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Research Article

The Efficacy of Sulfuryl Fluoride as a Fumigant against *Aspergillus niger* on Corn Seeds

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ABSTRACT

The importation of corn seeds may inadvertently introduce seed-borne fungi like *Aspergillus niger*. One potential method to control this pathogen was fumigation with sulfuryl fluoride. The objectives of this applied research were to obtain effective concentration and exposure time of sulfuryl flouride as a fumigant to control seedborne fungi and to observe its physiological impact on corn seeds. *Aspergillus niger* was isolated from corn seed samples, grew in potato dextrose agar, and used for *in vitro* studies. Parameters observed were isolates' growth inhibition. *In vivo* studies were conducted using corn seed samples infected by *Aspergillus niger*. Sulfuryl fluoride was applied at the concentration of 30, 40, 50, and 60 g/m^3 and 24, 48, 72, and 96 hour of exposure time. The results showed that sulfuryl fluoride concentration of 40 g/m^3 for 48 hours at 26–32°C is the only effective concentration against *A. niger in vitro* but all of the treatment did not significantly affect *A. niger in vivo*. Concentration of 30, 40, 50, 60 g/m^3 for 24 hours does not affect the quality of the seed.

Keywords: *Aspergillus niger;* corn; fumigation; growth inhibition; mortality; viability

INTRODUCTION

Corn is an important crop in Indonesia but its availability still depends on importation from other countries. This possesses the threat of soil-borne fungi pathogens invasion. The effort for Indonesia to self-sufficiently supply corn relies heavily on the availability of high-quality and pathogen-free corn seeds. Based on Regulations of Ministry of Agriculture No. 51 Year 2015, there are 12 fungi identified as seed-borne pathogens, including *Claviceps gigantean, Gaeumannomyces graminis* var. *graminis, Sphacelotheca reilliana, Sporisorium cruentum Gibberella zeae (Fusarium graminearum), Fusarium sporotrichioides, Gloeocercospora sorghi, Pyricularia setariae, Acremonium strictum (Sarocladium strictum), Stenocarpella maydis, Sclerophthora macrospora, Sclerospora graminicola* and they are categorized as quarantine plant organism (Organisme Pengganggu Tanaman Karantina, OPTK) level A1 that are not found in Indonesia. Meanwhile, known fungi pathogens on corn seeds are *Aspergillus flavus*, *Aspergillus niger*, *Fusarium sp*., and *Penicillium sp.* (Kurniawan *et al*., 2008).

Quarantine and non-quarantine pests of seeds can be controlled by using fumigants. Research have shown that the sulfuryl fluoride fumigant was toxic against *Ceratocystis fagacearum* (Woodward, 1995). Palencia *et al.* (2010) reported that *Aspergillus niger* produces mycotoxin ochratoxin on corn and cause kernel rot of corn seeds. Therefore, there is a merit to study the potency of sulfuryl fluoride as a fumigant against *Aspergillus niger* on corn seeds.

This study aimed to determine the effective concentration and the exposure time of sulfuryl fluoride to control *Aspergillus niger* and its physiological effect on corn seeds.

MATERIALS AND METHODS

A study was done at the Applied Research Institute of Agricultural Quarantine (ARIAQ) in Bekasi, West Java. This study was divided into 3 parts, the first was detecting and identifying the fungi on corn seeds, the second was measuring *in vitro* and *in vivo* inhibition rate of sulfuryl fluoride on *Aspergillus niger*,*r*, and the third was determining the effect of sulfuryl fluoride to seed vigor and seed germination.

Detection and Identifcation of Fungi on Corn Seed

Fungal detection on corn seeds was done using a blotter test by placing sterile water wetted filter paper in 5 petri dishes. Ten corn seeds were placed in Petri dishes and incubated for 7 days. Fungi identification was done under a stereo compound microscope and fungal appearances were compared based on the guide from "*A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain*" by Navi *et al.* (1999). The dominant species were then used for further testing.

In vitro *Inhibition Rate of Sulfuryl Fluoride against* Aspergillus niger *on Corn Seeds*

Isolates used in this study were obtained from pure *Aspergillus niger* cultures grown on potato dextrose agar (PDA). Isolate propagation was done by taking conidia from pure cultures using a isolating needle and placing them in the middle of a 9 cm diameter Petri dishes filled with PDA. Isolates were incubated for 2 days, lids were opened, and placed into plastic bags. Sulfuryl fluoride fumigation was done with concentrations of 30, 40, 50 60 g/m^3 and an untreated control for 24, 48, 72, and 96 hours at ≥ 26–32^{o}C with 5 replications for each treatment combination.

Observation on colonies were done 7 days after treatment. Parameters observed were growth and inhibition of colony diameter. Relative inhibiton (RI) were calculated using the formula from Hendricks *et al.* (2017):

$$RI = \frac{Dk - Dp}{Dk} \times 100\%$$

RI = Relative inhibition

Dk = Colony diamter from untreated control (cm)

Dp = Colony diameter from treatment (cm)

After 7 days, conidia were taken from treated isolates. As much as 10 ml of sterile water was added to isolates and 1 ml of the suspension were placed on PDA. Isolates were then incubated for 7 days and growth of *Aspergillus niger* conidia were observed.

In vivo *Inhibition Rate of Sulfuryl Fluoride against* Aspergillus niger *on Corn Seeds*

To obtain homogenic population density of *Aspergillus niger,* corn seeds were inoculated using pure isolates. Corn seeds were immersed in isolate suspensions made from 7 days-old *Aspergillus niger* cultures added to sterile water until density reached 10^4 cfu/ml. Tween 20, at rates of 2 ml/L, were added to *Aspergillus niger* suspensions to enhance spore's ability to stick to corn seeds. Corn seeds were immersed for 30 minutes, air-dried, and stored in a desiccator for 3 days.

Two days after innoculations, 500 g of corn seeds were placed in plastic containers and fumigated with sulfuryl fluoride at concentrations of 30, 40, 50, 60 g/m^3 and an untreated control for 24, 48, 72, and 96 hours at temperatures of ≥ 26–32^{o}C. Observations were done on percentage of infection on corn seeds. Observations were done on 500 fumigated and untreated control of seeds using a blotter test. Effective concentrations were then used as a generic concentration for the following test.

The Effect of Sulfuryl Fluoride on Corn Seed Vigor Germination

Treated and untreated corn seeds were planted into sterile sand placed on a plastic trays. In each tray, 100 seeds were planted with 5 replications. Seeds were watered every day. Vigor was observed 4 days after planting and germination was done 7 days after planting (Sadjad *et al.*,1999).

Vigor index (VI) were calculated from the percentage of seeds that germinated normally (KN) with the following formula:

$$VI = \frac{KN}{\text{Total seeds planted}} \times 100\%$$

Germination rates (GR) was calculated following methods from Sadjad *et al.* (1999) by counting the percentage of seeds that germinated normally on day 4 (KN I) (accordance to ISTA, 2018) and percentage of seed that germinated normally on day 7 (KN II) using the following formula:

$$GR = \frac{KN\ I + KN\ II}{\text{Total seeds planted}} \times 100\%$$

Data Analysis

Data were analyzed as a Complete Randomized Design with 2 factors. The first factor was the 5 sulfuryl fluoride concentration used, including 0 (untreated control), 30, 40, 50, 60 g/m^3, and the second factor was the exposure time 24, 48, 72, and 96 hours at temperatures of ≥ 26–32^{o}C. Each replication used 100 seeds and was replicated 5 times.

RESULTS AND DISCUSSIONS

Fungi on Corn Seeds

Results from the detection and identification process showed that *Aspergillus niger* was the dominant species. This is based on the black colony that grew on the medium (Elfita *et al.*, 2012). Kurniawan *et al.* (2008) reported that *Aspergillus niger* was one of the fungi isolated from corn seeds. Hussain *et al.* (2013) has reported that *Aspergillus niger* is a pathogen on corn seed germination.

In vitro *Inhibition Rate of Sulfuryl Fluoride against* Aspergillus niger *on Corn Seeds*

Colony inhibition results showed that colony growth and *Aspergillus niger* conidia on PDA were inhibited after treated with sulfuryl fluoride at concentration of 30 g/m^3 with exposure time of 72 hours and at concentration of 40 g/m^3 with exposure time of 48 hours (Table 1 and 2).

Fluoride content in sulfuryl fluoride affected pigment colors of *Aspergillus niger.* Fungal colonies treated with sulfuryl fluoride demonstrated yellowish zone on the perimeter of colonies compared to the grey blackish color from untreated controls (Figure 1). In normal conditions, *Aspergillus niger* colonies are black due to their ability to produce melanin (Oramahi & Haryadi, 2006). Sulfuryl fluoride treated at concentrations of 30, 40, 50 and 60 g/m^3 for 24 hours and concentration of 30 g/m^3 for 48 hours showed that fungal growth was inhibited compared to the control. Colonies in control were able to fill Petri dishes at day 5 whereas colony growth was inhibited respectively to the increase of treatment concentrations. For concentration 30 g/m^3 for 72 and 96 hours, 40, 50, and 60 g/m^3 for 48, 72 and 96 hours, no colony growth was observed after treatment due to the increase of sulfuryl fluoride concentration (Figure 2). Results from this study showed that sulfuryl fluoride can inhibit *Aspergillus niger* growth. Woodward & Schmidt (1995) showed *in vitro* that fumigation using sulfuryl fluoride at 80 g/m^3 for 48 hours effectively inhibited *Ceratocystis fagacearum*. A similar study done by Zhang (2006) at 30 g/m^3 for 72 hours, effectively inhibited *Cladosporium herbarum, Phlebiopsis gigantean, Schizophyllum commun, Armillaria novae-zelandiae, Botryodiplodia theobromae, Ophiostoma novo-ulmi, Phytophthora cinnamomi,* and *Sphaeropsis sapinea*.

Table 2. *In vivo* effects of sulfuryl fluoride concentrations on *Aspergillus niger* conidia growth

Exposure time (hours)	Sulfuryl fluoride concentration (g/m^3)				
	Control	30	40	50	60
24	G	G	G	G	G
48	G	G	NG	NG	NG
72	G	NG	NG	NG	NG
96	G	NG	NG	NG	NG

Note: G = grow, NG = did not grow

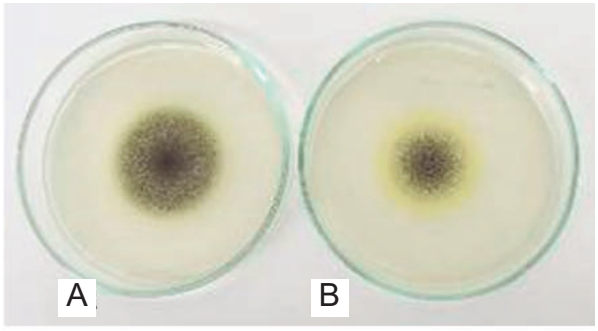

Figure 1. *Aspergillus niger* isolates after treated with sulfuryl fluoride; control (A), treated with sulfuryl fluoride at concentration of 30 g/m^3 for 24 hours (B)

Table 1. *In vitro* colony diameter and relative inhibition percentage of *Aspergillus niger* at 7 days

Exposure Time (hours)	Sulfuryl fluoride concentration (g/m)^3									
	Control		30		40		50		60	
	D (cm)	RI (%)	D (cm)	RI (%)	D (cm)	RI (%)	D (cm)	RI (%)	D (cm)	RI (%)
24	9^a	0^a	8.7^a	3.4^b	7.9^b	12.2^c	2.7^c	70^d	2.4^d	73.3^d
48	9^a	0^a	7.6^b	15.6^b	4.1^c	54.4^c	2.7^d	70^d	2.2^d	75.6^d
72	9^a	0^a	4.4^b	51.1^b	2.9^c	67.8^c	2.5^{cd}	72.2^{cd}	2.1^d	76.7^d
96	9^a	0^a	3.5^b	61.1^b	2.3^c	74.4^c	2.2^c	75.6^c	2^c	77.8^c

Notes: D = diameter; RI = relative inhibition. Different superscript letters on each column show significant differences ($p < 0.05$)

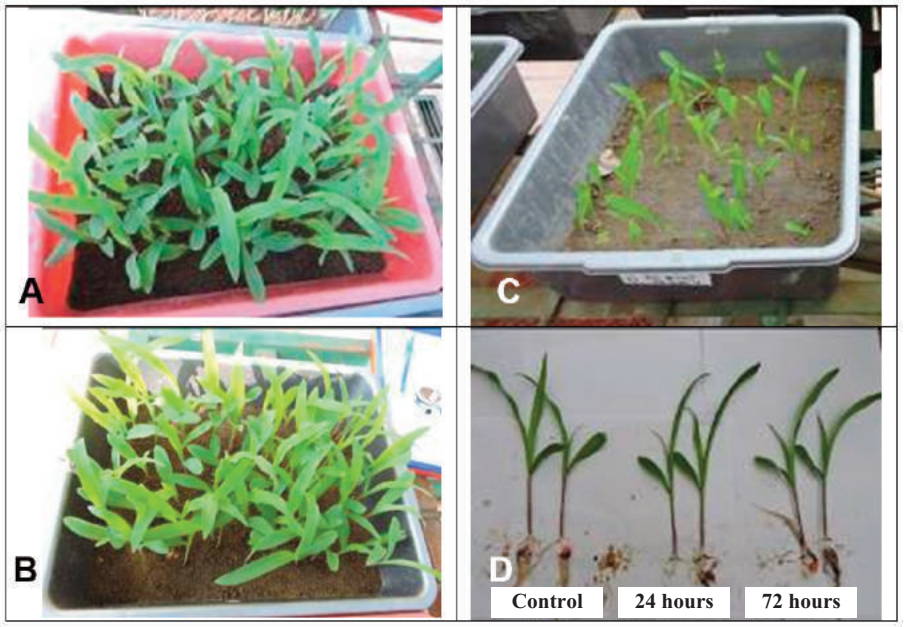

Figure 2. Corn seeds after 7 days; control (A), treated with sulfuryl fluoride at concentration of 30 g/m3 for 24 hours (B), treated with sulfuryl fluoride at concentration of 50 g/m^3 for 72 hours (C), physical growth of treated and untreated seeds (D)

In vivo *Effectiveness of Sulfuryl Fluoride against* Aspergillus niger *on Corn Seeds*

Aspergillus niger inhibition on corn seeds can be viewed at Table 2. Results showed that *Aspergillus niger* conidia started to be inhibited after treated with concentration of 40 g/m^3 for 48 hours (Table 3).

The highest concentration and longest exposure time did not show 100% fungal inhibition. Higher concentrations were not tested due the decrease of seed quality (vigor index and germination rate) based on following tests.

Tubajika & Barak (2008) showed that the use of sulfuryl fluoride as a quarantine treatments, completely inhibited growth (100% inhibition) of *Ceratocystis fagacearum* on birch, red pine and maple at concentration of 240 g/m^3 for 24 hours.

The Effects of Sulfuryl Fluoride on Corn Seed Vigor and Germination

The results showed that sulfuryl fluoride at concentrations of 30, 40, 50, 60 g/m^3 for 24 jam, did not decrease seed's vigor and germination ability, while exposure time for more than 24 hours (48, 72, 96 hours) decreased seed vigor and germination (Table 4 and 5).

Table 3. Inhibition percentage of *Aspergillus niger* on corn seeds after sulfuryl fluoride treatment

Exposure time (hours)	Sulfuryl fluoride concentration (g/m^3)				
	Control	30	40	50	60
24	0^a	0^a	0^a	0^a	0^a
48	0^a	0^a	0^a	0^a	0^a
72	0^a	0^a	88^b	92.6^{bc}	97.6^c
96	0^a	0^a	92.6^b	97.4^c	98.6^c

Note: Different superscript letters on each column show significant differences ($p < 0.05$)

Table 4. Corn seed vigor index after treated with sulfuryl fluoride at various concentration and exposure time

Exposure time (hours)	Sulfuryl fluoride concentration (g/m^3)				
	Control	30	40	50	60
24	97^a	88^b	83^{bc}	82^c	82^c
48	97^a	73^{bc}	80^b	71^c	70^c
72	97^a	75^b	52^c	43^d	38^d
96	97^a	40^b	27^c	26^c	23^c

Note: Different superscript letters on each column show significant differences ($p < 0.05$)

Table 5. Percentage of corn seed germination rate after treated with sulfuryl fluoride

Exposure time (hour)	Sulfuryl fluoride concentration (g/m^3)				
	Control	30	40	50	60
24	98^a	93^b	92^b	92^b	91^b
48	98^a	84^b	83^{bc}	76^c	76^c
72	98^a	78^b	58^c	46^d	42^d
96	98^a	59^b	40^c	31^d	27^d

Note: Different superscript letters on each column show significant differences ($p< 0.05$)

Prabhakaran *et al.* (2010) reported that fumigation using various concentration of sulfuryl fluoride can be used to inhibit potato sprouting depending on potato varieties. This research demonstrated that 200 g-hour/m^3 on Russet Burbank potato variety inhibit sprouting for 31 days without phytotoxic symptoms.

Germination rates of seeds should meet the standard of 80% (Kamil, 1979). Results from this study then implies that the maximum sulfuryl fluoride concentration that may be used is 40 g/m^3 for 48 hours. However, fumigation using sulfuryl fluoride for more than 48 hours reduces seed quality based on this standard.

The germination rates of corn seed treated with sulfuryl fluoride at concentrations of 30, 40, 50, and 60 g/m^3 for 24 hours, which were stored at 18–22^{o}C and humidity 65–70% for 2 months did not decrease compared to seeds that were immediately planted after aerated for 1 day. Their germination rates were still > 90%.

CONCLUSION

Sulfuryl fluoride concentration at 40 g/m^3 with exposure time of 48 hours and temperatures of 26–32^{o}C only inhibited *Aspergillus niger* growth when tested *in vitro,* while the fumigant was not effective when tested *in vivo*.

Sulfuryl fluoride treated at concentrations of 30, 40, 50, and 60 g/m^3 with exposure time of 24 hour and temperatures of 26–32^{o}C did not affect seed quality.

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