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Prospective of entomopathogenic fungi associated with *Helopeltis spp.* (Hemipter: Miridae) on cacao plantation

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KEYWORDS

Aspergillus sp., *Aspergillus flavus*, *Verticillium lecanii*, *Helopeltis* spp

A B S T R A C T

One of potential pests that attack the cocoa plant are *Helopeltis* spp. (Hemiptera: Miridae). This study aims to determine several kinds of entomopathogenic fungi associated with the pest *Helopeltis* spp. on the folk cocoa plantations and has the potential to be developed as a biocontrol agent of *Helopeltis* spp. The research was conducted in the village of Prosperous Palolo District of Sigi that served as center of cocoa production in Central Sulawesi. The research was undertaken from August 2013 to February 2014. The Village is geographically located at an altitude of 589 meters above sea level - 600 meters above sea level. Samples of infected *Helopeltis* spp. were surface sterilized and incubated on a potatos Agar (PDA). Isolates were then macroscopic and microscopic identified based on their morphological characteristics on Potato Dextrose Agar (PDA) medium, Czapek Dox Agar (CDA), peptone Malt Agar (MPA), Malt Extract Agar (MEA) and continued with the pathogenicity test of Koch's postulates in laboratory conditions and detection of aflatoxin. Based on the morphological characters, entomopathogenic fungal isolates associated with *Helopeltis* spp. on the cocoa plantations are identified as *Aspergillus* sp., *Aspergillus flavus*, and *Verticillium lecanii*. The result of Koch's postulates showed an identical symptom of dead insects which infected in the field and in the laboratory condition. Pathogenicity test of local fungal isolates in laboratory conditions revealed that the average mortality of *Helopeltis* spp. occurred on the second until the seventh day after inoculation, respectively. The percentages of mortality were 90% for application with *Aspergillus* sp., 80% with *Aspergillus flavus* and 77% with *Verticillium lecanii*.

Introduction

Biological pest control gain more great attention partly because of increasing public awareness of the dangers of the side effect use of chemical pesticides. According to Pemintel et al. (1978), the reduction of pesticide use in an agro-ecosystem, with an

alternative approach to ecological principles by using of natural enemies (predators, parasitoids, and pathogen). Biological control is a utilization of natural enemies to manage the existing of pests in the target area (augmentation, conservation) or bring in from outside of the area (introduction). The nature and the expected role of natural enemies is as a regulator, controlling and stabilizing a long-term population below the economic threshold. According to De Bach (1964) it is expected that natural enemies play an important role to suppress and regulate the abundance of the population below the economic threshold.

The use of biological agents like entomopathogenic fungi to control pests many developed and is an attempt to reduce the use of chemical pesticides. There are currently over 1500 of known types of pathogens that infect insects and that number may be only a small part of an insect pathogen on earth (Untung, 1996). Entomopathogenic fungi is one type of biopesticide that can be used for controlling pests, especially plant pests (Prayogo, 2011). The role of natural enemies to control pests as one of the more important biological agents in accordance with the concept of integrated pest management. Various regulations have been published on the conservation of natural resources and ecosystems such as UU No.5 / 1990, UU No.5 / 1994 and UU No.21 / 2004 (Rauf, 1989).

A number of microbes have been reported in various studies effectively as agents of biological control of pests and plant diseases including the Genus Agrobacterium, Arthobotys, Ascocoryne, Bacillus, Bdellovibrio, Chaetomium, Cladosporium, Fusarium, Glicocladium, Penicillium, Peniophora, Pseudomonas, Phytium, Trichoderma, and Verticillium (Hasanuddin, 2003).

Some entomopathogenic fungi have been used to control pests of tree crops and vegetables are *Metarhiziumv anisophilae, Beauveria bassiana, Verticillium* sp., *Penicillium* sp. *and Spicaria* sp. (Widayat & Rayati 1993; Boucias & Pendland 1998).
In general, entomopathogenic fungi included class of Hyphomycetes, Family Moniliaceae. Hyphomycetes fungi that widely used for pest control include *Beauveria bassiana, M. anisopliae, V. lecanii* (Ladja, 2009).

In Central Sulawesi, information research about entomopathogenic fungi associated with *Helopeltis* spp. has never existed. *Helopeltis* spp is a major pest on cocoa crops which can reduce cocoa production by 50-70%. Therefore, it is important to do research on entomopathogenic fungus associated with the pests *Helopeltis* spp. on cocoa plantations as one of the biological control efforts that are environmentally friendly and free of pesticide residues.
This study aims to determine the kinds of entomopathogenic fungi associated with *Helopeltis* spp. on the cocoa plantations how their potential to be developed as biological agents for controlling *Helopeltis* spp.

Materials and Methods

Early surveys were conducted at three locations in the District Palolo, Sigi of Central Sulawesi. Determining location for the observation based on the consideration that the site attacked by *Helopeltis* spp, and as a center for the cocoa production. The three sites are located at Kampung Baru, Pangana Makmur village, and Sejahtera village. Samples were obtained only from Pangana Makmur Village. At that location there are some cocoa farmers implemented

plant maintenance with integrated pest control and conditioned their farms for conservation of black ants. Observations of death *Helopeltis* spp. due to entomopathogenic fungi infection used magnifying glass.

Sampling of *Helopeltis* spp from the Field

Samples of *Helopeltis* spp. infected with fungi on cacao fruit collected by slicing the surface of the pod husks using a cutter, it was put into a petridish that has been given a pad of paper tissue. The samples were labeled according to the location and time of collection. Samples of *Helopeltis* cadaver or live *Helopeltis* were taken and collected separately in each container and in a labeled petri dish. Samples of *Helopeltis* spp. were then brought to the laboratory, collected and observed for morphological traits.

Each container is filled only one adult of *Helopeltis* spp. that already infected. For uninfected *Helopeltis* spp., insects were maintained separately and were observed every day if there infected. Infected *Helopeltis* were characterized by the presence of myceliums around of insect body. It was separated from the surface of the cocoa pods using a soft brush. Infected samples inserted into the collection bottle and each bottle is filled with only one sample. Before observation, the samples were stored in a refrigerator at a temperature of 10°C to maintain their freshness.

Isolation and Purification of entomopathogenic fungi

Isolation of fungi from *Helopeltis* spp. were carried out referring plating direct isolation and plating dilution methods (Soewarno et al., 2013), using Potato Dextrose Agar (PDA). *Helopeltis* cadaver cut into small pieces with a size of 1 mm, the cutting is done in the laminar flow, surface sterilized and then placed on PDA medium. Fungus that grows then subcultured to agar block for easy identification using a light microscope. After one week incubation, colonies were taken by using a sterile needle and re-purified on PDA medium to obtain single colonies. Single colonies, cultivate on the PDA slant medium, incubated at room temperature, and stored in the refrigerator at a temperature of 10° C for further test.

Identification of entomopathogenic fungi

The morphological identification of isolates performed in the laboratory of the Faculty of Agriculture UNTAD, Palu. The results confirmed with the identification that carried in the Integrated Biotechnology laboratory UNHAS, Makassar. Observation of the morphological characteristics include the shape and colony color, form of conidia, conidial mass, conidial chains, konidofor, and branching of conidiophore, phialide, fruiting bodies, and vescicle. Identification key used according to Barnett and Hunter (1972), Poiner and Thomas (1984), Klich (2002), Diba at al. (2007), Tzean and Wu (1997).

Pathogenicity test of isolates Field and In the Laboratory

The ability of fungal isolates to infects *Helopeltis* spp., conducted by Koch's postulat. *Helopeltis* spp. taken from the field and then maintained for one week and fed with 10% honey dripped on cotton and placed on young still fresh cocoa leaves. Maintenance of insects for one week in order to avoid if there is fungus carried from field. Pathogenicity of local isolates against *Helopeltis* spp. performed by the of Koch's postulates method.

Results and Discussion

Identification of fungi

Based on morphological characterization of fungal isolates that associated with *Helopeltis* spp. on cocoa crops, they belong to three species of fungi namely *Aspergillus* sp., *Aspergillus flavus*, and *Verticillium lecanii.*

Aspergillus sp. from Palolo grew well in several kinds of mediums, have septate hyphae, branched mycelium, clusterred colonies, conidiophores unseptate and arising from foot cells. The tip of hyphae appeared a bubble, from it appears sterigma with arranged conidium in the tip. Conidia arranged in a sequence resembling a string of pearls, conidium is black, brown, ocher, or green in color (Figure 1).

Isolate of *Aspergillus flavus* grow well in several mediums, colony is bluish-green with yellow sulfur on the surface area, and have a fine threadlike mycelium. *A. flavus* has conidiophores, legs and conidium head cell consists of a bubble. Sometimes phialid, metula and konidium can be formed directly on the bubble. Metula diseriat, conidium has head shaped with columnar or radial (Figure 2).

Colony morphology of *V. lecanii* on PDA culture medium, it is white or cream, resembling in a thin cotton hyphae, pale or dark yellow in colony reverse. Conidofor has phialid with V shaped, which is characteristic of *V. Lecanii*. Phialid size varies depending on the strain and age of the culture. Each conidophor supports 5-10 conidia encased in slime bag. Single conidia, develop in phialid end, cylindrical to elliptical and colorless (Figure 3).

Pathogenicity Test of entomopathogenic fungi

Group of fungi that infect insects called entomopathogenic fungi. Pathogenicity test carried out on the Koch's postulates test. Infected insect from the field have the same symptoms with symptoms that arise as a result of inoculation in the laboratory. The symptoms the body surface of the host insect is filled with mycelium. From the results of pathogenicity test showed the fourth local isolates of entomopathogenic fungi can cause insect mortality test on the second day until the seventh day. Table 1 show the mortality of *Helopeltis* spp. after artificially infection with fungal isolates in laboratory conditions. The results showed that all test isolates has the ability to infect more than 50% of the test insects. *Aspergillus* sp. caused 90% mortality of the test insects, *Aspergillus flavus* 80% and *Verticillium lecanii* 77% respectively.

Aspergillus species is widespread throughout the world, especially in warm regions (Soewarno, 2013). Some species of this fungus are pathogenic to humans and animals (Chang 2007). *Aspergillus flavus* strains can cause disease in plants and animals. *Verticillium lecanii* included in the division of Hyphomycetes. Most species of in this class of are capable to causing disease in insect pests (Ferron 1985).

According to Agrios (2005), Koch postulate test is successful if artificially inoculated plants produce symptoms similar to the nature infected plant. This is also applies on insects infecte with an entomopathogen fungus. From these observations prove that the infected Helopeltis spp. on cocoa pods was entomopathogenic fungi *Aspergillus* sp, *Aspergillus flavus* and *Verticilliun lecanii.*

Figure.1 Colony of *Aspergillus* sp. on four different Mediums, 7 dpi

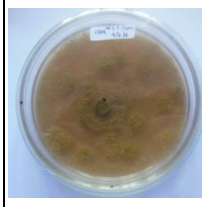
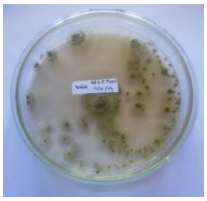
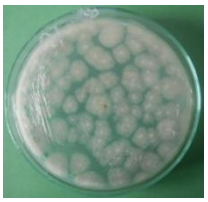
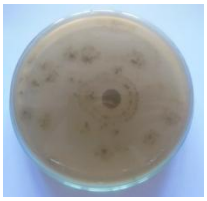
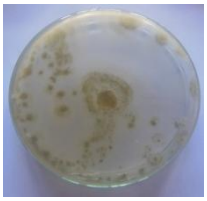
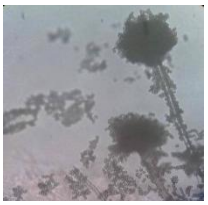
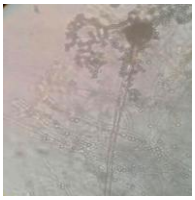
HS1 Isolate	PDA	CZA	MEA	MPA
Surface Colony				
Colony Reverse				
Mikroskopic features			Reference of *A. flavus* 	Reference of *A. flavus* 

Figure.2 Colony of *Aspergillus flavus* on four different Mediums, 7 dpi

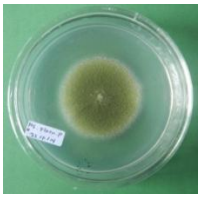
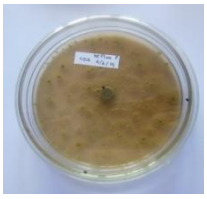
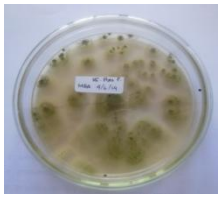
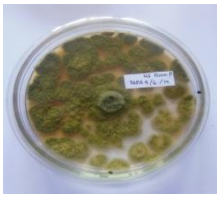
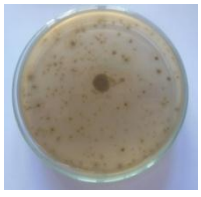
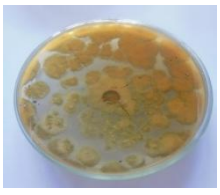
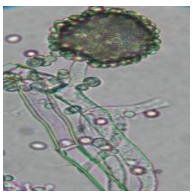
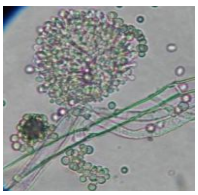
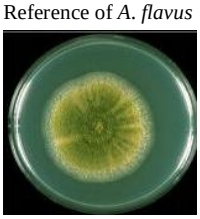
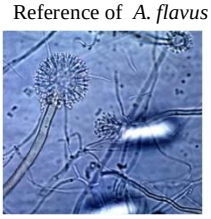
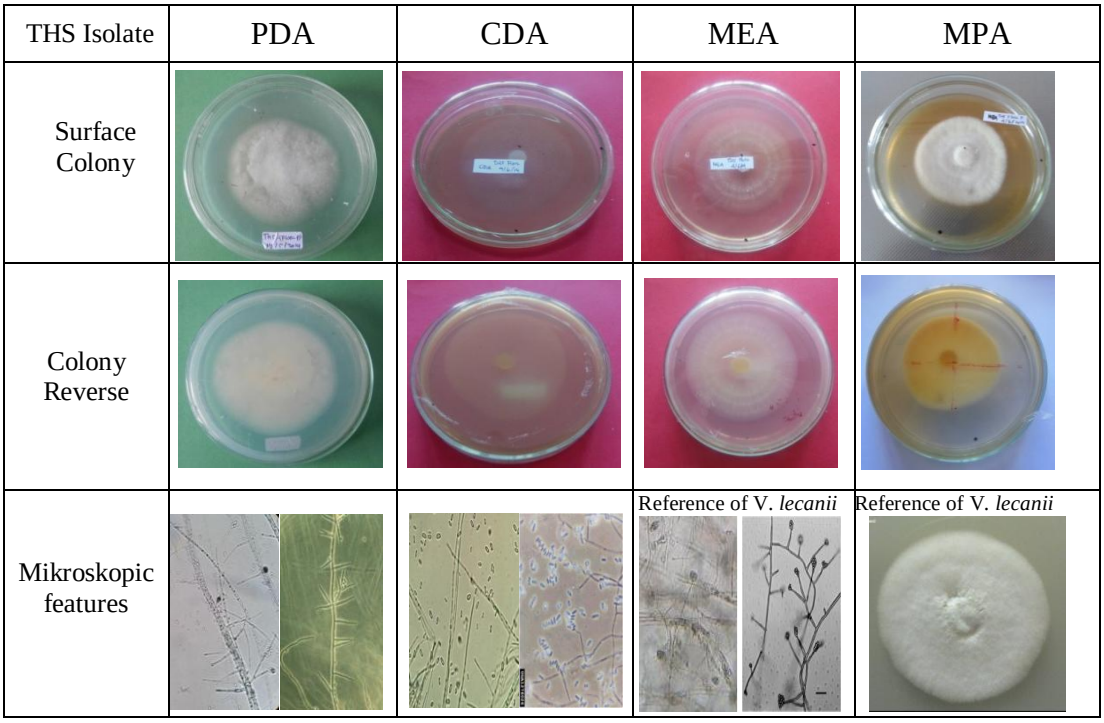
HS Isolate	PDA	CZA	MEA	MPA
Surface Colony				
Colony Reverse				
Mikroskopic features			Reference of *A. flavus* 	Reference of *A. flavus* 

Table.1 Percentage of *Helopeltis* spp mortality after infection with 3 fungal isolates, 7 dpi

Isolates	Percentage of Mortality of *Helopeltis* spp. 7 days post inoculation
Aspergillus sp.	90 %
Aspergillus flavus	80 %
Verticillium lecanii	77 %

Figure.3 Colony of *Verticillium lecanii* on four different Mediums, 7 dpi



Symptoms that appear in the test insects due to *Aspergillus* sp and *Aspergillus flavus* infection, has been seen at the second day, while *V. lecanii* infection appear after the third day. According to Cloyd (2003) entomopathogenic fungi require 24-72 hours to produce symptoms in insects after the fungus attaches to the insect's body, and forming germ tubes sooner than 10 hours after sticking in insects, and even then only occur when the humidity around 85% above the spores. It was revealed also by Rayati et al,. (1996), that infection of entomopathogenic fungi to can lead to death in insects within 3- 10 days. Mycelial growth seen in the second and third days. Mycelium initially appeared in the thoracic, limb segments, segment antenna and after the fourth day, the fifth to sixth day the entire surface of the insect's body filled with mycelium. Bodies of infected insects with *Aspergillus* sp. were covered with greenish yellow to dark yellow conidia. Infection with *A. flavus* isolate caused covering of insect body with yellow-green to dark brown mycelium, whereas *V. lecanii* caused pure white color suuround insect bodies, and no change in body shape of insect test.

Lecuona et al., (1997) stated that the infection is caused by an insect colony growth and development of fungi in the body of its host is not caused by an enzyme toxin produced by the fungus. If a toxin that kills its host, then the host will die sooner after application. Robert and Yendol (1971) suggested when appropriate environmental conditions fungus attached to the outer layer of the body wall of infected insects will start. Koch postulate test showed that pathogenicity of locally *Aspergillus* sp., *Aspergillus flavus*, and *Verticillium lecanii* isolates have potential to be developed as a biocontrol agent as a component of integrated pest control toward *Helopeltis* spp. Utilization of indigenous entomopathogens on local ecosystems is a major control tactic in Integrated Pest Management (IPM).

Conclusions

Based on the morphological characters fungal isolates associated with insect pest Helopeltis spp. on the cocoa plantations were *Aspergillus* sp., *Aspergillus flavus*, and *Verticillium lecanii*. Koch's postulate produces the same symptoms with field symptom as a result of fungal isolates infection in laboratory condition. The percentage of insect mortality was over 50% of all three kinds of entomopathogenic fungi.

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