

Studies on the Insecticide Sensitivity in some Yeasts

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Abstracts. The ascosporegenous Yeast *Hansenula anomala* together with anascosporegenous *Rhodotorula rubra* and *Candida guilliermondii* were grown aerobically on media containing different concentrations of insecticide, Trichlorophon "Dipterex" (2-10 mM).

The optimum culture temperature for all the tested yeasts was 30°C. Inclusion of Dipterex in glucose - yeast extract medium suppressed the growth of all organisms at higher concentrations. Also, at all pH values (4-8), there was a statistically significant inhibition with the high concentrations of Dipterex.

All the tested yeasts have an optimum growth at 30°C and pH 6. The presence of insecticide in the medium showed a statistically significant inhibition with the levels of cellular protein in the yeast.

Introduction

The inhibitory effect of pesticide on non-target soil and epiphytic microorganisms has long been attracted the attention of investigators [1-5]. They have reported the inhibitory effect of chlorinated hydrocarbons and organophosphorus insecticides on soil microorganisms.

Soil microorganisms play a significant role in insecticide degradation which is induced or produced by biological agents [6-10].

Many workers have studied the effect of Trichlorophon "Dipterex" (0-0-dimethyl - 2, 2, 2 - trichloro - 1 - hydroxyethyl phosphonate) insecticide on the growth and activity of soil microorganisms as well as its degradation by bacteria and fungi [11-15]. This insecticide, at concentrations of 3-5mM, was found to exert toxic effect on the growth of *Rhizobium leguminosarum* and *Rhizobium trifolii* and shifted the optimum temperature of growth from 30 to 25°C and optimum pH from 7 to 6.5. Adam [12] studied the effect of Dipterex on rhizosphere fungi and showed that high concentrations inhibited growth, while the lowest dose used was generally stimulat-

ory. However, no complete inhibition of growth was observed with any of the concentrations used.

El-Zawahry [11], Adam [12] and Salama *et al.* [15] have observed the formation of $^{14}\text{CO}_2$ from bacterial and fungal cultures containing radioactive Dipterex which was attributed to the oxidation of liberated methanol groups (during the breakdown of the Dipterex).

The sporogenous yeast *Hanseniaspora guilliermondii* and *Hansenula anomala*, together with asporogenous yeast *Rhodotorula rubra* were investigated by Mahmoud *et al.* [16] who found that the *akaricide* Takel was more toxic than the organophosphorus insecticide, Lebaycide and the carbamate fungicide, Antrolcol.

Mahmoud *et al.* [17] also studied the effect of water activity on pesticides sensitivity in some yeasts, and found that cells of *Saccharomyces cerevisiae* showed higher response to the water activity of growth medium in relation to pesticides sensitivity over the osmotolerant yeast *Saccharomyces rouxii*.

The aim of the present work is to investigate the effect of different concentrations of Trichlorophon "Dipterex" on the growth and cellular proteins of the ascosporogenous yeast, *Hansenula anomala* together with anascosporogenous *Rhodotorula rubra* and *Candida guilliermondii* at various incubation temperatures and pH values under aerobic conditions.

Materials and Methods

Test organisms

Three test organisms, *Hansenula anomala* (Hansen) H. et p. Sydow var *anomala*; *Rhodotorula rubra* (Domme), Lodder and *Candida guilliermondii* (Cast) Lengeron et guerra, previously isolated from the surface of orange, apricots and plums respectively, and identified by Mahmoud *et al.* [18] were used.

Growth medium

The test organisms were grown in glucose – yeast extract agar. The medium was prepared essentially as described by Crabtree and Hindstill [19] and consisted of (g/l); 5 peptone, 3 yeast extract, 10 glucose, 2 KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The pH was adjusted to 6 by using phosphate buffer.

Standard cell suspensions

Yeast cells were grown on slants of glucose – yeast extract agar for 48 hr at 30°C, and after centrifuging at 2400 r.p.m., were washed and suspended in distilled water. The optical density of the cell suspension was colorimetrically adjusted to 0.1 at 520 nm.

Insecticide treatments

The method described by Russel and Diann [20] was adopted. A 100-ml capacity Erlenmeyer flask containing 50 ml sterile distilled water having the desired insecticide concentration (2, 3, 5, 7 or 10 mM) was mixed using a magnetic stirrer at room temperature. The standard cell suspension (0.5 ml) was added. After 5 min, 0.5 ml portions were spread over the surface of the glucose-yeast extract agar media plates containing oxytetracycline, 100 p.p.m., to check for any bacterial growth [21]. The plates were prepared in triplicate and incubated at 20, 25, 30 and 35°C for 48 hr, then the colonies were counted. The same procedure was used to study the effect of the pH on the growth. The plates were incubated at 30°C and the media was adjusted to pH 4-8 with phosphate buffer [22] and addition of appropriate amounts of either 1N HCl or NaOH solution.

Estimation of cellular proteins

Cellular protein was estimated as nitrogen according to the techniques described by [23-24]. Proteins of washed compressed yeast cells, corresponding to about 75 mg of the dried yeast were used for analysis. The samples were run in triplicates. For statistical analysis of the data, the paired sample t-test was used according to Campbell [25] (Tables 1-3).

Table 1. Statistical analysis for viable yeast cells obtained at different temperatures and different Dip-terex concentrations.

Species	Temperature °C	Correlation r-value	Paired sample t-value
<i>H. anomala</i>	20 (n)	- 1.296*	a ver b = + 1.91
	25 (b)	- 0.997*	b ver c = - 2.99*
	30 (c)	- 0.948*	c ver d = + 2.30*
	35 (d)	- 0.984*	
<i>C. guilliermondii</i>	20 (a)	0.987*	
	25 (b)	- 0.921*	a ver b = + 2.62*
	30 (c)	- 0.931*	b ver c = - 3.92*
	35 (d)	- 0.950*	c ver d = + 3.63*
<i>Rh. rubra</i>	20 (a)	- 0.903*	a ver b = - 4.66*
	25 (b)	- 0.927*	b ver c = - 2.87*
	30 (c)	- 0.898*	c ver d = + 1.86*
	35 (d)	- 1.670*	

* Statistically significant values at 0.05 P-level.

Table 2. Statistical analysis for viable yeast cells obtained at different pH values and different Diptere concentrations (at 30 °C).

Species	pH	Correlation r-value	Paired sample t-value
<i>H. anomala</i>	4 (a)	- 1.046*	a ver b =+ 3.90*
	5 (b)	- 0.877*	b ver c = - 4.79*
	6 (c)	- 0.955*	c ver d =+ 1.28*
	7 (d)	- 0.829*	d ver e =+ 3.28*
	8 (e)	- 0.941*	
<i>C. guillier- mondii</i>	4 (a)	- 0.964*	a ver b =+ 2.10
	5 (b)	- 0.866*	b ver c =+ 1.36*
	6 (c)	- 0.931*	c ver d =+ 3.45*
	7 (d)	- 0.905*	d ver e =+ 1.04*
	8 (e)	- 0.843*	
<i>Rh. rubra</i>	4 (a)	- 0.904*	a ver b = - 3.87*
	5 (b)	- 0.911*	b ver c = - 3.83*
	6 (c)	- 0.895*	c ver d =+ 4.83*
	7 (d)	- 0.813*	d ver e =+ 2.63*
	8 (e)	- 0.918*	

* Statistically significant values at 0.05 P-level.

Table 3. Statistical analysis for protein contents in yeast cells grown under different condition of temperatures and Diptere concentrations.

Species	Temperature °C	Correlation r-value	Paired sample t-value
<i>H. anomala</i>	20 (a)	- 0.955*	a ver b = - 23.60*
	25 (b)	- 0.981*	b ver c = - 4.82*
	30 (c)	- 0.987*	c ver d =+ 58.08*
	35 (d)	- 0.932*	
<i>C. guillier- mondii</i>	20 (a)	- 0.979*	a ver b = - 27.23*
	25 (b)	- 0.995*	b ver c = - 2.70*
	30 (c)	- 0.960*	c ver d =+ 71.91*
	35 (d)	- 0.947*	
<i>Rh. rubra</i>	20 (a)	- 0.865*	a ver b = - 0.34*
	25 (b)	- 0.829*	b ver c = - 14.77*
	30 (c)	- 0.827*	c ver d =+ 27.87*
	35 (d)	- 0.905*	

* Statistically significant values at 0.05 P-level.

Results

The effect of temperatures on the growth of all those yeasts exposed for 5 min to different concentrations of the insecticide Dipterex is shown in Fig. 1. Growth of the yeasts with and without Dipterex gradually increased up to 30°C, above which growth was reduced. Without Dipterex, the growth of all yeasts was significantly greater.

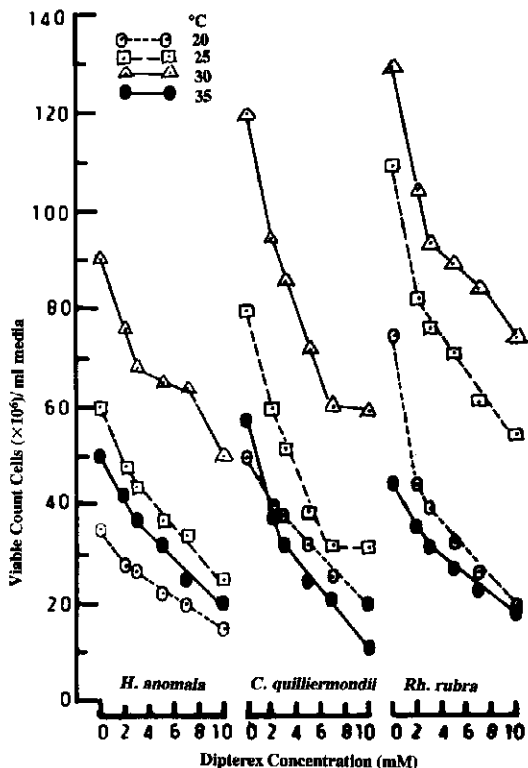


Fig. 1. Effect of the insecticide concentrations on the growth of yeasts *Hansenula anomala*, *Candida guilliermondii* and *Rhodotorula rubra* at different temperatures.

Figure 2 shows the effect of Dipterex concentrations on the growth of all yeasts under investigation at different pH values. It shows growth of yeasts increased with increasing pH values up to pH 6, and then decreased at pH 7 and 8. Dipterex produced significant inhibition of all those yeasts grown at 30°C and at various pH values, (Fig. 2 and Table 2). The growth of the yeasts at all pH values was significantly inhibited with all the concentrations of the insecticide.

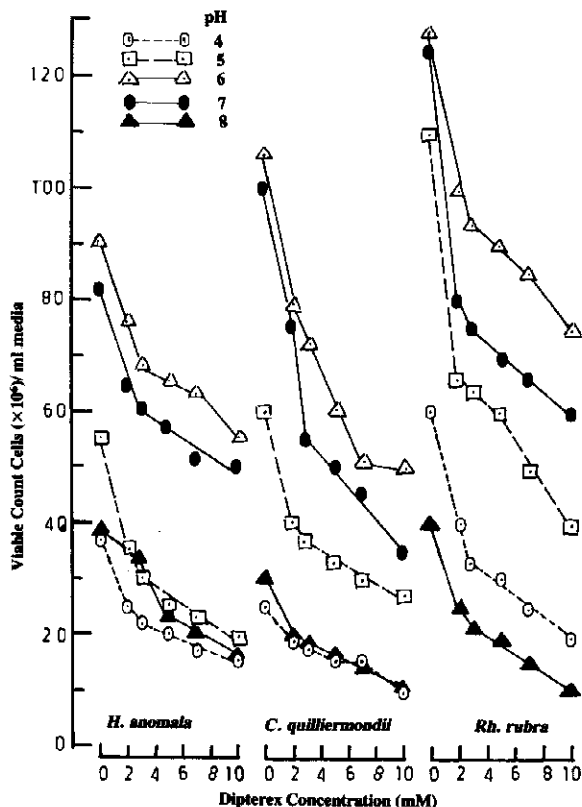


Fig. 2. Effect of the insecticide concentrations on the growth of yeasts *Hansenula anomala*, *Candida guilliermondii* and *Rhodotorula rubra* at different pH-values and constant temperature.

Fig. 3 indicates the effect of insecticide concentrations on the total protein content in all those yeasts at different temperatures. As regards the protein content of yeasts grown in media devoid of Dipterex at temperatures between 20 and 35°C, the results showed that the level of cellular protein of the tested organisms grown at 30°C were significantly higher than those grown at lower temperatures (*i.e.* 20°C and 25°C). Moreover, a remarkable significant decrease in levels of cellular protein occurred at 35°C. Supplementation of Dipterex to the medium generally caused statistically significant inhibition in the levels of cellular protein in all the yeast organisms at all levels of temperature tested (Table 3).

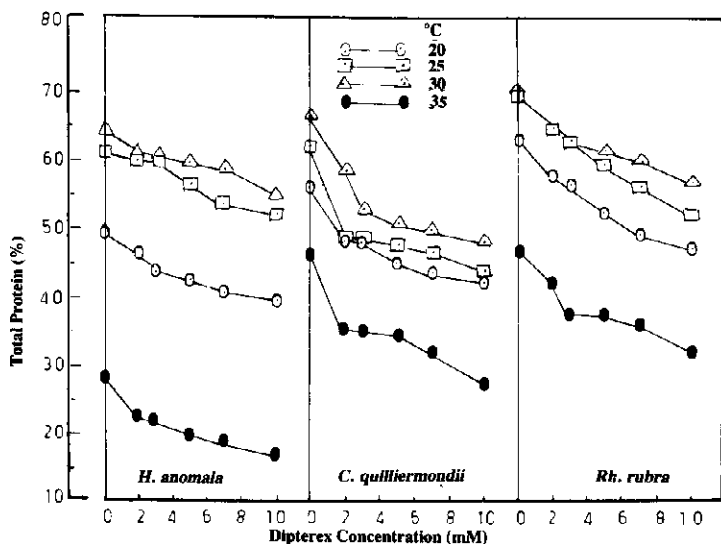


Fig. 3. Effect of the insecticide concentrations on the total protein contents in yeast cells of *Hansenula anomala*, *Candida guilliermondii* and *Rhodotorula rubra* at different temperatures and constant pH-value.

Discussion

Counts of viable yeast cells in the presence of Dipterex at all levels of the tested temperatures showed inhibition (Fig. 1), where no complete inhibition of growth was observed with any of the concentrations added. This result is in accordance with that

obtained by Khalf [26] who reported that the optimum temperature of the growth of the tested fungi; *Aspergillus* sp., *Fusarium* sp. and *Rhizoctonia solani* was 30°C. The recorded optimum temperature for the growth of other fungi is in accordance with literary data obtained by Allison and Minor [27]. They found that the optimum temperature was between 29–31°C.

Adam [12] and Salama *et al.* [13] studied the effect of Dipterex on the Rhizospheric fungi and found that higher concentrations of insecticide exert toxic suppressive effects on the growth of fungi. The lowest dose had an effect, but no complete inhibition was observed with any of the concentrations used.

It is known that the pH-value in a culture medium is one of the major factors affecting the growth of fungi as well as other microorganisms. In this study, the optimum pH obtained for growth of the tested yeasts at 30°C was 6 (Fig. 2). This indicates that the growth is more sensitive towards acidic media [11,13].

The depressive effect exerted by Dipterex on levels of cellular protein was also increased with increase of dose, indicating that the inhibitory effects, observed on cell counts (Figs. 1 and 2), were mainly affecting the cell metabolism.

Nagaib [28], while working with *Rhizoctonia solani*, reported that the total nitrogen of "Sevin" treated organisms decreased with increasing the dose level of the insecticide. Heikal [29] showed that the herbicides of the dimethyl phenyl urea derivatives lowered both soluble protein and non-protein nitrogen of the filamentous fungi *Fusarium oxysporum*.

In conclusion, the present paper has revealed certain fundamentals regarding suppressive effects exerted by the insecticide trichlorophon "Dipterex" on the viable of yeast counts grown at different temperatures and pH values. There have been few attempts to determine the amount of a pesticide required at the site of action to cause the desired effect. Trichlorophon (Dipterex) caused no complete inhibition for growth because much of this insecticide may not reach the site of action, i.e., it does not penetrate well into the cells, or because it is readily metabolised [30].

The results may be explained by the biochemical mode of action of pesticides on nucleic acids composition of organisms [31]. Therefore, in culture of yeasts, DNA synthesis became progressively inhibited after exposure to pesticides, and complete inhibition was not achieved until the DNA content per nucleus had nearly doubled [32-34].

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دراسة على حساسية بعض فطريات الخميرة للمبيدات الحشرية

زينب حسن خيرالله

مركز الدراسات الجامعية للنبات، قسم علم النبات، جامعة الملك سعود،
ص.ب. ٢٢٤٥٢ ، الرياض ١١٤٥٩ ، المملكة العربية السعودية

(أُستلم في ١٣ جمادى الآخرة ١٤٠٧هـ، قُبِلَ للنشر في ٢٨ جمادى الآخرة ١٤٠٨هـ)

ملخص البحث. نُميت خميرة هانسبول أنومالا (وهي خميرة زقية) مع كل من رودوتوريولا روبرا وكانديدا جويليير موندباي (وهما خميرتان غير زقيتان) تحت ظروف هوائية في بيئة تحتوي على تركيزات مختلفة للمبيد (الدييتركس) حيث وُجد أن درجة الحرارة المثلى للنمو لجميع الخمائر هي ٣٠م عند رقم هيدروجيني ٦ .

وقد لُوحظ أن نمو الخمائر ينخفض كلما تغيرت درجات الحرارة والرغم الهيدروجيني، وهذا الانخفاض يكون معنوياً عند زيادة تركيزات مبيد الدييتركس المضاف إلى بيئة النمو وينخفض تبعاً لذلك البروتين الخلوي في الخمائر الثلاث .