First record of entomopathogenic fungi on autumn leaf Caterpillar (Doleschallia bisaltide)

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Abstract. Caricature plant is one of the medicinal plants in Indonesia to cure hemorrhoids, menstruation, and others. The cultivation constraints of caricature plant is autumn leaf caterpillars (Doleschallia bisaltide). Utilization of synthetic insecticides is not allowed to avoid bioaccumulation of chemical residues. Entomopathogenic fungi is an alternative way to control D. bisaltide. The objective of the research was to obtain isolates of entomopathogenic fungi of D. bisaltide. The research conducted by two steps, which were exploration of infected D. bisaltide. The second step was identification of the fungi. Identification done by classify the microscopic and microscopic fungi isolate characteristic. One from five fungal isolates were entomopathogenic fungi from Verticillium genera.

1. Introduction
Caricature plant (Graptophyllum pictum L.) is one of the medicinal plants in Indonesia. The leaves are efficacious to cure diseases such as hemorrhoids, urinating, menstruation and others [8]. One of the constraints of caricature plant cultivation is a pest attack. Doleschallia bisaltide (autumn leaf caterpillar) is a main pest [10].

Pest controlled can be done with the application of synthetic insecticides. But, the use of synthetic pesticides has many negative impacts on ecology, especially the chemical residues. Therefore, applied synthetic pesticides is not permitted on medicinal plants to avoid bioaccumulation of chemical residues in the human body when it consumed. Biological control is one of the alternative pest control with environmentally friendly. One commonly used in biological control is to use entomopathogenic fungi [9].

Exploration is the first step in the implementation of biological control techniques. The first step in developing entomopathogenic fungi as the biological control agent that needs to be done is the collection of isolates from the host, the isolate characterization obtained and the pathogenicity test (Koch Posatultes) [5]. There are no research on entomopathogenic fungi of D. bisaltide before. Therefore, the preliminary research is needed to determine the species of potential entomopathogenic fungi to control D. bisaltide.
2. Methods
The research was conducted at B2P2TOOT Field (Central Research and Development of Medicinal and Traditional Medicinal Plants) located in Tawangmangu, Karangpandan and Karanganyar, Central Java, and also in Laboratory of Pest and Plant Pest Control. The study was conducted for 6 months, from November 2016 to May 2017. The research design was adapted from Rosmini and Lasmini [13]. The research was conducted in two phases, the first is exploration of *D. bisaltide* that infected by entomopathogenic fungi and the second stage is the identification.

The first phase using survey method to get infected *D. bisaltide* by entomopathogenic fungi in B2P2TOOT Karangpandan field with purposive sampling. The second stage is the isolation and identification of entomopathogenic fungi that infected *D. bisaltide*. Fungi identification by macroscopic and microscopic identification based on Humber [6], Ando [1] and Barnett & Hunter [3],[4]. The observed data were analyzed descriptively.

3. Results and Discussion

3.1. General condition of research location
The experiment was conducted in B2P2TOOT Field in Toh Kuning Village, Karangpandan Sub-district, Karanganyar Regency, Central Java Province. The average temperature is 25.3°C and an average humidity is 88.4% during the research.

3.2. Exploration and identification entomopathogen fungi
Exploration done by collecting *D. bisaltide* infected with fungi in the caricature plant on B2P2TOOT Karangpandan and Tawangmangu field. There are 16 pupae of *D. bisaltide* found during exploration. The similarity symptoms of infection is appears white hyphae that enveloped the surface of the pupae, and when the pupae dissected, it will appear white hyphae in the pupae body. Entomopathogenic fungi will penetrate into the host's body through the cuticle as an initial step of infection [15].

Infected *D. bisaltide* dissected using sterile tweezers and a scalpel. The inner hyphae of the pupa is drawn with an ose needle and isolated on a PDA medium. Isolation was performed several times until some fungi isolates grew. The isolation of the fungus was done to separate the entomopathogenic fungi from the infected host, to obtain a pure culture isolate [14]. The result of isolation, get 5 isolates, namely isolate 1, isolate 2, isolate 3, isolate 4 and isolate 5. Isolates of fungi subcultured into other PDA media. Subcultures were performed for purification and propagation of isolates. Isolates observed until it full in petridish. Observation of isolates was done macroscopically and microscopically. Macroscopic observations include patterns of fungal colony spreading, colony shapes and colony color from either the upper surface or the bottom. Pure fungi grown in media and then identified in macroscopic morphology and microscopic morphology.

| No. | Isolate Fungi | Color | Pattern of Colonies | Time Meets Petri |
|-----|---------------|-------|---------------------|------------------|
| 1.  | Isolate 1     | White Yellow | White Yellow | The Colony Spreads Flat | > 30 Days |
| 2.  | Isolate 2     | Dark Green Whites | Brown Chocolate | The Colony Spreads Flat | 9 Days |
| 3.  | Isolate 3     | White | White Tanned | Unfavorable Colony Edge | 9 Days |
| 4.  | Isolate 4     | White | White | The Colony Spreads Flat | 7 days |
| 5.  | Isolate 5     | White | White | The Colony Spreads Flat | 9 Days |
Microscopic identification based on conidia is an important component of identification. When the sexual structure associated with the conidial, then can be a valid taxonomic classification [3],[4]. Conidial morphology is required in the identification of imperfect fungi. Conidia shape in isolates 1 is oblong, fusiform-elliptical is isolates 2, isolates 3 is cylindrical, isolates 4 is catenate catenulate and isolates 5 is elliptic fusiform. The number of cells in conidia between 5 isolates also had diversity, but most isolates (isolates 2, 4 and 5) have more than one septa (Pragmospore). Isolate one does not have septa (Amerospore), whereas isolates 3 only have one septum (Didymospore). The size of the conidia on each isolate was different, but all isolates ranged from 7.6 to 16.4 μm.

**Table 2.** Data of microscopic morphology by conidia identification of fungi isolate

| No. | Isolate Fungi | Konidia                  | Number of Cells in Konidia | Konidia Distribution Pattern       | Size (μm)   |
|-----|---------------|--------------------------|----------------------------|-----------------------------------|-------------|
| 1.  | Isolate 1     | Oblong                   | Amerospore                 | Clustered on conidiofor            | ± 4 -12     |
| 2.  | Isolate 2     | Fusiform-elliptical       | Phragmospor                | Clustered on Conidiofor            | ± 18 -28    |
| 3.  | Isolate 3     | Cylindrical              | Didymospore                | Clustered on Conidiofor            | ± 5 - 10    |
| 4.  | Isolate 4     | Catenate catenulate      | Phragmospor                | Clustered on Conidiofor            | ± 6 - 13    |
| 5.  | Isolate 5     | Elliptic-fusiform         | Phragmospor                | Clustered on Conidiofor            | ± 5 - 19    |

The macroscopic and microscopic morphological diversity of fungal isolates indicates that there is a classification difference between one isolate and the other, whether the species, genus or family are different. Identification was carried out to the genus level. Identification of entomopathogenic fungi in rhizosphere in vegetable crops was carried out to the genus level by observing the characteristics of the fungus macroscopically and microscopically [16]. The morphology identification can not describe phylogeny to the species level, therefore molecular identification techniques are also performed [18].
Table 3. Data of macroscopic and microscopic morphological identification of entomopathogen fungi isolate

| Isolate fungi | Macroscopic Color | Konidia | Genus Fungus |
|---------------|-------------------|---------|--------------|
|               | Surface | Basic | Hypha | Form | Spread |               |
| Isolate 1     | White Yellow | White Yellow | Branch | Oblong | Clustered on conidiofor | Verticillium* |
| (Isolate)     |          |        |       |       |         |               |
| Isolate 2     | Dark Green Whites | Brown Chocolate | Branch | Fusiform-elliptical | Clustered on conidiofor | Cercosporella |
| (2.1)         |          |        |       |       |         |               |
| Isolate 3     | White   | White Tanned | No     | Cylindrical | Clustered on conidiofor | Monilochaetes |
| (B2.1)        |          |        |       |       |         |               |
| Isolate 4     | White   | White | No     | Catenate catenulate | Clustered on conidiofor | Thielaviopsis |
| (1.1A)        |          |       |       |        |         |               |
| Isolate 5     | White   | White | Branch | Elliptic-fusiform | Clustered on conidiofor | Fusarium |
| (A1 White)    |          |       |       |       |         |               |

*Entomopathogenic fungi

The results of identification based on macroscopic and microscopic morphology indicate that there is one isolate (isolate one) that has macroscopic color features on the surface and bottom of a yellowish white petri dish that turns into a pinkish brownish gray. Verticillium macroscopic morphology, the colony's color is pale yellowish white and becomes pink brown, red, green or gray. Microscopic observation, hyphae sectional, oblong-shaped and spreading conidia on conidiophores clustered so identified genus Verticillium [7]. The microscopic features of this fungus are slim conidiofor and circularly branched. Single or clustered Konidia [2].

There is one isolate which has morphology of surface color and base color is white. This is consistent with research Trizelia and Winarto [17], the results of the morphological characterization of fungi on PDA 14 days of incubation, colonies of Fusarium color is white. Isolates 5 white can grow full of petridish within 9 days, this is according to research Nuraida and Hashim [12], the rate of growth in colony diameter Fusarium exploration results shows that the growth of the colony faster than fungi Aschersonia spp., Paecilomyces, Metarhizium and Beauveria. At 7 days incubation, the
diameter of the colony has reached an average of more than 6 cm. Microscopic hyphae sectional, shape Elliptic-fusiform conidia and conidiophores conidia clustered on, so identified include the genus Fusarium. Macroconidia generally crescent-shaped sectional three and measuring 30-40 x 4.5-5.5 μm and microconida cells round-shaped or oval that is characteristic of Fusarium [11].

4. Conclusion and Suggestion
There are five fungi isolate from isolation, one of those isolates was from Verticillium genera. Symptoms of an infected D. bisaltide is pupa will become darker brown, pupa body hardened and stiff, and the nascent white dots (fungal hyphae that grow). Suggestion that can be given by authors about this research are Verticillium fungi pathogenicity test against D. bisaltide with several treatment conidia, stadia D. bisaltide and location of the research should be conducted.

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References
[1] Ando K. 1998. Workshop identifikasi fungi imperfekti dareah tropis 19-21 November 1998 in Yogyakarta.
[2] Arif A, Muin M, Kuswinanti T et al. 2008. J Perennial, 5 (1) 15-22.
[3] Barnett HL and Hunter BB. 1972. Illustrated genera of imperfect fungi third edition. America (US).
[4] Barnett HL and Hunter BB. 1972. Illustrated genera of imperfect fungi fourth edition. America (US).
[5] Hamdani, Trizelia and Yaherwandi. 2010. J Manggaro, 11 (2) 71 -76.
[6] Humber RA. 1998. ARS Plant protection Research Unit. America (US).
[7] Kidd S, Halliday C, Alexiou H et al. 2016. Australian and New Zealand Mycoses Interest Group., Australia (AUS).
[8] Lestari P, Khumaida N, Sartiiami D, et al. 2015. SABRAO J of Breeding and Genetics, 47 (2) 172-184.
[9] Lovett B, J. Raymond dan Leger ST. 2017. American Society for Microbiology Press. America (US)
[10] Mardiningsih TL, Sartiiami D, Sukmana C. 2008. Pros. Seminar Nasional Tumbuhan Obat Indonesia XXXV. Hal. 59-65. Serpong, Indonesia. 13-14 November 2008.
[11] Ngittu YS, Mantiri FR, Tallei TE, et. al. 2014. J Ilmiah Farmasi, 3 (3) 156 – 161.
[12] Nuraida dan Hasyim A. 2009. J Horticulutra, 19 (4) 419-432.
[13] Rosmini dan Lasmini SA. 2010. J Agroland, 17 (3) 205 – 212.
[14] Sanjaya Y, Ocampo VR dan Caoili BL. 2013. J Agrivita, 35 (1) 64 – 73.
[15] Shang Y, feng P dan Wang C. 2015. J PloS Pathog, 11 (8) 1-6.
[16] Trizelia, Armon N dan Jailani H. 2015. Prosiding Seminar Nasional Masyarakat Biodiversiti Indonesia, 1 (5) 998-1004.
[17] Trizelia dan Winarto. 2016. Prosiding Seminar Nasional Masyarakat biodiversity Indonesia, 2 (2) 277 – 281.
[18] Yusuf EV, Nuryani W dan Hanudin. 2016. J Hortikultura, 26 (2) : 2017-2222.