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Exploration and Inhibition Test of *Penicillium* sp. In Vitro by Trichoderma

Exploration and Inhibition Test of *Penicillium* sp. In Vitro by Trichoderma

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Abstract. *Penicillium* is an entomopathogenic fungus which can be used as a bio-bactericidal. Its existence in nature can be found together with other microbes including Trichoderma, a biocontrol agent that has the ability to damage the cell walls of other fungi due to the activity of the extracellular enzyme chitinase it produces. This study aims to obtain potential isolates of *Penicillium* biocontrol agents from vegetable growing fields and to determine their response to inhibition by *Trichoderma esperellum* in vitro. On PDA-chloramphenicol media, a suspension of 10^4 dilution was inoculated containing sample soil from vegetable crops to be isolated and purified and identified as to its species. The entomopathogenic isolates obtained were grown together with *T. esperellum* in dual culture and also grown in monoculture as a comparison. The isolation results obtained *Penicillium* sp. Pc-02. The in vitro test results showed that *Trichoderma* isolates could inhibit *Penicillium* sp. by 35.5 ± 1.9% and supported the growth of this entomopagen fungus by 15.7% at 24 and 72 hours after inoculation, respectively.
Keyword : Inhibition Test, ofPenicillium sp, Trichoderma**.**

1. Introduction

Efforts to realize national food security are not limited to strengthening food security in each region throughout Indonesia, but also strengthening the availability of all commodities needed to fulfill people's food, including the availability of horicultural crops. However, the production of healthy vegetables is often threatened by the activity of plant-disturbing organisms (PDO). Their attacks have been proven to often harm farmers and even cause crop failure.

One type of PDO disturbance in horticultural crops that often threatens the productivity and existence of vegetable crops is the disturbance of pathogenic bacteria or bacteria that are detrimental to plants. Attacks by pathogenic bacteria do not cause as great a loss as those caused by attacks by fungi and pests, but the dynamics of PDO attacks in the field is often unpredictable [1].

So far, attacks by insect pests and disease-causing pathogens from fungi have received the attention of researchers and pesticide manufacturers, but attention to bacterial attacks, especially on lowland vegetable crops, including mustard greens, has been lacking. On the other hand, the threat of an explosion of disease disorders can occur at any time and requires immediate treatment. With these considerations, the use of pesticides is unavoidable, even with high intensity. Until now, the use of chemical pesticides to protect vegetable production is highly reliable, despite the fact that they are not effective in controlling disease-causing pathogens [2]. On the other hand, the removal of pesticide and chemical fertilizer subsidies by the government must be accompanied by the availability of alternatives which also have benefits for increasing efficiency in farming [3]. The use of biological

agents as active ingredients in pesticides and biological fertilizers is one of the strategies in applying agronomic technology that is environmentally friendly and efficient in production costs.

With these considerations in mind, a series of effective microbial exploration activities are needed from agricultural land which can be used as active ingredients for the provision of materials that can play a role in providing plant protection so that they can grow and produce safely without interference from various plant-disturbing organisms including various bacteria that are detrimental to plants [4]; [5].

Penicillium is a type of fungus that has the ability to produce anti-metabolites such as adametizine, arisugacin, comazaphilones, communol, conidiogenone, and comazaphilones which can mainly suppress various types of bacteria in addition to several types of fungi [6]. Thus *Penicillium* can be used to provide protection against plant pathogenic bacteria. For this reason, exploration activities are needed on agricultural land to obtain *Penicillium* fungi which have the potential as biobactericidal active ingredients.

In the soil and around plant roots, various beneficial and detrimental microbes are found for plants. one type of fungus that is often found in the soil is *Trichoderma* which is a beneficial fungus by helping plants grow. One of the characteristics of this fungus is its ability to produce cellulolytic and chitinolytic enzymes extracellularly [7] which can potentially damage the cell walls of other types of fungi, including *Penicillium* fungi. For this reason, it is also necessary to examine the extent to which *Trichoderma*, which is native to the soil environment and rhizosphere of cultivated plants, can influence the activity of the *Penicillium* fungus which will be applied as a biobacterial in the soil around the roots.

2. Methods

2.1. Isolation and determination of Penicillium

For the isolation of fungi of potential biological agents, 5 g of the soil sample was taken and poured into a glass beaker, then 500 ml of distilled water was poured and stirred until evenly distributed. After dilution starting from 10-4, 1 ml was sampled using a syringe needle and sprayed into a cup that was filled with solid PDA-chloramphenicol. Then incubated for 48 hours. The emerging point which is the initial condition of the prospective colony was immediately isolated by growing it new PDA-c media. Pure culture isolates found at two weeks of age were sampled by propagules and placed on a glass object to be observed under a microscope at a magnification of 400 times. The observed microscopic structures, namely the shape, diameter, color, and branching of hyphae as well as the shape and size of the spores were compared with the descriptions shown in various scientific journals and publications of relevant research results..

2.2. Inhibiton test

Penicillium sp. inhibition test. by *T. esperellum* (collection of the Laboratory of Microbiology and Biotechnology, University of Muhammadiyah Sidoarjo) carried out using the dual culture method by placing 5 mm propagules *Trichoderma* and *Penicillium* sp. facing each other 25 mm from the edge of the petri dish. As a mono culture is to grow the propagule *Penicillium* sp. of the same culture alone in the middle of a petri dish. The overall position of the fungal propagule placement is schematically shown in Figure 1 [8]. During the incubation period, the growth of the colony radius was observed every 24 hours starting on the second day until the control filled the petri dish. The test was repeated four times.

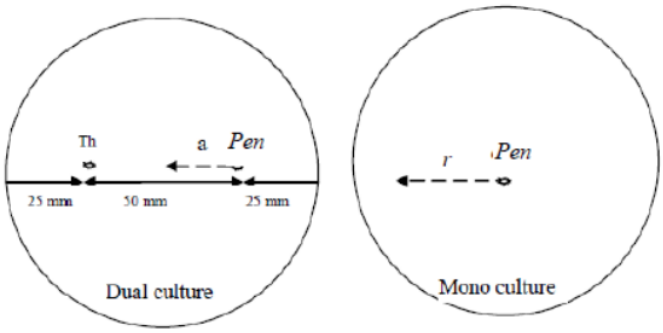

Figure 1. Placement of propagules in the inhibition test of *Trichoderma* against *Penicillium* sp. Th: *Trichoderma*, Pen: *Penicillium* sp.

To calculate the percentage of inhibition using formula (1) [9]:

$$Si = \frac{(r-a)100\%}{r} \quad \ldots\ldots\ldots\ldots (1)$$

with the following conditions: *Si*= Percentage of growth inhibition, *r* = growth radius of colonies of *Penicillium* sp. on monoculture media, and *a* = growth radius of colonies of *Penicillium* sp. on media in dual culture.

2.3. Data analysis

For exploration activities, the data obtained were in the form of colony descriptions on PDA media as well as the morphology and dimensions of the propagules of the isolates of *Penicillium* sp. Measurement result data. Inhibition (%) of *Penicillium* fungal colonies by *T. esperellum* was calculated on average and the deviation to show the strength of inhibition.

3. Results and Discussion

3.1. Penicillium exploration results

The results of macroscopic observations on the shape and color of *Penicillium* sp. and the shape and size of the spores are shown in Figure 2.

Figure 2. Macroscopic (left) and microscopic (right) observations of *Penicillium* sp. exploration results

Fungal colonies are white with slightly rough edges and colony surfaces (Figure 1, left); the shape and color show the characteristics of *Penicillium* sp. colonies. hyphae hyaline (arrow up) and branched (down arrow) with an average diameter of 2.58 ± 0.13.μm. Hyaline spores are rounded with an average diameter of 2.53 ± 0.28 μm. Likewise, the shape of the hyphae and phialids as well as the shape and size of the spores meet the general characteristics of *Penicillium* sp. [10]; [11]. In

accordance with the usual working procedure at the UMSIDA Microbiology and Biotechnology Lab, Penicillium isolates are coded Pc-02.

3.2. In vitro test results

Tests that have been carried out show the growth of *Penicillium* sp. colonies. both dual culture and monoculture showed that *Trichoderma* inhibition of this fungus started at 24 hours after inoculation (HAI) and decreased at 48 HAI (Table 1).

Table 1. Mean of colony growth and colony inhibition of *Penicillium* sp. by *T. esperellum*

Placement of propagules	Colony growth radius (mm)			
	24 HAI	48 HAI	72 HAI	96 HAI
Penicillium sp. *dual culture*	19,0±1,2	34,7±1,2	49,2±0,5	49,3±1,7
Penicillium sp. monokultur	25,3±1,2	36,2±0,4	42,5±0,3	49,7±4,4
Inhibition by *Trichoderma* (%)	35,5±1,3	4,1±0,9	(-) 15,7±1,0	2,4±1,9

3.3. Discussion

The hyphae and phialid forms as well as the size of the mushroom spores as a result of exploration in this study (Figure 1) can already be used to determine the fungal genus level. Therefore, this finding has not been able to determine the name of this fungus species. Among the isolates found by other researchers, it is possible that there are differences in characteristics, although morphologically, especially in the shape and size of hyphae and spores, they are relatively the same. Species determination should be perfected with identification based on molecular markers through a series of activities starting from DNA isolation, cutting and multiplying DNA pieces using PCR tools, and sequencing. The nucleide composition obtained will be matched with GenBank [12] to determine the level of similarity with isolates that have been found previously. Furthermore, by using the nucletide sequence information, the phylogenetic composition was arranged [13] to strengthen the statement of the name of the type of isolate found.

In Trichoderma inhibition experiments, up to 48 HAI period, *T. asperellum* inhibited *Penicillium* with a decreasing trend. *Trichoderma* is one of the fungi that is able to release the chitinase enzyme extracellularly [14], where the activity of this enzyme is able to disrupt the stability of the cell walls of other fungi, some of which are composed of chitinase molecules [15]; [16]. On the other hand, *Penicillium* also produces various extracellular compounds that will help its existence grow according to available food resources [17]. With Penicillium's intrinsic ability to utilize space and resources as well as its response to *Trichoderma* activity, then at 72 HAI inhibition began to appear (-) 15.7 ± 1.0%, which means that living together in one space and resources with *Trichoderma* have promotes the growth of *Penicillium*. *Trichoderma* activity can promote beneficial microbial activity [18]. This statement has been proven by the results of in vitro tests in this experiment, especially after 48 HAI. The percentage of inhibition is also the resultant between the activity of *Trichoderma* and *Penicillium*. *Penicillium*, besides being able to produce anti-bacterial compounds, was also able to produce compounds that were anti-fungal [19], however, this experiment did not measure how far this fungus affected *Trichoderma* activity.

4. Conclusion

The results of exploration and identification based on morphology obtained selected isolates taken from the soil of this vegetable growing area, namely Pc -02 as *Penicillium* sp. In vitro test results showed that *Trichoderma esperelllum* was able to inhibit the growth of *Penicillium* sp. by 35.5±1.3% and started to promote growth by 15.7±1.0% respectively at 24 and 72 hours after inoculation.

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