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ANTAGONISTIC EFFECT OF FOUR FUNGAL ISOLATES TO *GANODERMA BONINENSE*, THE CAUSAL AGENT OF BASAL STEM ROT OF OIL PALM

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ABSTRACT

Four fungal isolates from soils obtained from three sites of the oil palm plantations in North Sumatra were found antagonistic to *Ganoderma boninense*, the causal agent of basal stem rot of oil palm. *Penicillium citrinum* inhibited the growth of the pathogen and formed a zone of inhibition on the agar media. *Trichoderma harzianum* BIO - 1 as well as BIO - 2 and *T. viride* not only repressed the growth of the pathogen but also caused lysis of the hyphae, and the colony was totally overgrown by the antagonists.

INTRODUCTION

Ganoderma boninense Pat. is an important basal stem rot pathogen of oil palm *(Elaeis guineensis* Jacq.) in North Sumatra. Some control methods have been applied to overcome the disease but none seemed to be satisfactory (Parnata 1974; Suyoto & Djamin 1981; Sipayung & Purba 1986).

The use of antagonistic soil microorganisms to control the pathogen has not been investigated so far. Taking into consideration the statement asserted by Cook & Baker (1983) that certain species of *Penicillium* and *Trichoderma* have been reported to be antagonistic to plant pathogenic fungi, we at BIOTROP were curious to find out whether some antagonists could be isolated from soil samples taken from the oil palm plantations in North Sumatra and investigate their antagonistic properties against *Ganoderma boninense*. This paper describes the results of an investigation on the effect of four fungal isolates on the growth of the pathogen *in vitro*.

MATERIALS AND METHODS

Isolation of fungi from soil

Soil samples were taken randomly from three locations (Adolina, Gunung Bayu, and Tinjowan) of the oil palm plantations in North Sumatra. The dilution

plate method (Johnson & Curl 1972) was used to isolate the fungi at a concentration of 10^{-2} ml. Into each Petri dish, 1 ml of the soil suspension was transferred aseptically and added to it was 10 – 12 ml of melted selective medium containing chloramphenicol and rose bengal. The dishes were then incubated for 5 days at room temperature.

Isolates of *G. boninense*

Isolates of G. *boninense* used in this experiment were GA, GB and GD. They were isolated from basidiocarps obtained from the basal stem of oil palm in North Sumatra. The name of the isolates were based on the colour and morphology of the basidiocarp.

Antagonism between G. *boninense* isolate GB and the fungal isolates

The fungal isolates obtained were tested for their antagonistic property against the pathogen using the direct opposition method as recommended by Dennis & Webster (1971). The promising antagonistic molds were then put aside for further studies.

The direct opposition method was prepared by placing the pathogen (4 mm in diameter) on the PDA medium and 2 days later at a distance of 3 cm from the pathogen the antagonist was inoculated on the same dish (Figure 1). Each treatment was in three replications and incubated at room temperature.

Observations were made on the growth of the pathogen and the presence of an inhibition zone that might develop between the two colonies (Figure 2).

Antagonism between G. *boninense* isolates GA, GB and GD and the four promising isolates of fungi

In this study the four isolates of fungi that were found antagonistic to the pathogen were further tested individually for their effect on the pathogen when placed together using the direct opposition method.

The inoculation method of the pathogen and the antagonist is the same as previously mentioned. Observations were made 3 days after the inoculation of the antagonists on the inhibition of mycelial growth of the pathogen by the antagonist using the formula of Fokkema (1973):

$$I = \frac{r_1 - r_2}{r_1} \times 100\%$$

where I = percentage of inhibition
r_1 = radius of the pathogen away from the antagonist
r_2 = radius of the pathogen towards the antagonist

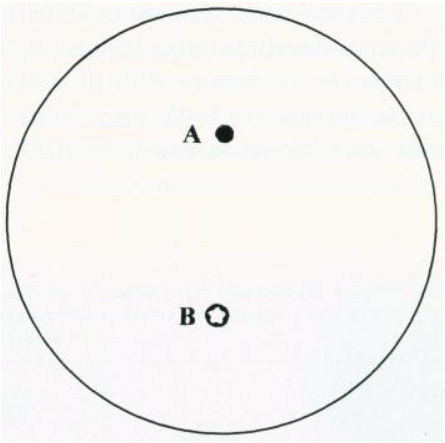

Figure 1. Antagonistic test between *G. boninense* and the fungal isolate. A, inoculum of G. *boninense;* B, inoculum of the fungal isolate tested.

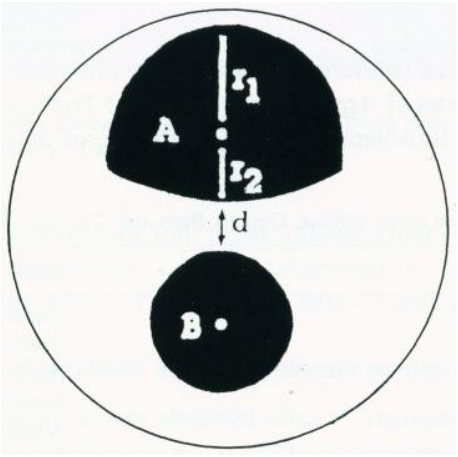

Figure 2. Colony measurement of G. *boninense* (A) to calculate the percentage of inhibition by the antagonist (B). Radius of the pathogen (r, and r_2) and the zone of inhibition (d) were also noted.

For *P. citrinum,* the distance (d) of the zone of inhibition was also measured. Hyphal interaction in the zone of confrontation between the colonies was observed by direct examination under the microscope with 10 x 200 magnification 2 days after the inoculation of the antagonist (AIA). Interactions observed between the pathogen and antagonist were scored according to the categories presented in Table 1.

Table 1. Score of interaction between *Ganoderma boninense* and *Trichoderma*.

Score	Criteria/pathogen hyphae lysed
1	±25%
2	±50%
3	±90%
The scores were added by 1	If pathogen hyphae were smaller than the normal ones
The scores were reduced by 1/2	If ±10% of the antagonist hyphae were also lysed
The scores were reduced by 1	If ± 25% of the antagonist Hyphae were also lysed

RESULTS AND DISCUSSION

The fungi isolated from the soil samples are presented in Table 2: 4 isolates of *Penicillium, 2* isolates of *Aspergillus,* 3 isolates of *Trichoderma,* and 1 unidentified isolate, totaling 10 isolates obtained by the use of dilution plate method.

Table 2. The fungi isolated from Adolina, Gunung Bayu and Tinjowan, North Sumatra.

No.	Fungal isolate	Origin of the soil sample
1.	*Penicillium* sp. 1	Adolina, Tinjowan
2.	*Penicillium* sp. 2	Adolina, Tinjowan
3.	*Penicillium* sp. 3	Adolina, Tinjowan
4.	*Penicillium* sp. 4	Gunung Bayu, Tinjowan
5.	*Aspergillus* sp. 1	Adolina, Gunung Bayu
6.	*Aspergillus* sp. 2	Gunung Bayu, Tinjowan
7.	*Trichoderma* sp. 1	Gunung Bayu, Tinjowan
8.	*Trichoderma* sp. 2	Adolina
9.	*Trichoderma* sp. 3	Gunung Bayu
10.	Unidentified	Adolina, Gunung Bayu

Cook and Baker (1983) stated that *Penicillium* and *Trichoderma* have long been recognized as antagonists to plant pathogenic fungi. All of the ten isolates obtained were further tested on the possibility of inhibiting the growth of the pathogen (isolate GB) *in vitro* using the direct opposition method as recommended by Dennis & Webster (1971). Of the 10 isolates, only four showed promising result, that is *Penicillium* sp. 2, *Trichoderma* sp. 1, *Trichoderma* sp. 2, and *Trichoderma* sp. 3 (Figure 3).

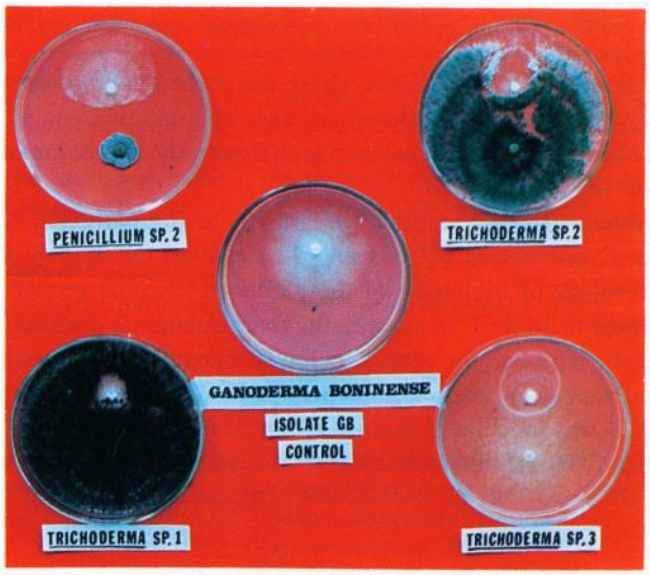

Figure 3. Antagonism between *Ganoderma boninense* isolate GB and *Penicillium* sp. 2, *Trichoderma* sp. 1, *Trichoderma* sp. 2 and *Trichoderma* sp. 3.

Further identification of the four isolates showed that *Penicillium* sp. 2, *Trichoderma* sp. 1, *Trichoderma* sp. 2, and *Trichoderma* sp. 3 were respectively *P. citrinum*, *T. harzianum* BIO-1, *T. harzianum* BIO-2 and *T. viride*.

Antagonism between P. *citrinum* and *G. boninense* isolates GA, GB, and GD

A zone of inhibition (d) was observed when *P. citrinum* was paired with the pathogen. The growth of the pathogen towards the antagonist was inhibited since the 2nd day AIA and it stopped to grow on the 6th day AIA. The distance (d) of the zone of inhibition on the 3rd day AIA were 11.25 mm, 12.88 mm and 10.88 mm for isolates GA, GB and GD, respectively. It was assumed that an antibiotic diffused into the medium.

Statistical analysis using F indicated that I on the 3rd day AIA was not significantly different among the three isolates of the pathogen: 29.95% for isolate GA, 25.28% for isolate GB and 19.08% for isolate GD, respectively.

Direct examination under the microscope showed that the hyphae of the pathogen were not lysed, but they were abnormal, i.e. they had more septa and their cells were shorter than the normal ones.

Antagonism between *Trichoderma* isolates and *G. boninense* isolates GA, GB and GD

Having examined all the dishes where the pathogen colonies and the antagonists were grown, it was noted that there were no inhibition zones. For all treatments, it was observed that mycelial contact between the two colonies on the same dish started on the 2nd day AIA.

T. harzianum BIO - 2 stopped the growth of the pathogen on the 3rd day AIA, whereas *T. harzianum* BIO- 1 and *T. viride* on the 4th day AIA.

The percentage of inhibition of the pathogen mycelial growth by the antagonist is presented in Table 3. In general, the three isolates of *Trichoderma* inhibited the growth of the pathogen *in vitro.* The lowest percentage occurred when isolate GB was placed against *T. harzianum* isolate BIO-2, while the highest was noted when isolate GD was inoculated against *T. harzianum* BIO-2. It is interesting to report here that *T. harzianum* isolate BIO - 1 was able to inhibit at statistically the same degree, the growth of all isolates GA, GB, and GD, whereas the inhibition

Table 3. Average percentage inhibition of mycelial growth of *Ganoderma boninense* isolate GA, GB and GD by *Trichoderma harzianum* (isolate BIO - 1 and BIO - 2) and *T. viride.*

Combination of treatment (Pathogen vs. antagonist)	Average of inhibition*) (%)
Ganoderma boninense vs. *Trichoderma harzianum* isolate GB isolate BIO - 2	28.96 a
G. boninense isolate GD vs. *T. viride*	37.71 b
G. boninense isolate GB vs. *T. viride*	38.57 b
G. *boninense* isolate GA vs. *T. harzianum* isolate BIO- 1	39.53 be
G. *boninense* isolate GA vs. *T. harzianum* isolate BIO-2	39.62 be
G. boninense isolate GD vs. *T. harzianum* isolate BIO- 1	40.29 be
G. *boninense* isolate GB vs. *T. harzianum* isolate BIO- 1	41.57 be
G. boninense isolate GA vs. *T. viride*	41.84 be
G. *boninense* isolate GD vs. *T. harzianum* isolate BIO-2	45.18 c

*) Means within columns followed by the same letter are not significantly different. LSD 0.05 = 6.14.

of *T. harzianum* BIO - 2 and *T. viride* was determined by the type of isolate of the pathogen.

Direct examination under the microscope showed that the pathogen hyphae underwent lysis; the percentage of which was determined by the type of isolate of the antagonist (Table 4). In any case, the hyphae of the antagonist were also lysed in certain treatment, e.g. *T. harzianum* BIO- 1 vs. G. *boninense* isolates GA and

Table 4. Microscopic examination of hyphal interaction between *Ganoderma boninense* isolates GB and GD with *Trichoderma harzianum* (isolates BIO -1 and BIO - 2) and *T. viride.*

Combination of treatment (Pathogen vs. antagonist)	Description	Effectivity score of antagonist
Ganoderma boninense isolate GA vs. *Trichoderma harzianum* isolate BIO – 1	*G. boninense* hyphae lysed about 90% *T. harzianum* hyphae lysed about 10%	2 1/2
G. boninense isolate GB vs. *T. harzianum* isolate BIO – 1	*G. boninense* hyphae lysed about 90%	3
G. boninense isolate GD vs. *T. harzianum* isolate BIO – 1	*G. boninense* hyphae lysed about 90% *T. harzianum* hyphae lysed about 10%	2 1/2
G. boninense isolate GA vs. *T. harzianum* isolate BIO – 2	*G. boninense* hyphae lysed about 50% starting from the tip of hyphae, the hyphae tended to become small. *T. harzianum* hyphae lysed about 25%	2
G. boninense isolate GB vs. *T. harzianum* isolate BIO – 2	*G. boninense* hyphae lysed about 90% and hyphae tended to become small	4
G. boninense isolate GD vs. *T. harzianum* isolate BIO – 2	*G. boninense* hyphae lysed about 50% starting from the tip of hyphae. *T. harzianum* hyphae lysed about 10%	1/2
G. boninense isolate GA vs. *T. viride*	*G. boninense* hyphae lysed about 25%, the tips of hyphae were normal. *T. viride* hyphae lysed about 25%	0
G. boninense isolate GB vs. *T. viride*	*G. boninense* hyphae lysed about 25%, the tips of hyphae were normal. *T. viride* hyphae lysed about 10%	1/2
G. boninense isolate GD. vs. *T. viride*	*G. boninense* hyphae lysed about 90%, and hyphae tended to become small. *T. viride* hyphae lysed about 25%	3

GD, *T. harzianum* BIO-2 vs. *G. boninense* isolates GA and GD, *T. viride* vs. *G. boninense* isolates GA, GB and GD.

In general *T. harzianum* BIO - 1 had the same effectivity score against the three pathogen isolates. The ef fectivity score of *T. harzianum* BIO - 2 and *T. viride* was determined by the type of isolate of the pathogen.

If we look at either the percentage of inhibition of mycelial growth of the pathogen by the antagonist or the capability of the antagonist to cause hyphal lyses of the pathogen, *T. harzianum* BIO- 1 was the most potential antagonist. Chet *et al.* (1979) found that *T. harzianum* could control damping-off disease on bean, peanuts and egg plants caused by *Sclerotium rolfsii* and *Rhizoctonia solani.* Sivan and Chet (1986) also found that *T. harzianum* could control *Fusarium* spp. in cotton, wheat and muskmelon.

CONCLUSION

P. citrinum, T. harzianum (isolate BIO- 1 and BIO-2), and *T. viride* isolated from oil palm plantation in North Sumatra were antagonistic to *G. boninense. T. harzianum* isolate BIO - 1 was the most potential antagonist. *P. citrinum* probably produces an antibiotic substance that diffuses into the medium, while *Tricho-derma* causes lyses of the pathogen's hyphae.

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