The Effect of Aromatic Nitro Compound on the Growth of Mycosis- Causing Fungi

Agus Aulung,drs; Jandi R. Sulaeman,dr

Abstrak

Penelitian yang dilakukan di Laboratorium Parasitologi Fakultas Kedokteran Universitas Indonesia ini bertujuan untuk mengetahui potensi komponen nitro aromatik (ANC) dalam mempercepat pertumbuhan koloni jamur in vitro untuk maksud diagnostik. Spesies jamur sebanyak 18 buah diperoleh dari persediaan biakan Laboratorium Mikologi bagian Parasitologi . Koloni jamur dibiak pada media agar Sabouraud dekstrose yang ditambah ANC sebagai bahan penelitian, sedangkan media agar tersebut tanpa ANC digunakan sebagai media kontrol. Tampak jelas adanya pengaruh ANC terhadap kecepatan pertumbuhan yang bervariasi tergantung pada konsentrasi ANC yang ditambahkan. Konsentrasi paling efektif yang menyebabkan stimulasi adalah 0,01%.

Abstract

This study was carried out at the Parasitology Laboratory of the Faculty of Medicine, University of Indonesia, to explore the potential of the fertilizer Aromatic Nitro Compound (ANC) for accelerating the growth of fungus colonies in vitro for diagnostic purposes. The 18 fungal species studied were obtained from the culture stocks of the Mycology Laboratory of the Department of Parasitology . The colonies were cultured in Sabouraud dextrose agar medium to which ANC was added in increasing amount for research purposes, while Sabouraud dextrose agar without ANC was used as a control. It was found that the influence of ANC on growth rates was marked, varying considerably according to the concentration of ANC added, the most effective concentration for stimulation being 0,01%.

Keywords *: Fungal growth, Sabouraud dextrose agar medium, Orthonitrophenol, Natriumparantrophenol, Dinitrophenol, Nitroguaicol.*

INTRODUCTION

Aromatic Nitro Compound (ANC) consists of 0.2 % orthonitrophenol, 0.3% natrium paranitrophenol, 0.5% dinitrophenol, and 0.1% 5-nitroguaicol. In Indonesia, ANC is available on the open market and is popular with farmers and gardeners because it improves the growth rate of plant roots and stimulates plant development by improving cytoplasmic circulation. A preliminary study of the effect of ANC on the growth of Candida showed a promising result.[1]

Since examination of culture is an effective specific diagnostic tool, but often extremely time - consuming,[2-5] the aim of this study was to establish whether ANC could speed up the growth rate of fungi in Sabouraud dextrose agar, a very widely used medium, in order to save time.

MATERIALS AND METHODS

Eighteen species of fungi from the culture stocks of the Mycology Laboratory of the Department of Parasitology, University of Indonesia , were studied, including five dermatophyte species. The medium used in this study was Sabouraud dextrose agar to which was added 1 ml of 0.01 %, 0.05%, and 0.1% ANC solution. Sabouraud dextrose agar without ANC was used as a control media.

Department of Parasitology, Faculty of Medicine, University of Indonesia

The eighteen species fell into two groups, fast growing, i.e *Candida albicans, Rhizopus sp, Aspergillus fumigatus, Penicillium sp, Blastosporus meristosporus, Hormodendrum sp, Absidia sp, Cephalosporium sp, Syncephalostrum sp,* and *Cryptococcus neoformans*, and slow growing dermatophyte, i.e. *Trichophyton mentagrophytes, Microsporum gypseum, Epydermophyton floccosum, Tricophyton rubrum, Microsporum canis, Histoplasma capsulatum* and *Sporotrichum schenckii.*

All these species were cultured in the Sabouraud dextrose agar medium containing ANC and incubated at room temperature, three days for the fast growing species , and 14 for the slow growing ones. The control groups were given the same treatment. Growth was monitored daily.

FINDING AND DISCUSSION

The growth rates of the fungi in the different media varied widely (Table 1). Table 2 summarized observations of the growth of the fast growing fungi on day 2. The Anova statistical test (Table 3) showed that addition of ANC to the Sabouraud dextrose agar media affected the growth rates of these fungi significantly ($p<0.01$). Fungi of the dermatophyte group such as *Tricophyton rubrum, Tricophyton mentagrophytes, Microsporum canis, Microsporum gypseum*, and *Epidermophyton floccosum* are known to grow slowly in Sabouraud dextrose agar. Culturing clinical material requires 14 days, the dermatophyte being keratinophylic and generaly host specific. The findings on the effect of ANC on slow growing fungi on day 6 are shown in Table 4 . Analysis of this data with the Anova statistical test (Table 5) showed that addition of ANC has a significant effect ($p < 0.01$). To identify the most effective concentration of ANC for fast growing fungi, the Scheffe test was applied . This test showed that a 0.01 % ANC concentrationwas the most effective concentration for the stimulation of fungi growth. A comparison of the growth rates in the different media is shown in table 6. The 0.01% and 0.05% concentration of ANC showed significant inreases of growth rates in comparison with the control, while the concentration of 0.1% showed no significant difference. The same test was done on slow growing fungi (Table 7) . On the basis of these results, the writer maintains that ANC promotes the multiplication process as well as the growh of fungal colonies and that the 0.01 % concentration of ANC is the most optimal stimulant of fungal growth among the three concentrations tested.

Table 1. Growth rate of fungi in Sabouraud dextrose agar with and without ANC

Fungi Species	Growth fo fungi (cm) at ANC concentration of :				Observation on day
	0.01 %	0.05 %	0.1%	0.0%	
C. albicans	0.6	0.5	0.2	0.4	2
Rhizopus sp	2	1.6	0.7	0.7	2
Fusarium sp	0.6	0.6	0.4	0.4	2
A. fumigatus	1.3	0.9	0.4	0.6	2
Penicillium sp	1.3	0.8	0.3	0.6	2
B.meristoporus	1.3	0.7	0.4	0.5	2
Hormodendrum sp	0.6	0.4	0.3	0.6	2
Absidia sp	1.9	1.6	0.5	0.6	2
Cephalosporum sp	1.0	0.8	0.4	0.6	2
Syncephalostrum sp	1.1	0.8	0.4	0.5	2
C.neoformans	0.7	0.5	0.3	0.4	2
T.mentagrophytes	1.6	0.9	0.7	0.9	6
M.gypseum	1.2	1.0	0.7	0.9	6
E.floccossum	1.1	1.0	0.7	0.7	6
T.rubrum	1.5	1.0	0.7	0.8	6
M.canis	1.6	1.2	0.8	0.9	6
H.capsulatum	0.6	0.4	0.2	0.4	6
S.Schenckii	0.8	0.5	0.3	0.4	6

Table 2. Growth rate of fast-growing fungi on Sabouraud dextrose agar with and without ANC

Fungi Species	Fungi growth (cm) at ANC concentration of :				Observation on day
	0.01%	0.05%	0.1%	0.0%	
C.albicans	0.6	0.5	0.2	0.4	2
Rhizopus sp	2.0	1.6	0.7	0.7	2
Fusarium sp	0.6	0.6	0.4	0.4	2
A.fumigatus	1.3	0.9	0.4	0.6	2
Penicillium sp	1.3	0.8	0.3	0.6	2
B.meristosporus	1.3	0.7	0.4	0.5	2
Hormodendrum sp	0.6	0.4	0.3	0.3	2
Absidia sp	1.9	1.6	0.5	0.6	2
Cephalosporum sp	1.0	0.8	0.4	0.6	2
Syncephalostrum sp	1.1	0.8	0.4	0.5	2
C.neoformans	0.7	0.5	0.3	0.4	2

Table 3. Anova for table 2

Source of Variation	df	SS	MS	F	F table
Average	1	22.55	-		
Block	10	3.08	0.308		
Treatment	3	3.65	1.21	30.25	4.51
Error	30	1.39	0.04		

Table 4. Growth rate of slow-growing fungi on Sabouraud dextrose agar with and without ANC

Fungi Species	Fungi growth(cm) at ANC concentration of : 0.01 %	0.05%	0.1%	0.0%	Observation on day
T.mentagrophytes	1.6	0.9	0.7	0.9	6
M.gypseum	1.2	1.0	0.9	0.9	6
E.floccosum	1.3	1.0	0.7	0.7	6
T.rubrum	1.5	1.0	0.7	0.8	6
M.canis	1.6	1.2	0.8	0.9	6
H.capsulatum	0.6	0.4	0.2	0.4	6
S.schencekii	0.8	0.5	0.3	0.6	6

Table 5. Anova for table 4

Source of Variation	df	SS	MS	F	F table
Average	1	13.93	-		
Block	6	2.08	0.34		
Treatment	3	4.28	1.42	28.4	5.09
Error	18	0.99	0.05		

Table 6. Comparison of the growth effect of different concentrations of ANC in the medium on fast growing fungi (Scheffe test)

MEDIUM : II	III	IV
ANC CONC : (0.05%)	(0.1%)	(0.0%)
I (0.01%)	F>F' = 28.22>16.68	F>F' = 181.056>16.68	F>F' = 127.308>16.68
II (0.05%)		F>F' = 66.30> 16.68	F>F' = 35.64>16.68
III (0.1%)			F<F' = 4.72<16.68*

* no significant difference

Table 7. Comparison of the growth effect of different concentration of ANC in the medium on slow growing fungi (Scheffe test)

MEDIUM	II	III	IV
ANC CONC :	(0.05%)	(0.1%)	(0.0 %)
I (0.01%)	F<F' = 13.68<16.68 *	F>F' = 70.56>16.68	F>F' = 59.29>16.68
II (0.05%)		F>F' = 22.09>16.68	F<F' = 16.0<16.68 *
III (0.1%)			F<F' = 0.49<16.68 *

* no significant difference

CONCLUSIONS

The effect of ANC on the growth of mycosis-causing fungi can be summarized as follows :

1. The addition of ANC in certain concentrations to Sabouraud dextrose agar stimulates the growth rate of fungi.
2. The most approriate concentration of ANC of the three studied in the medium for stimulating fungal growth is 0.01 %.

RECOMMENDATION

Since ANC in low concentrations appears to have a favourable effect on the growth of fungal cultures, it is recomended that :

1. Further tests be conducted at still lower concentrations and
2. The stimulant effect of ANC on fungal growth be further tested on cultures of clinical material.

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