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Detection of *Peronospora manshurica* in imported soybean and analysis of oospore viability in storage warehouses in Ternate City, Indonesia

Deteksi keberadaan *Peronospora manshurica* pada kedelai impor dan analisis daya tumbuh oospore di gudang penyimpanan Kota Ternate, Indonesia

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ABSTRACT
The soybean needs of the people in Ternate are fulfilled by imported soybeans that enter through Surabaya. The process of transporting imported soybean seeds from Surabaya to the port of Ternate and storage in warehouses may lead to contamination with *Peronospora manshurica* fungus, which poses a risk of spreading to soybean crops in Ternate. This study aims to detect the presence of *P. manshurica* fungus, viability, and growth of *P. manshurica* oospores in the imported soybean storage warehouses in Ternate. The study was conducted using a combination of qualitative and quantitative methods. The qualitative method was carried out through an exploratory approach, while the quantitative method used a randomized block design (RBD) consisting of four treatments, i.e., G.Tbsi, G.Jbla, G.Tfra, and G.Tfrb, with five replications. The observation variables included disease occurance, presence, and characteristics of oospores, as well as oospore growth and viability percentage. The results showed that the average disease occurance in the soybean storage warehouses was highest in the sample of soybeans from the G.Tfrb warehouse at 1.88%, followed by G.Jbla, G.Tfra, and G.Tbsi warehouses at 1.44%, 1.41%, and 1.24%, respectively. The study also found *P. manshurica* fungus in all four soybean storage warehouses in Ternate, with a viability percentage of G.Tbsi, G.Jbla, G.Tfra, and G.Tfrb were 25.8%, 28.8%, 27.4%, 26.5% respectively. These findings indicate the potential for disease spread to soybean crops in Ternate, thus necessitating appropriate preventive measures to address this problem.

ABSTRAK
Kebutuhan kedelai masyarakat Kota Ternate dipenuhi dari kedelai impor yang masuk melalui Kota Surabaya. Proses pengangkutan biji kedelai impor dari Surabaya ke pelabuhan Kota Ternate hingga masa penyimpanan di gudang memungkinkan kedelai terkontaminasi cendawan *Peronospora manshurica* dan berisiko menyebar ke pertanaman kedelai di Kota Ternate. Penelitian ini bertujuan untuk mendeteksi keberadaan cendawan *P. manshurica,* viabilitas dan pertumbuhan oospora *P. manshurica* di gudang penyimpanan kedelai impor Kota Ternate. Penelitian dilakukan dengan kombinasi metode kualitatif dan kuantitatif. Metode kualitatif dilakukan melalui pendekatan eksplorasi. Metode kuantitatif menggunakan rancangan acak kelompok yang terdiri atas 4 perlakuan yaitu G.Tbsi, G.Jbla, G.Tfra, dan G.Tfrb, dengan ulangan 5 kali. Variabel pengamatan meliputi kejadian penyakit, keberadaan dan karakteristik oospora, serta pertumbuhan dan persentase viabilitas oospora. Hasil penelitian menunjukkan bahwa rata-rata kejadian penyakit di gudang penyimpanan kedelai paling tinggi terdapat pada sampel biji kedelai dari G.Tfrb sebesar 1.88%, diikuti G.Jbla, G.Tfra, G.Tbsi berturut-turut 1.44%, 1,41%, dan 1.24%. Hasil penelitian menunjukkan bahwa terdapat cendawan *P. manshurica* di 4 gudang penyimpanan kedelai Kota Ternate dengan persentase viabilitas G.Tbsi 25.8%, G.Jbla 28.8%, G.Tfra 27.4%, dan G.Tfrb 26.5%. Hal ini menunjukkan adanya potensi penyebaran penyakit ke pertanaman kedelai di Kota Ternate, sehingga perlu adanya tindakan pencegahan yang tepat untuk mengatasi masalah ini.

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INTRODUCTION

Soybean (Glycine max (L) Merril) holds a significant position in the food industry of Indonesia owing to its exceptional nutritional value (Childs et al., 2018). It is extensively employed as a fundamental ingredient for manufacturing processed food products such as soy sauce, tofu, tempeh, and milk (Hu et al., 2020) and also has therapeutic applications (Bilir et al., 2017). Despite the national demand for soybean being 1.7 million tons per annum, the domestic production amounts to only 424.19 thousand tons annually (Setyawan & Huda, 2022). The present domestic soybean production is merely capable of meeting 24% of the national demand, which has resulted in the implementation of import policies by the government (Destasari et al., 2015). The import volume of soybean in the last five years has totaled 6.88 million tons per year, with the United States being the highest importer of 2.5 million tons annually (Kementerian Pertanian, 2020).

In North Maluku Province, the yearly consumption of soybean in the form of tofu, tempeh, and soy sauce is estimated at 1.96 kg per capita (BPS, 2022). Meanwhile, in Ternate city, entrepreneurs producing tofu and tempeh require 600-1,000 kg of soybean daily to meet the demand of the local community. Most of the soybeans used as raw materials for the production of tofu and tempeh are imported through Surabaya, which serves as the entry point, and then transported between regions to Ternate City via the Ahmad Yani Port. According to data from the Ternate Class II Agriculture Quarantine Center in 2022, the total amount of soybean imported to Ternate City through the Ahmad Yani Port in 2021 was 278,700 kg. Subsequently, the soybeans are transported by trucks or open-back vehicles to storage warehouses belonging to the tofu and tempeh processing entrepreneurs in Ternate City.

The importation of soybeans from the United States carries the risk of bringing Plant Pests Organisms (PPOs), including *Peronospora manshurica* which causes downy mildew disease (Lim, 2022). This disease is widely spread in soybean production centers worldwide with a damage rate of 8-14% (Silva et al., 2016). *Peronospora manshurica* is one of the fungi that can easily be carried through the air over long distances with a very high sporulation rate (Hayati et al., 2022). The intensity of *P. manshurica* attack reaches 30.28% in East Java, making it a disease with important status (Susanti et al., 2021). Based on the Ministry of Agriculture Regulation No. 25 of 2020 concerning Types of Plant Quarantine Pests Organisms, *P. manshurica*'s distribution is in West Java and East Java, while North Maluku Province is still free from it.

The presence of *P. manshurica* in West Java and East Java indicates that this fungus can grow in the tropical climate of Indonesia. Therefore, research is needed to detect the presence and growth potential of *P. manshurica* in several storage warehouses for imported soybeans, including those in Ternate City which have not been previously studied. This study aims to detect the presence, viability, and growth of *P. manshurica* oospores in the storage warehouses of imported soybeans in Ternate City. The results of this study will serve as a basis for anticipating the risk of the entry and spread of *P. manshurica* fungus and avoiding the infection of soybean crops in North Maluku Province.

MATERIALS & METHODS

In this study, both qualitative and quantitative research methods were employed. The qualitative method was utilized to detect the existence and proliferation of *Peronospora manshurica* oospores through an exploratory approach. Meanwhile, a Randomized Complete Block Design (RCBD) was employed for the quantitative method, which consisted of four storage warehouse (G) treatments: Treatment 1 (G. Tbsi), Treatment 2 (G. Jbla), Treatment 3 (G. Tfra), and Treatment 4 (G. Tfrb). All the treatments were storage warehouses located in Ternate City and were assessed for their technical suitability as agricultural storage facilities based on the Minister of Agriculture Regulation No. 73 of 2012. The regulation stipulates that the floor construction must be made of cement hardening, ceramics, or tiles, the warehouse must have good ventilation and adequate lighting, and must be free of flooding and standing water. Based on these criteria, the soybean storage warehouses in Ternate City were classified according to their conditions as presented in the Table 1.

The soybean seeds used in this study are imported soybean seeds from the United States (varieties). Meanwhile, the chemicals used are analytical grade from Merck, Germany. The Mikro 185 centrifuge (Hettich, Germany) was used to separate particles in the solution, and the IKA MS1 mini shaker (IKA Works Inc, USA) was used to homogenize the solution.

Meanwhile, the Nikon SMZ745T trinocular stereo microscope was used for low magnification observation of relatively large objects, and the Olympus CX33 trinocular compound microscope was used for small specimen observation and high magnification. In addition, the Marienfeld Superior Germany hemocytometer was also used to count the number of spores, the Beurer HM16 thermohygrometer was used to measure air humidity and temperature, the Mastech MS6612 luxmeter was used to measure light intensity, and the General MMG608 grain moisture meter was used to measure soybean moisture content.

Table 1. The criteria for the suitability of soybean storage warehouses in Ternate City.

Warehouses code	Parameter				Suitability criteria
	Cement/tile/ceramic flooring	Ventilation	Sufficient lighting	Free of standing water	
G. Tbsi	+	+	+	+	Very good
G. Jbla	+	+	-	+	Good
G. Tfra	+	-	-	+	Less good
G. Tfrb	+	-	-	-	Not good

The research was conducted from December 2021 to June 2022. The research procedure was carried out in three stages. The first stage involved sampling, which was done by taking ± 1,000 soybean seeds per sample point diagonally using a stainless steel seed trier (Pathak et al., 1978; Zhang & Scarcelli, 2021; Hayati et al., 2022). Five sample points were taken diagonally for each treatment. The second stage involved measuring the moisture content of soybeans and the environmental parameters of the storage warehouse (temperature, humidity, and light intensity) which were measured at a uniform time and condition. The third stage involved sample testing, which was conducted using the method recommended by the International Seed Testing Association/ISTA. The viable spore count was determined by taking 0.1 mg of oospore crust among 1,000 symptomatic soybean seeds, which was then dissolved in 0.1 ml of distilled water. The sample testing was carried out in the Laboratory of the Class II Agricultural Quarantine Center in Ternate. The observation variables in this study were the disease occurrence (DO), the percentage of *P. manshurica* oospore viability, and the growth of *P. manshurica* oospores.

The occurrence of disease refers to the presence of the fungus *P. manshurica*, which is indicated by the appearance of white crust on the surface of soybean seeds (Suryani & Cahyanto, 2022). The number of symptomatic seeds was calculated to determine the disease occurrence in soybean storage facilities in accordance with equation (1) (Sutarman et al., 2020). Meanwhile, the percentage of viable *P. manshurica* oospores was calculated using equation (2) (Sutarman et al., 2020), and observations of *P. manshurica* oospore growth were carried out using the blotter test method, as indicated by the appearance of hyphae. Data analysis was performed using Analysis of Variance (ANOVA) with Statistical Analysis System (SAS) software version 9.1.3 and followed by Tukey's test at a significance level of 5%. Regression analysis was also conducted to determine the relationship between warehouse feasibility criteria and disease occurrence in this study.

$$DO = \frac{n}{N} \times 100\% \quad (1)$$

where:
DO = percentage of disease occurrence (based on the symptoms)
n = the number of samples showing symptoms of the disease
N = the number of total samples

$$V = \frac{g}{(g+u)} \times 100\% \quad (2)$$

Notes:
V = percentage of oospore viability
g = the number of viable oospores
u = the number of nonviable oospores

RESULTS & DISCUSSIONS

Disease occurance of P. manshurica

The presence of *P. manshurica* fungus is indicated by the occurrence of oospores in the form of white-grayish crusts on the surface of soybean seeds (Hayati et al., 2022). The number of soybean seeds that have crusts at each sampling point is counted, resulting in the percentage of disease occurance as shown in Table 2.

Table 2. Average occurance of *P. manshurica* disease

Warehouses code	Avarage (%)
G. Tbsi	1.24 b
G. Jbla	1.44 ab
G. Tfra	1.41 ab
G. Tfrb	1.88 a

[a]The numbers in the same column followed by the same letter indicate no significant difference based on the Tukey post hoc test at a significance level of α=5%.

The research results showed that the highest average occurance of disease in soybean storage warehouses was found in samples from the Tfrb warehouse at 1.88%, followed by Jbla, Tfra, and Tbsi warehouses, 1.44%, 1.41%, and 1.24%, respectively. Statistical analysis results showed that the occurance of disease in soybean samples from the Tbsi warehouse differed significantly from the Tfrb warehouse but did not differ significantly from the Jbla and Tfra warehouses. Furthermore, the regression analysis results showed that the coefficient of determination (R-square) for the dependent variable, the occurance of disease, was 0.6524, indicating that the warehouse as a storage place had a moderate influence of 65.24% on the occurance of disease. According to Chin et al. (1998), an R-square value is categorized as moderate if it is greater than 0.33, strong if it is greater than 0.67, and weak if it is greater than 0.19 but less than 0.33.

The disease symptoms observed using a stereo microscope showed that at all test points, imported soybean seeds with white or dull-colored mold were found. The presence of white mold on the surface of imported soybean seeds is consistent with the findings of Suryani & Cahyanto (2022) when examining imported soybean seeds. The white mold on imported soybean seeds indicates the presence of the *P. manshurica* oospore mass, as presented in Figures 1 and 2.

Figure 1. *P. manshurica* (white crust) attached to the surface of imported soybean seeds in Ternate City

The calculation of disease occurance shows a significant difference between storage warehouses. This indicates that the warehouse condition affects the occurance of disease. The measurement results of environmental parameters in import soybean storage warehouses are presented in Table 3. Based on Table 3, it can be seen that the highest soybean moisture content, which is 14.86, and the highest air humidity (%) of 69.8% are found in Warehouse G.Tfrb, which correlates positively with the highest occurance of disease in Warehouse Tfrb at 1.88%. Fungi thrive in moist environments. *P. manshurica* grows optimally in high humidity and low temperature at 20-22 °C (Dong et al., 2018). However, fungi can adapt and survive in dry environmental conditions, so in a study (Silva et al., 2016), *P. manshurica* spores were found to contaminate other seeds in the same sample plot. The results of Sudjud et al.'s research (2013) showed that independent variables (temperature, humidity, and spore count) have a significant influence on the occurance of fruit rot disease in cocoa, while dependent variables (disease occurance) vary in size. Furthermore, according to Sudjud et al. (2020), plants with the same genetic relationships tend to have the same resistance to disease occurance.

Table 3. The results of measuring the average moisture content of soybeans and other environmental parameters

Warehouses Code	Moisture content of soybeans in the warehouse (%)	Room temperature (°C)	Humidity (%)	Light intensity (lux)
G. Tbsi	13.44	31.2	62.4	45.53-104.04
G. Jbla	14.58	31.0	66.2	1.08-3.96
G. Tfra	14.78	31.2	62.8	1.99-3.93
G. Tfrb	14.86	31.4	69.8	0.23-1.15

The temperature in the storage warehouses ranged from 31°C to 31.4°C. According to Suryani & Cahyanto (2022), the optimum temperature for fungal growth varies among different species, but generally, fungi can grow at temperatures between 0°C and 35°C, with the best temperature range being 20-30°C. In addition, Suryani & Cahyanto (2022) suggested that light intensity can either stimulate or inhibit the formation of reproductive structures and spores, oospores, in fungi. The relatively good light intensity for fungal reproduction is around 380-720 lux. High light intensity can inhibit the growth of fungal populations, as it can hinder the formation of reproductive structures and the production of spores or oospores. The results of the study showed that the light intensity in the storage warehouses varied significantly, ranging from 0.23 to 104.4 lux. Light can also be a factor that stimulates or inhibits the formation of reproductive structures and spores, oospores, in fungi (Suryani & Cahyanto, 2022).

The occurance of disease in the Tbsi warehouse was the lowest and significantly different from the Tfrb warehouse due to the sufficient ventilation in the Tbsi warehouse, which allows for good air circulation and lower air humidity compared to other warehouses. The warehouse environment and good sanitation conditions will affect the quality of soybean seeds. This can be seen from the moisture content of soybean seeds in the lowest Tbsi warehouse, which was 13.44%, while it reached 14.86% in the Tfrb warehouse. The occurance of disease in the four soybean storage warehouses in Ternate City was 1.24-1.88%. According to research findings from Pathak et al. (1978) and Dong et al. (2018) on the percentage of disease occurance based on symptoms in imported soybean seeds from 17 countries entering India, the occurance rate of disease was 1.8-6.9%.

Detection of Oospora P. manshurica

The detection of *P. manshurica* oospores was performed through direct observation of the crust on the surface of soybean seeds. This was confirmed by direct observation under an Olympus CX33 trinocular compound microscope of white crust scrapings from the surface of soybean seeds, as shown in Figure 2. The same image was also shown in another study (Agarwal et al., 2006) which found that oospora *P. manshurica* is round-shaped with thick cell walls. The oospore formed is the result of the fusion of anteridium and oogonium in the sexual cycle of *P. manshurica* (Tambunan, 2018). Oospore is a resting spore with a thick cell wall that can survive on beans in dry or less favorable conditions (Pinaria & Assa, 2017). Fungi need a moist environment to germinate, grow, and reproduce. However, the fungus *P. manshurica* can survive in dry conditions by forming oospores or resting spores.

The dry conditions of storage warehouses cause *P. manshurica* to survive in the form of resting spores or oospores on the surface of soybeans, which appear as a white crust. Research (Dunleavy, 1971) revealed that the white crust was found on the surface of stored soybeans in storage warehouses in the United States. According to Dunleavy (1971), the white crust is a mass of *P. manshurica* oospores. The observation results showed the presence of round-shaped oospores with thick cell walls in *P. manshurica*. The diameter of the oospores found in each warehouse can be seen in Table 4. The research results showed that the diameter of *P. manshurica* oospores in soybean samples stored in the Tbsi warehouse ranged from 20.03 to 35.82 μm, in the Jbla warehouse ranged from 27.87 to 46.86 μm, in the Tfra warehouse ranged from 27.80 to 43.04 μm, and in the Tfrb warehouse ranged from 28.69 to 42.88 μm. The difference in oospore diameter is influenced by the health and maturity level of the oospores (Müller et al., 2019). The characteristics of *P. manshurica* oospores found in the four imported soybean storage warehouses in Ternate City are uniseptate, spherical in shape, with a diameter ranging from 20.03 μm to 46.86 μm (Table 4), and hyaline in color. These characteristics of *P. manshurica* oospores are similar to the findings of Lin et al. (2022), which are uniseptate, spherical in shape, with a spore diameter ranging from 32 to 42.5 μm, a thick or double-layered wall, and a hyaline to light brown color. Oospores have a thick wall, allowing them to survive in dry conditions during soybean maturation and storage in warehouses. The outer surface of the oospores is irregular due to the attachment of hyphae, remnants of antheridia or oogonia (Silva et al., 2016; Müller et al., 2019).

Table 4. Diameter of oospora *P. manshurica*

Warehouses code	Diameter of oospora
G. Tbsi	20.03 – 35.82 μm
G. Jbla	27.87 – 46.86 μm
G. Tfra	27.80 – 43.04 μm
G. Tfrb	28.69 – 42.88 μm

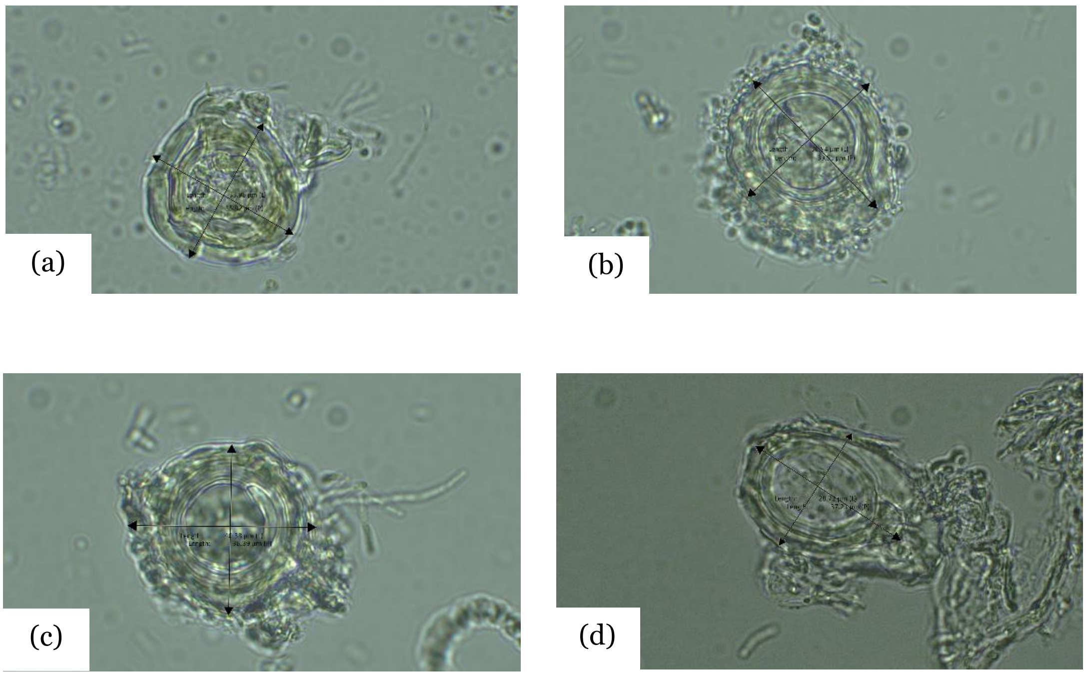

Figure 2. Oospora *P. manshurica* (magnification 1,000x) on imported soybean seeds: (a) G.Tbsi; (b) G. Jbla; (c) G.Tfra; (d) G.Tfrb, respectively.

Viability test of oospora P. manshurica

The spore viability indicates the ability or the vitality of the spores to grow normally under optimum conditions. In this study, the viability of *P. manshurica* oospores in soybean storage warehouses can be seen in Table 5. The research findings indicate that the highest average viability of oospores in soybean storage warehouses was found in the Jbla warehouse at 28.8%, followed by the Tfra warehouse at 27.4%, the Tfrb warehouse at 26.5%, and the Tbsi warehouse at 25.8%. Statistical analysis shows that the viability of oospores in soybean samples from all warehouses did not differ significantly. Viable oospores are indicated by a change in the color of the cytoplasm to orange. In this study, the cytoplasm of some oospores changed color to yellow, while others remained hyaline. This is due to the reduction process of the TTC reagent by dehydrogenase enzyme produced by living cells, which produces a red or pink-colored triphenyl formazan (Rajkumari et al., 2019).

Table 5. Viability of oospora *P. Manshurica* in soybean storage warehouse.

Warehouses code	Viability of oospora (%)
G. Tbsi	25.8
G. Jbla	28.8
G. Tfra	27.4
G. Tfrb	26.5

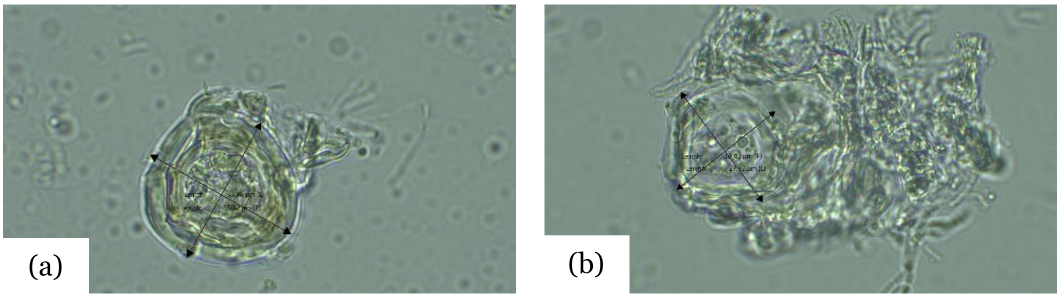

Figure 3. Oospora *P. manshurica* (magnification 1,000x) on imported soybean seeds: (a) cytoplasm changes color to yellow; (b) cytoplasm remains hyaline.

Based on the technical eligibility requirements for buildings used as storage facilities for agricultural commodities as stipulated in the Minister of Agriculture Regulation No. 73 of 2012, it was found that the eligibility criteria for warehouses did not significantly affect spore viability. This means that spore viability is more influenced by other factors, such as imported soybean seeds that have already been infected with *P. manshurica* before entering the storage facility and still able to survive. Under dry storage conditions, pathogens can survive in the form of resting spores or oospores, which can then germinate when the environment becomes favorable, becoming a source of inoculum that causes disease spread (Roongruangsree et al., 1988). The viability of this fungus's oospores must be watched out for because they can germinate and grow rapidly when they encounter a suitable living environment. *P. manshurica* is a seed-borne pathogen, which means that the fungus that has infected the embryo will then be carried when the soybean seeds are planted. Oospores of *P. manshurica* are also easily carried by the wind and can pose a threat to soybean cultivation if infected seeds are used as a seed source for planting (Susanti et al., 2021).

The growth of *P. manshurica* oospores can be observed based on the hyphae growth on five soybean seeds placed on a petri dish with wet filter paper and incubated for seven days. The results of the Blotter Test method showed that oospores from soybean seeds taken from Tbsi warehouse, Tfra warehouse, and Tfrb warehouse could survive and grow, as indicated by the appearance of hyphae (Figure 4). Meanwhile, in the Jbla warehouse, no signs of *P. manshurica* oospore growth were found, although *P. manshurica* oospores were observed in the warehouse. This is because the oospores in the Jbla warehouse were damaged or unhealthy. Unhealthy oospores will have difficulty growing again, even if the environmental conditions support their growth and development. Roongruangsree et al. (1988) stated that oospores with abnormal appearance, indicated by irregular inner walls and irregular cytoplasm content, are characteristics of oospores that are damaged or dead.

Oospora *P. manshurica* is a resting spore that serves as a survival structure in dry or unfavorable environments. This spore will become active again if the environmental conditions are supportive (Silva et al., 2016). The incubation conditions on a petri dish with moist filter paper provide a humid environment that triggers the reactivation of the cells that cause the oospore to germinate. In this study, the oospores were grown together with imported soybean seeds because *P. manshurica* is an obligate parasitic fungus with a specific host and can only grow in living organisms and cannot be cultured on artificial media. The appearance of senescent hyphae that dichotomize is an indication that the oospores of the *P. manshurica* fungus can become active again and resume their life activities (Figure 4). Hyphae are fungal structures that function in the absorption of nutrients from the environment and will later produce reproductive organs in their life cycle. Hyphae can emerge from the inner layer of germinating oospores or sclerenchyma mycelium that becomes active again (Dong et al., 2018; Müller et al., 2019). Furthermore, Thakur (2016) found thick-walled mycelia composed of sclerenchyma cells that can survive along with oospores in the form of crusts on the surface of soybean seeds in storage. On the other hand, thin-walled mycelia can also survive in the skin tissue of soybean seeds, especially in the spongy layer located between palisade and parenchyma tissues.

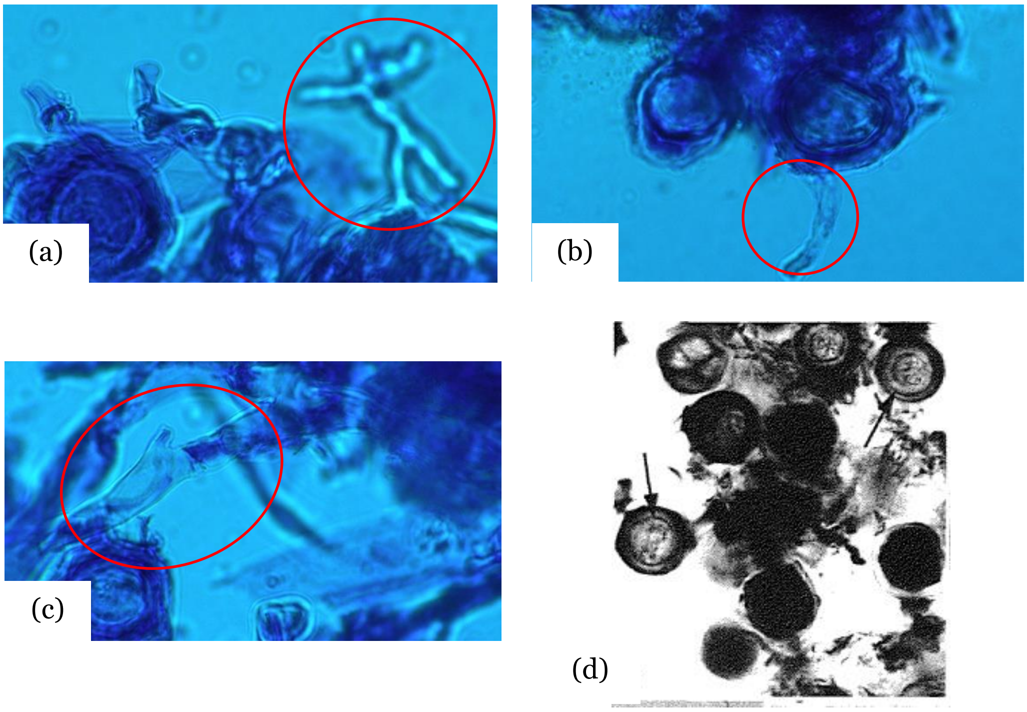

Figure 4. Growth of senescent hyphae with the appearance of *P. manshurica* on imported soybean seed samples (magnification 1,000x, methylene blue staining) in: (a) G.Tbsi, (b) G. Tfra, (c) G. Tfrb, (d) oospores and hyphae of P. manshurica according to Roongruangsree et al. (1988), Copyright (1988) John Wiley and Sons.

The emergence of hyphae indicates the potential of *P. manshurica* spores to survive, grow, and spread in plantations. *P. manshurica* has the potential to live and thrive in Ternate City due to the suitability of its abiotic environment and the adaptive characteristics of this fungus to climatic conditions. *P. manshurica* thrives well in high humidity of 80-90% and a temperature range of 20-24°C (Dong et al., 2018). Sporulation occurs at temperatures between 20-30°C. Based on BMKG data (2022) and Umasugi et al. (2021) research, the average humidity in Ternate City is around 85.03-97.42% with an average temperature ranging between 27.42-32.83°C. The climatic conditions in Ternate City enable the sporulation of *P. manshurica*. This is supported by the distribution map of P. manshurica, which is widely distributed in many tropical and subtropical countries.

CONCLUSION

The conclusion of this study is that the average occurance of *P. manshurica* disease in soybean storage warehouses is 1.24-1.88%, with soybean moisture content of 13.44-14.86%, air temperature of 31-31.4°C, and humidity of 62.4-69.8% found in four imported soybean storage warehouses in Ternate. The viability of oospores is 25.8%-28.8%. The diameter of *P. manshurica* oospores in soybean samples from the four storage warehouses in Ternate ranges from 20.03 µm - 46.86 µm. Oospores on soybeans taken from the Tbsi, Tfra, and Tfrb warehouses were able to live and grow, indicated by the emergence of hyphae, while in the Jbla warehouse, *P. manshurica* oospores did not grow due to damaged oospores.

REFERENCES

Agarwal, P. C., Singh, B., Dev, U., Rani, I., Chand, D., & Khetarpal, R. K. 2006. Interception of *Peronospora manshurica* in soybean germplasm imported during 1976–2005. *Current Science*, *91*(3) 347-350.

Bilir, B., Sharma, N. V., Lee, J., Hammarstrom, B., Svindland, A., Kucuk, O., & Moreno, C. S. (2017). Effects of genistein supplementation on genome-wide DNA methylation and gene expression in patients with localized prostate cancer. *International Journal of Oncology*, *51*(1), 223–234. https://doi.org/10.3892/ijo.2017.4017

BMKG. (2022). Data iklim di maluku utara 2021. UpTodate. Dikutip 28 Maret 2023, from https://bbmkg4.com/public/dokumen/pdf/Profil__Artikel__2021_12_30_11_47_25_BUKU_TREN_HUJAN_MALUKU_DAN_MALUKU_UTARA.pdf

BPS. (2022). Data produksi kedelai: Maluku Utara dalam angka. In *Badan Pusat Statistik Provinsi Maluku Utara*. UpTodate. Dikutip 28 Maret 2023, from https://malut.bps.go.id/publication/2022/02/25/9ed6001bfafc78be63d71fdf/provinsi-maluku-utara-dalam-angka-2022.html

Childs, S. P., Buck, J. W., & Li, Z. (2018). Breeding soybeans with resistance to soybean rust (Phakopsora pachyrhizi). *Plant Breeding*, *137*(3), 250–261. https://doi.org/10.1111/pbr.12595

Chin, W. W., Chinn, W. W., & Chin, W. W. (1998). *The partial least squares approach to structural equation modelling. In Marcoulides G. A.* (1st Ed.). In Modern Methods for Business Research. https://doi.org/10.4324/9781410604385

Destasari, A. N., Suharyono, & Yulianto, E. (2015). Pengaruh produksi kedelai dalam negeri dan harga kedelai dunia terhadap volume impor kedelai di Indonesia (studi terhadap volume impor kedelai tahun 1996-2013). *Jurnal Administrasi Bisnis (JAB)*, *1*(1), 1–8. https://media.neliti.com/media/publications/85458-ID-pengaruh-produksi-kedelai-dalam-negeri-d.pdf

Dong, H., Shi, S., Zhang, C., Zhu, S., Li, M., Tan, J., Yu, Y., Lin, L., Jia, S., Wang, X., Wu, Y., & Liu, Y. (2018). Transcriptomic analysis of genes in soybean in response to *peronospora manshurica* infection. *BMC Genomics*, *19*(1), 1–13. https://doi.org/10.1186/s12864-018-4741-7

Dunleavy, J. M. 1971. Races of *peronospora manshurica* in the united states. *American Journal of Botany*, *58*(3), 209-211. https://doi.org/10.1002/j.1537-2197.1971.tb09964.x

Hayati, I., Susanti, A. A., Marwan, H., & Mapegau, M. (2022). Uji viabilitas cendawan *peronospora manshurica* pada biji kedelai impor penyakit bulai (downy mildew). *Jurnal Ilmiah Ilmu Terapan Universitas Jambi JIITUJ*, *6*(1), 23-31. https://online-journal.unja.ac.id/JIITUJ/article/view/19328

Hu, C., Wong, W. T., Wu, R., & Lai, W. F. (2020). Biochemistry and use of soybean isoflavones in functional food development. *Critical Reviews in Food Science and Nutrition*, *60*(12), 2098–2112. https://doi.org/10.1080/10408398.2019.1630598

Kementerian Pertanian. (2020). Outlook Kedelai: Komoditas Pertanian Subsektor Tanaman Pangan. Pusdatin Kementan. https://satudata.pertanian.go.id/assets/docs/publikasi/OUTLOOK_KEDELAI_2020.pdf

Lim, S. M. (2022). *Epidemiology of soybean downy mildew* (Taylorfrancis.com (ed.); 1st Edition). CRC Press.

Lin, F., Satish, S., Caio, C., Vieira, C., Paulo, M., Silva, D., Rojas, A., Lee, D., Liu, N., Mariano, E., Lee, P. Y., Dong, Z., Baldin, J., Leonardo, P., Ploper, D., Rupe, J., Chen, P., Wang, D., Nguyen, H. T., Lee, D. (2022). Breeding for disease resistance in soybean : a global perspective. In *Theoretical and Applied Genetics*, *135*, 3773-3872. https://doi.org/10.1007/s00122-022-04101-3

Müller, M. A., Klosowski, A. C., Pria, M. D., Mazaro, S. M., & Mio, L. L. M. De. (2019). Bacillus thuringiensis combined with fungicide applications in the management of soybean leaf diseases. *Journal of Agricultural Science*, *11*(13), 226. https://doi.org/10.5539/jas.v11n13p226

Pathak, V. K., Mathur, S. B., & Nergaard, P. (1978). Detection of *peronospora manshurica* (Naum.) syd. in seeds of soybean, glycine max. *EPPP Bulletin*, *1*, 1032. https://doi.org/https://doi.org/10.1111/j.1365-2338.1978.tb02144.x

Pinaria, A. G., & Assa, B. H. (2017). Jamur patogen tanaman terbawa tanah. In *Media Nusa Creative : Malang*. https://www.scribd.com/document/485242418/buku-Jamur-Patogen-Terbawa-Tanah-pdf

Rajkumari, J., Magdalane, C. M., Siddhardha, B., Madhavan, J., Ramalingam, G., Al-Dhabi, N. A., Arasu, M. V., Ghilan, A. K. M., Duraipandiayan, V., & Kaviyarasu, K. (2019). Synthesis of titanium oxide nanoparticles using Aloe barbadensis mill and evaluation of its antibiofilm potential against pseudomonas aeruginosa PAO1. *Journal of*

Photochemistry and Photobiology B: Biology, *201*, 111667. https://doi.org/10.1016/j.jphotobiol.2019.111667.

Roongruangsree, U. T., Kjerulf-Jensen, C., Olson, L. W., & Lange, L. 1988. Viability tests for thick walled fungal spores (ex: oospores of *Peronospora manshurica). Journal of Phytopathology*, *123*(3), 244-252. http://dx.doi.org/10.1111/j.1439-0434.1988.tb04474.x

Setyawan, G., & Huda, S. (2022). Analisis pengaruh produksi kedelai, konsumsi kedelai, pendapatan per kapita, dan kurs terhadap impor kedelai di Indonesia. *Jurnal Ekonomi Dan Manajemen*, *19*(2), 215–225. https://doi.org/10.29264/jkin.v19i2.10949

Silva, O. C., Santos, H. A. A., Pria, M. D., & de Mio, L. L. M. (2016). Damage to soybean caused by downy mildew. *Ciencia Rural*, *46*(3), 389–392. https://doi.org/10.1590/0103-8478cr20150093.

Sudjud, S., Mahmud, S. A., & Djafar, C. A. (2020). Exploration of morphological diversity of cacao plant (Theobroma cacao L.) in Bacan Island. *Advances in Engineering Research*, *194*, 89–93. https://doi.org/10.2991/aer.k.200325.018

Sudjud, S., Sastrahidayat, I. R., Mudjiono, G., & Muhibuddin, A. (2013). The intensity distribution of cacao pod rot disease (Phytophthora palmivora Butl) in smallholder plantation in North Maluku Indonesia. *Journal of Biology, Agriculture and Healthcare*, *3*(7), 131–139. https://core.ac.uk/download/pdf/234658911.pdf

Suryani, Y. & Cahyanto, T. (2022). *Pengantar jamur makroskopis*. In Gunung Djati. http://digilib.uinsgd.ac.id/49533/1/Buku%20Pengantar%20Jamur%20Makroskopis.pdf

Susanti, A. A., Marlina, M., Marwan, H., & Mapegau, M. (2021). Uji patogenesitas oospora *peronospora manshurica* asal kedelai impor pada tiga kultivar kedelai. *Jurnal Media Pertanian*, *6*(1), 23. https://doi.org/10.33087/jagro.v6i1.112

Sutarman, Prihatiningrum, A. E., & Miftakhurrohmat, A. (2020). *Pengelolaan penyakit tanaman terpadu*. Umsida Press. https://press.umsida.ac.id/index.php/umsidapress/article/view/978-623-6833-08-7

Tambunan, J. T. K. (2018). Uji toksisitas ekstrak daun sirih hijau (piper battle L) terhadap sclerotium rolfsii penyebab rebah kecambah pada tanaman Kedelai. *In Fakultas Pertanian Universitas Brawijaya, Malang,* 1(1). http://repository.ub.ac.id/id/eprint/13048/

Thakur, A. (2016). Evaluation of biological control strategies against a range of plant pathogens. *Laboratory of plant* pathology. http://dspace.aua.gr/xmlui/bitstream/handle/10329/6508/Thankur_A.pdf?sequence=5

Umasugi, B., Prijono, S., Soemarno, & Ariffin. (2021). Soil moisture analysis and rainwater management at clove plantation to meet the water requirement of clove plants during dry season. *International Journal on Advanced Science, Engineering and Information Technology*, *11*(6), 2197–2203. https://doi.org/10.18517/ijaseit.11.6.15030

Zhang, J., & Scarcelli, G. (2021). Mapping mechanical properties of biological materials via an add-on Brillouin module to confocal microscopes. *Nat Protoc*, *16*(2), 1251–1275. https://doi.org/10.1038/s41596-020-00457-2.Mapping

