390	0	0
60	402	73	390	2	0.51
Total	2421		1950	2	0.1

TABLE 6. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Diazinon (0.1 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	350	0	0
15	512	93	350	0	0
30	473	86	350	0	0
45	391	71	350	2	0.57
60	336	61	300	3	1
Total	1750		1700	5	0.29

TABLE 7. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Diazinon (0.15 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	350	0	0
15	501	91	350	3	0.86
30	391	71	350	4	1.14
45	341	62	320	0	0
60	248	45	220	0	0
Total	2031		1590	7	0.44

TABLE 8. Survival and recovery of auxotrophic mutants resulting from treated conidia of *Aspergillus terreus* by Diazinon (0.2 ml/10 ml)

Treatment (min)	Survivors		Number of colonies tested	Auxotrophic mutants	
	(No)	(%)		(No)	(%)
0	550	100	350	0	0
15	451	82	350	2	0.57
30	380	69	350	3	0.86
45	337	61	320	1	0.31
60	231	42	220	0	0
Total	1949		1590	6	0.38

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

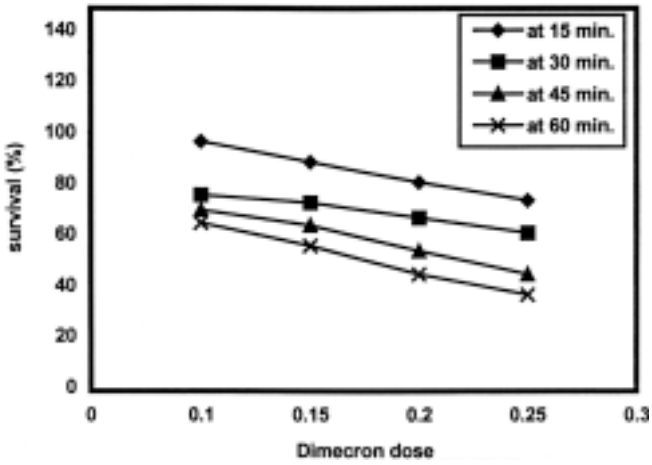

FIG. 1. Effect of Dimecron dose and exposure time on survival percentage.

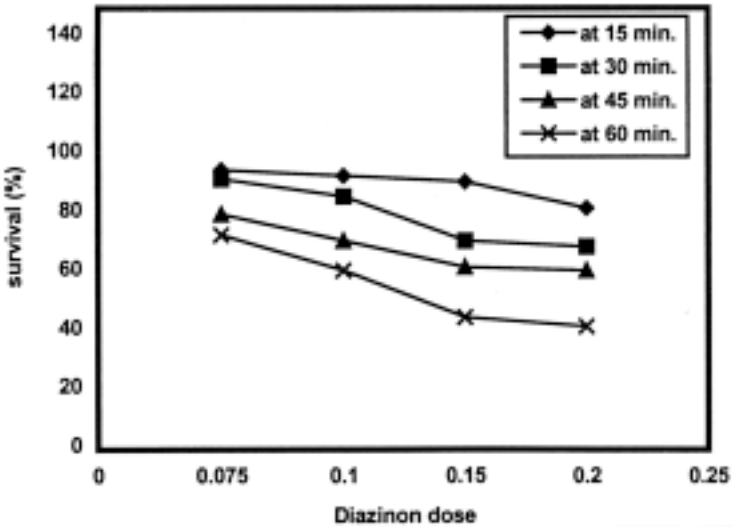

FIG. 2. Effect of Diazinon dose and exposure time on survival percentage.

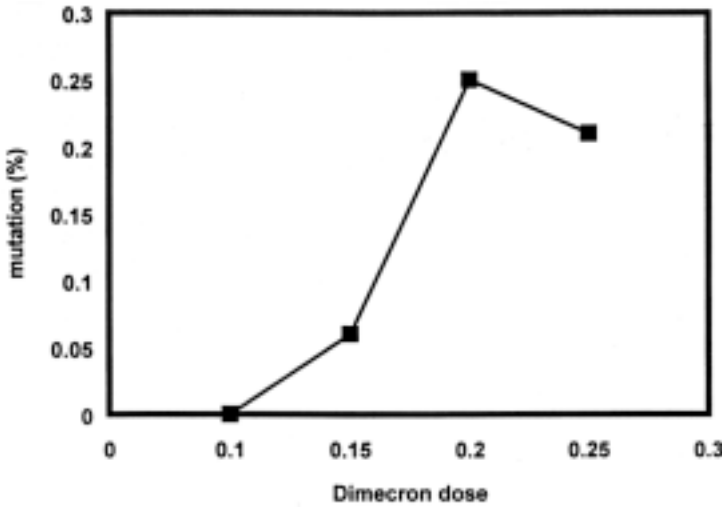

FIG. 3. Effect of Dimecron dose on mutation percentage.

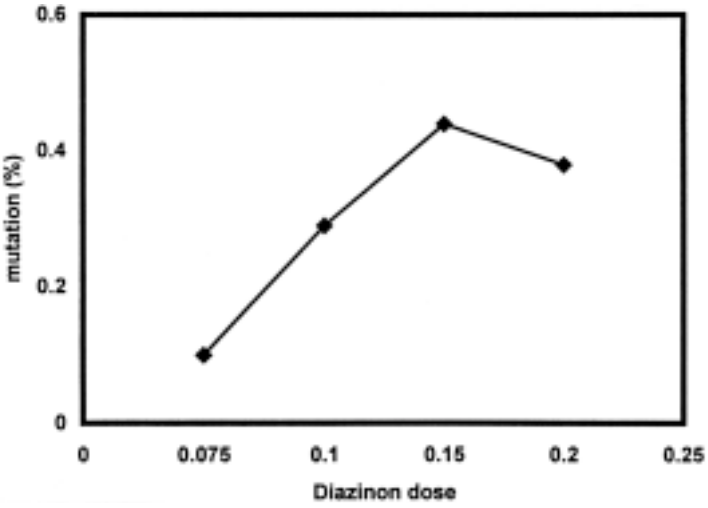

FIG. 4. Effect of Diazinon dose on mutation percentage.

The highest possible percentage of survivals and mutation was achieved with the dose 0.2 ml/10 ml of Dimecron for 30 min. of exposure and 0.15 ml/10 ml of Diazinon for 30 min. of exposure, as shown in Tables 3 and 7. Therefore, these doses would be the optimal doses for induction of mutation in this fungus with these pesticides.

Discussion

This study revealed that the organophosphorous pesticides Dimecron and Diazinon are genotoxic. It was found that the pesticide dose is inversely proportionate to survival percentage and that the survival percentage is inversely proportionate to the exposure time, thus, the mutation percentage was found to be increased to a certain limit as dose of both pesticides and exposure time increased. These results are in general agreement with the rule mentioned by Fincham *et al.*[16], 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 results of the present study showed that both of pesticides Dimecron and Dizinon were positive for induction of mutation in *Aspergillus terreus,* which is in accordance with the several pesticides of the organophosphorous group that have been reported to induce mutations in microbial systems. These include the well-studied insecticide Dichlorvos for which alkylation of DNA bases in *Escherichia coli* has been shown (Wild[1]). Compared with Dimecron, Diazinon has a slightly greater genotoxic potential in this fungus since the highest auxotrophic mutants percentage obtained by Dimecron in the present study was 0.86% while 1.14% auxotrophs were obtained by Diazinon. This may due to the phosphorothioate (P = S) which is oxidised to oxon (P = O) microsome oxidase (Benke and Murphy[17]). The oxons are highly toxic compounds which account for the profound cytotoxic effect of organophosphorous pesticides. Further study on the mutagenicity of the oxons should be carried out with this fungus.

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السمية الوراثية لاثنين من المبيدا الحشرية الفسفورية العضوية : الدايمكرون والدايازينون في فطر أسبرجيلس ترس

جمال صابر
قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبدالعزيز
جـــــدة – المملكة العربية السعودية

المستخلص . في محاولة لمعرفة السمية الوراثية وطريقة عمل إثنين من المبيدا الفسفورية العضوية وهما الدايمكرون والدايازينون ، تم معاملة الجراثيم الكونيدية لفطر أسبرجيلس ترس بالمبيدين كل بمفرده وذلك على النحو التالي :

تم معاملة الكونيديا بالمبيد الحشري الدايمكرون وبأربعة تركيزا مختلفة منه هي : ١,٠ مل / ١٠ مل ، ١٥,٠ مل / ١٠ مل ، ٠و ٢ مل / ١٠ مل ، ٢٥,٠مل / ١٠مل معلق جرثومي ولفترا زمينة مختلفة ، ثم حساب النسبة المئوية للبقاء والنسبة المئوية للطفرا وذلك لمعرفة التركيز الأمثل لإحداث الطفرة إن أمكن والذي بواسطته يمكن الحصول على أعلى نسبة من البقاء يقابلها أكبر نسبة من طفرا العوز الغذائي . وبالطريقة نفسها ، تم معاملة الجراثيم الكونيدية للفطر بأربعة تركيزا مختلفة من المبيد الحشري الديازينون وهي : ٠,٠٧٥ مل / ١٠مل ، ١,٠ مل / ١٠ مل ، ١٥,٠مل / ١٠مل و ٢,٠مل / ١٠مل معلق جرثومي ولفترا زمنية مختلفة .

وقد بينت هذه المعاملا المختلفة أن للمبيدين الحشريين المقدرة على إحداث الطفرة في هذا الفطر ، فقد وجد أن الزيادة في تركيز المبيد وفترة التعريض تقابل دائماً بانخفاض للنسبة المئوية للبقاء وزيادة إلى حد معين للنسبة المئوية للطفور .