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Protection the flavonoids, rutin and proto chatechuic acid, against mitotic crossing over, gene conversion and reverse mutation induced by (chlorpyritos) in *Saccharomyces cerevisia* D7.

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Abstract

Introduction: Protection by the flavonoids , rutin and protochatechuic acid , against insecticide chlorpyrifos induced mitotic crossing over , gene conversion and reverse mutation were investigated in *Saccharomyces cerevisia* D7 .

Results: The results indicate that Rutin and Protochatechuic acid have some antimutagenic potential against mutagenicity of chloropyrifos.
There for, the flavonoids contained in Senna seem to be important as antimutagenic and antioxidants.

Introduction

In many genetic investigation the organophosphorus insecticides has been reported as a potent genotoxic agents (Abdallah *et al.* (1973); Nafei *et al.* (1984); Salam *et al.* (1984); Mansour *et al.* (1988) and Rahaman *et al.* (2002) . The induction of mitotic crossing over in diploid yeast *Saccharomyces cereviciae* is strongly correlated with the mutagenic effects. These tests very sensitivily react with compounds which induce base-pair substitution as well as fram-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) .

Flavonoids are widely distributed in the plant Kingdom and are strong antioxidants (NaKatani, 1990 and KayoKo *et al.,* 1996).

The antimutagenic activity of some of the isolated flavonoids from Senna species against mutation in yeast, caused by insecticide, will be studied. The short term tests have been used to detect the various physical, chemical, and biological agents (Anuradha *et al.,* 1996).More recently these same tests have been used to study the antimutagenicity of certain single chemical and complex chemical mixtures. We selected the diploid strain D7 of *Saccharomyces crevisiae* which was constructed by Zimmermann, (1975) Specifically to detect, mitotic gene conversions, revertants and mitotic crossing over. Many, naturally occurring compounds can have important effects on the consequences of exposure to mutagens and carcinogense.Food ingredients like flavonoids have been claimed to have antimutagenic or anticarcinigeneic potential (Steinmetz and Potter, 1991).

In this study the influence of quercetin glycoside,namely rutin also 3,4-dihydroxy benzoic acid (protochatechuic acid) on insecticide chlorpyrifos induced mutations in *Saccharomyces cerevisiae* strain D7 are investigated.

Materials and methods

1- Yeast strain

The D7 strain of *Saccharomyces* cereviciae was used as a test organism (Courtesy of F.K. Zimmermann.Darmstad, Germany). This strain 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. Chlorpyrifos is an organ-ophosphorus insecticide, its chemical is:
O, O-diethylo-3, 5, 6 trichloro-2 pyridyl phosphorothioate.

b-Rutin and protochatechuic acid are isolated compounds from Senna spp (Cassia) (Leguminosae),obtained from Dr. Aisha Mohamed,Ali Khogli, Faculty of Science, King Abdelazizi University .

3- Medium

a. Complete medium

This medium was used for routine culture growth , it contains : peptone 5 mg/L,yeast exract 10g/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 rutin and protochatechuic acid are 5 μl per ml media.

c- Combined trearment the used concentration of chlorpyriphos, rutin and protochatechuic acid for combined treatments was 5 μ l/ml media.

Treatment protocol

1. 10 ml of liquid complete medium were inoculated with about 5 x 10 cells/ml in 50 ml conical flask.
2. The culture was incubated on an orbital shaker water bath at 24 c^o for 6 hrs.
3. The sample of the cell 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^o on a water bath shaker for 18 hrs.
5. After appropriate dilution , the cells were plated onto :

i. Complete medium with cyclohexim-ade 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 conversion, 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 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 (Brusik,1980).

Results and discussion

The result in table (1) show the genetic activities in such chlorpyriphos in *Saccharos-myces cereviciae* D7. Chlorpyriphos exhibited 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 l μl/ml where the induced frequency of mitotic crossing over at the cyclohexamide (Cyh) locus was 4.7 times the spontaneous frequency , While the same concentration showed negative result 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 2μl/ ml which resulted in mitotic crossing over in frequency 3.6, 4.1 and 9.6 times the spontaneous ones respectively. Chlorpy-riphos 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 result suggest the mutagenic effect of chlorpyriphos in the induction of conversion of convertant, revertant and mitotic crossing over in *Saccharos-myces cereviciae,*strain D7. This is in agreement with the results obtained by many reports used pesticides in *Saccharos-myces cereviciae* , El-Adawy *et al.*(1998);Salam *et al.*(1993 and 1995); Ahmed *et al*. (1999) and Al-twaty (1999). Results of rutin one of the quercetin glycosides are shown in table (2) exhibited the genetic activities of rutin in S.*cerevisiae* strain D7 .Rutin exerted a weak recornbinogenic activity. which resulted in revertants in frequency 2.3 times as the control levels. While, using the same concentration it did not induce gene conversion and mitotic crossing over. Mean While, moderate mutagenic activity was obtained at the combined treatment of rutin and chlorpyrifos, resulted in mitotic gene conversion and mitotic crossing over in frequencies of 3.3 and 2.6 times the spontaneous ones respectively while the same treatment was inducing revertants in a frequency of 10.3 the control level. This results suggests that rutin was capable of inducing only revertants in a weak frequency of 2.3 the control level, but did not induce mitotic gene convrsion or mitotic crossing, over. The combind treatment with the two substances led to an anti mutagenic effect, whereas a frequencies of gene conversion, revertants and mitotic crossing over with the combined treatment was slightly lower as compared with chlorpyrifos alone.

A satisfactory contribution to the understanding of the antimutagenic effect of rutin was obtained by using the D7 strain of *S. cenrevisiae.* The effect of rutin in eukaryotic systems was not clear and the results were contradictory. In strain D7 rutin reduces mitotic gene conversion and mitotic crossing-over induced by chlorpyrifos. The most likely hypothesis is that rutin exerts its effect in repair processes of the DNA. The results obtained with rutin in strain D7 of *S. cerevisiae* are in agreement with Giorgio *et al.* (1992) who found that the spermine reduces point mutation and mitotic gene conversion induced by agents with different mechanisms of action using the D7 strain of *S.cerevisiae*. Also Bear and TeeL (2000) found that heterocyclic amines (Melqx and G'lu-p-1) induced mutagensis in *Salmonella typhimurium* , were significantly inhibited by flavonoid (rutin).Moreover, Horcajada-Molteni *et al.*(2000) reported that rutin inhibtes ovariectomy induced osteopenia in rats . Edenharder and Grunhage (2003). conclude that in the *Salmonella*/ reversion assay , antimutagenic activities of rutin against the peroxide mutagens are caused by radical scavenging effects. In human Lymphocytes rutin displayed protective effects on DNA damage induced by mitomycin C (Undeger *et al.*, 2004).Also , Stagos *et al* ., (2005) reported that protovatechuic acid and rutin act as chemopreventive agents by inhibiting mitomycin C-induced DNA damage .

Results in table (3) showed the genetic activities of 3.4-dihydroxybenzoic acid and its combined with chlorpyrifos.3.4- dihydroxybenzoic acid exerted a weak recombinogenic activity when applied at the concentration of 5ul/ml,which resulted in mitotic gene conversion and mitotic crossing-over in frequencies of 3.8 and 6.8 times as the control levels, respectively. While, using the same concentration of 3.4-dihydroxy benzoic acid showed strong positive indications of mutagenic activity where the induced frequency of reversion was 12 times the spontaneous frequency.

The combined treatment was slightly lower as compared with chlorpyrifos alone , whereas a frequencies of gene conversion and mitotic crossing-over was 7 and 6.5 times the control level in combined treatment , but the treatment of chlorpyrifos a frequencies of gene conversion and mitotic crossing-over were 13.4 and 20.5 times the control ones respectively .

The result of the present study show that rutin and 3,4-dihydroxybenzoic acid may prevent binding of metabolicaly

activated of insecticide chlorpyrifos with DNA and inhibit its mutagenicity . Also synergism is an effect which intensifies the antimutagenic activity during simultaneous application of two or more compounds in genetic tests. It is important to elucidate the possibility of strengthening the antimutagenic potency of already known substances (Andrew,1997 and Shimoi e*t al*. (1996) the defence systems of organisms that restrict the level of undesirable mutation processes . Flavonoids may be essential substancec for these roles . Moreover, organism defense systems may be protecting organisms from exogenous and endogenous DNA defeating factors,synergism may be the maim principle of defense system organization .

To summarize, the present results indicate that rutin and 3,4-di hydroxybenzoic acid have some antimutagenic potential . In the future , more studies are needed to establish more firmly the possible antimutagenic effects of flavonoids .

Table (1) Response of Saccharomyces cerevisiae D7 to treatment with different concentration of chlorpyrifos

		Χονωερταντ			Ρεωερταντ			Χροσσινγ – οωερ		
Χον. μ /μλ	Νυμβερ οφ χελλσ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ
Χοντρολ	17084	14.1(24)	1	–	11.7 (20)	1	–	16.4(28)	1	–
1μλ	10930	25.6(28)	1.8	–	21.9(24)	1.8	–	76.8(84)	4.7	+
2 Μλ	7570	52.6(40)	3.6	+	47.5(36)	4.1	+	158.5(120)	9.6	+
5 Μλ	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 ++=>10control level
- =non significant
D. of Act = Degree of activity, numbers between parentheses represents actual colony counts

Table (2) Response of *Saccharomyces cerevisiae* D7 to treatment with rutin and its combined with chlorpyrifos

		Χονωερταντ			Ρεωερταντ			Χροσσινγ – οωερ		
Χον. μ /μλ	Νυμβερ οφ χελλσ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ
Χοντρολ	17084	14.1(24)	1	–	11.7(20)	1	–	16.4(28)	1	–
Χη. 5 μ λ/μλ	4642	189.6(88)	13.1	++	155.2(72)	13.2	++	336.2(156)	20	++
Ρυτιν 5 μ λ/μλ.	18512	16.8(31)	1.2	–	27.55(51)	23	+	26.47(49)	1.6	–
Χομ Τρ.	10180	46.17(47)	3.3	+	120.8(123)	10.3	++	42.2(43)	2.6	+

Key : Con.= Concentration ,Mut.=Mutation;
D. of Act = Degree of activity ;Numbers. between Parentheses represents actual colony counts;C= Control value;T=Treatment value;+=2-10 control Chlopyrifos;com. Tr. = combined treatment .

Table (3) Response of *Saccharomyces cerevisiae* D7 to treatment protochatechuic acid and its combined with chlorpyrifos

		Χονωερταντ			Ρεωερταντ			Χροσσινγ – οωερ		
Χον. μ /μλ	Νυμβερ οφ χελλσ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ	Μυτ Φρεθ	Τ/χ	Δ. οφ Αχτ
Χοντρολ	17084	14.1(24)	1	–	11.7(20)	1	–	16.4(28)	1	–
Ποσ. Χ. Χηλο.	4642	189.6(88)	13.4	++	155.2(72)	13.2	++	336.2(156)	20.5	++
Προτ.	13650	53(73)	3.8	+	140.6(192)	12	++	112(153)	6.3	+
Χομβινεδ	9852	93.5(97)	7	+	151.2(149)	12.9	++	106.5(105)	6.5	+

Key : Cons.= Concentration ,Mut.=Mutation; D. of Act = Degree of activity ;Number between Parentheses represents actual colony counts;C= Control value ;T=Treatment value;+=2-10 control Level;++=>10 control level;-=non significant;prot= Protocatechuic acid;Pos.c.chlo=positive control (chlcrpyrifos).

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ألية الحماية بواسطة الفلوفونيرات ، راتين وحمض البروتوكاتكويك من تأثير المبيد الحشرى كلورپايرفوس على إحداث العبور الوراثى الجسمى و التحول الجينى والطفرة المرتدة فى فطر خميرة الجباز السلالة D7

إكرام صلاح الدين أحمد
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الية الحماية بواسطة الفلوفونيدات ،رايتن وحمض البروتوكاتكويك من تأثير المبيد الحشرى كلور بايرفوس على إحداث العبور الوراثى الجسمى والتحول الجينى والطفرة المرتدة تم بحثها فى سلالة D7 لفطر خميرة الخباز . وكانت النتائج المتحصل عليها أشارت الى احتمال وجود بعض التاثير المضادة للطفور للفلوفوثيرات ما أحدثه المبيد الحشري كلورپايرفوس من طفرات لذلك الفلوفونيرات المستخرجة من نبات السنا قد تكون لها أهمية كمضادات للطفور ومضادات للأكسدة .