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THE EFFECT OF ENVIRONMENTAL FACTORS ON CONIDIAL GERMINATION, SPORULATION AND GROWTH OF *TRICHODERMA HARZIANUM IN VITRO*

PENGARUH FAKTOR-FAKTOR LINGKUNGAN TERHADAP PERKECAMBAHAN KONIDIUM, SPORULASI DAN PERTUMBUHAN TRICHODERMA HARZIANUM IN VITRO

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INTISARI

Penelitian dilakukan untuk mengevaluasi pengaruh suhu, pH media, dan lama penyinaran terhadap perkecambahan konidium, sporulasi, dan pertumbuhan Trichoderma harzianum *di media PDA (*Potato Dextrose Agar*). Penelitian dilakukan dengan menggunakan Rancangan Acak Lengkap dengan 3 ulangan. Hasil pengamatan menunjukkan bahwa perkecambahan konidium tertinggi terjadi pada suhu 30°C, demikian pula untuk sporulasi dan pertumbuhan* T. harzianum. *Lama penyinaran tidak berpengaruh nyata terhadap perkecambahan konidium akan tetapi sporulasi akan terpacu oleh perlakuan 12 jam terang-12 jam gelap dan diameter koloni* T. harzianum *nyata lebih besar pada perlakuan gelap bila dibandingkan dengan perlakuan 24 jam terang ataupun 12 jam terang-12 jam gelap. Keasaman (pH) media berpengaruh nyata terutama terhadap perkecambahan konidium dan sporulasi. Perkecambahan konidium tertinggi terjadi pada media dengan pH 8 sedangkan yang terendah terjadi pada pH 5. Sporulasi akan terpacu pada pH tinggi. Pengaruh pH terhadap pertumbuhan* T. harzianum *terjadi pada pH 9 dimana panjang diameter koloni nyata lebih kecil dibandingkan dengan perlakuan lainnya.*

Kata kunci: Suhu, pH media, lama penyinaran, Trichoderma harzianum

ABSTRACT

This experiment was conducted to study the influence of temperature, pH of media, and length of radiaton on conidial germination, sporulation and growth of *Trichoderma harzianum* on PDA (*Potato Dextrose Agar*). It was conducted in a Completely Randomized Design (CRD) with 3 replications. The results indicated that the highest conidial germination occurred at 30°C, and so the sporulation and the growth of *T. harzianum*. Length of radiation did not significantly affect conidial germination. However sporulation was enhanced by 12 hrs light-12 hrs dark treatment and colony diameter of *T. harzianum* was significantly greater at dark. Acidity influenced mainly the conidial germination and the sporulation of *T. harzianum*. The greatest of conidial germination occurred at pH 8, whereas the smallest occurred at pH 5. Sporulation was also enhanced by higher pH. The influence of acidity on the growth of *T. harzianum* occurred at pH 9 which its diameter colony was significantly smaller compared to the other treatments.

Key words: Temperature, pH of medium, length of radiation, *Trichoderma harzianum*

INTRODUCTION

Trichoderma sp. have been known as potential biological control agents of a wide range of plant pathogens. These microorganisms are easily isolated from soil and most biological control trials were done against soil borne pathogens. *Trichoderma* sp. fulfill the demands as biological control agents for the high reproductive capacity, the ability to survive in unfavorable environmental conditions, and the aggressiveness to colonize plant roots. The effectiveness of *Trichoderma* sp.

as the biological control agents depend on its ability to colonize and maintain its effective population (Tronsmo, 1986).

The practical use of *Trichoderma* sp. for biological control will relate when further trials are carried out to determine the stability, ability to adapt and survive under different fields conditions (Huang, 1990). The effect of environmental factors such as temperature, humidity, radiation, and pH to *Trichoderma* sp. must be understood in order to succeed the treatment. For example Harman *et al.* (1981) showed that of *T. hamatum* was effective to control seed rot of pea caused by *Rhizoctonia solani* at 22°C whereas Elad *et al.* (1980) showed that *T. harzianum* was effective to control *Sclerotium rolfsii* under low temperature and high soil pH level. This study was intended to examine factors affecting the conidial germination, the sporulation, and the growth of *T. harzianum in vitro*.

MATERIALS AND METHODS

Fungal culture. *T. harzianum* culture was obtained from Dr. G. Bedlan (Bundesamt und Forschungzentrum fur Landwirtschaft, Vienna, Austria) was isolated from biofungicide Trichodex.

Influence of temperature on conidial germination, sporulation, and fungal growth. Ten ml of sterilized water was poured onto 3 days cultures of *T. harzianum* on a PDA plate incubated at 30°C and conidia were scraped with a glass rod. Conidial suspension was filtered by using a sterile cheesecloth and 0.2 ml of conidial suspension was dropped onto a water agar plate, incubated for 24 hrs at 10°C, 20°C, 30°C, and 40°C after which a drop of lactophenol cotton blue was pipetted on it. The germinating conidia were counted under 400 × magnification.

The fungal growth was observed by placing a disk of pure culture of *T. harzianum* onto a PDA plate and incubated for 7 days at 10°C, 20°C, 30°C, and 40°C. The colony diameter was measured everyday starting at one day after plating. After 7 days of incubation, 10 ml of sterilized water was poured onto the culture, conidia were scraped with a glass rod, and conidial suspension was filtered with a cheesecloth. The number of conidia was observed with a haemocytometer under 400 × magnification.

Dry weight of fungal mycelium was observed after 7 days of incubation. A 10 ml of HCl 0.1 N was dropped onto a fungal culture and the agar was melted on a water bath. Fungal mycelium was removed into filter paper, washed with tap water, dried in an oven for 24 hrs at 50°C and weighted with analytical scale.

Influence of pH on conidial germination, sporulation and fungal growth. Ten ml of sterilized water was poured onto 3 days cultures of *T. harzianum* on a PDA plate incubated at 30°C and conidia were scraped with a glass rod. Conidial suspension was filtered by using a sterile cheesecloth and 0.2 ml of conidial suspension was dropped onto a water agar plate which pH were adjusted to 5, 6, 7, 8 and 9, incubated for 24 hrs at 30°C after which a drop of lactophenol cotton blue was pipetted on it. The germinating conidia were counted under 400 × magnification.

The fungal growth was observed by placing a disk of pure culture of *T. harzianum* onto a PDA plate which pH was adjusted to 5, 6, 7, 8 and 9, and incubated for 7 days at 30°C. The colony diameter was measured everyday, starting at one day after plating. After 7 days of incubation, 10 ml of sterilized water was poured onto the culture, conidia were scraped with a glass rod, and conidial suspension was filtered with a cheesecloth. The number of conidia was observed with

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Influence of length of radiation on conidial germination, sporulation, and fungal growth. Ten ml of sterilized water was poured onto 3 days cultures of *T. harzianum* on a PDA plate incubated at 30°C and conidia were scraped with a glass rod. Conidial suspension was filtered by using a sterile cheesecloth and 0.2 ml of conidial suspension was dropped onto a water agar plate, incubated in a growth chamber which length of radiation was adjusted to 24 hrs dark, 24 hrs light and 12 hrs dark–12 hrs light for 24 hrs at 30°C after which a drop of lactophenol cotton blue was pipetted on it. The germinating conidia were counted under 400 × magnification.

The fungal growth was observed by placing a disk of pure culture of *T. harzianum* onto a PDA plate and incubated in a growth chamber which length of radiation was adjusted to 24 hrs dark, 24 hrs light and 12 hrs dark–12 hrs light for 7 days at 30° C. The colony diameter was measured everyday. After 7 days of incubation, 10 ml of sterilized water was poured onto the culture, conidia were scraped with a glass rod, and conidial suspension was filtered with a cheesecloth. The number of conidium was observed with a haemocytometer under 400 × magnification.

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Statistical analyses. The experiments were conducted in Completely Randomized Design (CRD) with 3 replications. The effects of treatments were assessed by analyses of variance (ANOVA). Differences among treatments were evaluated by the Least Significant Different (LSD) at 5% level of significant ($P < 0.05$).

RESULTS AND DISCUSSION

The result of this study showed that temperature influenced the conidial germination of *T. harzianum* which the greatest percentage of conidial germination was obtained at 30°C. Table 1 shows that at 30°C, the percentage of conidial germination of *T. harzianum* was 79.7% whereas at 20°C the conidial germination was only 44.7%. At 10°C and 40°C the conidia of *T. harzianum* did not germinate.

Table 1 also shows that temperature influenced both fungal growth and sporulation. At 30° C, 7 days after incubation the colony diameter was 8.5 cm and mycelium dry weight was 0.095 g. Although *T. harzianum* still grew at 10°C, the colony diameter was only 1.7 cm with mycelium dry weight of 0.022 g. At 40°C the growth of *T. harzianum* was eliminated. The greatest fungal sporulation was at 30°C where the number of conidium was 4.2×10^6 cells/ml, but at 20°C and 10°C the number of the conidium was only 1.0×10^5 cells/ml and 3.8×10^5 cells/ml, respectively. Fungal sporulation might be affected by fungal growth. In this experiment, as the fungus grew best at 30°C, sporulation was exhibited.

Tabel 1. The conidial germination, sporulation, and the growth of *T. harzianum* at different level of temperature.

Treatment	Conidial germination (%)		No. of conidium (cells/ml)		Colony diameter (cm)		Mycelium Dry weight (g)	
10°C	0	c	3.8×10^5	b	1.7	c	0.022	c
20°C	44.7	b	1.0×10^5	c	6.8	b	0.042	b
30°C	79.7	a	4.2×10^6	a	8.5	a	0.095	a
40°C	0	c	0	d	0	d	0	d

Means followed by the same letters were not significantly different at 5% level of significant.

However as the fungal growth was slower at 20° and 10°C, sporulation was inhibited. At 40°C, the fungus did not grow and the conidia were not formed.

Temperature has significant effect on conidial germination of various fungi. For example Agnihotri & Vaartaja (1967) showed that germination of sporangia of *Pythium ultimum* was exhibited at 15–25°C. At this temperature region, the percentage of sporangia germination was 93%. However at higher temperature (30° and 35°C) the percentages of sporangia germination were only 40% and 26%, respectively. Heat treatment influenced the physiological and cytological basis of spore activation of fungi. Heat treatment that activated spores might caused changes in constituent proteins and lipids, regulatory protein, and alterations in the plasma membrane which increased osmolarity and permeability of spores (Ruben *et al.*, 1980). This result also confirmed previous study conducted by Harman *et al.* (1981) who showed that on PDA plates, *T. hamatum* grew well at 20–30°C, but at higher or lower temperature growth was much lower.

Table 2 shows that radiation of 24 hrs light, 12 hrs light - 12 hrs dark and 24 hrs dark did not affect conidial germination. However length of radiation affected the sporulation of *T. harzianum*. At 24 hrs light the number of conidium was 6.17 x 10^6 cells/ml whereas at 12 hrs light-12 hrs dark and 24 hrs dark the number conidium was only 2.83 x 10^6 and 2.28 x 10^5 cells/ml respectively.

Length of radiation also affected the fungal growth. There was no significantly different of fungal growth exposed to 24 hrs light and 12 hrs light-12 hrs dark (6.9 cm after 7 days of incubation). However when the culture was exposed to 24 hrs light, the colony diameter was significantly greater (8.0 cm). Although there was significantly difference of the colony diameter, the dry weight of mycelium between treatments were not significantly different.

This result confirmed the experiment conducted by Moore-Landecker (1982) who showed that the sporulation of *T. lignorum* occurred under light, whereas Dharmaputra & Suwandi (1989) mentioned that light limitation caused the growth of *Trichoderma sp.* on its substrate was inhibited.

Acidity has been found to affect conidial germination of fungi. Table 3 shows that low pH inhibited the conidial germination of *T. harzianum*. At pH 9, 99.2% of conidia were germinated, but at pH 5 the conidial germination was significantly reduced to 89.0%. The smallest number of *T. harzianum* conidium was at pH 6 (2.6×10^5 cells/ml). However, the number of conidium significantly increased at pH 7, 8 and 9 which were 6.8 x 10^4, 1.9 x 10^5 and 1.0 x 10^5 cells/ml, respectively. The number of conidium obtained at pH 5 (5.7 x 10^4 cells/ml) was also greater than that obtained at pH 6.

Table 2. The conidial germination, sporulation, and fungal growth of *T. harzianum* at different length of radiation.

Treatment	Germination (%)		No. of conidium (cells/ml)		Colony diameter (cm)		Dry weight of Mycelium (g)	
24 h light	91.5	a	6.2×10^6	a	6.9	b	0.062	a
12 h light 12 h dark	93.7	a	2.8×10^6	b	6.9	b	0.072	a
24 h dark	92.8	a	2.3×10^6	c	8.0	a	0.091	a

Means followed by the same letters were not significantly different at 5% level of significant.

Table 3. The conidial germination, sporulation, and the fungal growth of *T. harzianum* at different level of pH of the medium.

Treatment	Conidial germination (%)		No. of conidium (cells/ml)		Colony diameter (cm)		Dry weight of mycelium (g)	
5	89.0	c	5.7×10^4	c	8.48	a	0.074	a
6	92.6	bc	2.6×10^4	d	8.50	a	0.074	a
7	96.4	ab	6.8×10^4	bc	8.50	a	0.072	a
8	99.7	a	1.9×10^5	a	8.50	a	0.062	a
9	99.2	a	1.0×10^5	ab	7.49	b	0.052	a

Means followed by the same letters were not significantly different at 5% level of significant.

The fungal growth were not significantly different at pH 5, 6, 7, and 8 whereas at pH 9 the colony diameter was significantly smaller (7.5 cm, 7 days after incubation). However the dry weight of mycelium at pH 5, 6, 7, 8, and 9 were not significantly different.

Ruben *et al.* (1980) showed that acidity affected the rate of oospore germination of *Pythium aphanidermatum*. Twelve hrs after incubation, the oospore germination rate was the highest (more than 90%) at pH 7. However at pH 5 the oospore germination rate was greatly reduced to less than 40%. Acid conditions might also favor the activity and development of *Trichoderma* spp. (Harman *et al.*, 1981; Chet & Baker, 1981). Isolation of *Trichoderma* sp. at soil with lower pH (5.1) showed that its population density was 8×10^5 propagules per gram soil and it was significantly greater than that at soil with higher pH (8.1) which was 1×10^2 propagules per gram soil (Chet & Baker, 1981).

CONCLUSION

It can be concluded that the conidial germination, sporulation and growth of *Trichoderma harzianum* were affected by temperature, radiation, and pH of the medium. At 30°C, conidial germination, sporulation and fungal growth were the greatest. The length of radiation did not affect conidial germination and the dry weight mycelium. However at 24 hrs light the sporulation was enhanced. The conidial germination was the greatest at pH 8, and 7 for sporulation. At pH 9, the fungal growth was inhibited.

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