JURNAL
FITOPATOLOGI
INDONESIA
ISSN: 0215-7950

Volume 18, Nomor 6, November 2022
Halaman 264–268
DOI: 10.14692/jfi.18.6.264–268

SHORT COMMUNICATION

Reducing the Risk of Seed-Borne Fungi Development of Foxtail Millet (*Setaria italica*) from Buru Island Through Hot Water Treatment

Penurunan Risiko Perkembangan Cendawan Terbawa Benih Hotong (*Setaria italica*) Asal Pulau Buru Melalui Perlakuan Air Panas

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ABSTRACT

The use of fungi-free seeds is an important factor to increase the production of foxtail millet (*Setaria italica*). This study aims to develop a technique to eliminate seed-borne fungi of foxtail millet seeds from Buru Island through hot water treatment. Identification of seed-borne fungi showed that *Boeremia* sp. was the dominant fungus on foxtail millet seeds from Buru Island. Hot water treatment with a combination of temperature (50 °C, 52 °C, 54 °C) and treatment period (15, 20, 25 minutes) was examined to determine the most effective treatment in suppressing the growth of fungi but not affecting seed germination. This study showed that hot water treatment at 50 °C for 25 minutes reduced fungal infestation *in vitro* and *ad planta* by 57% and 55%, respectively. This indicates that this method can be relied upon to save foxtail millet seed production losses due to the development of pathogenic fungi.

Keywords: *ad planta* treatment, dominant fungi, elimination technique, healthy seed, *in vitro* treatment

ABSTRAK

Penggunaan benih sehat yang bebas cendawan terbawa benih merupakan faktor penting untuk meningkatkan produksi hotong (*Setaria italica*). Penelitian ini bertujuan untuk memperkaya teknik eliminasi cendawan terbawa benih melalui perlakuan air panas terhadap benih hotong asal Pulau Buru. Identifikasi cendawan terbawa benih menunjukkan bahwa *Boeremia* merupakan cendawan dominan pada benih hotong asal Pulau Buru. Perlakuan air panas dengan kombinasi suhu (50 °C, 52 °C, 54 °C) dan lama perlakuan (15, 20, 25 menit) diujikan untuk mencari perlakuan yang paling efektif menekan pertumbuhan cendawan tetapi tidak mengganggu perkecambahan benih. Penelitian ini menunjukkan bahwa perlakuan air panas pada suhu 50 °C selama 25 menit mampu menurunkan infestasi cendawan pada perlakuan *in vitro* dan *ad planta* berturut-turut sebesar 57% dan 55%. Hal ini mengindikasikan bahwa metode ini dapat diandalkan untuk menyelamatkan kerugian produksi benih hotong akibat perkembangan cendawan patogen.

Kata kunci: benih sehat, cendawan dominan, perlakuan *in vitro*, perlakuan *ad planta*, teknik eliminasi

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Foxtail millet has long been cultivated for a specific purpose in eastern Indonesia, especially in Buru Island, Maluku, i.e. as an alternative food source for local communities (Herodian 2008). Recent studies regarding foxtail millet origin from Buru have discussed agronomic aspects such as *in vitro* regeneration and molecular characterization (Iriawati *et al*. 2017; Yulita and Ridwan 2018). The risk of seed-borne pathogens associated with the seeds of foxtail millet needs to be studied as well as the solutions to reduce the risk. The availability of pathogen-free seeds is crucial to expand and spread foxtail millet cultivation.

Seed-borne fungal pathogens may significantly reduce seed quality and seedling performance in the field. Khairani *et al*. (2022) reported that of 100 foxtail millet seeds tested, 100% were contaminated with seed-borne fungi with 34% of them being dominated by *Fusarium* spp. Earlier, Khairani *et al*. (2021) also found several disease symptoms in foxtail millet plants in the field. Ear-rot symptoms found in three foxtail millet genotypes were confirmed caused by *F. incarnatum* and *F. verticilloides*. Furthermore, *Penicillium* sp. was successfully isolated and identified from the abnormal symptoms of young leaves and panicle development.

Recognizing the potential losses that arise from seed-borne fungi, specific treatments that can suppress the development of seed-borne fungi without adverse effects on seed quality are needed. Alkemade *et al*. (2022) reported that treatment with four biocontrol agents from the bacteria and fungi group reduced anthracnose disease severity in seeds of white lupin (*Lupinus albus*). Chemical treatment using systemic fungicide as seed dressing has been successfully controlling *Fusarium* spp. infection in maize (Capo *et al*. 2020). Hot water treatment has also been reported to be effective as a physical seed treatment (Bennett and Colyer 2010; Sharma *et al.* 2015). According to Piñeros-Guerrero *et al*. (2019), thermal treatment at 55 °C (both hot water and dry heat treatment) is the most effective method in suppressing the development of seed-borne fungi from the genus *Fusarium* without affecting seed germination of maize. Kim *et al.* (2022) reported that hot water treatment is one of the best alternative methods for suppressing phytopathogenic fungi, especially in oat, sorghum, wheat, and foxtail millet. The combination of optimum water temperature and treatment period must be optimized for each plant species or the same species grown in different agroecological origins. Appropriate methods must be applied to avoid damage to seed embryos and lower seed germination rates.

This research was undertaken in the Laboratory of Plant Mycology and Laboratory of Plant Bacteriology, Department of Plant Protection, IPB University from December 2021 to February 2022. As many as 400 foxtail millet seeds that have been stored for 2 years with no visual abnormalities were subjected to *in vitro* fungal examination on potato dextrose agar (PDA) medium. Isolated fungal colonies were identified morphologically and a pathogenicity test for each fungal colony was conducted using seedlings of foxtail millet (Watanabe 2010). The hot water treatment experiment involved combinations of temperature (50, 52, 54 °C) and treatment period (15, 20, and 25 min). Variables to determine the most appropriate treatment include suppression rate of fungal colonization on seeds (%) and seed germination (%). Selected treatment was then confirmed by further examination. *In vitro* and *ad planta* examination for 7 and 14 days was conducted involving 10 seeds and 20 seeds per tray, respectively. Each treatment was replicated 4 times and statistical analyses were performed by Tukey test and linear regression (α 5%).

A high infestation (60%) of seed-borne fungi on foxtail millet seeds from Buru Island was evidenced. As many as 241 fungal colonies were detected from the seeds and they can be differentiated into 7 genera based on morphological identification (Table 1). Two genera, *Boeremia* (prev. *Phoma*) and *Curvularia* were considered the most frequent fungi found and both caused necrotic on seedlings 7 days after inoculation. Five other genera, i.e. *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, and *Pyricularia* were found as minor seed-borne fungal pathogens on foxtail millet although they showed a more rapid growth compared to *Boeremia* (Figure 1). Based on the pathogenicity test, it was found that 3.3% of seed-borne fungi were not pathogenic (Table 1). This group of fungi might belong to endophytic fungi which has the potential to be used as a biocontrol agent for seed-borne fungal pathogens.

Hot water treatment with a combination of temperature and treatment period was examined in this study to determine the most efficient combination to suppress fungal development on the seed of foxtail millet. The higher temperature tends to increase fungal suppression, similarly the longer the treatment period the higher the fungal suppression rate. On the other hand, the higher temperature and the longer treatment period tend to cause a lower seed germination rate (Table 2). It was obvious on the combination of the highest temperature (54 °C) with the longest treatment period (25 min) which caused the highest fungal suppression (82.5%) and low seed germination rate (54.3%). Singh *et al*. (2019) explained that high temperatures might reduce the growth of seedlings, increase the risk of

Table 1 Seed-borne fungi associated with foxtail millet seeds from Buru Island

Fungal genus	% Colonization		Type of seedlings abnormalities
	Of a total number of seeds (n=400)	Of a total number of infested seeds (n=241)	
Boeremia	24.3	40.2	Necrotic
Curvularia	23.0	38.2	Necrotic
Alternaria	3.5	5.8	Germination failure
Fusarium	1.8	2.9	Germination failure
Penicillium	1.3	2.1	Stunt growth
Cladosporium	1.5	2.5	Stunt growth
Pyricularia	1.5	2.5	Necrotic
Unknown*	2.0	3.3	No symptoms

*The fungi were unidentified, but they were non-pathogenic based on the pathogenicity test

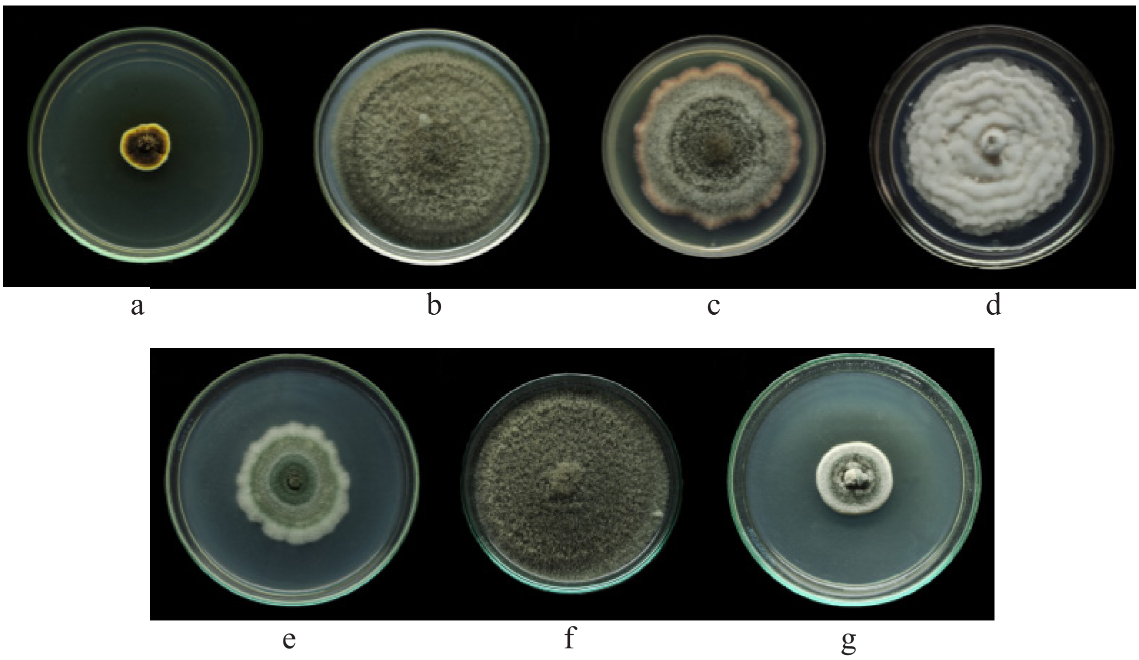

Figure 1 Growth of pathogenic fungi isolated from seeds of foxtail millet from Buru Island on potato dextrose agar at 7 days after inoculation. a, *Boeremia*; b, *Curvularia*; c, *Alternaria*; d, *Fusarium*; e, *Penicillium*; f, *Cladosporium*; and g, *Pyricularia*.

Table 2 Effect of hot water treatment to suppression of fungal colonization and germination rate of foxtail millet seeds from Buru Island

Treatment	Fungal suppression *in vitro* (%)	Germination rate (%)
Control	0.0 a	67.5 abc
50 °C for 15 min	37.5 b	90.0 a
50 °C for 20 min	48.3 bc	85.0 a
50 °C for 25 min	57.3 bcd	85.0 a
52 °C for 15 min	67.5 cde	75.0 ab
52 °C for 20 min	71.8 cde	72.5 ab
52 °C for 25 min	73.8 de	62.5 abc
54 °C for 15 min	79.3 de	67.5 abc
54 °C for 20 min	81.0 de	52.5 bc
54 °C for 25 min	82.5 e	54.3 c

Numbers in the same column followed by similar letters are not significantly different by Tukey test (α 5%)

Table 3 Correlation analysis between temperature and treatment period against fungal suppression and seed germination

Variable	Unit of intercept and R^2 value[a]	
	Fungal suppression (%)	Seed germination (%)
Temperature (°C)	7.22 (0.93)	-8.35 (1.0)
Treatment period (min)	-1.5 (1.0)	0.73 (0.69)

[a]Provided by regression analysis with α 5%

disease infection and solubility of excess salts, and result in damaged/dead seedlings.

A separate regression analysis to investigate the single correlation between temperature or treatment period and fungal suppression or seed germination was conducted. This analysis suggested that higher temperatures increase fungal suppression but decrease seed germination. On the other hand, longer treatment periods increase seed germination but decrease fungal suppression (Table 3). Considering the importance of seed germination besides fungal suppression, a treatment combination of 50 °C and 25 min treatment period is recommended for seed treatment, specifically for seeds from Buru Island to lower the risk of yield loss due to seed-borne pathogens. Following the *in vitro* fungal suppression (57%), 55% suppression *ad planta* was demonstrated as a significant result of seed-borne disease transmission control. Efficient seed treatment might depend on the species or/and cultivar of target seeds. A previous report by Khairani and Ardie (2020) recorded that hot water treatment of 52 °C for 20 minutes was able to suppress seed-borne disease by 64% on ICERI-6 genotype infested by *Fusarium* spp.

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