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Study of the single and combined genotoxic effects of chlorpyrifos and quercetin in *Saccharomyces cereviciae*

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Abstract

The genotoxic effects of chlorpyrifos and quercetin either single or combined were tested in terms of their ability to induce reverse mutation, gene conversion and mitotic crossing over in *Saccharomyces cereviciae* D7. The results indicated that all single and combined treatments induced reverse mutation, gene conversion and mitotic crossing over in *Saccharomyces cereviciae* D7. Combined treatment was more effective than the single treatment of quercetin. The insecticide (chlorpyrifos and quercetin which are common flavonoids) proved to be mutageneic in *Saccharomyces cereviciae*.

Introduction

In many genetic investigation the organophosphorus insecticides has been reported as a potent genotoxic agents (Abdallah *et al*. (1973); Villani *et al*. (1983); Nafei *et al*. (1984); Salam *et al*. (1984); and Mansour *et al.* (1988)). The induction of mitotic crossing over in diploid yeast *Saccharomyces cereviciae* is strongly correlated with the mutagenic effects. These tests very sensitivity react with compounds which induce base-pair substitution as well as from-shift mutations. This system has revealed the genetic activity of large number of carcinogens, pesticides, radiation and many other chemical mutagens (Siebert and Elsenbrand, (1974); Zimmermann *et al.*, (1975); Altwaty, (1999); Anjaria and Rao, (2001) and Buschini *et al*., (2003 and 2004)).

Quercetin is one of the most common flavonoids in plants, widely distributed in natural foods, consumed by humans in a range of 50 mg per day (Brown and Dietrich, 1979 and Caria *et al*., 1995). Quercetin was shown to be mutagenic in the Ames assay (Bjeldames and Chang 1977; MacGregor and Jurd 1978; Brown and Dietrich 1979 and Rueff *et al*., 1986). Also it has been shown to be carcinogenic in rats (Pamukcu *et al.,* 1980), to be mutagenic in a variety of genotoxicity tests (Muller *et al.,* 1991 and Caria *et al*., 1995). The conflicting inform-ations on the genotoxicity of chlorpyrifos and quercetin from previous studies reported in the literature led to us to study the genotoxic effects of both of them in single and combined forms.

Materials and methods

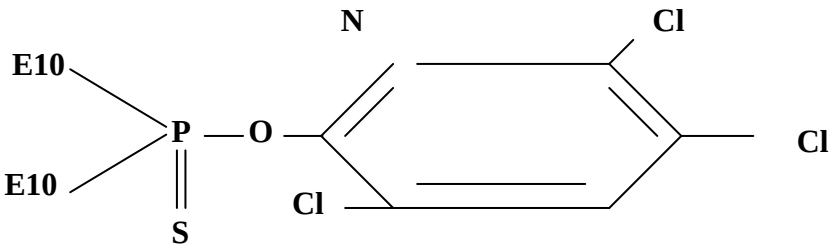
1- Yeast strain:

The D7 strain of *Saccharomyces cereviciae* was used as a test oragnism (Courtesy of F. K. Zimmermann. Darmstad, Germany). This strains has the following genotype: ade2-40 / ade2-119, trp5-12 / trp5-27, ilvl-92 / ilvl-92. It is used for the simultaneous detection of induced reverse mutation, mitotic gene conversion, and mitotic crossing over (Zimmermann *et al*., 1975).

2- Chemicals

a. The insecticide chlorpyrifos was obtained from Hanoo Agricultural, the sole agent in K.S.A.,P.O. Box.4894 Riyadh 114412. Manufactured by Chemac-Agriphar / Rue De Renory, 261B-41020 Ugree/Belgium.

Formula of chlorpyrifos



Chlorpyrifos is an organophosphorus insecticid, it's chemical is:

O.o-diethylo-3,5,6 trichloro-2 pyridyl phosphorothioate.

b.Quercetin: is one of the flavonoids in Senna spp (Cassia) (Leguminosae), obtained from Dr. Aisha Mohamed, Ali Khogli, Faculty of Science, King Abdelaziz University.

3- Medium

a. Complete medium

This medium was used for routine culture growth, it contains : peptone 5 mg/L, yeast extract 10 g/L, glucose 20 g/L and Agar 20 g/L

b. Minimal medium

The medium components have been described in detail by Zimmermann et al. (1975).

4- Testing assay:

a- Three concentrations were prepared from chlorpyriphos, these concentrations were 1, 2, 5 µl per ml media.

b- The used concentration of quercetin was 5 µl per ml media

c- Combined treatment

The used concentration of chlorpyriphos and quercetin for combined treatment was 5 µl/ml media

Treatment protocol:

1. 10 ml of liquid complete medium were inoculated with about 5 x 10 cells/ml in a 50 ml conical flask.
2. The culture was incubated on an orbital shaker water bath at 24 c for 6 hrs.
3. The sample of the cells was examined under the microscope, the proper culture must be in experimental phase (at least 90 % of the cells have buds).
4. Concentration series for treatment were inoculated cache with 1 ml sample cells and incubated at 28 c on an water bath shaker for 18 hrs.
5. After appropriate dilution, the cells were plated onto:
   - Complete medium with cycloheximide to detect mitotic crossing over
   - Synthetic complete medium without tryptophan to detect gene conversion
   - Synthetic complete medium without isoleucine to detect point mutation

Analysis and evaluation of the data

The frequencies of gene convertion, reverse mutation and mitotic crossing over were computed by dividing the number of convertant, revertant and mitotic crossing over colonies. The general consensus was

that the increase in an end point under investigation up to two folds or more of the mean of control frequency is biologically considered as a significant response (Brusick, 1980).

Results and discussion

The results in table (1) show the genetic activities in such chlorpyriphos in *Saccharo-myces cereviciae D7*. Chlorpyriphos exhib-ited moderate toxicity at the lower concentration which proportionally increased by increasing the treatment dose (1-5 µl/ml). Survival percentages ranged from 70 % at the lowest concentration (1 µl/ml) to 27 % at highest one (5 µl/ml). Weak positive mutagenic activity was obtained using the concentration 1 µl/ml where the induced frequency of mitotic crossing over at the cycloheximide (Cyh) locus was 4.7 times the spontaneous frequency, while the same concentration showed negative results in the induction of gene conversion at the tryptophan-5 (Trp-5) locus and reversion at isoleucine (il) locus. Also, moderate mutagenic activity was obtained at the three loci under study when chlorpyriphos applied at the concentration 2 µl/ml which resulted in mitotic gene conversion, reversion and mitotic crossing over in frequency 3.6, 4.1 and 9.6 times the spontaneous ones respectively. Chlorpyr-iphos as a mutagen proved to be more potent at the concentration 5 µl/ml which caused 27 % survival and resulted in mitotic gene conversion, reversion and mitotic crossing over in frequencies 13.1, 13.2 and 20 times of control ones respectively. These results suggest the mutagenic effect of chlorpyriphos in the induction of convertion of convertant, revertant and mitotic crossing over in *Saccharomyces cereviciae strain D7*. This is in agreement with the results obtained by many reports used pesticides in *Saccharo-myces cereviciae*, El-Adawy *et al*. (1998); Salam *et al*. (1993 and 1995); Ahmed et al. (1999) and Al-twaty (1999). The results shown in table (2), showed the genetic activities of quercetin. Its positive indications of mutagenic activity were obtained at the concentration of 5 µl/ml of quercetin, where the induced frequency of mitotic gene conversion and mitotic crossing over was 5.1 and 4.7 times the control ones respectively. Also, the same concentration showed a strong activity at the (ilv) locus, causing revertants in a frequency 11.9 times the control level. Meanwhile, the combined treatment of quercetin and chlorpyriphos, showed a cumulative mutagenic effects when compared with quercetin alone which resulted in mitotic gene conversion, revetant and crossing over in frequencies at 11.7, 11.2 and 5.4 times as the control levels, respectively. This result suggests that quercetin and the combined treatment with chlorpyriphos showed mutagenic effect in induction of convertant, revertant and mitotic crossing over in *Saccharomyces cereviciae D7*. This is in agreement with Caria *et al*. (1995), who reported that quercetin induced micronuclei in human lymphocytes. Moreover, quercetin was shown to be mutagenic in the Ames assay (Brown and Dietrich, 1979 and Rueff *et al*., 1986). Induced safe our sheep (SoS) functions (Rueff *et al*., 1992). The results of the present study show that chlorpyriphos (organoph-osphorus insecticides) and quercetin (one of the common flavonoids in plants) were capable to induce the three genetic end points and to reveal genetic activity at the three loci under study. Chlorpyriphos shoed obviously high genotoxicity to strain D7 of *Saccha-romyces cereviciae* when compared with quercetin. Moreover, the treatment of chloep-yriphos and quercetin was slightly lower as compared with chlorpyriphos alone.

Table (1): Response of Saccharomyces cereviciae D7 to the treatment with different concentrations of chlorpyrifos

		Convertant			Revertant					
Con. M/ml	Number of cells	Mut Freq	T/C	D. of Act.	Mut Freq	T/C	D. of Act.	Mut Freq	T/C	D. of Act.
Control	17084	14.1 (24)	1	-	11.7 (20)	1	-	16.4 (28)	1	-
1 Ml	10930	25.6 (28)	1.8	-	21.9 (24)	1.8	-	76.8 (84)	4.7	+
2 Ml	7570	52.6 (40)	3.6	+	47.5 (36)	4.1	+	158.5 (120)	9.6	+
5 Ml	4642	189.6 (88)	13.1	++	155.2 (72)	13.2	++	336.2 (156)	20	++

Key: Con = Concentration Mut = Mutation
C = Control value T = Treatment value
+ = 2 – 10 control level ++ = > 10 control level - = non significant
D. of Act = Degree of activity, numbers between parenthese represents actual concoly counts

Table (2): Response of Saccharomyces cereviciae D7 to the treatment with quercetin alone on combined with chlorpyrifos

		Convertant			Revertant					
Con. M/ml	Number of cells	Mut Freq	T/C	D. of Act.	Mut Freq	T/C	D. of Act.	Mut Freq	T/C	D. of Act.
Control	17084	14.1 (24)	1	-	11.7 (20)	1	-	16.4 (28)	1	-
Ch. 5 Ml/ml	4642	189 (88)	13.1	++	155.2 (72)	31.2	++	336.2 (156)	20	++
Quer 5 Ml/ml	9482	72.8 (69)	5.1	+	139.2 (132)	11.9	++	77 (73)	4.7	+
Com. Tr.	9197	166.3 (153)	11.7	++	155.2 (72)	11.2	++	89.2 (82)	5.4	++

Key: Con = Concentration Mut = Mutation
C = Control value T = Treatment value
+ = 2 – 10 control level ++ = > 10 control level - = non significant
Ch. = chlorpyrifos quer. = quercetin com Tr. = combined treatment
D. of Act = Degree of activity, numbers between parenthese represents actual colony counts

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دراسة مفردة ومشتركة للسمية الوراثية للكلوربرفوس والكيورستين فى فطر خميرة الخباز السلالة D7

ندى حسن على التواتى
قسم علوم الأحياء – جامعة الملك عبدالعزيز

تمت دراسة التأثير السمى الوراثى للكلوربيرفوس والكيورستين فى معاملة مفردة ومشتركة لكل منهما لمعرفة مقدرتهما على إحداث كل من الطفرة المرتدة والتحول الجينى والعبور الوراثى الجسمى على سلالة D7 لفطر خميرة الخباز وأوضحت النتائج المتحصل عليها أن كل المعاملات المفردة والمشتركة استحدثت طفرة مرتدة وتحول جينى وعبور وراثى جسمى فى سلالة D7 لفطر خميرة الخباز. المعاملات المشتركة كان لها تأثير أكبر من المعاملات المفردة للكيورستين.

وقد أوضحت النتائج المتحصل عليها أن كلاً من المبيد الحشرى كلوربيرفوس وكيورستين (وهو الفلافوتيدات المعروفة) والمستخدمة فى مقارنة الحشرات الموجودة على النباتات لهما تأثير طفرى على فطر خميرة الخباز.