Fungi diversity in the rhizosphere of corn and *Spodoptera frugiperda* larvae

**W H Lubis¹, Lisnawita*² and M C Tobing²**

¹Magister Program of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.
²Program Study of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *lisnawita@usu.ac.id

**Abstract.** Fungi are heterotrophic organisms that can find in many habitat like soil rhizosphere, and insect larvae in the field. The purpose of this study was to determine the diversity of fungi in the soil rhizosphere of corn and *Spodoptera frugiperda* larvae that are attacked by fungi in the field. Sampling of soil and insects infested by fungi in this study was carried out in a corn cropping in Gedung Johor Sub-district, Medan Johor District, North Sumatra Province, Indonesia. Isolation and identification were conducted at the Biology Laboratory, Universitas Negeri Medan. The results showed that the number of fungi found from the soil rhizosphere of corn plants was higher than from *S. frugiperda* larvae that were attacked by the fungus. There were 16 fungal isolates found from the soil rhizosphere of corn, namely: Penicillium (1 isolate), Trichoderma (2 isolates), Fusarium (3 isolates), Mucor (1 isolate), Aspergillus (3 isolates), and 6 isolates have not identified yet. Meanwhile, 9 fungal isolates found from *S. frugiperda*, consisting of 6 isolates from Aspergillus, and 3 other isolates have not been identified.

1. **Introduction**

Corn (*Zea mays* L.) is one of global food crop commodities that has an important role in the agricultural sub-sector in Indonesia. Apart from rice, corn is also used as a staple food, especially in Eastern Indonesia, such as Maluku and East Nusa Tenggara [1]. Currently, Indonesia is ranked 7th as the largest corn cultivation producer in the world. Corn cultivation provides benefits in increasing the income of environment, farmers, society, and the state. The use of corn as food, animal feed, and other industrial materials has resulted in increased consumer demand for corn commodities. Based on data from the Ministry of Agriculture of the Republic of Indonesia in 2018, the harvested area for cornfield in Indonesia reached 5.73 ha with corn production of 30.055 tonnes. The development of corn production in Indonesia is influenced by the area of harvest, seed, and use of hybrid seeds [2]. In addition, microorganisms one of them is fungi can affect the development of corn production [3]. Fungi are heterotrophic organisms that can affect the plant growth and development of a plant. The habitat of the fungus varies, some are in the ground, some are in insects, and others are even in a crop. The diversity of fungi types is due to the existence of different substrates that cause various types of fungi to grow, besides environmental conditions such as humidity, soil moisture, temperature, soil
acidity (pH), and light intensity [4]. There are fungi that are beneficial and/or harmful. One of the beneficial fungi is the entomopathogenic fungi [5].

Entomopathogenic fungi are bioinsecticides that can infect insects through natural holes in insects, such as skin, digestive tract, spiracles, or other natural holes in insects. The inoculum of the entomopathogenic fungi that enters or attaches to insects will develop into a sprout tube, then it will penetrate the insect’s skin mechanically or chemically by removing enzymes or toxins [6]. Furthermore, the fungus will develop and the fungus mycelium will cover the insect’s body. Entomopathogenic fungi generally can be obtained from around the plant rhizosphere or from insects attacked by entomopathogens. Furthermore, the population of fungi usually originating from the rhizosphere is more easily to be observed and obtained than the population of fungi that do not come from the rhizosphere [7]. It happens because microbial development is influenced by the metabolic activity of plant basal stem or roots. Plant roots carry out metabolic activities so that they release metabolite compounds called exudates into the soil [8].

The fungal isolates were obtained by various methods, one of which was the exploration method for various types, such as soil and insects attacked by fungi in the field. Exploration of fungi from the soil rhizosphere and larva is a very interesting research. Therefore, this study aims to explore fungi in the rhizosphere and larva S. frugiperda that are attacked by fungi in the corn cropping in Gedung Johor.

2. Materials and methods

2.1. Sampling technique

Soil samples were obtained from the rhizosphere of 5 healthy corn plants grown in Gedung Johor Village, Medan Johor District, North Sumatra Province. Soil sample was collected along from 5 points around the rhizosphere of each corn plant. At each sampling point, 200g of soil with a depth of 15-20 cm near the active growing roots were taken, then the samplings were put in a plastic bag labeled with the date and location of the sample. The soil samples are taken to the laboratory and were composite before further processing.

Samples of S. frugiperda larvae infected with the fungus in the corn plants were taken with a surgical tweezers and then put in a polyethylene plastic bag, then samples were labeled with the date and location of the sample. The samples are taken to the laboratory for further processing.

2.2. Exploration and isolation of fungi in the rhizosphere of corn

Exploration of fungi obtained in the rhizosphere soil samples of corn was carried out by serial dilution following the method of [9] using Potato Dextrose Agar (PDA) media. An amount of 10g of the soil sample were combined with 90 ml of sterile water, then 1ml of the suspension was put into a test tube containing 9 ml of sterile water. Serial dilutions are carried out up to 10⁻⁴. After that, an amount of 1ml of the suspension was taken and then grown on PDA media. The suspension was then incubated at a temperature of 22-25°C for 5-7 days of measurement period. Observations were made every day. Each colony appeared was reisolated to get a pure culture.

2.3. Exploration and isolation of fungi in the S. frugiperda larvae infected by fungi

Exploration for fungi was carried out by cutting the infected larvae to a size of ± 0.5 cm, then the cutting infected larvae was surface-sterilized by immersing the pieces of larvae in 0.25% of sodium hypochlorite solution for 30 seconds, then they were rinsed with sterilized water 2 to 3 times. Furthermore, the pieces of S. frugiperda larvae were dried with filter paper and grown on PDA media. Observations were made every day. Each colony appeared was isolated again to get a pure culture.

2.4. Fungi identification

Fungi identification was carried out by observing the macroscopic characteristics of each isolate obtained in the form of the colony color due to the upper surface, the lower surface, and the texture. Meanwhile, microscopic observations were made with methyl blue staining and observed under a
compound microscope by observing the shape of hyphae, conidia, and spore arrangement. Fungi were identified based on *Morphologies of Cultured Fungi and Key to Species* [10].

3. Results and discussion

Diversity relates to the variability of life among plants, animals, and microorganisms. Fungi are a major component of biodiversity that play an important role in ecological processes. The results of exploration and isolation of fungi in the rhizosphere soil of corn plants and *S. frugiperda* larvae infected in the corn field showed that the number of isolates from rhizosphere soil was greater than the number of isolates from *S. frugiperda* larvae infected. There were 16 fungal isolates in the rhizosphere soil. Meanwhile, there were 9 isolates from *S. frugiperda* larvae infected.

The fungal analysis was conducted with identified all isolates by observing macroscopic and microscopic characteristics. Of the 16 fungal isolates from rhizosphere soil, it was found that they consisted of 1 isolate from the genus Penicillium, 2 isolates from the genus Trichoderma, 3 isolates from the genus Fusarium, 1 isolate from the genus Mucor, and 3 isolates from the genus Aspergillus, while 6 other fungal isolates have not been identified yet (figure 1). Furthermore, 9 fungal isolates from *S. frugiperda* larvae infected by fungi consisted of 6 fungal isolates from the genus Aspergillus while 3 other fungal isolates had not been identified (figure 2). Macroscopic and microscopic characteristics from the each genus that have been identified can be described as follows (table 1a and 1b, and table 2).

| Isolate Code | Upper Surface | Color | Shape | Texture |
|--------------|---------------|-------|-------|---------|
| RJAJ1        | Moss green    | Round and spread | Cottony |
| RJAJ2        | Moss green    | Round and spread | Valvety |
| RJAJ3        | Moss green    | Round and spread | Valvety |
| RJAJ4        | Moss green    | Round | Valvety |
| RJAJ5        | Moss green with white edges | Round | Valvety |
| RJAJ6        | Light green with white edges | Round upright | Cottony |
| RJAJ7        | Moss green with white edges | Round and spread | Cottony |
| RJAJ8        | Light green in the center with moss green edges | Round with upright spores | Valvety |
| RJAJ9        | Dark green    | Spread | Valvety |
| RJAJ10       | Moss green    | Round and spread | Grainy |
| RJAJ11       | Moss green    | Round and spread | Valvety |
| RJAJ12       | White         | Spread | Valvety |
| RJAJ13       | Light green   | Round and spread | Valvety |
| RJAJ14       | Broken white  | Round | Cottony |
| RJAJ15       | Broken white  | Round | Cottony |
| RJAJ16       | Dark green    | Spread | Valvety |
Table 1b. Macroscopic and microscopic characteristics of fungal isolates from the rhizosphere soil of corn pants (Continue).

| Isolate Code | Lower Surface | Color | Genus |
|--------------|---------------|-------|-------|
| RJAJ1        | Yellow        | Semi Round | Hyaline | *Penicillium* |
| RJAJ2        | White         | Slightly oval | Hyaline | *Trichoderma* |
| RJAJ3        | White         | Slightly oval | Hyaline | *Trichoderma* |
| RJAJ4        | Broken white  | Round slightly oval | Hyaline | Unknown |
| RJAJ5        | Cream         | Round | Hyaline | *Mucor* |
| RJAJ6        | Cream         | Round | Hyaline | Unknown |
| RJAJ7        | Cream         | Crescent moon with a slightly blunt tip | Hyaline | *Fusarium* |
| RJAJ8        | Yellow        | Round | Hyaline | Unknown |
| RJAJ9        | Cream         | Round | Hyaline | *Aspergillus* |
| RJAJ10       | White         | Round slightly oval | Hyaline | Unknown |
| RJAJ11       | White         | Round | Hyaline | Unknown |
| RJAJ12       | Greenish white | Round slightly oval | Hyaline | Unknown |
| RJAJ13       | Cream         | Round | Hyaline | *Aspergillus* |
| RJAJ14       | Broken white  | Crescent moon with a slightly blunt tip | Hyaline | *Fusarium* |
| RJAJ15       | Broken white  | Crescent moon with a slightly blunt tip | Hyaline | *Fusarium* |
| RJAJ16       | Greenish yellow | Round | Hyaline | *Aspergillus* |

Table 2. Macroscopic and microscopic characteristics of fungal isolates from the *Spodoptera frugifera* larvae infected with fungi.

| Isolate Code | Upper Surface | Color | Shape | Texture |
|--------------|---------------|-------|-------|---------|
| LTJ1         | White         | Round | Round | Threaded |
| LTJ2         | Light green with white edges | Round and spread | Valvety |
| LTJ3         | Black         | Round and spread | Valvety |
| LTJ4         | Light green with white edges | Round and spread | Valvety |
| LTJ5         | Light green   | Round and spread | Valvety |
| LTJ6         | Grayish green | Round and spread | Valvety |
| LTJ7         | Black         | Round and circular | Valvety |
| LTJ8         | Dark green    | Round | Round | Valvety |
| LTJ9         | White         | Round | Round | Cottony |

| Isolate Code | Lower Surface | Color | Genus |
|--------------|---------------|-------|-------|
| LTJ1         | White         | Round | Hyaline | Unknown |
| LTJ2         | White         | Round | Hyaline | *Aspergillus* |
| LTJ3         | White         | Round | Hyaline | *Aspergillus* |
| LTJ4         | White         | Round | Hyaline | *Aspergillus* |
| LTJ5         | White         | Round | Hyaline | *Aspergillus* |
| LTJ6         | Greenish      | Round | Hyaline | *Aspergillus* |
| LTJ7         | White         | Round | Hyaline | *Aspergillus* |
| LTJ8         | Cream         | Round | Hyaline | Unknown |
| LTJ9         | White         | Round slightly oval | Hyaline | Unknown |
3.1. Genus Aspergillus
Fungi from the *Aspergillus* genus have colonies ranging from light green and dark green with white or white borders to black, which are round and diffuse in color. The average macroscopic features of the fungus *Aspergillus* sp. have a velvety texture (valvety). The conidia are spherical to semi-spherical and have an ornamentation of irregular, thorn-like projections. The conidiophores are upright.

Several species of *Aspergillus* sp. cause disease in plants and some are saprophytic. Whereas if in a wide range of species, the fungus *Aspergillus* sp. also can infect insects. The results of this research were in line with the previous research related to *Aspergillus* sp. by [11] show that *Aspergillus flavus* can infect the corncob borer (*Helicoverpa armigera*).

3.2. Genus Fusarium
From the results obtained, the surface of some *Fusarium* sp. obtained have white color, round shape, and cottony texture while some have a moss green colony surface with white edges with a round shape spread and have a cottony texture. They have branched conidiophores. Characteristics of *Fusarium* sp. are varied, it has macroconidia that are bent slightly like a crescent moon with slightly blunt tips and generally has septa. Several species of *Fusarium* sp. have pink, purple, and yellow color.
Fusarium sp. fungus generally is included in entomopathogenic. It means that it can be pathogenic to insects. This is in line with [12] reported that there were 3 genus of fungi, namely Aspergillus sp., Beauveria sp., and Fusarium sp. which are pathogenic to insects.

3.3. Genus Mucor
Mucor sp. fungi were obtained in moss green colonies and white edges on PDA media. On the upper surface of the isolates, the Mucor sp. Has round and velvety texture (velvety). On the bottom surface, the isolates were cream-colored and the conidia were round. When the fungi were still young, the columella will be round [13]. The hyphae are hyaline in color and has no septa. Mucor sp. is a very common fungus that is easily isolated from soil, animal manure, nuts, and others. The optimum growth of Mucor sp. and the sporulation ranges from 5-30°C.

Based on the results of research conducted by [14], the Mucor sp. has the highest ability to inhibit pathogenic fungi and fast growth. This is in accordance with [15] stating that usually biological agents are able to grow faster than pathogens so that they can dominate the available space and pathogenic fungi are unable to grow and develop.

3.4. Genus Penicillium
The fungal genus Penicillium sp. has moss green color with a round shape. They spread and have a texture like cotton (cottony). On the lower surface, Penicillium sp. is yellow-colored. The spores of Penicillium sp. are semi-spherical with hyaline hyphae and septa. The spores were arranged like a chain at the end of the fialids. According to [13], the research stated that Penicillium sp. conidiophores were mononematous, but when the isolates were younger, the conidiophores were synnematous, which branched off near the apex and ended up forming fialids.

Genus Penicillium sp. fungi are mostly found in tropical or subtropical areas [13]. Several species of Penicillium sp. can cause pathogens in humans and in food/livestock [16]. Whereas some Penicillium sp. species can also act as entomopathogens because they produce penicillin [17]. The resulting penicillin can weaken the cell walls of fungi or bacteria and can kill metabolite compounds in insects.

3.5. Genus Trichoderma
Trichoderma sp. was initially white in color, then it changed to greenish white and over time it will change its color to moss green, especially in areas that have a large number of conidia. The shape and texture of Trichoderma sp. were spherical spread and velvety in texture and a white in under surface. Conidia are slightly oval in shape with hyaline hyphae with septa. The conidiophores were three-branched. The obtained fialids appeared slender and long, especially at the apex of the branches alongside the stem [13]. The fungus Trichoderma sp. interacts with plant roots so that it is used as a biocontrol agent that can control plant diseases with several antagonism mechanisms [18]. This fungus can grow at optimum temperature (15-30°C) [13]. Several research results on Trichoderma sp. that has been done, including what has been done by [19], stated that Trichoderma sp. isolates were able to inhibit the growth of Fusarium sp. with the entrapment mechanism and hyphal intervention. [18] found that Trichoderma sp. were effective in suppressing the growth of R. lignosus.

The results of this study showed that the genus Aspergillus was the dominant genus found in the rhizosphere soil of corn plants and from S. frugiperda larvae infected. This indicates that the genus has a wide distribution and is able to associate with corn plants. [20] stated that the presence of a fungus is highly dependent on the nature of the substrate and the temporal region that supports colonization, growth and ownership of the fungal substrate. The dominance of the Aspergillus genus can also be caused because this fungus produces toxins and antibiotics that can prevent the growth of other fungal species [21].
4. Conclusions

The number of fungi found from the rhizosphere soil of corn plants was more than the number of fungi found from *S. frugiperda* larvae infected. The 16 fungal isolates found from the rhizosphere soil of corn plants consisted of several genus, namely: *Penicillium* (1), *Trichoderma* (2), *Fusarium* (3), *Mucor* (1), and *Aspergillus* (3), while six other isolates have not been identified yet. There were 9 fungal isolates found from *S. frugiperda* larvae infected fungus, consisting of 6 isolates from the Aspergillus genus and 3 other isolates have not been identified yet.

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