INTRODUCTION

Corn is an important crop in Indonesia but its availability still depends on importation from other countries. This poses 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, and the third was determining the effect of sulfuryl fluoride to seed vigor and seed germination.
**Detection and Identification 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/m³ and an untreated control for 24, 48, 72, and 96 hours at ≥ 26–32°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 inhibition (RI) were calculated using the formula from Hendricks et al. (2017):

\[ RI = \frac{D_k - D_p}{D_k} \times 100\% \]

where:
- \( RI \) = Relative inhibition
- \( D_k \) = Colony diameter from untreated control (cm)
- \( D_p \) = 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 10⁴ 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/m³ and an untreated control for 24, 48, 72, and 96 hours at temperatures of ≥ 26–32°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:

\[ VI = \frac{KN}{Total\ seeds\ planted} \times 100\% \]

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:

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

**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/m³, and the second factor was the exposure time 24, 48, 72, and 96 hours at temperatures of ≥ 26–32°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*.

| Exposure time (hours) | Sulfuryl fluoride concentration (g/m$^3$) |
|-----------------------|------------------------------------------|
| Control 30            | G                                        |
| 48                    | G                                        |
| 72                    | NG                                       |
| 96                    | NG                                       |

Note: G = grow, NG = did not grow

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

| Exposure Time (hours) | Control | Sulfuryl fluoride concentration (g/m$^3$) |
|-----------------------|---------|------------------------------------------|
|                       | D (cm)  | RI (%)                                   |
|                       | 30      | 40                                       |
|                       | 50      | 60                                       |
| 24                    | 9$^a$   | 0$^a$                                    |
|                       | 8.7$^b$ | 3.4$^b$                                  |
|                       | 7.9$^b$ | 12.2$^c$                                 |
|                       | 7.7$^c$ | 2.7$^c$                                  |
|                       | 70$^d$  | 2.4$^d$                                  |
| 48                    | 9$^a$   | 0$^a$                                    |
|                       | 7.6$^b$ | 15.6$^b$                                 |
|                       | 4.1$^c$ | 54.4$^c$                                 |
|                       | 2.7$^d$ | 70$^d$                                   |
|                       | 2.2$^e$ | 75.6$^e$                                 |
| 72                    | 9$^a$   | 0$^a$                                    |
|                       | 4.4$^b$ | 51.1$^b$                                 |
|                       | 2.9$^c$ | 67.8$^c$                                 |
|                       | 2.5$^d$ | 72.2$^d$                                 |
|                       | 2.1$^e$ | 76.7$^e$                                 |
| 96                    | 9$^a$   | 0$^a$                                    |
|                       | 3.5$^b$ | 61.1$^b$                                 |
|                       | 2.3$^c$ | 74.4$^c$                                 |
|                       | 2$^e$   | 77.8$^e$                                 |

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

**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            | G                                        |
| 48                    | G                                        |
| 72                    | NG                                       |
| 96                    | 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)**
**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/m³ 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/m³ 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/m³ 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).

![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/m³ for 72 hours (C), physical growth of treated and untreated seeds (D)](image)

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

| Exposure time (hours) | Sulfuryl fluoride concentration (g/m³) |
|-----------------------|----------------------------------------|
|                        | Control  | 30 | 40 | 50 | 60 |
| 24                    | 0        | 0 | 0 | 0 | 0 |
| 48                    | 0        | 0 | 0 | 0 | 0 |
| 72                    | 0        | 88 | 92.6 | 97.6 | 98.6 |
| 96                    | 0        | 92.6 | 97.4 | 98.6 |

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/m³) |
|-----------------------|----------------------------------------|
|                        | Control  | 30 | 40 | 50 | 60 |
| 24                    | 97        | 88 | 83 | 82 | 82 |
| 48                    | 97        | 73 | 80 | 71 | 70 |
| 72                    | 97        | 75 | 52 | 43 | 38 |
| 96                    | 97        | 40 | 27 | 26 | 23 |

Note: Different superscript letters on each column show significant differences (p< 0.05)
Prabhakaran et al. (2010) reported that fumigation using various concentrations 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°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°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°C did not affect seed quality.

ACKNOWLEDGEMENT

We thank Ir. Banun Harpini, Dr. Ir. Antarjo Dikin, M.Sc., Dr. Ir. Ummu Salamah Rustiani M.Si., Dr. Ir. Idham Sakti Harahap M.Si., Siti Fadillah M.Si., and M. Soleh for the critics, suggestions, and correction when the study was performed.

LITERATURE CITED

Elfita, Muharni, Munawar, & S. Aryani. 2012. Secondary Metabolite from Endophytic Fungi Aspergillus niger of the Stem Bark of Kandis Gajah (Garcinia griffithii). Indonesian Journal of Chemistry 12: 195–200.

Hendricks, K.E., M.C. Christman, & P.D. Roberts. 2017. A Statistical Evaluation of Methods of in-Vitro Growth Assessment for Phylosticta citricarpa: Average Colony Diameter vs Area. PloS One 12: e0170755.

Hussain, N., A. Hussain, M. Ishtiaq, S. Azam, & T. Hussain. 2013. Pathogenicity of Two Seed Borne Fungi Commonly Involved in Maize Seeds of Eight District of Azad Jammu and Khasmir, Pakistan. African Journal of Biotechnology 12: 1363–1370.

[ISTA] International Seed Testing Association. 2018. International Rules for Seed Testing, Chapter 7, i–7-6 (12).

Kamil, J. 1979. Teknologi Benih. Angkasa Raya, Padang. 227 p.

Kurniawan, S., A. Widiastuti, & Y.M.S Maryudani. 2008. Pengaruh Perlakuan Uap Air Panas dengan Sistem Pemanasan Terbuka terhadap Kesehatan dan Viabilitas Benih Jagung. Jurnal Perlindungan Tanaman Indonesia 14: 63–69.

Navi, S.S., R. Bandyopadhyay, A.J. Hall, & P.J. Bramel-Cox. 1999. A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain. Information Bulletin (59). International Crops Research Institute for Semi Arid Tropics, Patancheru, Andhra Pradesh, India. 128 p.

[NPIC] National Pesticide Information Centre. 2011 1.800.585.7378. Sulfuryl Fluoride Technical Fact Sheet. http://npic.orst.edu/factsheets/sulfurylfluoridetech.pdf, modified 20/11/2016.

Palencia, E.R., D.M. Hinton, & C.W. Bacon. 2010. The Black Aspergillus of Maize and Peanuts and their Potential for Mycotoxin Production. Toxins 2: 399-416.

Prabhakaran, S., D. Jenkins, & A. Ratterman. 2009. Use of Sulfuryl Fluoride as a Sprout Inhibition Agents. http://www.google.com/patents/WO2009061862A?cl=en, modified 15/11/2016.

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$^a$  | 40$^c$  | 31$^a$ | 27$^a$ |

Note: Different superscript letters on each column show significant differences (p< 0.05)
Sadjad, S., E. Murniati, & S. Ilyas. 1999. *Parameter Pengujian Vigor Benih, dari Komperatif ke Simulatif*. PT Grasindo Gramedia Widiasarana Indonesia, Jakarta. 185 p.

Tubajika, K. & A. Barak. 2008. Methyl Iodide and Sulfuryl Fluoride as Quarantine Treatments for Solid Wood Packing Material. *Phytopathology* 98: S159.

Woodward, R.P. & E.L. Schmidt. 1995. Fungitoxicity of Sulfuryl Fluoride to *Ceratocystis fagacearum* in vitro and in Wilted Red Oak Log Section. *Plant Disease* 79: 1237–1239.

Zhang, Z. 2006. Use of Sulfuryl Fluoride as an Alternative Fumigant to Methyl Bromide in Export Log Fumigation. *New Zealand Plant Protection* 59: 223–227.