Effects of physical and chemical treatments on seed germination and soybean seed-borne fungi

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Abstract. Soybean is an important food commodity in Indonesia after rice and maize. Plant pathogens still constrain the increase in soybean productivity. One of the plant pathogenic infections can occur during the seed phase. Therefore this study aimed to determine the effect of physical and chemical treatments as control of seed-borne fungi and their impact on soybean seed germination. This study used a completely randomized design consisting of 9 treatments, namely physical therapy by heating the seeds in a microwave at a temperature of 40 °C for 10, 20, 30, and 40 seconds and chemical treatment by soaking the seeds in a fungicide with active ingredient difenoconazole with a concentration of 0.5%, 1%, 2%, and 3%. Seeds without heating and as control are soaking fungicides. Each treatment was repeated three times. The seeds that have been given treatments are then planted using the growing test technique and incubated for seven days. Furthermore, the seed viability and the growth of pathogenic fungi were observed at the end of incubation. The results showed that soybean seed germination was not affected by physical and chemical treatments. The seed viability of 100% with or without treatment. This was confirmed by the findings of seed-borne fungi (Curvularia, Fusarium, Aspergillus) with a low infection rate of 0.01-0.19%. Chemical treatment with concentrations of 0.5%, 2%, and 3% had a significant effect on the Fusarium infection level, which was higher than the control, which was 0.18%, 0.17%, and 0.19%. Meanwhile, for Curvularia and Aspergillus, physical and chemical treatments did not have a significant effect.

1. Introduction
Soybean is one of the essential commodities after rice and corn. Soybeans are also an important and cheap source of vegetable protein. Therefore, the government prioritizes soybeans to increase production. Indonesia is currently a soybean importer because the national need for soybeans has not been met. Moreover, Indonesia’s soybean production decreased by 7.16% during the 2011-2017 period [1].

Quality and certified seeds are one of the main components to increase soybean production. One aspect of seed quality is high pathological quality, which means that the seeds are free from infection by seed-borne pathogens, including fungi, bacteria, viruses, and nematodes [2]. The seed-borne fungus can be present on surfaces, in tissues, or freely mixed with seeds. The fungus inoculum source comes from the field, contamination during harvesting, processing, packaging, storage, or distribution of seeds [3]. Soesanto et al. [4] reported that the soybean seed-borne fungi identified were Aspergillus flavus, Aspergillus niger, Cladosporium oxysporum, Colletotrichum demotion, Curvularia pallescens, Fusarium solani, Melanospora zamiae, and Nigrospora sp. Seed-borne fungi can cause loss of viability and seed weight. In addition, it can also harm mammals with the production of mycotoxins.
that can cause poisoning. Amza [5] reported some of the mycotoxins are Aflatoxin, Ochratoxin, Trichothecence, Zearalenone, Fumonise, Ergot alkaloin, and sterigmatysin.

Controls that are carried out to suppress seed-borne pathogens are physical and chemical treatments. Govindaraj et al. [6] have reviewed physical treatment techniques on seeds in the form of magnetic fields, gamma irradiation, electric fields, laser irradiation, sound, healing energy, light, and heat. Meanwhile, seed treatment used chemicals consist of Hexaconacole, Mancozeb, Carbendazim, Thiphanate Methyl, Difenoconazol [7]. Apart from suppressing seed-borne fungi’ growth, physical and chemical treatments have also succeeded in increasing seed viability and dormancy [8]. Therefore, this study will examine the physical and chemical treatments of the viability and fungi of soybean seeds.

2. Methods

2.1. Time and Place of Research
The research was carried out from December 2019 to January 2020 at the Middle Agrotechnology Laboratory, Agrotechnology Departement, Faculty of Industrial Technology, Gunadarma University Campus F7, Ciracas, East Jakarta.

2.2. Soybean-seeds Treatments
The soybean seeds used are commercial seeds that are sold in bulk. Soybean seeds are prepared and separated in advance from damaged seeds and dirt. The seeds in good condition (not damaged and not dirty) as ten seeds for each treatment were randomly selected [9]. This research consisted of physical treatment using a microwave for 10, 20, 30, and 40 seconds with the heating temperature at 40 oC to provide dry heat conditions. In addition, chemical treatment was also given by immersing in a fungicide solution (active ingredient diphenaconazole) with a concentration of 0.5%, 1%, 2%, and 3% for 15 minutes. The seeds without heating in the microwave and soaked in fungicide were used as controls, so there were 9 treatments (Table 1).

2.3. Testing the viability and health of seeds using the Growing on Test Method
The growing on test testing technique follows the method of Ramdan and Kalsum [10] with fan paper media. A total of 3 sheets of frosted paper folded into a fan, then placed on a plastic tray and moistened with water. A total of 10 soybean seeds were placed between the folds of the paper. Then the trays were wrapped in clear plastic so that moisture was maintained. After incubation for 7 days, the seeds were observed for their germination capacity and examined in a stereo microscope to see the presence of pathogens carried by the seeds. Germination using the formula;

\[
\text{Seed germination} = \frac{\sum \text{seed germinated}}{\sum \text{total seeds}} \times 100\%
\]

while the percentage of infection by seed-borne pathogens was calculated using the formula:

\[
\text{Infection percentage} = \frac{\sum \text{infected seed}}{\sum \text{total seeds}} \times 100\%
\]

2.4. Fungus Identification from Seed Samples
Each seed observed under a stereo microscope identified a seed-borne fungus. Identification is also carried out under a microscopic compound by placing a glass object containing fungal scrapings on the seeds. Fungi were determined by following the identification key book from [11] and [12].

2.5. Experiment Design and Data Analysis
The experimental design used in this study was a non-factorial, completely randomized design consisting of 10 treatments (Table 1). Each treatment was repeated three times so that there were a total of 30 experimental units. The data obtained were then analyzed using variance fingerprints. If there is a significant difference, then further tested using the Tukey test with a level of 5%.
Table 1. Treatment of soybean seeds

| No | Treatment Code | Information |
|----|----------------|-------------|
| 1  | K0             | Kontrol     |
| 2  | F1             | Physical treatment with dry heating for 10 seconds |
| 3  | F2             | Physical treatment with dry heating for 20 seconds |
| 4  | F3             | Physical treatment with dry heating for 30 seconds |
| 5  | F4             | Physical treatment with dry heating for 40 seconds |
| 6  | K1             | Chemical treatment by immersing difenoconazole with a concentration of 0.5% |
| 7  | K2             | Chemical treatment by immersing difenoconazole with a concentration of 1% |
| 8  | K3             | Chemical treatment by immersing difenoconazole with a concentration of 2% |
| 9  | K4             | Chemical treatment by immersing difenoconazole with a concentration of 3% |

3. Results and Discussion

3.1. Viability of Soybean Seeds in Physical and Chemical Treatment

The results of the analysis of variance showed that physical and chemical treatments did not significantly affect seed viability. This is indicated by the level of soybean seed viability of 100% in each treatment (Table 2). In contrast to the report of Ramdan et al.[13] stated that physical treatment has higher viability than the chemical treatment of corn seeds. Based on these results, it is suspected that the physical and chemical treatments do not interfere with plant physiological processes. In addition, physical treatment with dry heating at 40°C with 10-40 seconds is still safe and does not experience damage. Several studies have reported that dry heat treatment will reduce seed viability due to damage to seed membranes and leakage of electrolyte solutions [14],[15], but Izle and Isik [16] have reported that the optimum temperature in physical treatment that supports the viability of soybean seeds is 45 °C. In this study, the chemical treatment also did not affect seed viability. Nalis et al. [17] reported that chemical treatment could cause symptoms of poisoning in sprouts so that their viability decreases.

Table 2. Seed viability under physical and chemical treatment

| Treatments | Seeds Viability (%) |
|------------|---------------------|
| K          | 100a                |
| F1         | 100a                |
| F2         | 100a                |
| F3         | 100a                |
| F4         | 100a                |
| K1         | 100a                |
| K2         | 100a                |
| K3         | 100a                |
| K4         | 100a                |

The numbers followed by the same letter in the same column show no significant difference based on Tukey's test at the level of α = 5%
Figure 1. Growth of soybean sprouts on a) Physical and b) Chemical treat

3.2. Seed-borne Pathogen Identification
The pathogens carried by jangung seeds that were identified consisted of 3 groups of fungi. Identification was carried out by observing the morphology microscopically. The identification results were microscopic morphological features and fungal colonies on the surface of the seeds (Table 3).

Table 3. Results of identification of soybean seeds fungus

| Result    | Macroscopic Observation | Microscopic Observation |
|-----------|-------------------------|-------------------------|
| Curvularia sp. | ![Image](image1.png) | ![Image](image2.png) |
| Aspergillus sp. | ![Image](image3.png) | ![Image](image4.png) |
| Fusarium sp. | ![Image](image5.png) | ![Image](image6.png) |

3.2.1. *Curvularia* sp. Colony characteristics of *Curvularia* sp. on the seeds' surface, which is characterized by strong conidophore, brownish color. Meanwhile, microscopic observation showed that conidia were light brown to dark brown, mostly straight and slightly curved, with three baffles and four cells.

3.2.2. *Aspergillus* sp. Colony characteristics of *Aspergillus* sp. on the surface of the white seed, which will turn green over time. On the surface of the seeds, there are also green powder-like grains. These green grains are the heads of conidia. Meanwhile, on microscopic observations, *Aspergillus* sp. shows the hyphae structure that has septa, clear (hyaline), with green conidia.

3.2.3. *Fusarium* sp. Colony characteristics of *Fusarium* sp. on the surface of the seeds, which is white like cotton. Meanwhile, on the microscopic observations of *Fusarium* sp. characterized by hyphae with septa and colorless with conidia in the form of macroconidia in the form of a crescent moon.

The three pathogens have been identified as being carried by soybean seeds apart from Alternaria and Rizoctonia [18]. The association of *Fusarium* and *Aspergillus* on soybeans has been reported by Ramdan and Kalsum [10] respectively, at 38% and 14%.
3.3. *Infection Power of Seed-Carried Pathogens After Physical and Chemical Treatment*

The results of the analysis of variance showed that the treatment of K1, K3, and K4 had a significant effect on the infectivity of Fusarium sp. (Figure 2), while the fungus Curvularia sp. and Aspergillus sp. physical and chemical treatment had no significant effect. In general, physical and chemical treatments show a decrease in fungal infection power. This is in line with the high seed viability (100%) in physical and chemical treatments (Table 2). Even though the K1, K3, and K4 treatments were not able to suppress Fusarium sp growth, the infection power is still low 0.17-0.19%. This is consistent with Sonhaji et al. [19], who reported that seed treatment with synthetic fungicides could suppress *Peronosclerospora maydis* in soybean seeds than other seed treatments. Nurahmi et al. [20] explained that fungicides in seed treatment could work systemically or in contact. Systemic mechanisms work to inhibit pathogens in the seed tissue, while the contact mechanism works to inhibit pathogens on the seeds' surface.

![Figure 2. Infection power of seed pathogens in physical and chemical treatments](image)

The use of fungicides as seed treatments has been reported to suppress pathogenic infections, particularly fungi, during 3-4 weeks of storage [21]. The presence of pathogens in seeds causes a decrease in viability, a musty odor, and a change in seed color. Damage to seeds can be exacerbated if stored in a location that supports the development of the pathogen. Factors that support the development of pathogens in seeds include the physical condition and moisture content of the seeds and the storage area's humidity.

4. **Conclusion**

1. Physical and chemical treatments did not significantly affect seed viability. All treatments showed that the seed viability was up to 100%.
2. The identification results show that the fungi carried by soybean seeds are *Curvularia* sp., *Fusarium* sp., and *Aspergillus* sp.
3. In general, physical and chemical treatments can suppress the growth of fungi. Chemical treatment with a concentration of 0.5%, 2%, and 3% significantly affected the Fusarium infection rate, which was higher than the control, namely 0.18%, 0.17%, and 0.19%, respectively.

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