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Exploration of Endophytic Fungi in Sweet Maizes (*Zea mays saccharata* L.) and Their Potential as Entomopathogenic Fungi

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ABSTRACT

Maize is one of the horticultural commodities with high economic value and it was the second source of carbohydrate food after rice. The attack of pests and pathogens on the cultivation of maize leads to a decrease in crop productivity. To control pest attacks can be done by utilizing the antagonism ability of endophytic fungi on sweet maize plants. This study was a descriptive study, namely by identifying endophytic fungi found in parts of sweet maizes and testing their potential as entomopathogenic fungi against armyworms (*Spodoptera litura*). To test the correctness of this potency, Koch's Postulate Test was carried out on dead larvae that had been transmitted by endophytic fungi. Based on the results of the study, there were five isolates of endophytic fungi found in sweet maize consisting of the genera *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Cladosporium* sp. and *Rhizoctonia* sp. The results of endophytic fungal tests on armyworms (*Spodoptera litura*) showed that the five isolates can varying degree mortality larvae such as changing body color and drying out, especially *Trichoderma* sp. However, by testing Koch's Postulates on these five isolates, these five fungi did not have the ability to parasitize to cause disease symptoms in *S. litura*, but larva can killed by fungi carried out to the production of insecticide secondary metabolites, antifeedant compounds and repellent metabolites.
Key words: endophytic fungi, entomopathogen, *Spodoptera litura*, *Zea mays saccharata* L.

ABSTRAK

Jagung merupakan salah satu komoditas hortikultura yang bernilai ekonomi tinggi dan merupakan sumber pangan berkarbohidrat kedua setelah beras. Serangan hama dan patogen pada budidaya jagung menyebabkan penurunan produktivitas tanaman. Untuk mengendalikan serangan hama tersebut dapat dilakukan dengan memanfaatkan kemampuan antagonisme jamur endofit yang terkandung dalam tanaman jagung manis. Penelitian ini merupakan penelitian deskriptif yaitu dengan mengidentifikasi jamur endofit yang terdapat pada bagian jagung manis dan menguji potensinya sebagai jamur entomopatogen terhadap ulat grayak (*Spodoptera litura*). Untuk menguji kebenaran potensi tersebut, dilakukan Uji Postulat Koch terhadap larva mati yang ditularkan oleh jamur endofit. Berdasarkan hasil penelitian, terdapat lima isolat jamur endofit yang ditemukan pada jagung manis yang terdiri dari famili *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Cladosporium* sp. dan *Rhizoctonia* sp. Hasil uji jamur endofit terhadap ulat grayak menunjukkan bahwa kelima isolat tersebut mampu mematikan larva dengan tingkat kematian yang berbeda-beda seperti perubahan warna tubuh dan kekeringan khususnya *Trichoderma* sp. Namun berdasarkan pengujian postulat Koch pada kelima isolat tersebut, kelima jamur tersebut tidak mempunyai kemampuan parasitisasi hingga menimbulkan gejala penyakit pada *S. litura*, namun larva tersbut dapat terbunuh oleh jamur tersebut dikarenakan adanya produksi metabolit sekunder insektisida, senyawa antifeedant dan metabolit repelent yang dimiliki oleh jamur endofit tersebut.

Kata kunci: jamur endofit, entomopatogen, *Spodoptera litura*, *Zea mays saccharata* L.

INTRODUCTION

Sweet maize (*Zea mays saccarata* L.) was included in food commodities that have great potential for the benefit of the feed and food industry. In addition to human consumption, maize was also used as feed for poultry and ruminants. According to the Department of Food Crops and Horticulture East Kalimantan, maize food crops have been realized in East Kalimantan reaching 180%. The decrease in productivity that occurred in sweet maize plants in East Kalimantan one of them is caused by armyworm pests. New ways to control these diseases and pests need to be constantly researched and developed. An alternative way of environmentally friendly control can be used, namely by utilizing biological agents in the form of endophytic fungi that are antagonistic.

Biological control is control that is carried out without the use of chemicals and is friendly to the environment. The excessive use of chemicals can disrupt the environment so other innovations are needed as alternatives in protecting the environment. The use of antagonistic microbes needs to be pursued and one of them is utilizing endophytic fungi.

Antagonistic microbes are found in endophytic fungi that produce mycotoxins, enzymes and antibiotics, therefore endophytic entomopathogenic fungi are needed as an alternative in plant pest control.

Endophytic fungi are fungi found in the system of plant tissues, such as leaves, stems, flowers or plant roots. Endophytic fungi are currently receiving more attention because of the usefulness of endophytes in protecting host plants from attacks by plant-disturbing organisms such as pests and pathogens. Endophytic fungi produced by plants can have influences such as increasing resistance to pests and diseases, increasing the availability of nutrients and producing plant growth hormones themselves.

Based on the description above, it is necessary to conduct research on the exploration of endophytic fungi in sweet maizes (*Zea mays saccarata* L.) and their potential as entomopathogenic fungi. This study aims to explore endophytic fungi found in sweet maize plants and test endophytic fungi that have the potential as entomopathogens.

MATERIALS AND METHODS

The study was conducted for approximately 6 months, from March to August 2023 starting from sampling to the testing process for armyworm pests. The research was conducted at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, Mulawarman University, Samarinda. The sampling location was on the land of the Samarinda State Development Agricultural School, South Sempaja subdistrict, North Samarinda District, Samarinda City.

The materials used in this study were Potato Dextrose Agar (PDA) media, samples of healthy maize plants (45-50 Days Afetr Planting/DAP), armyworm pests instar 3, mustard leaves, alcohol 90%, NaOCl 5%, plastic cling wrap, aquadest, methilene blue, chloramphenicol, spirits, cotton, aluminum foil and tissues.

The ingredients for making PDA media consist of 200 g potatoes, 20 g powdered sugar, 20 g agar, 2 chloramphenicol capsules and 1 L of distilled water. The boiled potato water is then filtered and added agar-agar, refined sugar and chloramphenicol alternately and stirred evenly. After boiling, the PDA is put into a sterile Erlenmeyer and covered using cotton and aluminum foil tightly. Then insert into the autoclave media with a pressure of 1.5 atm for 15 minutes. The media is ready for use.

Isolation carried out from the healthy roots, stems, leaves and petals of maize plants that have been cut 1 cm begins with washing plant parts using aquadest solution for 1 minute and then soaking in alcohol 90% for 1 minute and repeated 2 times, then the sample pieces are dried on sterile tissue. After cleaning and drying, the sample pieces are planted on the PDA media provided on the petri dish. Then observations are made after 5 days.

Purification was carried out on different colonies of fungi. After the fungus was planted and grown on other media, the fungus was then identified under a compound microscope. Preparations are made by taking a small number of colonies using a loop needle, then placing them on a glass object and then covering it with a cover glass. Identification is carried out by matching macroscopic or microscopic morphological characters based on the guidelines of (Barnett and Hunter 1998; Watanabe 2002).

The identified fungus was then propagated to obtain endophytic fungi for application to armyworms. Fungal purified are then mixed with 5 mL of aquadest and homogenized using a vortex. The available solution was sprayed on mustard leaves as feed for armyworm pests. Five plastic containers were provided, each containing 10 armyworms that have been provided with mustard leaves sprayed with mushroom liquid. Then observe the death that occurs in armyworm pests for 2 weeks.

RESULTS AND DISCUSSION

A. Endophytic Fungi Found in Maize Plants *Trichoderma* sp.

Isolates of the fungus *Trichoderma* sp. found in petri dish have colony growth that spreads evenly over PDA media, colonies shaped like mosquito repellent and dark green (Fig. 1A). Microscopically with 400x magnification *Trichoderma* sp. It has ovoid hyaline conidia, perpendicular conidiophores, 1-celled, much branched and septatic, as well as single phialids or groups (Fig. 1B). This was shown by Barnett and Hunter (1998) that *Trichoderma* have description such as conidiophores hyaline, much branched, not verticillate; phialides single or in groups; conidia (phialospores) hyaline, 1-celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches or cushions of conidia. Thus, *Trichoderma* isolates that developed profuse fluffy mycelium and two to three fine-defined concentric mycelium (white) and conidia (green) rings (García-Núñez *et al*. 2017). *Trichoderma* are a fast growth in culture medium and development of conidia with green-yellow color (Chaverri *et al.* 2015).

Aspergillus sp.

The isolate of the fungus *Aspergillus* sp. found in petri dishes has colony growth that spreads evenly over the PDA medium, the surface of the colony is smooth and dry pollinated, and the colony is yellowish-green (Fig. 1C). Microscopically with 400x magnification *Aspergillus* sp. It has globose conidia and non-septaic conidiophores, 1-celled (Fig. 1D). Barnett and Hunter (1998) said that *Aspergillus* morphological characters have conidiophores upright, simple, terminating in a globose or clavate swelling, bearing phialides at the apex or radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains.

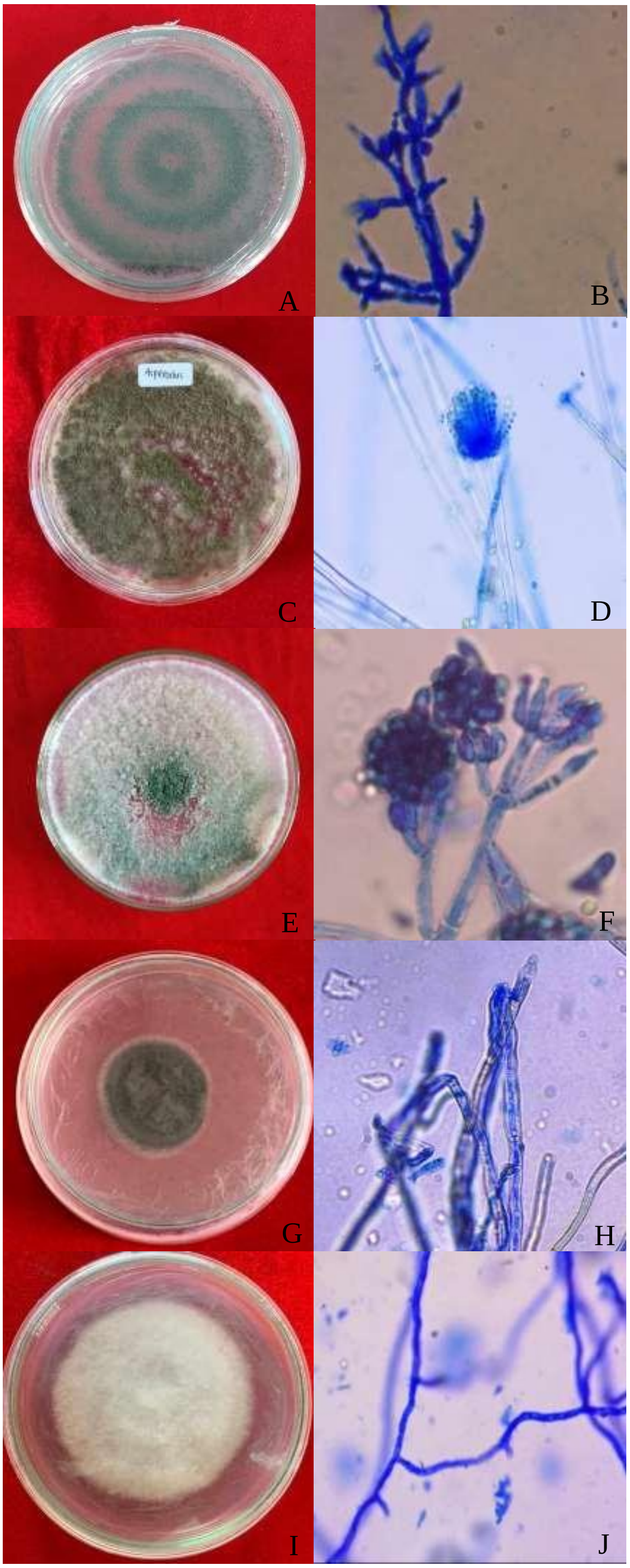

Figure 1. A. Macroscopic of *Trichoderma* sp.; B. Microscopic of *Trichoderma* sp. (400x magnification); C. Macroscopic of *Aspergillus* sp.; D. Microscopic of *Aspergillus* sp. (400x magnification); E. MACRoscopic of *Gliocladium* sp.; F. Microscopic of *Gliocladium* sp. (400x magnification); G. Macroscopic of *Cladosporium* sp.; H. Microscopic of *Cladosporium* sp. (400x magnification); I. MACroscopic of *Rhizoctonia* sp.; J. Microscopic of *Rhizotocnia* sp. (400x magnification)

***Gliocladium* sp.**

Isolate of the fungus *Gliocladium* sp. what is found in petri dishes is white in the early days of growth and will turn pale to green, dense, circular, smooth and flocculose (Fig. 1E). On observation using a compound microscope with a magnification of 400x, *Gliocladium* sp. It has conidia oval and erect, conidiophores have septa, 2-5 phialides (Fig 1F). As described by Sreekanth *et al.* (2011), *Gliocladium* has white in front and pale cream on reverse in PDA. Circular, smooth, flocculose and dense, Erect, branched to unbranched verticillium-like, with 2-5 phalides on conidiophores, cylindrical, oval, and curved on conidia.

***Cladosporium* sp.**

Isolate of the fungus *Cladosporium* sp. what is found in petri dishes is slightly blackish gray at the base and the colony is black, round colony shape and has a velvety appearance and raised elevation (Fig. 1G). Microscopically with a magnification of 400x *Cladosporium* sp. has septal hyphae (Fig. 1H). The complete description is stated by Barnett & Hunter (1998) that *Cladosporium* has conidiophores tall, dark, upright, branched variously near the apex, clustered or single; conidia (blastophores) dark, 1 or 2-celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon-shaped; often in simple or branched acropetalous chains. Then, Bensch *et al.* (2012), added the hyphae are consistently septate, mostly branched, smooth, occasionally somewhat rough-walled, and subhyaline, lightly pigmented to dark brown, thin-walled, but sometimes becoming thick-walled with age; conidiophores usually arise from internal or external hyphae, from small to large stromatic hyphal aggregation, cylindrical, subcylindrical or filiform shape, formation of the conidia in chains or solitary is a useful feature.

***Rhizoctonia* sp.**

Rhizoctonia sp. in petri dishes have colony growth that spreads evenly over PDA media, the surface of the colony is smooth that resembles thin white thread fibers and can change color to brownish Fig. 1I). Microscopically with a magnification of 400x *Rhizoctonia* sp. does not produce spores, has insulated hyphae with perpendicular branching almost forming an angle of 45 - 90°, at the point of branching forming an indentation (Fig. 1J). As described by Barnett & Hunter (1998) that *Rhizoctonia* has mycelium hyaline in some species to dark in others (such as *R. solani),* the most common species; cells of mycelium usually long, septa of branches usually set off from the main hyphae; asexual fruit bodies and conidia absent; sporodochium-like bodies and chlamydospore-like cells in chains produced in some species; sclerotia light colored and poorly formed in some species or brown or black and well formed in other.

B. Endophytic Fungi as Bioagents

Biological control is control carried out by utilizing natural enemies as pest and plant disease control consisting of predators, parasitoids, pathogens, and antagonists has long been proclaimed as one of the components of integrated pest and disease control. Endophytic fungi are fungi in plants found in tissue systems such as leaves, twigs and roots that do not cause symptoms of disease (Fitria 2014). The compounds produced by endophytic fungi have the potential to be biological controllers. Endophytic fungi can increase the resistance of host plants and can control growth against the growth of pathogenic fungi.

The role of endophytes as biological agents began to be widely studied since the phenomenon of the ability of plants to deal with biotic and abiotic stress related to the presence of endophytes in their tissues (Sturz and Nowak 2000). Rodriguez *et al.* (2009) report that endophytes produce various bioactive compounds that help plants deal with biotic and abiotic stress.

Endophytic fungi are biological agents that are antagonistic in producing alkaloid compounds and mycotoxins that function in increasing plant resistance to disease (Kusumawardani *et al.* 2015). Each plant has endophytic microorganisms such as bacteria and fungi that can produce compounds that function as antibiotics, antivirals, anti-insects, antidiabetics, antimalarials, growth regulators and producers of hydrolytic enzymes such as amylase, cellulase, xylanase, ligninase, chitinase. The benefits obtained by host plants, protect themselves from pest attacks and are resistant to disease and drought (Kurnia *et al.* 2014).

C. The Role of Endophytic Fungi as Entomopathogen

According to (Pracaya 2011) *Spodoptera litura* is called armyworm because this caterpillar in very large numbers until thousands attack and eat plants at night so that plants will run out in a short time. *S. litura* armyworms include insects that are polyphagous or have a wide host range so that they have the potential to become pests on various types of food crops, vegetables, fruits and plantations. Armyworm pests are spread in tropical and subtropical regions (Sari *et al.* 2013).

Entomopathogenic fungi are one of the fungi that are used as natural enemies for insects. Entomopathogenic fungi are heterotrophs, due to the heterotrophic nature of these fungi live as parasites on insects (Permadi *et al*. 2019). The use of entomopathogenic fungi to control insects has advantages in high production capacity, the cycle of entomopathogenic fungi is relatively short and is able to form spores that are resistant to adverse environmental conditions (Rosmayuningsih *et al.* 2014). Fungi acts directly as an entomopathogen through parasitism and the production of insecticidal secondary metabolites, antifeedant compounds and repellent metabolites.

Based on the fungus found, endophytic fungal tests were carried out on armyworms to determine the potential of fungi in killing larvae. Based on 14 days of observation, among the fungi *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Cladosporium* sp., and *Rhizoctonia* sp. Some fungi have the potential to kill armyworms. The mortality graph can be seen in Figure 2.

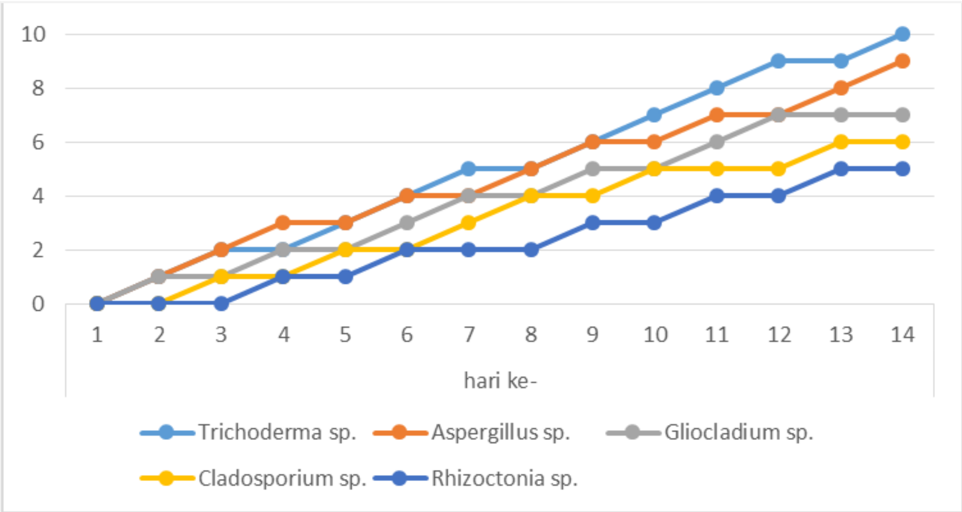

Figure 2. Mortality of *Spodoptera litura* during 14 days of observation

The test was carried out on armyworm larvae, there are four stages of entomopathogenic fungi infecting insects, namely inoculation, penetration, infection and invasion. After being attacked by entomopathogenic fungi, insects will turn dry and blackish in color. In general, fungi enter the body of insects through the cuticle where fungal conidia attach and penetrate the intergument. The fungus in the cuticle then damages the tissue and absorbs larval body fluids so that the larval body dries out (Masyitah *et al.* 2017). In this study, armyworms that experienced various mortality such as black and fragile burnt, remained brown but dry and only died unchanged physically.

Based on observations made for 14 days, there was a death in 10 armyworms. The body of the larva turns blackish-brown and dries out. Armyworm mortality can be seen immediately on the second day and there is an increase in mortality during the 14-day observation period.

Based on tests on *Spodoptera litura* larvae during 2 weeks of observation proved that the fungus *Aspergillus* sp. can kill larvae due to feed sprayed with a solution containing the fungus *Aspergillus* sp. Not all but 1 left alive. The fungus *Aspergillus* sp. can produce mycotoxin compounds such as aflactoxin. Mycotoxin compounds are known to cause disruption to human or animal health by undergoing clinical and pathological changes. (Miskiyah *et al.* 2010).

CONCLUSION In the Koch Postulate Test, it was proved that none of the isolates grew hyphae around the larval body area. That is why the five fungal isolates found in sweet maize samples, which were found as an endophyte and used in experiments, their ability is not entomopathogenic through parasitism. The fungi that are obtained and have been detected have the potential to kill insects but need to re-examine the content contained in these fungi. *Trichoderma* can act directly as an entomopathogen through parasitism and the production of insecticide secondary metabolites, antifeedant compounds and repellent metabolites (Poveda 2021). In another case, there is the potential for *Trichoderma* to have a level of virulence against *S. frugiperda* larvae and cause larval mortality of up to 71% 10 days after application in the laboratory (Afandhi *et al.* 2022). This is due to *Trichoderma* can produce metabolites such as citric acid and ethanol. Enzymes produced by *Trichoderma* sp. fungi such as urease enzymes, cellulase, glucanase and chitinase (Carlile *et al.* 2001) can have a bad effect on insects and even kill them when they enter their bodies.

Based on the results of research that has been done, it can be concluded that endophytic fungi found in maize samples taken on the land of the Samarinda State Development Agricultural School are *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Cladosporium* sp. and *Rhizoctonia* sp. The results of endophytic fungal tests on armyworms (*Spodoptera litura*) showed that the five isolates can varying degree of mortality larvae such as changing body color and drying out, especially *Trichoderma* sp.

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