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Viability and Virulence of *Fusarium oxysporum* f. sp. *zingiberi* Isolates from Boyolali and Temanggung Preserved for 17 Years in Sterile Soils

Viabilitas dan Virulensi *Fusarium oxysporum* f. sp. *zingiberi* Asal Boyolali dan Temanggung Setelah Disimpan Tujuh Belas Tahun di Dalam Tanah Steril

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ABSTRACT

Fusarium oxysporum f. sp. *zingiberi* is a soil-borne plant pathogen causing rhizome rot on ginger. This pathogen can survive in the soil for several years without a host plant. This study aimed to examine the viability and virulence of 21 isolates of *F. oxysporum* f. sp. *zingiberi* after being preserved for 17 years in sterile soil. Fungal viability was determined by descriptive method, while the experiment using randomized block design was conducted to examine the virulence of fungal isolates. The treatments consisted of control, 21 isolates of *F. oxysporum* f. sp. *zingiberi* from Boyolali and Temanggung, each treatment was replicated three times. The variables consisted of colony colors and diameters, macroconidia and microconidia shapes, growth time, dry weight of mycelia, conidia density, incubation period, affected area, rhizome wet weight difference, and waste index. The results showed that all fungal isolates which were stored in sterile soil for 17 years still had the ability to grow well on PDA medium and fill up petri dishes in 11–36 days. Moreover, all the isolates caused infection and disease symptoms development in ginger rhizome var. Gajah. Less virulence isolate was characterized by a long incubation period (6–12 days after inoculation) and smaller affected area of the rhizome.

Keywords: ginger, incubation period, macroconidia, microconidia, rhizome rot

ABSTRAK

Fusarium oxysporum f. sp *zingiberi* merupakan cendawan penyebab busuk rimpang pada jahe. Patogen ini dapat bertahan di tanah selama bertahun-tahun tanpa tanaman inang. Penelitian bertujuan untuk menguji viabilitas dan virulensi 21 galur *F. oxysporum* f. sp. *zingiberi* setelah disimpan tujuh belas tahun di dalam tanah steril. Variabel yang diamati adalah warna dan diameter koloni, bentuk makrokonidium dan mikrokonidium, waktu pertumbuhan, berat kering miselium, kepadatan konidium, masa inkubasi, luas serangan pada rimpang, selisih bobot basah rimpang, dan indeks sampah. Hasil penelitian menunjukkan bahwa semua galur *F. oxysporum* f. sp. *zingiberi* asal Temanggung dan Boyolali yang disimpan di tanah steril selama 17 tahun mampu tumbuh baik pada medium PDA dan mengisi penuh cawan petri antara 11–36 hari. Selain itu, semua galur menyebabkan gejala penyakit pada rimpang jahe var. Gajah. Isolat dengan tingkat virulensi yang rendah ditandai oleh masa inkubasi yang panjang (6–12 hari setelah inokulasi) dan luas area rimpang terserang yang terkecil.

Kata kunci: busuk rimpang, jahe, makrokonidium, masa inkubasi, mikrokonidium

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INTRODUCTION

Fusarium oxysporum f. sp. *zingiberi* is one of the causes of ginger rhizome rot in the world (Stirling 2004; Li *et al*. 2014), including in Indonesia (Soesanto *et al*. 2003; Wahyu *et al.* 2012). The distribution of rhizome rot disease in ginger has been mapped in eight regencies in Central Java Province; two of them are Boyolali and Temanggung Regencies (Soesanto *et al*. 2003). Rhizome rot disease decrease ginger production (Soesanto *et al*. 2005b), and cause yield loss from 50% to 90% (Acharya *et al.* 2016). The fungus *F. oxysporum* is difficult to control because it can form a resting or resistant structure, which can survive in the soil for up to ten years in the absence of a host plant (Agrios 2005). Therefore, it is necessary to observe how long the fungus can be stored with good viability and virulence, to preserve *F. oxysporum*.

Preservation of microbial isolates is generally associated with survival and growth ability on artificial media, such as liquid nitrogen for nine years (Dahmen *et al*. 1983), PDA at -70 °C for up to 13 years (Pasarell and McGinnis 1992), distilled water for 12 years (Qiangqiang *et al.* 1998; Diogo *et al.* 2005), silica gel (Perez-Garcia *et al*. 2006), and glycerol at 4 °C for 24 months (Paul *et al*. 2015). The preservation method of fungi should be adjusted, for examples in term of the number of collections and costs in order to meet the situation in developing countries that may have economic constraint.

A cheaper and easier alternative to fungal preservation that does not require expensive equipment is the use of sterile soil (Bakerspigel 1953). The storage of *Fusarium* sp. is rarely performed in sterile soil. However, Windels *et al*. (1993) explained that *Fusarium* sp. can be preserved in sterile soil for ten years. The preserved fungal should be retested to determine its viability and virulence. Previous research by Cahyaningrum *et al*. (2017) has revealed that *F. oxysporum* f. sp. *zingiberi* preserved for four years in sterile soil has the viability to grow on potato dextrose agar (PDA) and showed its virulence by causing symptoms in ginger rhizome when inoculated through the wound. In addition, the research by Riyadi *et al*. (2008) has discovered that *F. oxysporum* f. sp. *zingiberi* from Boyolali and Temanggung which was preserved in sterile soil for six years has good viability in PDA; however, the virulence of this fungus decreases which indicated by the longer incubation period on ginger rhizome. Based on the aforementioned statements, this research aims to examine the viability and virulence of 21 isolates of *F. oxysporum* f. sp. *zingiberi* from Boyolali and Temanggung that has been preserved in sterile soil for 17 years.

MATERIALS AND METHOD

Preparation of pathogen inoculum

F. oxysporum f. sp. *zingiberi* were isolated from rhizome rot symptoms on ginger plants from Boyolali and Temanggung Regencies (Soesanto *et al*. 2003) that had been stored in bottles filled with sterile soil for 17 years. Each of fungal isolates was taken around 10 g and spread directly on PDA in a separate Petri dish. Then, the fungus was incubated at room temperature. The growing *F. oxysporum* f. sp. *zingiberi* was purified in PDA enriched with streptomycin sulfate (0.2 g L^{-1}) (Iqbal *et al*. 2017). *F. oxysporum* f. sp. *zingiberi* from Temanggung consisted of 14 isolates; namely TKO1, TKO2, TKO3, TKO4, TKO5, TKO6, TKO7, TKO8, TKb, TPO1, TPO2, TPO3, TPO4, and TPO5. Meanwhile *F. oxysporum* f. sp *zingiberi* from Boyolali consisted of seven isolates; namely BAO1, BAO2, BAO4, BAO6, BAO7, BAO8, and BAC. This research employed a randomized block design with three replications and two samples for each replication. The variables observed included colony colors and diameters, macroconidia and microconidia shapes, growth time, mycelia dry weight, and conidia density.

Preparation of *F. oxysporum* f. sp. *zingiberi* conidia suspension

Three mycelial plugs (5 mm in diameter) of individual fungal colonies in PDA were prepared and placed into a 125 mL erlenmeyer

flask, containing 100 mL of potato dextrose broth (PDB) solution. The solution was shaken (Daiki Orbital Shaker) at a speed of 150 rpm for seven days at room temperature (Supriyanto 2020). The conidia density of the solution was calculated with a hemocytometer to the density of 10^7 conidia mL^{-1} before being used (Soares *et al*. 2017).

Preparation of ginger rhizome

The ginger rhizome var. Gajah was obtained from farmers. It was subsequently washed in running water with 1% Na-hypochlorite solution until the surface was cleaned and sterilized. The ginger rhizome was inoculated by *F. oxysporum* f. sp. *zingiberi* with a sterile preparatory needle at a depth of 1 mm for 30 punctures in an area of 25 mm^2. One drop of *F. oxysporum* f. sp. *zingiberi* suspension (density 10^7 conidia mL^{-1}) was dripped to the wound. The inoculation area was covered with a damp cotton swab. The ginger rhizome was placed in a plastic bag and then incubated for three weeks (Riyadi *et al*. 2008) at room temperature (± 25 °C) (Cahyaningrum *et al*. 2017). The variables observed at this stage were incubation periods, affected areas of the rhizome, the differences in the wet weight of the rhizome, and the index of waste. The affected area was measured using millimeter blocks with observation intervals of two days for three weeks. The waste index was calculated using a formula by Mattsson *et al.* (2018):

$$\text{Waste index} = \frac{\text{diseased tissue weight}}{\text{weight total of tissue}} \times 100\%$$

The data from the viability test were analyzed descriptively, while the data from the virulence test were analyzed using the F-test. Further analysis using DMRT at an error rate of 5% will be proceeded when the data showed significant differences.

RESULTS

The viability of *F. oxysporum* f. sp. *zingiberi* isolates

All fungal isolates preserved in sterile soil for17 years could grow well on PDA (Figure 1; 2). The fungal colonies on PDA shows various colony colors, including white, broken white, purplish-red, and reddish-orange. Macroconidia of *F. oxysporum* f. sp. *zingiberi* are generally long curved or crescent-shaped with blunt ends and have 1–4 septa; while its microconidia has an oval shape.

The growth of fungal viability was also diverse. All isolates could grow to fill the petri dish although with different time period, i.e. between 11 to 36 days. The fastest growth occurred in TKO4 isolate (11 days), while the slowest growth occurred in TKO7 isolate (36 days) (Table 1). The highest and lowest colony diameter after 11 days in PDA was found in TK04 and TP02, respectively (Figure 2).

Mycelial dry weight and conidia densities of fungal isolates was varied (Table 1). Significant differences on mycelial dry weight between isolates was evidenced, with the largest and the lowest values was found in BAO1 isolate from Boyolali (0.457 g) and TKO7 isolate from Temanggung (0.329 g), respectively. Similarly, conidia density among fungal isolates was also different significantly, with the highest and the lowest value was found in TPO3 isolate (5.285×10^6 conidium mL^{-1}) and BAC isolate (0.029×10^6 conidium mL^{-1}), respectively (Table 1).

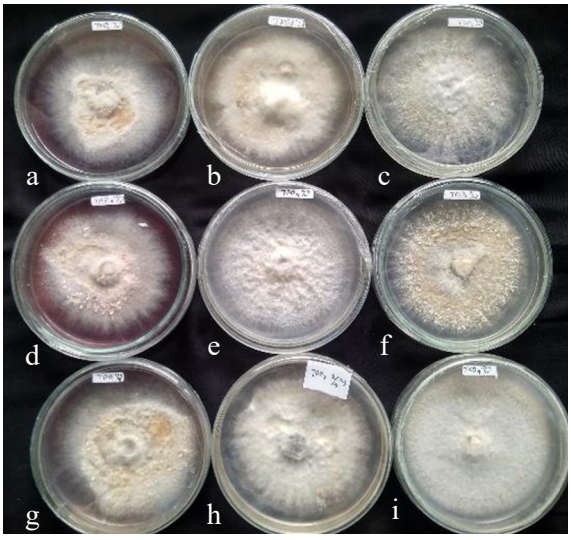

Figure 1 Colony color and shape of *Fusarium oxysporum* f. sp. *zingiberi* isolates. a, TKO1; b, TKO8; c, TKO7; d, TPO3; e, TPO4; f, TPO5; g, TPO1; h, TPO2; and i, TKO4.

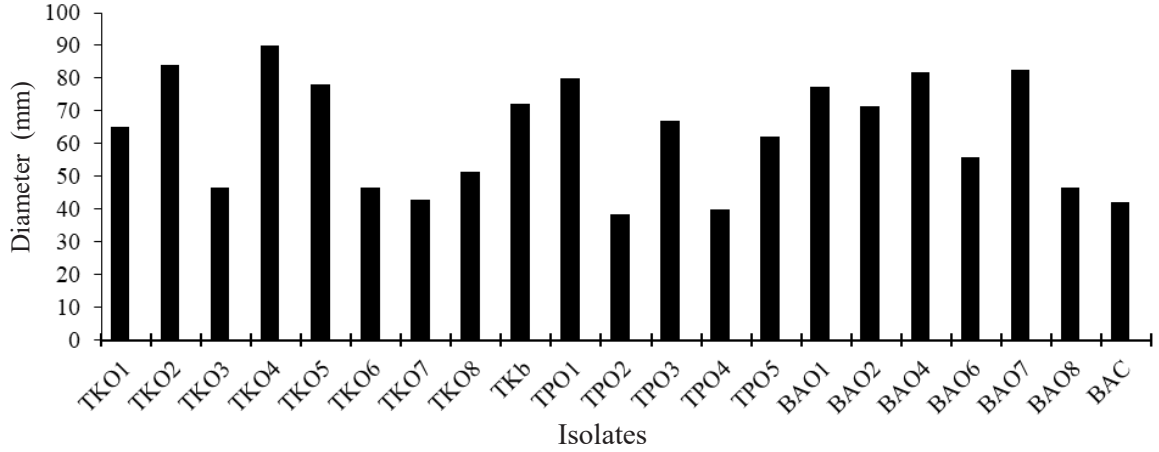

Figure 2 Colony diameter of *Fusarium oxysporum* f. sp. *zingiberi* isolates at 11 days on PDA.

Table 1 Viability of *Fusarium oxysporum* f. sp. *zingiberi* isolates preserved in sterile soil for 17 years

Isolates	Growth time (days)	Mycelia dry weight (g)	Conidia density (conidia mL^{-1})
TKO1	21 efg	0.387 ab	2.234×10^6 cd
TKO2	16 ghij	0.378 ab	2.233×10^6 cd
TKO3	29 bc	0.383 ab	2.864×10^6 c
TKO4	11 j	0.392 ab	2.470×10^6 c
TKO5	16 ghij	0.439 ab	2.218×10^6 cd
TKO6	22 def	0.424 ab	4.028×10^6 b
TKO7	36 a	0.329 ab	2.325×10^6 cd
TKO8	30 bc	0.411 ab	3.840×10^6 b
TKb	17 fghi	0.413 ab	1.289×10^6 ef
TPO1	20 efgh	0.396 ab	2.382×10^6 cd
TPO2	34 ab	0.359 ab	1.267×10^6 ef
TPO3	29 bc	0.395 ab	5.285×10^6 a
TPO4	23 de	0.393 ab	2.640×10^6 c
TPO5	27 cd	0.364 ab	2.525×10^6 c
BAO1	15 hij	0.457 a	0.573×10^6 fg
BAO2	17 fghi	0.448 ab	1.637×10^6 de
BAO4	16 ghij	0.445 ab	2.193×10^6 cd
BAO6	23 de	0.416 ab	1.113×10^6 ef
BAO7	13 ij	0.429 ab	0.285×10^6 g
BAO8	22 def	0.449 ab	2.399×10^6 cd
BAC	34 ab	0.395 b	0.029×10^6 g

Note: The numbers followed by the same letter in the same column show no significant difference at DMRT 5%.

The Virulence of *F. oxysporum* f. sp. *zingiberi*

F. oxysporum f. sp. *zingiberi* isolates preserved in sterile soil for 17 years was able to cause rhizome rot symptoms after being inoculated into the ginger rhizome. The visual symptoms of the diseased involved slight wrinkled, brown, and sunken rhizomes. The symptoms will become more severe showing wider and deeper wound, and dry rot (Figure 3).

The incubation period of fungal isolates was 6–12 days after inoculation (Table 2). The fastest incubation period was six days which occurred in TKO1, TKO2, TPO1, TPO2, BAO4, BAO6, and BAO8 isolates.

Meanwhile, the longest incubation period was 12 days or 44.42%, which occurred in TPO5 isolates; this number is longer than the control's number. The area of rhizome affected by fungal infection was varied among isolates and it was different significantly (Table 2). The largest and the lowest area affected was found in TKO7 isolate (86.95% wider than control treatment) and TPO5 isolate (73.27% smaller than control treatment), respectively. TPO5 isolate consistently showed the lowest virulence with a slower incubation period (12 days) and affected area of rhizomes. Similarly, there was significant differences of wet weight of rhizomes and waste index among isolates (Table 2). The largest and the lowest wet weight of rhizome was found in TKO4 isolate (80.02% wider than control treatment) and BAO2 isolate (24.09% smaller than control treatment), respectively. The highest and the lowest waste index was found in TKO2 isolate (125.69% wider than control

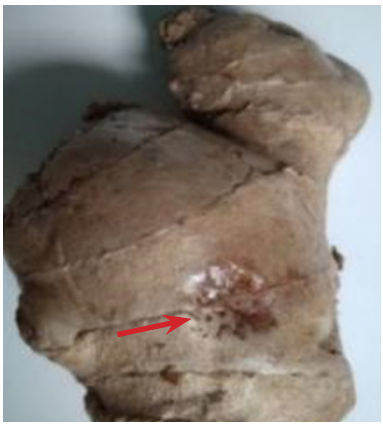

Figure 3 Ginger rhizome infected by *Fusarium oxysporum* f. sp. *zingiberi*. The symptoms involved slight wrinkled, brown, deep and wide sunken area and dry rot.

Table 2 Virulence of *Fusarium oxysporum* f. sp. *zingiberi* isolates preserved in sterile soil for 17 years

Isolates	Incubation period (dai)	Affected area (mm^2)	Rhizome wet weight (g)	Waste index (g)
KO (control)	6.67 b	18.00 d	1.66 b	0.06 e
TKO1	6.00 ab	99.00 abc	1.89 b	0.90 cde
TKO2	6.00 ab	97.67 abc	1.95 b	2.18 a
TKO3	10.00 a	96.33 abc	6.66 ab	1.41 abc
TKO4	6.67 ab	94.00 abc	8.31 a	1.90 ab
TKO5	10.00 a	70.67 cd	1.82 b	0.83 cde
TKO6	8.00 ab	116.33 abc	1.58 b	1.01 bcde
TKO7	6.67 ab	138.00 a	1.55 b	1.02 bcde
TKO8	7.33 ab	88.33 abc	2.26 ab	0.66 cde
TKb	6.67 ab	127.33 ab	3.25 ab	0.61 cde
TPO1	6.00 ab	88.67 abc	4.38 ab	0.73 cde
TPO2	6.00 ab	93.00 abc	1.67 b	1.11 bcd
TPO3	6.67 ab	89.00 abc	3.07 ab	1.00 bcde
TPO4	6.67 ab	85.00 abc	2.08 b	0.83 cde
TPO5	12.00 a	67.33 cd	1.46 b	0.33 cde
BAO1	8.67 ab	69.33 cd	2.92 ab	0.62 cde
BAO2	8.00 ab	78.67 bc	1.26 b	1.07 bcde
BAO4	6.00 ab	108.00 abc	3.67 ab	1.33 abcd
BAO6	6.00 ab	118.67 ab	2.66 ab	0.62 cde
BAO7	6.67 ab	97.00 abc	1.94 b	0.63 cde
BAO8	6.00 ab	126.00 ab	2.06 b	0.99 bcde
BAC	7.33 ab	73.33 bc	1.37 b	1.14 bcd

Note: The numbers followed by the same letter in the same column show no significant difference at DMRT 5%. Incubation period data were transformed to $\sqrt{(x+0.5)}$.

treatment) and TPO5 isolate (81.81% smaller than control treatment), respectively.

DISCUSSION

F. oxysporum f. sp. *zingiberi* preserved in sterile soil for 17 years still has good viability. However, its growth tends to be slower than the same fungal isolates preserved in sterile soil for six years (Riyadi *et al.* 2008). Senanayake *et al.* (2020) stated that the preservation of fungi in sterile soil for a long time can change the physiology, morphology, and variations of fungus. These changes require a long adaptation time when the fungus is grown on new media. The colony of fungal isolates developed different colors. Similar result was reported by Mekuria and Alemu (2020) whom found white colony of *Fusarium* on PDA and later on the color changed into beige or pale yellow, or in a certain circumstance into slightly purplish pink. Likewise, Soesanto *et al.* (2003) reported that the *Fusarium* isolates originating from Temanggung and Boyolali have various colony forms, such as concentric, non-concentric, thick, rather thick, thin, aerial, starchy, and fingered forms. Hafizi *et al.* (2013) stated that the type of *Fusarium* colony was dominated by two, i.e. cotton and thin type. Furthermore, Sempere and Siurana (2009) informed that the microconidia and macroconidia of the *F. oxysporum* isolates were oval or kidney-like microconidia formed on fialids, some are single, grouped at the end of the fialids, and arranged like a chain. Meanwhile, the shape of macroconidia is similar to crescent moon with 1–3 septa.

The viability test has revealed that *F. oxysporum* f. sp. *zingiberi* has various mycelia dry weight and conidia density. This diversity occurs due to different incubation times of the isolates on PDA. Genetic variations of the *Fusarium* sp. also influence differences in fungal morphology (Vieira *et al.* 2008; Debbi *et al.* 2018; Rahman and Vaheed 2018). The conidia densities of *F. oxysporum* f. sp. *zingiberi* are not in accordance with the growth time and dry weight of the mycelia. Each isolate has different characteristics due to its genetic differences. The isolates' different conidia densities indicate the genetic diversity of *F. oxysporum* f. sp. *zingiberi* (Riyadi *et al.* 2008; Debbi *et al.* 2018).

Pancasiwi *et al.* (2013) found that the incubation period of *F. oxysporum* f. sp. *zingiberi* on ginger rhizome var. Gadjah was 2.60 days. This incubation period was faster than the incubation period of *F. oxysporum* f. sp. *zingiberi* stored for six years in sterile soil for 5.0–5.7 days (Riyadi *et al.* 2008). The long preservation period of fungi will affect the reduction of the pathogens' ability to cause infection. Isolates preserved for a long time in sterile soil can change fungal physiology which will affect spore production (Senanayake *et al.* 2020). The incubation period is also determined by the decomposing enzymes or toxins produced by pathogens during infection (Armesto *et al.* 2019).

Virulence test proved that fungal isolates are still infectious and were able to cause diseases on ginger rhizome. The shape of ginger rhizome's changes into wrinkled, whitish in color, and dry; these symptoms are similar with previous study by Soesanto *et al.* (2005a). The virulence test shows that the rhizome wet weight of all treatments has decreased. This occurrence occurs due to an increased respiration rate of ginger rhizome inoculated by *Fusarium*. Farahani-Kofoet *et al.* (2020) claimed that inoculation of pathogenic fungi will accelerate metabolism and decrease the weight of inoculated asparagus roots and shoots.

The incubation period is related to the development of pathogens and damages on ginger rhizomes. The area of the rhizome affected during fungal infection is also influenced by pathogenicity levels of the pathogen and supported by favorable environmental conditions. Furthermore, environment adaptation and different pathogenic factors such as spore and toxin production are influenced by genetic differences of the fungal isolate (Riyadi *et al.* 2008; Cahyaningrum *et al.* 2017; Rauwane *et al.* 2020). The area of the rhizome affected by fungal infection will affect wet weight of

the rhizome. Infection of the fungal isolates may cause an increased rate of respiration and evaporation, which in turn influenced the rhizome shoot growth. Ullah *et al*. (2017) proposed that high respiratory activity occurs at high temperatures and lower endogenous ABA content. Therefore, the rhizomes cannot maintain a dormancy period which causes the appearance of the shoots. The waste index in this study is not related to the incubation period, affected area, and different wet weights of the rhizomes inoculated with *F. oxysporum* f. sp. *zingiberi*. According to Velásquez *et al.* (2018), the development of symptoms is determined by virulent pathogenic factors, the susceptibility of the host plant to infectious pathogens, and the suitability of environmental conditions.

This research concludes that the isolates of *F. oxysporum* f. sp. *zingiberi* from Temanggung and Boyolali which has been stored in sterile soil for 17 years could still grow well on PDA medium and filled up petri dishes. Moreover, the isolates were able to cause infection and developed disease symptoms in var. Gajah; although virulence level was decreased, characterized by a long incubation period and a small affected rhizome area.

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