Viability And Conidial Production Of Entomopathogenic Fungi Penicillium SP.

Nurariaty Agus, Annie P. Saranga, Ade Rosmana, Ade Sugiarti

Abstract: Penicillium sp. (order Eurotiales, class Eurotiomycetes, family Trichocomaceae) is one of the entomopathogenic fungi that have the potential to be developed as biological control agent of pests.The study aims to determine the viability and spora production of Entomopathogenic fungi Penicillium sp. Experiments was conducted in Pests Identification and Biological Control laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University. The fungus Penicillium sp. cultured in a liquid medium and then added chitin as treatment and others without chitin., The spora viability of fungi was observed on 12th and 24th hours, while spora production on 3nd, 6th, 9th and 12th days after application.The results showed that conidial viability of the fungus Penicillium sp. at 24 hours after application was higher if the medium given chitin than without chitin. The conidial production was higher if given chitin than without chitin. It was highest on 12th day, reached 143.4 x 10^6 conidia/ml if media given chitin and on 6th day if without chitin (0.50 x 10^6 conidia/ml).

Index Terms: viability, conidial production, chitin, entomopathogenic fungi, Penicillium sp.

1.INTRODUCTION

In agricultural ecosystems there are variation of microbial diversity, both as entomopathogenic and antagonistic as well as plant pathogenic. During this time, in cacao plantation, the fungus Beauveria bassiana is only known as a entomopathogenic on CPB (Conopomorpha cramerella Snellen), but it turns out that the role of the fungus Penicillium sp. is higher than B. bassiana and Aspergillus sp. (Nurariaty, 2006) [8]. There are about 300 species of Penicillium that different roles (Anonymous, 2013) [2]. The fungi were known as entomopathogenic fungi because its found attacking certain pests such as Spodoptera sp. (Tanada and Kaya, 1993) [14], on Plutella xylostella (Anaisie, Eziah and Owusu, 2011) [1] and the CPB pupae (Nurariaty, 2006; Sulityowati and Junianto, 2002) [8,12]. There are known constraints in the propagation of entomopathogenic fungi in vitro, including Penicillium sp. such as the decline in the quality of the spores and virulence. Taborsky (1997) [13] reported that the sub-culture of entomopathogenic fungi Metarhizium anisopliae more than five generations can reduce the density of spores. In addition, it will also decrease carbon sources such as glucose, glucosamine, chitin, starch, and nitrogen effect on the growth of hyphae which eventually will result in a decrease of viability (Tanada and Kaya, 1993) [14].

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The fungi produce toxins, among others penicillic acid, breviana mid, ochratoxin and citrinin that can infect insects (Tanada and Kaya 1993) [14], peptide nephrotoxin, viomellin, xantomegin X, cyclopiazonic acid, isofumigaclavine A, penitrem A, decumbin, patulin citerviridin, griseofalvin , verruculogen, ochratoxin, chrysogine and melegrin (Anonymous, 2013) [2]. Virulence of the fungus is influenced by the physiological character of fungi, such as colony growth rate, size, conidial production, conidial germination, conidial sensitivity to temperature, the character of the host insect colonies and mortality. Sub culture repetitive and the nutrient of media also determine the entomopathogenic fungi growth. Rosalind (2000) [10] argue that the media are less protein content may reduce the ability of spores germination. In general, South Sulawesi community are seafood consumers like fish, shrimp, crab, etc.. which of course would leave the waste of shrimp and crab shell. Waste shrimp and crab shell contains the main building blocks consisting of protein, calcium carbonate, chitin, pigments, ash, and others. The content of chitin from shrimp shells less that 42% -57% compared with the skin or shell crab which is about 50% -60%. Chitin can be utilized in the fields of health, agriculture, food processing, textiles, etc. Chitosan is a chitin derivatives have more advantages when viewed in terms of economy as well as its application. The main sources that can be used for further development are chitin crustaceans and crabs (Subadiyasa, 1997) [11]. Hirano (1996) [6] reported that chitin and chitosan have various biological functions, for istance; antimicrobial activity, growth inhibitor of some pathogens, elicitor of phytoalexins, inducer of chitinase including accelerator of lignifications in plants. Information on the utilization of chitin in the areas of crop protection is still less so as the beginning of the research study was conducted to determine the effect of chitin addition against on the growth of entomopathogenic fungi, Penicillium sp.

2. MATERIAL AND METHODS

The research was conducted at the Pests Identification and Biological Control Laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Makassar.

2.1 Preparation of fungus Penicillium sp. and its media

The Penicillium sp. isolates was obtained from cadaver of CPB pupae. Preparation of the fungus, inoculum was transferred to

petri dishes containing Potato Dextrose Agar (PDA) and allowed to grow at 28°C for 2 days. Media PDA can be made by boiling 200 g of sliced potatoes, washed but unpeeled in 1 L distilled water for 30 minutes and then decanting or straining the broth through cheesecloth. Distilled water is added such that the total volume of the suspension is 1 L, added 20 g sugar and 15 g agar powder. Then the medium is sterilized by autoclaving for 15 minutes

2.2.Preparation of Chitin

Cleaned crab shell, dried, and blend until smooth or using a mortal. Furthermore, the shell is smooth then filtered and dried in an oven temperature of 50-55 ^{0}C for 24 hours. Product obtained is called chitin, a reddish white flour. Chitin as much as 1 g put in to erlenmeyer containing liquid medium.

2.3 Observations

The subcultures spore in liquid media was obtained from Penicillium sp. spores were cultured on PDA. Spores taken using corkborer then inserted into the erlenmeyer containing liquid medium with chitin and without chitin given as treatment. The liquid suspension was shaken with a shaker and the supernatant discarded. The conidia mass retained in the bottom of the erlenmeyer was also incubated in an oven at 60ºC until constant weight. The difference in the weight gave the biomass produced. Three replications were maintained. The growth of fungi was observed on 3nd, 6th, 9th and 12th days by counting the conidia viability, conidia production, wet weight and dry weight. Further, dilution to obtain a suspension with a density of 10^6 / ml. The number of spores was calculated by the formula:

$$S = \frac{t}{N \times 0.25} \times 10^6$$

where:

t =number of spores in the observed box
N = number of boxes were observed in haemocytometer
0.25 = volume of spore suspension in haemocytometer
10^6 = Constant

Conidia viability of Penicillium sp. was known by preparing three pieces of glass preparations that each placed on the lid of a petri dish coated with moist sterile filter paper. Glass preparations then spilled gelatinous liquid with a pipette, after freezing colony Penicillium sp. on petri dishes of PDA and PDA media were mixed chitin is placed upside down on the lid of the cup petri dish. After 12 hours of observed spores germinated and were not germinated. Conidial germination was calculated by the formula:

$$G = \frac{a}{a+b} \times 100\%$$

where:

G = percentage of germination
A = number of germinated conidia
B = number of conidia do not germinated

3. RESULTS AND DISCUSSION

The growth of the fungus Penicillium sp. note of the conidia viability and conidia production.

3.1 Conidia viability

The average of conidia viability of the fungus Penicillium sp. on liquid media with chitin and without chitin can be seen in Table 1.

Table 1. Average of Conidia Viability of Penicillium sp.

Treatments	Mean of conidia viability (%) on (hours)	
	12	24
Penicillium sp. without chitin	59.36	74.39
Penicillium sp. with chitin	83.26	85.79
T test	3.30*	4.12*

T table 5 %: 2.35

Table 1 shows that the fungus Penicillium sp. both were given chitin and without chitin begin to germinate at 12-24 hours after application. At the 24 hours after application, it seems that the conidia viability was maximal on media that given chitin. The T test analysis showed that there were significant differences between the media were given chitin and without chitin on viability of conidia. Viability of Penicillium sp. higher if given chitin than without chitin either the 12 and 24 hours which 83.26% and 59.36%. At 24 hours after application was 85.79% and 74.39%, respectively. This is presumably because the nutrient content of the chitin contained are made from the shells of crabs. Chitin is a polymer, a large natural molecule compose of repeating units of a simple sugar molecules, next to cellulose, it is most abundant polymer on earth (Benkeblia, 2014) [4]. Chitosan is a chitin derivative products with the formula Nasetil- D-Glucosamine, a cationic polymer that has a number of monomers around 2000-3000 monomers and not toxic. Chitosan is produced by deacetylation process of chitin layers contained in the shell Crustaceae animals (crustaceans) such as shrimp, lobster, and crab. Skin crab protein (15.60 to 23.90%), calcium carbonate (53.70 to 78.40%) and chitin (18.70 to 32.20%), depend on the habitat of crab. The entomopathogenic fungi requires media with high sugar content in addition to protein.

2. 2 Conidial Production

The average conidia number of the Penicillium sp. can be seen in Table 2.

Table 2. Average Number of Conidia of The Fungus Penicillium sp.

Treatments	Number of conidia (ml) on (days)			
	3rd	6th	9th	12th
Penicillium sp. without chitine	0.18 x 10^6	0.50 x 10^6	0.48 x 10^6	0.24 x 10^6
Penicillium sp. with chitine	3.48 x 10^6	4.89 x 10^6	143.4 x 10^6	109 x 10^6
T test	4.94**	4.51**	11.34**	8.63**
T table 5 %: 2.92				

The results was showed the number of conidia increased over time and was significantly higher when the fungus Penicillium sp. added chitin (Table 2). The T test analysis showed that there were significant differences between the media were given chitin and without chitin on the number of conidia. The number of conidia of Penicillium sp. higher if given chitin than without chitin, since the 3nd day until 12th day. It also appears that the number of conidia increased until 12th day (143.4 x 10^{6}) if media given chitin but only until 6th day in the media without chitin. The number of conidia produced if given chitin reaches 143.4 x 10^{6} on the 9th day. It was mean that is very supportive capacity to be developed as biological agents for pest control. The optimal of conidia density for pests control depends on the kinds of insect to be controlled. Baehaki and Noviyanti (1993) requires 10^{5} conidia/ml to control the adults of brown planthopper, while Luz et al. (1998) [7]; Wang and Powell (2001) [15] only requires of 10^{5}-10^{6} conidia/ml to control of Triatoma infestans. This suggests that the nutrients contained in Penicillium sp. were given chitin affect to viability of Penicillium sp. The high viability determine the effectiveness of entomopathogenic fungi (Prayogo and Tengkano, 2002) [9]. In addition, kinds of media also affect to physiological character of Penicilium sp., which in this study was initially grown on PDA were subsequently subcultured in liquid media. The entomopathogenic fungi requires media with high sugar content in addition to protein. Guerrero et al. (2007) [5] reported that the comparison of the effect of chitosan at 2 mg/ml in the three different media (PDA, CMA and WA) for all fungi and the oomycete showed that plant pathogen sand mycoparasitic fungi were more affected by chitosan than nematophagous and entomopathogenic fungi.

4. CONCLUSIONS

The conidial viability of the fungus Penicillium sp. at 24 hours after application was higher if the medium given chitin than without chitin. The conidial production was higher if given chitin than without chitin. It was highest on 12th day, reached 143.4 x 10^{6} conidia/ml if media given chitin and on 6th day if without chitin (0.50 x 10^{6} conidia/ml).

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