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Inventory of Nematophagous Fungi in Sumatera Utara, Indonesia

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ABSTRACT

An inventory of nematophagous and entomophagous fungi in Sumatera Utara, Indonesia was conducted between 2010 and 2011. As much as 105 soil samples were collected from vegetable field and plantation areas of Sumatera Utara. The combine method, consist of Pour method (Larsen *et al* 1988) and sprinkle method (Jafee *et al* 1996) were used to isolate the nematophagous and entomophagous fungi from the soil. Two species of nematophagous fungi identified were *Paecilomyces fumosoroseum* (Wize) Brown & Smith and *Paecylomyces lilacinum* (Thom) Samson, only one species of entomophagous fungi identified was *Lecanicillium lecanii* (Zimm.) Zare & W. Gams.

Key word: Nematophagous, entomophagous, springkle method.

1. Introduction

Antagonistic interactions between fungi and nematodes have been described for some mycologist (Clay 1996; Kuldau & Bacon 2008; Saikkonen *et al* 2010; Baynes *et al* 2012). About 70 genera and 160 species of fungi are known to have been associated with nematodes, and more than 50 species of them are predators in nature (Ghahfarokhi *et al*. 2005). Based on their mechanisms for infecting and attacking nematodes, the nematophagous fungi can be divided into:

(i) endoparasitic fungi that use their spores (conidia or zoospore) to infect nematodes. The propagules adhere to the nematode cuticle, and spore contents are then injected into the nematode, or spores are swallowed by the host. Most of these fungi are obligate parasites of nematodes and live their entire vegetative stages inside infected nematodes.

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(ii) Egg and female-parasitic fungi infect nematode females and the eggs they contain, using appressoria or zoospores.

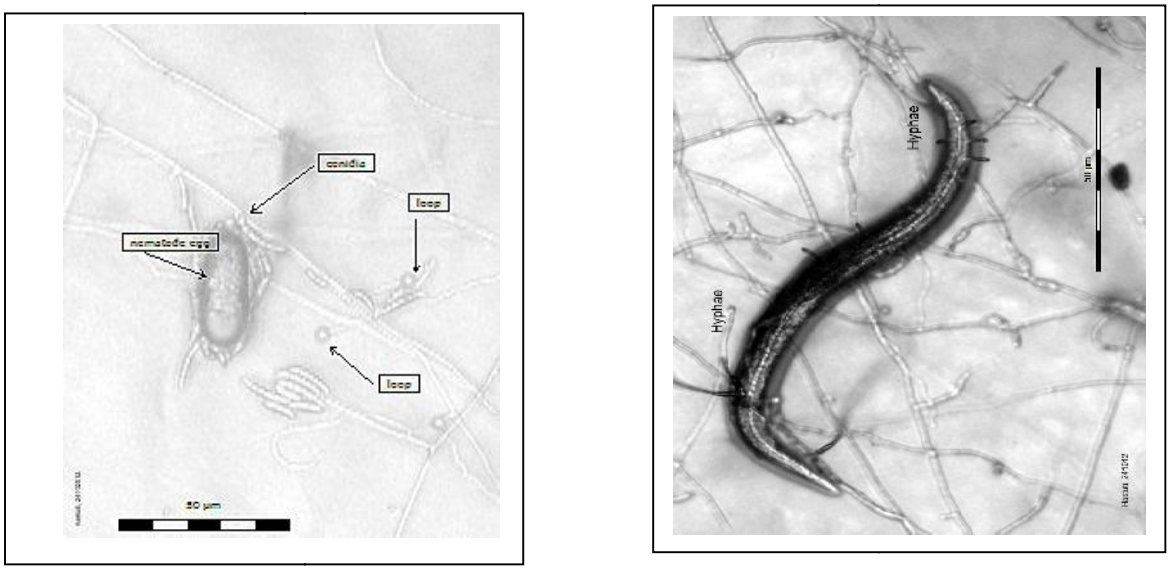

Fig.6. Penetrating conidia and hyphae of egg- and female-parasitic fungi

(iii) Toxin-producing fungi (Barron, 1977; Lopez *et al* 2002).

(iv) Nematode-trapping (formerly sometimes called predacious or predatory fungi).

1.1. Nematode problems in Indonesia

Nematodes are serious pests of many cultivated crops around the world and also in Indonesia. Nematodes parasites penetrate and feed on the roots of growing plants, creating a drain on the vital nutrients for plant growth and exposing roots to attack by other soil pathogens. Infected plants show typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency (Wiratno *et al* 2009).

Nematodes pathogens are usually present in plant in family *Solanaceae* like tobacco, tomato, potato and also aubergine (egg plants). These nematodes cause infection by feeding on the root tissue of the plant causing a primary infection that damages the root tissue, creating opportunities, for infection by secondary pathogens. These susceptibilities are used by other pathogen that common live in the soil such as *Xanthomonas* (*Pseudomonas*) *solanacearum* (black

shank disease) and *Fusarium* (wilt desease), *Rhizoctonia* sp. and *Pythium* sp. which cause rotting as secondary infection on the root of plant making an early plant death is more certain (Utomo and Pardede (1993); Lambert *et al*. 2002).

The nematode is not only able to attack the tobacco plant, but it is infectious to the other plants such as soybean. Nematode pathogens are the limiting factor in soybean production in many agricultural systems (Adnan, 1997). Results of surveys carried out in East Java, Madura and Lombok showed that soybeans in these areas could be infected nematodes at the level of 400-900 nematodes per gram of roots (Adnan, 1997). A field visit in Ngablak, Central Java Province (Suri and Jayasinghe, 2003) revealed the presence of root knot nematodes in the fields of farmers that are seed producers for a nearby cooperative.

1.2. Management to Control Pest Nematode

Pest management, especially for plant nematodes, has usually been achieved by using chemical pesticides such as nematicides. Chemical nematicides are associated with environmental hazard, high costs, limited availability in many developing countries and diminished effectiveness following repeated applications (Dong and Zhang, 2006).

Based on the facts mentioned above, it is very important to know the number of nematophagous fungi present in Indonesia. Some nematophagous fungi are species that live inside the nematodes from the beginning of their life cycle by infection with spores from the cuticle of their body wall and the egg shell of Nematode or even larva of insects. Another genus of fungus can catch the nematode Juvenile 2 stage mostly with the sticky traps. Another technique is to stun the nematodes using toxins, *Paecilomyces* sp for example can be used as a bio-nematicide to predisposing chemical nematicide and offering the kind environmental agent.

Thus it has become imperative to all concerned to seek alternate methods of pest management and to evaluate nematode control with biological control agents.

2. General Material and Methods

2.1. Soil Samples

Random soil samples (using thrown 0.5 m quadrats) were chosen based on Duddington's (1955) observations that nematode trapping fungi can be found in the area where the numbers of nematode are abundant. He predicted that a large number of nematode-trapping fungi may be found in composts and soils and in the dung of herbivores where many nematodes are present.

2.2. Maintenance Nematode as bait.

The nematode *Caenorhabditis elegans* was obtained from Davies, K.G (University of Nottingham) and was used as bait for nematode trapping fungi. *C. elegans* was maintained and produced in the laboratory using the method of Strienagle (2006), either on nematode growth medium (NGM) plates to which *Escherichia coli* OP 50 were applied or to obtain a large quantity of nematodes, plates were supplied with 0.5 g peanut butter (Mendoza, 1999).

2.3. **Isolation of Nematophagous Fungi from soil and faecal samples.**

Isolation of nematophagous fungi was by the method of Larsen *et al*. (1988). Primary isolation of nematophagous fungi was achieved using Chloramphenicol-2% water agar (CHF-WA) medium. The sprinkle technique as described by (Wachira *et al*. 2009) was also used. A small sub-sample (1g) of each soil was sprinkled on to tap water agar plates (with 0.1g L streptomycin added after autoclaving). Nematode worms were added to the inoculated plate to act as bait for the nematode trapping fungi. Plates were incubated at 20^{o}C and monitored 3-4 times a week for 6 weeks for growth of nematode trapping fungi. Any fungal isolated demonstrating nematohagous fungal characteristics were sub-cultured on to PDA until they were in pure culture, and then the fungal isolates were maintained in potato dextrose agar (PDA).

3. RESULTS

Three species of nematophagous fungi was found in soil collected from Sumatera Utara are described below:

1. *Lecanicillium lecanii* (Zimm.) Zare & W. Gams, Nova Hedwigia, 73(1-2):10.

   Colonies thin, cottony with reverse colourless to pale when young and with white, cream in colour or deep yellow when mature, after 10 days of incubation on potato dextrose agar colonies were white or cream in colour, or deep yellow. Hyphae 1-2 µm wide. Phialides formed either singly, in pairs or in whorls of 3 or 4 on poorly developed conidiophores much like the vegetative mycelium, delicate, of very variable size depending on both the

strain and the age of the culture, from 8,5-16 x 1µm. Conidia produced singly and aggregating in heads at the tips of the phialides, ellipsoidal to cylindrical with rounded ends. Chlamydospores absent. Blastospores are formed in submerged culture.

Substrate: Rhizosphere soil of Strawberry plantation Karo Regencey, Sumatera Utara Province, Indonesia

2. *Paecilomyces lilacinus* (Thom) Samson, Studies in Mycology, 6:58, 1974.

Microscopy, colonies compact, velvet with pinkies colours when young and purple when mature. Conidiophores erect, 400-600 µm in length, mostly arising from submerged hyphae, septate, occasionally forming tufts up to 2 mm high, bearing branches with densely clustered phialides; conidiophore stipes 3-4 µm wide, yellow to purple, rough-walled. Phialides consisting of a swollen basal part, tapering into a thin neck. Conidia rounded to fusiform, smooth-walled to slightly roughened, hyaline, purple in mass, 2.5-3.0 x 2.0-2.2 µm, in divergent chains. **Substrate**: Soil (Sugar cane Plantation) Sumatera Utara Indonesia

3. *Paecilomyces fumosoroseus* (Wize) Brown & Smith, Trans. Br. mycol. Soc. 40: 67. 1957.

Host attached to substratum by pulvinate, felted mycelium, covered by sparse, floccose mycelium, orange white to pale orange (6A2-3), or reddish white (7A2), reddish orange (7A5-6), bearing several synnemata. Hyphae hyaline, septate, branched, smooth-walled, 1.4-4.0 µm wide. Synnemata erect, simple, up to 1.2 mm long, 400 µm in diam., white, reddish white (7A2) to pastel red (8A4-5), consisting of loosely longitudinal interwoven septate hyphae. Conidiophores mono- or synnematous, erect, 1.6-3.6 µm wide, smooth-walled, hyaline, bearing solitary, or whorls of 2-7 metulae, around or beneath the septa. Metulae cylindrical, clavate, smooth, 4.4-8.7 × 2.4-4.8 µm, sometimes bearing verticils of 4-9 phialides, phialide solitary, or in whorls of 2-5, arising terminally or subterminally from the conidiophore. Phialides smooth-walled, 4.8-9.1(-15.1) × 2.0-4.0 µm, with a globose or ellipsoidal base, tapering into a distinctly thin neck, 0.6-1.7 × 0.6-1.0 µm. Conidia subglobose, ovoid, ellipsoidal to cylindrical, or regularly to irregularly campanulate, occasionally fusiform, smooth-walled, hyaline, (2.4-) 3.2-5.6 (-6.4) × 1.9-3.6 µm. Chlamydospores absent.

Substrate: Soil (rizhosphere) of Palm tree and decay of wood from Sumatera Soil .

4. Discussion

There are three nematophagous fungi found in soil collected from Sumatera Utara which may have important value as bio-control agents to control nematode pathogen in plants and pest control agent especially larva of insects.

1. *Lecanicillium lecanii* was found from soil substrate in the rhizosphere of strawberry plant in Berastagi area, Karo Regency Sumatera Utara Province. Previously, this fungi was placed into genus *Verticillium* which divide in to three ecologically 1. Mycopathogens; 2. Entomopathogens 3. Plant pathogen. However, recently the genus has undergone some revision into which most entomopathogenic and mycopathogenic isolates fall into a new group called *Lecanicillium* (Zare and Gams, 2001; Barbara and Clewes, 2003).
2. *Paecilomyces lilacinus* (Thom.) Samson principally infects and assimilates eggs of root-knot (*Meloidogyne* spp) and cyst nematodes (*Globodera* spp.). The fungus has been the subject of considerable biological control research following its discovery as a biological control agent in 1979. *P. lilacinus* has been considered to have "the greatest potential for application as a bio-control agent in sub-tropical and tropical agricultural soil (Jatala *et al* 1979; Jones and Kabana, 1988).
3. *Paecilomyces fumosoroseus* known as insect pathogen. This fungi infects and kill insect with the wide host range that includes insects in over twenty five different families and many species of mites (Anonimus, 2002). When a blastopores (an asexual fungal spore produced by budding) of *Paecilomyces fumosoroseus* lands on a suitable host, it produces enzymes to penetrate the insects cuticle. A comparison made between several entomopathogenic hyphomycetes showed that *Paecilomyces fumosoroseus* provided more effective control of the cabbage-heart caterpillar (Hashim and Ibrahim, 2003).

4. CONCLUSION

Three nematophagous fungi isolate from Sumatera Utara have been found from natural area such as Taman Hutan Raya Sibolangit and un-nature areas such as tobacco and palm tree plantation, vegetable field, dairy farm and dung of herbivore. Nematophagous fungi isolate from Sumatera Utara has been identified as *Lecanicillium lecanii, Paecilomyces fumosoroseus and P. lillacinus.*

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