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

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

Arif Wihowo
Agriculture Faculty, Gadjah Mada University

INTISARI

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 radiation 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 darkness. Acidity influenced mainly the conidial germination and the sporulation of T. harzianum. The greatest of conidial germination occurred at pH 7, 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 (Tromso, 1986).

The practical use of Trichoderma
sp. for biological control will relate when
future traits are carried out to determine
the stability, ability to adapt and survive
under different field 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, Harnan et al.
(1981) showed that of T. harzianum 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 Sclerotinia rossii 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. Bedian (Bundesamt
und Forschungszentrum für Landwirtschaft,
Vienna, Austria) was isolated from
biofungicide Trizohex.

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 x 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 x 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 x magnification.

The fungal growth was observed by
placing a disk of pure culture of T.
harzianum onto a PDA plate which pH
were 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
a haemocytometer under 40× 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 weighed with analytical scale.

**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.

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 weighed with analytical scale.

**Statistical analyses.** The experiments were conducted in Completely Randomized Design (CRD) with 3 replications. The effects of treatments were assessed by analysis of variance (ANOVA). Differences among treatments were evaluated by the Least Significant Difference (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 × 10^7 cells/ml, but at 20°C and 10°C the number of the conidium was only 1.0 × 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.
Table 1. The conidial germination, sporulation, and the growth of *T. harzianum* at different level of temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conidial germination (%)</th>
<th>No. of conidium (cells/ml)</th>
<th>Colony diameter (cm)</th>
<th>Mycelium Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>0</td>
<td>3.8 x 10⁵</td>
<td>1.7</td>
<td>0.022</td>
</tr>
<tr>
<td>20°C</td>
<td>44.7</td>
<td>0.0 x 10⁵</td>
<td>6.8</td>
<td>0.042</td>
</tr>
<tr>
<td>30°C</td>
<td>79.7</td>
<td>4.2 x 10⁶</td>
<td>8.5</td>
<td>0.095</td>
</tr>
<tr>
<td>40°C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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 Agaricom & Vaaraja (1967) showed that germination of sporangia of *Phytophthora ultima* was exhibited at 15–25°C. At this temperature region, the percentage of sporangia germination was 95%. 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 alternations in the plasma membrane which increases osmolality 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. harzianum* grew well at 20–30°C, but at higher or lower temperature germination 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 10⁵ cells/ml whereas at 12 hrs light - 12 hrs dark and 24 hrs dark the number conidium was only 2.53 x 10⁶ and 2.28 x 10⁷ 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 x 10⁵ cells/ml). However, the number of conidium significantly increased at pH 7, 8 and 9 which were 6.8 x 10⁷, 1.9 x 10⁸ and 0.1 x 10⁹ cells/ml, respectively. The number of conidium obtained at pH 5 (5.7 x 10⁵ 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 incubation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>No. of conidium (cells/ml)</th>
<th>Colony diameter (cm)</th>
<th>Dry weight of mycelium (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h light</td>
<td>91.3 a</td>
<td>$6.2 \times 10^4$ a</td>
<td>6.9 b</td>
<td>0.062 a</td>
</tr>
<tr>
<td>12 h light</td>
<td>95.7 a</td>
<td>$2.5 \times 10^5$ b</td>
<td>6.9 b</td>
<td>0.072 a</td>
</tr>
<tr>
<td>24 h dark</td>
<td>92.8 a</td>
<td>$2.3 \times 10^5$ c</td>
<td>8.0 a</td>
<td>0.091 a</td>
</tr>
</tbody>
</table>

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

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

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

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

The fungal growth was not significantly different at pH 5, 6, 7, and 8 whereas at pH 9 the colony diameter was significantly smaller (5.5 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 conidial germination of *P. aphidermannii*. Twelve hrs after incubation, the conidial germination rate was the highest (more than 90%) at pH 7. However at pH 5 the conidial 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 $8 \times 10^4$ propagules per gram soil and it was significantly greater than that at soil with higher pH (8.1) which was $1 \times 10^5$ 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.

**LITERATURE CITED**


