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Detection and Morphological Identification of Seed-Borne Fungi in Six Mung Bean Varieties

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ABSTRACT

Seed health testing needs to be carried out to get quality seeds, increase crop yields, and get seeds that are free from disease attacks. The purpose of this study is to detect and identify types of seed-borne fungi in six varieties of mung beans (*Vigna radiata*) by the Plating of Seeds (Blotter Test) and Seedling Symptom Test methods. The results of the study found six species of seed-borne fungi, namely *Aspergillus* sp., *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium* sp., *Rhizopus* sp., and six other unidentified species, named Isolate 1, Isolate 2, Isolate 3, Isolate 4, Isolate 5, and Isolate 6. From the Plating of Seeds (Blotter Test) method testing, 4 fungi were found in the varieties Vima 1, Vima 3, Vima 5, and Kutilang, with 1 fungal isolate found for each variety. The best germination index is found in the Vima 2 and Vima 4 varieties, and the best germination length is found in the Kutilang variety. From the testing of the Seedling Symptom Test method, 16 fungal isolates were found, with the most fungal isolates found in the Vima 5 variety, which was 5 isolates. The best germination power is found in the Vima 1 variety.

Keywords: fungi, mung beans, seeds, varieties

INTRODUCTON

Seed-borne diseases are important because they are detrimental to the quality and quantity of plant production or the food industry made from seeds. Seed-borne pathogenic inoculum can reduce seed germination, increase disease development in the field, increase seedling and young plant mortality, decrease yield, and change seed chemical components. The seeds become carriers of a new fungus to a place, so that it will cause an explosion of disease in that place. Seeds that are infected or carry fungi will be contaminated by toxins produced by fungi and can change the nutritional value of seeds (Soekarno 2003; Agarwal & Sinclair 1996). Symptoms of seed disease are visible visually when the seeds are germinated, generally in the form of seed rot, seedling collapse (damping-off), or dead plants, causing a decline in the plant population in the field (Malvick 2002).

The production of mung beans achieved by farmers is still relatively low. The low yield of mung beans is caused by the attack of seed fungus. Moreover, most of the mung bean seeds are stored in bound sacks and stored inside the warehouse. Unsuitable temperature conditions will cause fungi to grow and attach to mung bean seeds (Manurung & Setiawan 2014). Seed-borne fungi can attach to seeds, get into seeds or seed sheets, and penetrate into the embryo. According to Rahayu (2016), seed-borne fungi can mix and spread through propagules, sclerotia, and spores that live between seed individuals; this usually occurs during seed management in the field. The active period of fungi that cause seed disease occurs when the seeds grow in the soil, especially in a fairly humid environment.

Geetanjali *et al.* (2014), Rahayu (2016), Datta *et al.* (2016), and Bakr *et al.* (1998) stated that fungi species carried by mung bean seeds were found, namely *Aspergillus niger*, *A. flavus*, *Fusarium* sp., *F. oxysporum*, *F. solani*, *F. equiseti*, *Penicillium* spp, *Macrophomina phaseolina*, *Rhizoctonia bataticola*. *Alternaria* sp., *Myrothecium roridum*, *Cercospora* spp, *Rhizopus* spp, and *Drechslera* sp. According to Sari (2020), *Fusarium* sp. is one of the seed-borne fungi that was detected and identified using the Blotter Test method. It is a soil-borne pathogen that can survive relatively long in the soil by forming mycelium or spores without a host.

Detection and identification of seed-borne fungi need to be carried out because pathogenic inoculum has the opportunity to develop into adverse diseases in the field, thereby reducing the commercial value of seeds. Quarantine and seed health certification are very useful to prevent the spread of diseases, on a national and international scale. Seed health testing aims to explain the factors that cause low germination of seeds in the field. The results of seed health testing

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can show whether or not treatment is necessary in a seed lot related to disease control efforts or reduce the risk of disease spread (Pamekas 2013). The purpose of the study was to detect and identify the types of seed-borne fungi in six varieties of mung beans.

METHODS

The research was carried out from December 2023 to March 2024 at the Plant Protection Laboratory, Faculty of Agriculture, University of Bengkulu. The study used 6 varieties of mung beans, namely Vima 1, Vima 2, Vima 3, Vima 4, Vima 5, and Kutilang from the Research Institute for Beans and Tubers (Balitkabi). Each treatment was repeated 4 times so that 24 experimental units were obtained. The methods used are the Platting of seeds (Blotter Test) method and the Seedling Symptom Test method.

The Platting of seeds (blotter test) method is carried out by planting 10 seeds on 3 sheets of sterile filter paper. Previously, the mung bean seeds are dipped in 1% NaOCl for 30 seconds, then rinsed with sterile aquades 3 times. The filter paper is moistened using a spray bottle containing sterile water. Petri dishes are placed in the incubation chamber for 14 days with 12 hours of dark and 12 hours of light (Budiarta 2017). The variables observed in this method include:

a. Germination indeks (ISTA 1972 in Kuswanto 1996)

   = (Σ Normal sprouts)/(Σ Seeds tested) x 100 %

b. Infected sprouts

   = (Σ Infected Sprout)/(Σ Normal Sprout) x 100 %

c. Sprout length (cm)

   = (Longest sprout length+Shortest sprout length)/2

d. Observation, reisolation and identification of pathogens

The Seedling Symptom Test method was carried out by planting 5 seeds in a 1.5 L bottle containing sterile river sand and manure with a ratio of 2:1. Previously, the seeds were soaked in a 1% NaOCl solution for 30 seconds and rinsed with sterile aquades 3 times. The bottles are placed in a laboratory room for 30 days of the growing period. Watering is done regularly. The variables observed are:

a. Germination index (ISTA 1972 in Kuswanto 1996)

   = (Σ Normal sprouts)/(Σ Seeds tested) x 100 %

b. Symptoms and signs that appear during the 30 days of observation

c. Isolation and identification of invading pathogens

All data obtained were analyzed in a descriptive qualitative manner, presented in the form of tables and figures.

RESULT AND DISCUSION

From the plating of seed method, it can be seen that the germination index of mung bean seeds ranges from 80%-100% with the percentage of infected sprouts ranging from 0%-17%, and the average sprout length ranges from 10.62 cm to 13.87 cm (Table 1). The germination index is lower than that of Balitkabi. The difference in germination index is suspected to be due to the length of seed storage. Kolo and Tefa (2016) stated that a decrease in the average value of germination index can occur along with an increase in seed storage time, so that the seeds experience a decrease in germination power during the storage process.

The criteria for good seed quality are seeds that have a germination percentage of >80% (ISTA 2006 in Rulvi *et al.* 2022). Based on this statement, it can be concluded that the six varieties of mung bean seeds have an excellent germination index, so that they can make plants able to grow normally in all conditions and can produce maximum production results. This is supported by the fact that the average percentage of infected sprouts is relatively small and the length of sprouts is normal.

The distribution of the types and numbers of fungi found in the Plating of Seeds (Blotter test) method is presented in Table 2. In this method, 4 fungal isolates were found in the seeds, namely *A. flavus*, *Penicillium* sp., and 2 species that have not been identified. Figure 1 shows 4 seed-borne fungi isolates obtained from the platting of seed (blotter test) method. All fungi colonies were photographed at the age of 7 days.

Table 1 Germination index, infected sprouts, and sprouts length fouon platting of seeds (blotter test) methode

Variety	Germinaton index (%)	Infected sprouts (%)	Sprouts length (cm)
Vima 1	80.00	6.00	10.62
Vima 2	100.00	0.00	13.12
Vima 3	90.00	17.00	11.87
Vima 4	100.00	0.00	11.75
Vima 5	93.00	11.00	12.87
Kutilang	95.00	6.00	13.87

The fungi found in the Plating of Seeds (Blotter test) method are fungi that live in seeds and use endosperm as food because endosperm contains carbohydrates and glucose that are very preferred by fungi. Endosperm that has been eaten up will result in embryos in the seed not being able to germinate due to the unavailability of energy. Fungi that attack seeds also cause the embryos to rot and turn black, so that

Table 2 Distribution of the numbers and types of fungi found in platting of seeds (blotter test) method

Variety	Number of fungi isolates	Type of fungi isolates
Vima 1	1	*A. flavus*
Vima 2	-	-
Vima 3	1	*Penicillium* sp. 1
Vima 4	-	-
Vima 5	1	Isolate 1
Kutilang	1	Isolate 2

Information: no fungi found

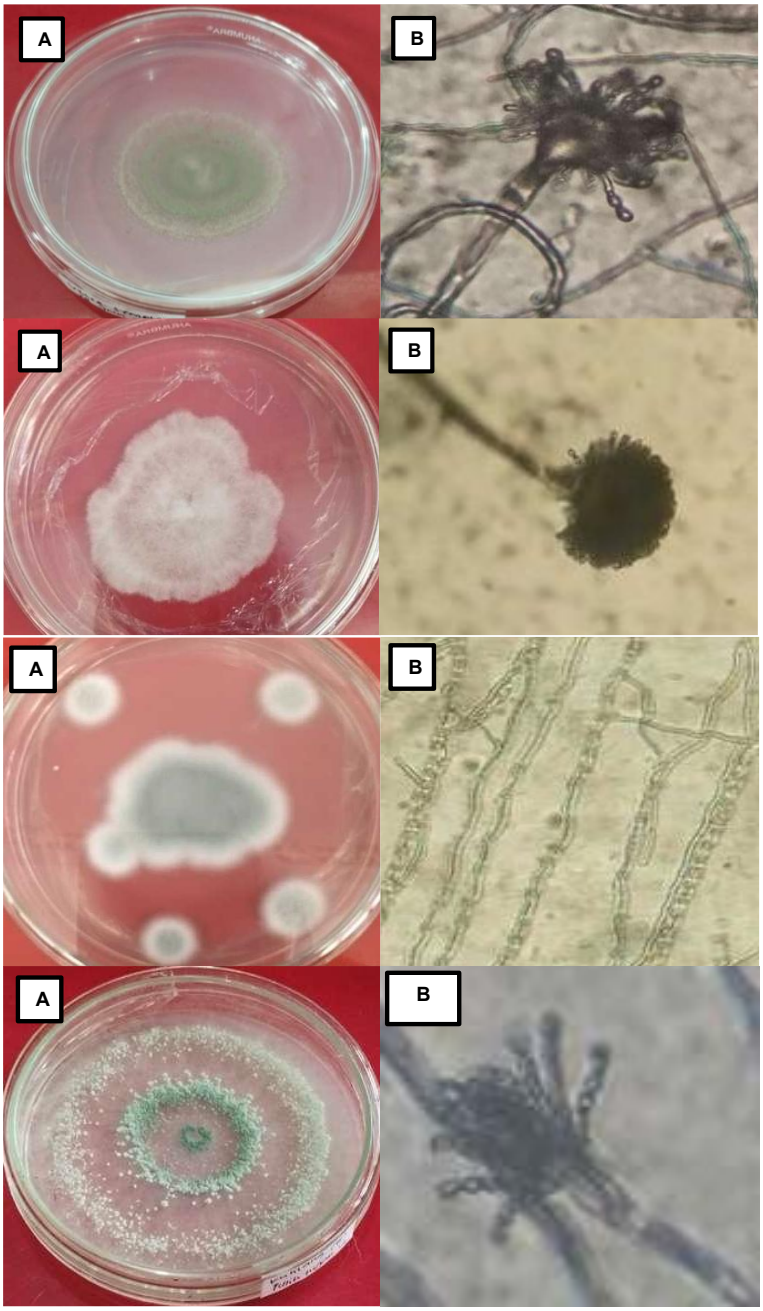

Figure 1 Characteristic macroscopic dan microscopisc of 4 fungi isolates found on Platting of seed (blotter test) methode. Information: line 1- 4= *Aspergillus flavus*, *Penicillium* sp. 1, isolate 1 and isolate 2

they can affect the growth of seeds in the next planting period (Husman *et al.* 2019). Pathogens that are embedded in the radicle will grow and attack the roots of the plant. Symptoms that appear in the embryo are caused by pathogens that grow and attack the cotyledons. Cotyledons attacked by pathogens can cause seeds to be unable to germinate (Anggraeni *et al.* 2014).

The results of the germination test of six varieties of mung beans in the Seedling Symptom Test method showed that the percentage of germination index ranged from 60%-95%. (Table 3). Each variety of mung beans has a different average percentage of germination index because each variety has different seed quality. A decrease in the average value of germination index can occur along with an increase in seed storage time, so that the seeds experience a decrease in germination index during the storage process (Kolo & Tefa 2016). Agung (2012) stated that the difference in germination index and plant growth is caused by genetic factors inherited from each different elder. According to Rahmawati *et al.* (2022), in addition to being influenced by seed quality, germination index is also influenced by physiological quality. Two important aspects of physiological quality are the ability of seeds to grow in both optimal and suboptimal conditions, and the ability to grow even if it has been a long time.

The high or low germination index does not always correlate with the number of fungi carried by the seeds. A large number of fungi should cause a decrease in germination, but not all fungi can cause a decrease in germination. Pratama and Nugraha (2023) stated that not only fungi can cause a decrease in germination, but it can also be caused by other microorganisms such as bacteria and viruses. Meanwhile, storing seeds in a warehouse with poor temperatures can also cause a decrease in germination index.

During the growth period, some mung bean plants die by showing symptoms of rotting at the base of the stem, and there are signs of mildew growing on the plant. Fungi that attack during the planting period have a long disease development, so the disease can live dormant in the seeds until it finally grows following the development of the plant. Symptoms and signs in the seedling symptom test method can be seen in Figure 2.

As many as 9 isolates of fungi carried by seeds were found in the Seedling Symptom Test method, namely *Aspergillus flavus*, *Penicillium* sp. 2, *Penicillium* sp. 4, *Penicillium* sp. 5, *Penicillium* sp. 6, *Penicillium* sp. 9, *Rhizopus* sp, Isolate 5, and Isolate 6. The distribution of types and numbers of fungi found in the Seedling Symptom Test method is shown in Table 4.

The difference in the distribution of the number and types of fungi found is due to internal factors, such as the growth rate of fungi, and external factors in the field (seedling stage, planting stage, maintenance stage, and harvesting stage), as well as during storage (temperature, humidity, moisture content, storage period, and lighting). Figure 3a and Figure 3b explains the existence of 9 seed-borne fungal isolates found in the seedling symtomp test method. The nine isolates have a lifespan of 7 days.

Kono (2021) stated that the high contamination of seed-borne fungi can occur due to infection in the field and then development in the seeds, poor post-harvest handling, and dirty room air during storage. Containers at the time of storage can support the growth of fungi that carry seeds from the field and can attack seeds of

Table 3 Germination index of mung bean seeds on Seedling Symptom Test method

Variety	Germination index (%)
Vima 1	95.00
Vima 2	75.00
Vima 3	75.00
Vima 4	65.00
Vima 5	87.00
Kutilang	60.00

Figure 2 Symptom and sign of disease on mung bean seedlings.

varieties that are not resistant to disease. The life of fungi depends on the environment, temperature, and climate. Open storage will cause high seed damage, decrease germination, and the storage capacity of seeds will not last long.

The life of fungi in storage depends on the presence of fungi. Fungi that are inside the seed will survive longer because the fungus is protected by the seed tissue, while the fungus that is on the surface of the seed can be eliminated immediately because it is often a type of saprophytic fungus (which decomposes dead organic matter). Fungal infection of seeds will affect seed germination and seed vigor derived from the seeds, cause staining and abnormalities in the seeds, and reduce seed quality, such as the degradation of carbohydrates, proteins, and fats as well as mycotoxin contamination (Pamekas 2013).

Based on the results of the seed health examination from the two methods, it was found that the fungi isolate was carried by quite a lot of seeds, so that post-harvest disease prevention must be carried out. One of the methods for preventing post-harvest diseases that can be done through seed sorting by removing cracked or wounded seeds to eliminate the appearance of

Table 4 Distribution the numbers and types of fungi found on Seedling Symptom Test method

Variety	Number of fungi isolates	Types of fungi isolates
Vima 1	4	*A. flavus* *Penicillium* sp. 2 *Penicillium* sp. 4 *Penicillium* sp. 5
Vima 2	3	*A. flavus* *Penicillium* sp. 5 *Penicillium* sp. 6
Vima 3	2	*A. flavus* *Penicillium* sp. 5
Vima 4	-	-
Vima 5	5	*A. flavus* *Rhizopus* sp *Penicillium* sp. 5 Isolat 5 Isolat 6
Kutilang	2	*Rhizopus* sp *Penicillium* sp. 9

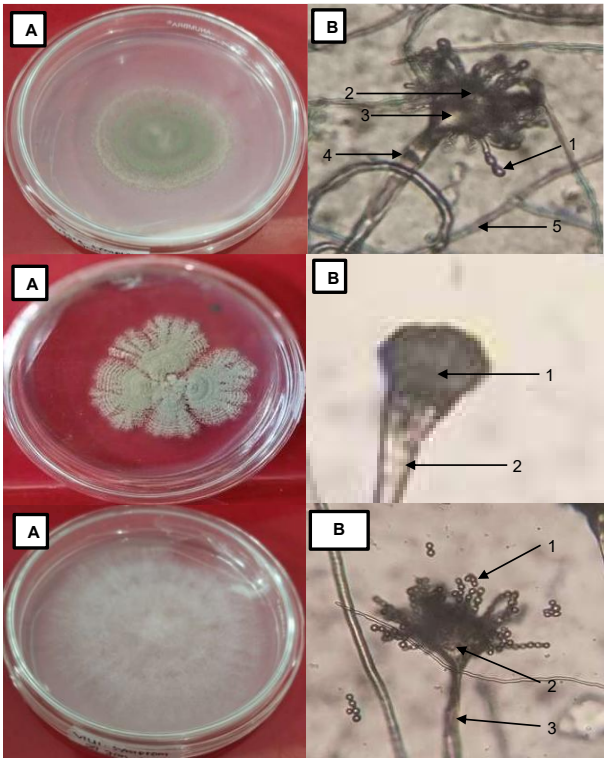

Figure 3a Characteristic macroscopic and microscopic of 3 fungi isolates found on seedling symptom test. Information: line 1–3 = *Aspergillus flavus, Penicillium* sp. 2, and *Penicillium* sp. 4.

contaminants on seeds in storage. The condition of seeds that experience mechanical damage, namely cracks or injuries due to harvesting activities and the seeding process, often facilitates fungal attacks (Agarawal & Sinclair 1997).

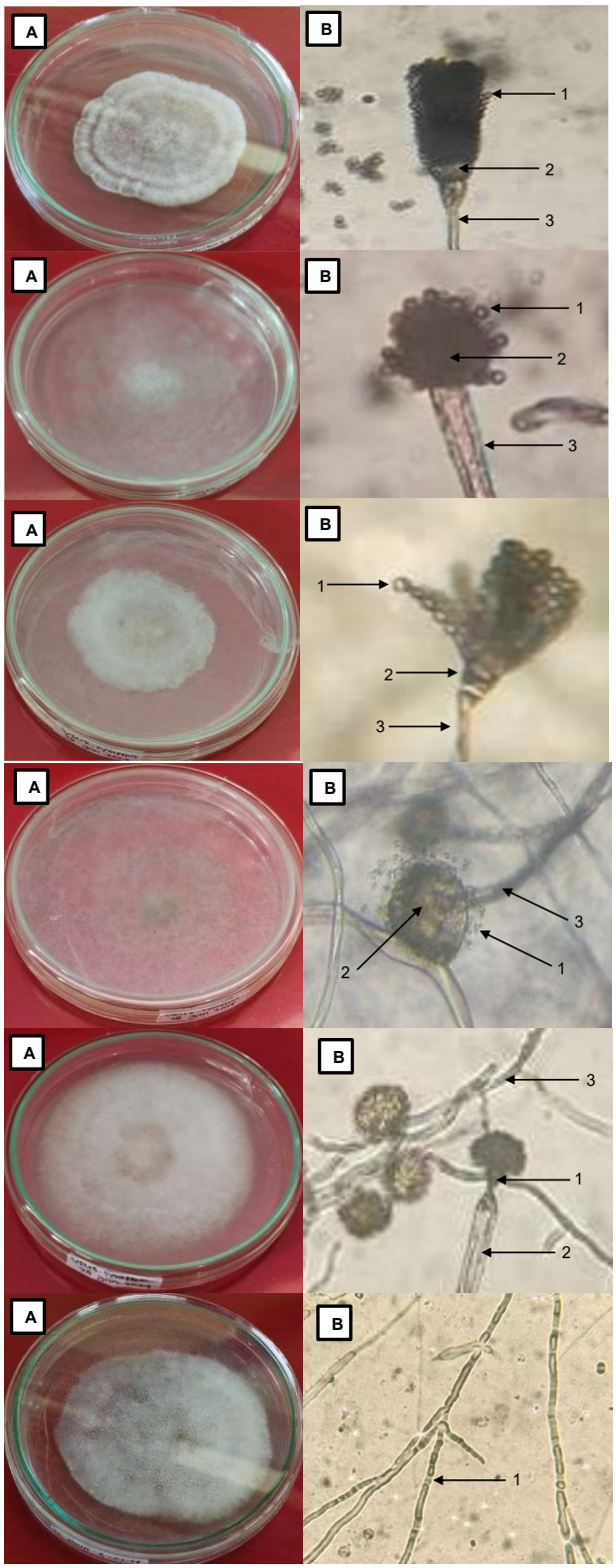

Figure 3b Characteristic macroscopic and microscopic of 6 fungi isolates found on seedling symptom test. Information: line 4–9 = *Penicillium* sp. 5, *Penicillium* sp. 6, *Rhizopus* sp., Isolate 3, Isolate 4, and *Penicillium* sp. 9.

Seed treatment that is often carried out before planting or when the seeds will be stored is by mechanical, physical, and chemical means. Mechanical treatment aims to remove the source of the disease that is mixed in the seed lot, or the fungus is outside the seed. The seeds need to be cleaned manually by removing any kind of contamination such as moldy seeds, infected plant organs, soil, and insects. Physical treatment generally uses hot temperatures such as solarization; namely, seeds are exposed to the heat of sunlight (sun-dried), soaked in warm water, hot air, hot steam, or microwave radiation. Chemical treatment using pesticides (fungicides, antibiotics, insecticides) and disinfectants is generally applied in the seed industry. Disinfectants only act as protectors to remove contaminants mixed or attached to the surface of seeds (Grum *et al.* 1998).

CONCLUSION

The Vima 2 variety has the best germination index in the blotting test) testing method, and no fungi were found on seeds that grew in the seed tissue. Meanwhile, the Vima 1 variety showed the best germination index in the seedling symptom test method, and no seed-borne fungi were found growing on the Vima 2 and Vima 4 varieties during the 30-day planting period.

From the seed health testing method carried out, six species of seed-borne fungi were found, namely *Aspergillus flavus*, *Penicillium* sp., *Rhizopus* sp., and six other unidentified species named Isolate 1, Isolate 2, Isolate 4, Isolate 5, and Isolate 6.

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