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THE EXISTENCE OF ENTOMOPATHOGENIC FUNGI ON RICE PLANTS RHIZOSPHERE

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ABSTRACT

Enthomophatogenic fungi are one of bioinsecticides that can be used as pests control. This research aimed to identify and analyze the existence of fungi on semi-organic and inorganic rice plants rhizosphere and prove the role as entomophatogenic fungi. The research method isolation of fungi was conducted in Takalar Regency, semi-organic and inorganic rice plants rhizosphere and continued in Biological Laboratory, Plant Pests and Diseases Department, Faculty of Agriculture, Universitas Hasanuddin, Makassar. This research using a modified insect bait method, larvae mortality calculated, insect testing were infected in reisolation and fungal diversity index. The result showed that the average *Tenebrio molitor* larvae mortality infected of enthomopathogenic fungi on semi-organic rhizosphere was around 24% and 8% on inorganic rhizosphere. The percentage of larvae mortality on semi-organic rhizosphere were infected by *Fusarium* sp., range the percentage was around 20-60 %, *Aspergillus* sp. 60%-80% and *Trichoderma* sp. 80%, while in inorganic rhizosphere up to 100% were infected by *Aspergillus* spp.

Keywords: Semi-organic and inorganic, Rhizosphere, Entomopathogenic

INTRODUCTION

All microorganisms are present in rhizosphere, affect the growth of the plant, nutrient supply for plants, as biological control for pests. Enthomophatogenic fungi as biological agens are commonly found on rhizosphere. But the existence of microorganisms in the soil especially for this time has decreased. This is due to environmental pollution caused by agricultural activity continues that rely on chemical fertilizers (inorganic) and synthetic pesticides. Nutrients secretion into the rhizosphere of plants were influenced by environmental factors and further affect the abundance and diversity of microorganisms in the area (Kuswinanti *et al*, 2014).

There are about 700 types of entomopathogenic fungi, which is of 90 genera are represented (Goettel et al., 2010). Several types include *Metharizium anisopliae* (Priwiratama and Susanto, 2014), *Beauveria bassiana* (Malekan et al., 2015; Herlinda, 2010), *Aspergillus* (Bawin *et al*., 2016) *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma* (Assaf *et al*., 2011).

Several studies of soil fungi were isolated from rhizosphere and potentially as biological control Trizelia *et al*., (2011) showed that, isolates *Metarhizium* spp. which

is isolated from cabbage rhizosphere, virulent to eggs and larvae of *Spodoptera litura* instar I of larvae, and also Trizelia *et al*., (2015) showed that there were 3 genera of entomopathogenic fungi in various vegetable crops rhizosphere, *Metharizium*, *Beauveria* and *Aspergillus* with the highest diversity of entomopathogenic fungi were found in the tomato plant rhizosphere. Nurariaty *et al*., (2013) showed that mortality pupae and imago of cocoa pod borer infected enthomophatogenic fungi genera of *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. Refer to Hamdani *et al*., (2011) there are 6 genus of entomopathogenic fungi were isolated from cocoa rhizosphere in different agroecosystems were *Aspergillus*, *Metarhizium*, *Beauveria*, *Paecilomyces*, *Fusarium* and *Penicillium* effective as controlling *C.cramerella* insects. However, no previous study has investigated the existence diversity of entomopathogenic fungi in semi organic and inorganic rice rhizosphere in South Sulawesi, therefore this study was conducted for determining control strategy in Integrated Pest Management (IPM) system.

MATERIALS AND METHODS

Soil samples

The present studies were conducted at Plant Pests and Diseases Department, Faculty of Agriculture, Universitas Hasanuddin, Makassar, during April- July. Soil samples were collected from semi-organic and inorganic rice ecosystem belonging to farmers in North Polongbangkeng Sub-District Takalar Regency. Determined diagonally 5 points on the semi-organic and inorganic rice plants rhizosphere. Each point was taken in the rhizhosphere, at a depth 20-30 cm of soil with a volume of approximately 300 g.

Insect Bait Method

The fungi were isolated by insect bait method (Zimmerman, 1986) modified. Larvae *Tenebrio molitor* as a test insect, each of 5 larvae were inserted into plastic cups (measuring 10 cm x 16 cm) containing 100 g of filter soil with a sieve 1100 mesh. Then covered with white cloth size 3 cm x 3 cm and were incubated at 22-25 °C. Larvae that were infected of fungi then placed in sterile filter paper were moistened. Larvae were infected of entomopathogenic fungi characterized by the appearance of mycelium of the fungus in each of the insect segment body. Surfaces sterilized using 70% alcohol for 1 minute and then rinsed with sterile distilled water, again kept in sterile distilled water and dry it on sterile filter paper, and then isolated larvae in fresh sterilized petri dish containing PDA and incubated for 3-7 days at 22-25 °C . Thereafter, identified the fungi that grew on PDA.

Bioassay Test of Entomopathogenic Fungi

The fungi that were identified then tested to determine their role as entomopathogen. The fungus was used in this experiment all the fungus obtained. The preparation suspension of spores for each fungus was obtained by the addition 10 ml of sterile water into the petri dish containing the culture of the fungus for 8 days , then was homogenized by using spatula for 1 minute. Drop suspension on Haemocytometer then the number of spores counted by the formula :

$$S = \frac{t \times d}{n \times 0{,}25} \times 10^6$$

S = number of spores

T = total number of spores observed in square cell sample

D = dilution factor

N = number of square cell sample observed

0.25 = correction factor

application of suspense in a petri dish (diameter 20 cm), placed 5 larvae of *T. molito*r as treatment. Next sprayed as much as 0.1 ml of the suspension of the fungal spores were selected on test insect. Thereafter 24 hours carried out observations of percentage mortality of each larvae. Morphological changes to insect stadia observed discoloration, texture, activity larvae. The percentage mortality of *T. molitor* larvae was calculated by the formula:

$$P = \frac{\text{Larvae infected by fungi}}{\text{number of larvae observed}} \times 100\%$$

RESULTS AND DISCUSSION

Identification of Enthomophatogenic Fungi

Identification of the fungi isolates were found based on macroscopic and microscopic morphology characteristics. The result showed that 6 fungi isolates from semi-organic rhizosphere and 2 isolates from inorganic rhizosphere have various characteristic. Microscopic and macroscopic characteristics for each fungus on (Table 1 and Table 2.)

Table 1. Cultural Characteristics of Various Isolates Fungi on PDA Isolated from Semi-organic Rhizosphere

Isolates	Upper Surface			Colony Reverse	Conidia / Spores	Hyphae		Genera
	Color and Cultural aspect	Density	Zonation			Color	Setae / None	
RSOT 1	Light greenish, powdery, white halo (margin) and cotton-like	High	Concentric zones	Cream	Globose	Hyaline	Setae	*Aspergillus*
RSOT 2	Light green, powdery, white halo	High	Concentric zones	Cream	Globose	Hyaline	Setae	*Apergillus*
RSOT 3	White, cotton-like	High	None	White	Macroconidia slightly curve, microconidia bent	Hyaline	Setae	*Fusarium*
	White to dark green, light green halo, cotton-like	High	Concentric zones	White	Ovoid	Hyaline	Setae	*Trichoderma*
RSOT 4	White to creamish, creamish halo, cotton-like	High	Concentric zones	Yellow	Macroconidia slightly curve, microconidia ovoid	Hyaline	Setae	*Fusarium*
	White to creamish, creamish halo, cotton-like	High	Concentric zones	Yellow	Macroconidia slightly curve, microconidia ovoid	Hyaline	Setae	*Fusarium*

Note: Identification based on determination keys of the identification books (Barnett and Hunter, 1972; Watanabe, 2002).

Table 2. Cultural Characteristics of Various Isolates Fungi on PDA Isolated from Inorganic Rhizosphere

Isolates	Upper Surface			Colony Reverse	Conidia / spores	Hyphae		Genus
	Color and Texture	Density	Zonation			Color	Setae/ None	
RIOT1	Black spores, powdery, white halo	High	Single concentric zone	White	Globose	Hyaline	Setae	*Aspergillus*
	Light green, powdery, white halo	High	Concentric zones	Cream	Globose	Hyaline	Setae	*Aspergillus*

Note: Identification based on determination keys of the identification books (Barnett and Hunter, 1972; Watanabe, 2002).

Table 1 showed that 3 genera morphological identification were *Fusarium*, *Trichoderma* and *Aspergillus*, while on Table 2 showed that one genera, thas was *Aspergillus*. The macroscopic and microscopic characteristics of the three types of fungi on (Figures 1-4).

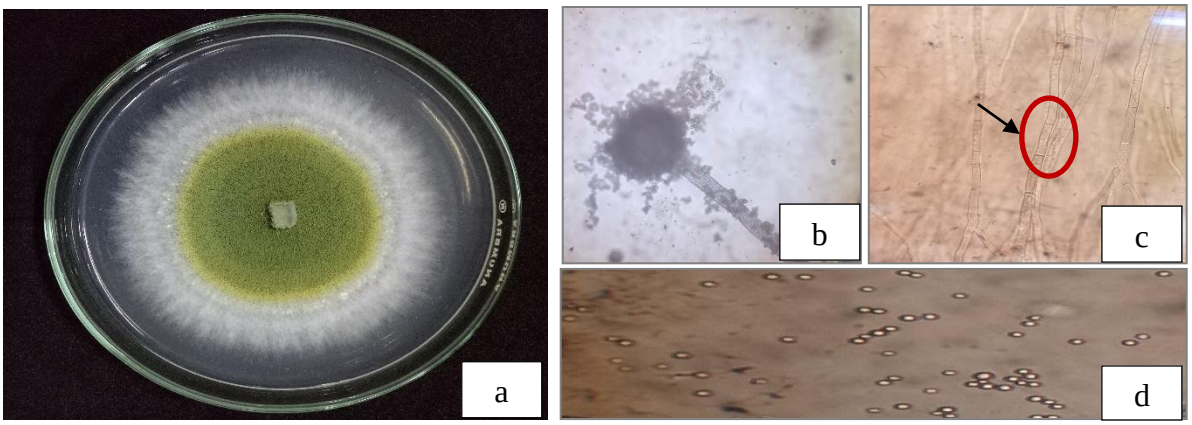

Fig. 1. Macroscopic (a) and microscopic of *Aspergillus*, conidiophore (b), hypha with setae (c), conidia (d)

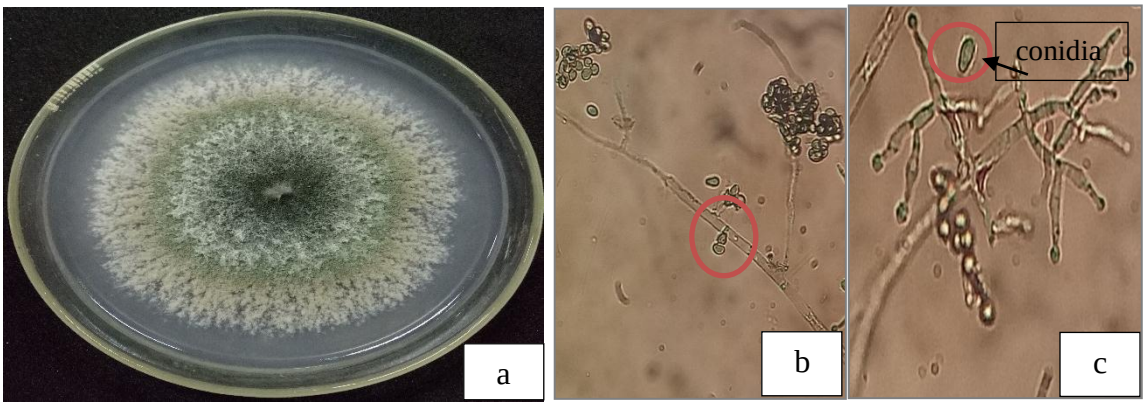

Fig. 2. Macroscopic (a) and microscopic of *Trichoderma*, hypha with setae (b), conidia and conidiophores (c)

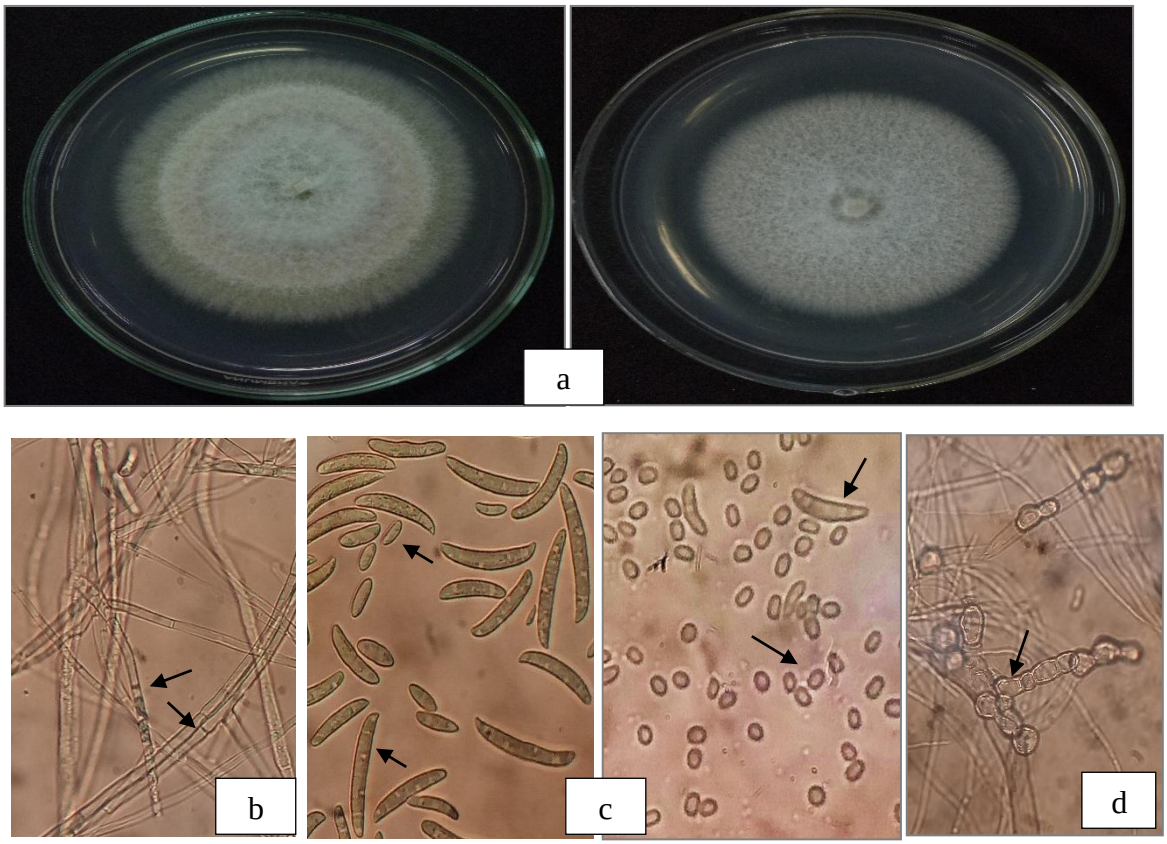

Fig. 3. Macroscopic (a) and microscopic of *Fusarium*, hypha with setae (b), microconidia and macroconidia (c), chlamydospores (d)

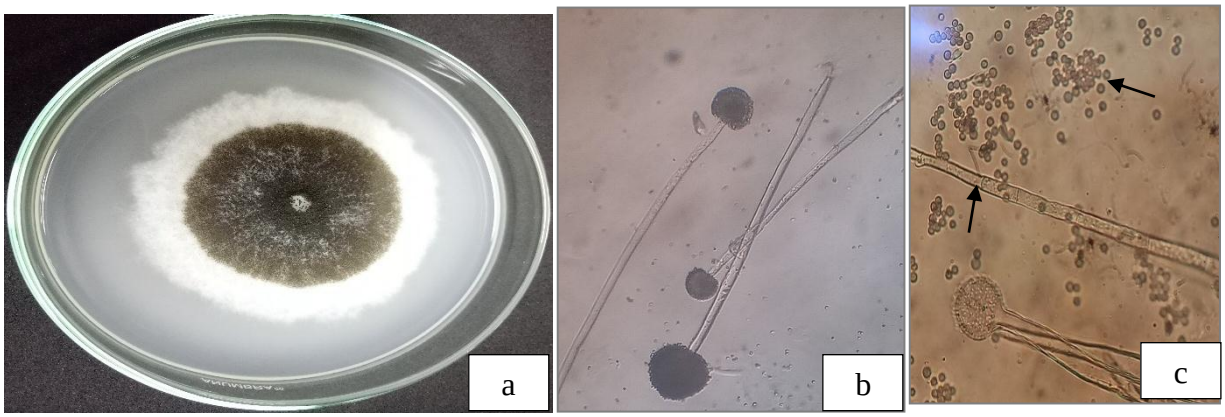

Fig. 4. Macroscopic (a) and microscopic of *Aspergillus*, conidiophores (b), conidia and hypha with setae (c).

Bioassay Test of Enthomophatogenic Fungi

Six isolates were isolated from semi-organic rhizosphere, there were three isolates of *Fusarium* sp, two isolates of *Aspergillus* sp., one isolate of *Trichoderma* sp. Each of the fungi was found in application on test insects, the average mortality of test insects on (Table 3).

Table 3. Mortality of *T. molior* Larvae Infected of Fungi on Semi-organic Rhizosphere

Isolates	Genera	% Mortality of Larvae
RSOT1	*Aspergillus*	80
RSOT2	*Aspergillus*	60
RSOT3 (1)	*Fusarium*	20
RSOT3 (2)	*Trichoderma**	80
RSOT4 (1)	*Fusarium*	60
RSOT4 (2)	*Fusarium*	60

Note: *) Test insects of dead larvae, but the mycelia did not exit through the insect host and the numbers in brackets on the isolates showed that the larvae to-

Table 3 showed that the percentage of larvae mortality were infected of enthomophatogenic fungi on semi-organic rhizosphere, the percentage mortality infected by genera of *Aspergillus* sp. range the percentage was around 60-80 % isolates of RSOT1 and RSOT2, *Fusarium* sp. 20%-60% isolates of RSOT3 (1), RSOT4 (1) and RSOT4 (2), *Trichoderma* sp. 80% isolate of RSOT3 (2).

Two isolates isolated from inorganic rhizosphere were *Aspergillus* spp., the average mortality of test insects on (Table 4).

Table 4. Mortality of *T. molitor* Larvae Infected of Fungi on Inorganic Rhizosphere

Isolates	Genera	% Mortality of Larvae
RIOT1 (1)	*Aspergillus*	100
RIOT1 (2)	*Aspergillus*	60

Table 4 showed that the percentage of larvae mortality were infected of enthomophatogenic fungi on inorganic rhizosphere, the percentage mortality infected by genera of *Aspergillus* spp. were isolate of RIOT1 (1) 100%, while isolate of RIOT1 (2) percentage of larvae mortality around 60 %. Entomopathogenic fungi that were infected and application *T.molitor* larvae on (Figure 5).

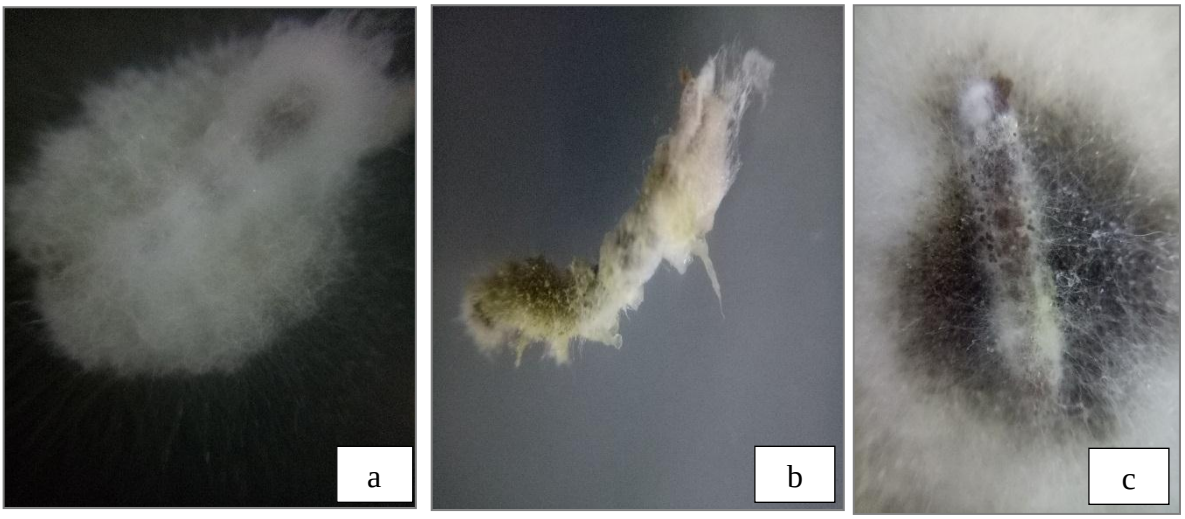

Fig. 5. Larvae *T.molitor* were infected of *Fusarium* sp. (a) and *Aspergillus flavus* (b) *Aspergillus niger* (RIOT1 isolate) (c)

The growth of fungi in culture media for 7 days isolated from semi-organic rhizosphere showed that as long as incubation period increase the growth of fungi colony diameter. This was occured in all type of fungi, which were isolates of RSOT3(2) genera of *Trichoderma* sp. has rapid growth on PDA, followed by isolates of RSOT4 (1), RSOT4 (2), RSOT2, RSOT3 (1) and RSOT1 on (Figure 6) and from inorganic rhizosphere were isolates RIOT1 (1) and RIOT1 (2) on (Figure 7).

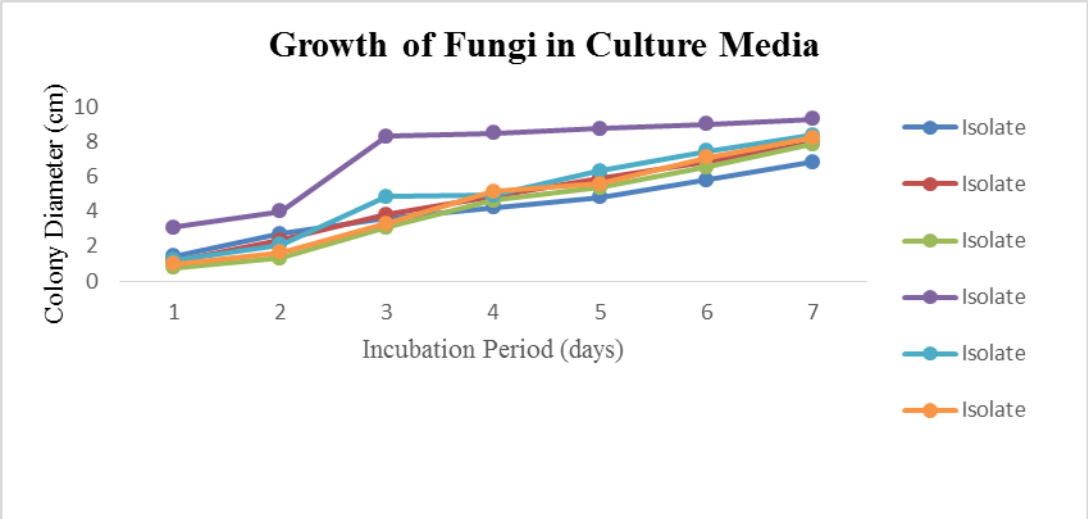

Fig. 6. The average growth of fungi in PDA media for 7 days on semi-organic rhizosphere

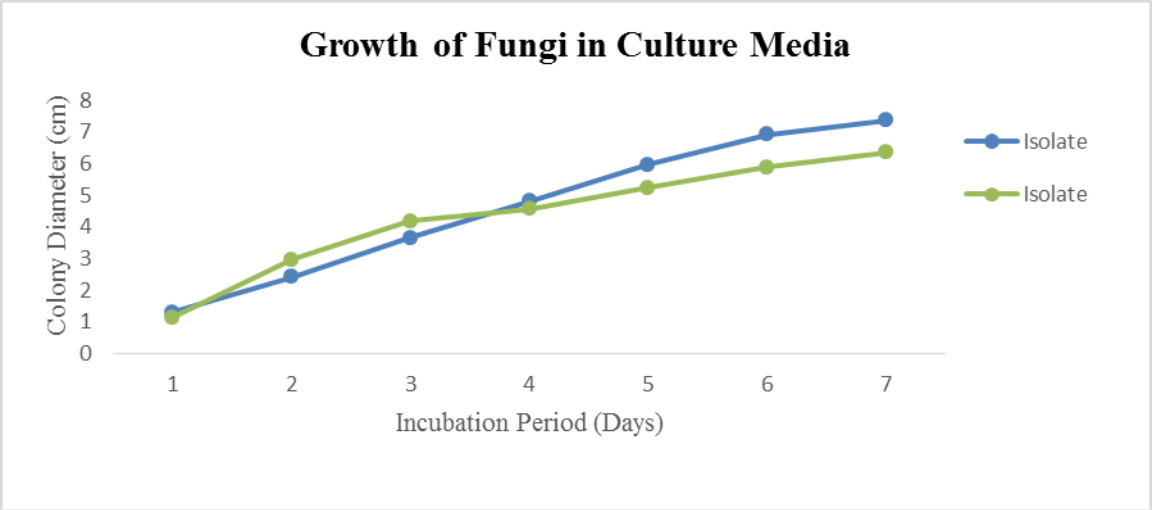

Fig. 7. The average growth of fungi in PDA media for 7 days on inorganic rhizosphere

Percentage of larvae mortality infected by enthomophatogenic fungi were different between semi-organic rhizosphere and inorganic rhizosphere. Semi-organic rhizosphere were found 6 isolates from 3 genera of enthomophatogenic fungi while in inorganic rhizosphere were found 2 isolates from one genera. It caused of the conidial density of each entomopathogenic fungus on semi-organic rhizosphere as well as favorable environmental conditions. It was knew that on semi-organic rhizosphere were treated with organic materials were used fertilizer, biological pesticides, prioritize using organic materials that organic materials were an important ingredient for soil fertility, physically, chemically and in terms of soil biology. Refer to Doeswono (1983), the organic materials were a source of energy and food for microorganisms living in the soil. Soil microorganisms interact with organic material because their provide carbon as a source of energy to grow. The high percentage of larvae mortality infected on semi-organic rice plants rhizosphere because total of conidia and spores present in the rhizosphere. Tanada and Kaya (1993) showed that the density of pathogens or inoculum is one important factor of infection in insects. Soil microorganisms have an important role in biogeochemical processes that determine the productivity of the plant as

a microbial inoculant and affect on soil health. The dominant genera in all the agricultural crop fields were *Aspergillus*, *Penicillium* and *Mucor* species (Chandrashekar *et al*., 2014).

On semi-organic and inorganic rhizosphere were found genera of *Aspergillus* spp. (Tables 1 and 2.), refer to Hidayat (2010) showed that enthomopathogenic fungi were isolated from agricultural fields genera of *Aspergillus* the most commonly were found. *A. flavus* subsists as mycelium, conidia, or sclerotia is one of the soil components associated with the soil organic materials (Hedayati, *et al*. 2007). *Aspergillus* spp. are able to assimilate minerals from minimal medium and survive on simple carbon and nitrogen sources with no vitamin requirements (Tarrand *et al.* 2005).

Previous studies have reported 11 isolates of fungi were found in two different locations on the chasew plants rhizosphere were *Aspergillus flavus, A. niger, A. parasiticus*, *Botrytis cinera*, *Cladosporium sphacospermum*, *Fusarium sporotrichioides*, *Penicillium brevicompactum*, *P. citrinum*, *P. chrysogenum*, *Rhizopus stolonifer* and *Synchephalastrum racemosum* (Wulandari *et al*., 2011). Genera of fungi were isolated on the rhizosphere soil and rizoplan cassava plants cultivar TME 419 were *Alternari*, *Aspergillus*, *Acremonium*, *Brettanomyces*, *Botrytis*, *Byssochlamys*, *Cladosporium*, *Doratomyces*, *Geotrichum*, *Humicola*, *Moniliella*, *Monascus*, *Neurospora*, *Oidiodendron*, *Penicillium*, *Pyricularia*, *Papulaspora*, *Rhodotorula*, *Rhizopus*, *Saccharomyces*, *Sporothrix*, *Trichothecium and Trichoderma* (Sule and Oyeyiola, 2012).

The larvae mortality by discoloration of the insect cuticle from light brown to dark brown and some subsequently were transformed into black color, characterized of conidia enthomophatogenic fungi penetrating the cuticle entry of insect host and absorbing insect host fluids. Then the mycelium exit through on host surface (e.g. the caput and abdomen) covered all of host surface and growth on the host surface. Refer to Humber

(2008) showed that the life cycle of entomopathogenic fungi against the insect cuticle with spore germination and penetration, thereafter proliferation of cells in the fungus that eventually are causing the death of its host, death of the insects followed by the production of infective spores entry the host immediately to repeat it life cycle. The pathogenicity of fungal species was thought related to produce enzymes and mycotoxins during infection of the insect on contact with the cuticle and in hemosoel (Tanada and Kaya, 1993).

PDA has significant effect on the growth of fungal colony diameter, the growth of eight isolates on PDA are presented on (Figs. 6 and 7), PDA is a good medium for the culture of *Fusarium*, *Aspergillus* and *Trichoderma*, the growth of all isolates remained significantly high on this medium. PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi (Sharma and Pandey, 2010).

CONCLUSIONS

Semi-organic rice plants rhizosphere were found six isolates genera of *Fusarium* sp., *Trichoderma* sp. and *Aspergillus* sp., while and on inorganic rice plants were found 2 isolates genera of *Aspergillus* spp. have a role as enthomophatogenic fungi.

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