Pyrolysis of Oil Palm Kernel Shell into Liquid Smoke and its Application to Control Anthracnose Disease on Chili (*Capsicum annuum* L.)

M. Faisal, A. Gani, H. H. Baihaqi and H. Daimon
1Department of Chemical Engineering,
2Department of Agri Technology,
3Department of Agri Business, Syiah Kuala University, Banda Aceh, Aceh, Indonesia
4Department of Environmental and Life Science, Toyohashi University of Technology, Toyohashi, Aichi, Japan

**Abstract:** This research is an investigation of the application of liquid smoke produced from the pyrolysis of Palm Kernel Shell (PKS) controlling anthracnose, a fungal disease caused by *Colletotrichum capsici* on chili. The experiments for liquid smoke production were carried out in a batch reactor apparatus on a range temperature of 100-350°C. The results showed that pyrolysis temperature and liquid smoke concentration significantly influenced the fungus spot caused by *C. capsici*. A chemical compound present in liquid smoke in treatment T1 was able to impair a growth of the fungal mycelium of *C. capsici*. The smallest spot diameter was found at concentration K1 with an average value of 0.454 cm; although it was not significantly different from the sizes of treatment K2, K3 and K4. However, the best treatment was obtained from a combination of treatment T1K1 (Pyrolysis temperature of 200-250°C and liquid smoke concentration of 8%). A long period of incubation in treatment T1K1 and 4.5 day after incubation showed that there was a resistance characteristic in the treatment which caused the disease symptoms to appear in long time, making the pathogens to evolve longer.

**Key words:** Liquid smoke, oil palm kernel shell, pyrolysis, *Colletotrichum capsici*, anthracnose disease, chili

**INTRODUCTION**

Until now, the potential from the variety of agricultural biomass waste has not been well explored. Indonesia is an agriculturally vast country that has a lot of agricultural biomass. One of the biggest industries in Indonesia is the oil palm industry which produces a lot of waste biomass such as, palm kernel shells, frond and empty fruit bunches whose resources are not properly utilized. One of the methods to exploit the potential biomass of oil palm waste in Indonesia is the pyrolysis of Palm Kernel Shell (PKS) to produce liquid smoke product. Liquid smoke is a solution containing mixture of oxidized organic compounds, such as ketones, aldehydes, phenols and carboxylic acids. It is obtained from the condensation of steam pyrolysis process (burning without oxygen) on plant or timber at a temperature of about 400°C (Soldera *et al.*, 2008). Liquid smoke contains compounds that function as an anti-bacterial, anti-fungal and antioxidant which can be natural ingredients for preventing pests and diseases. Based on the properties of several components, these compounds can also serve as a botanical insecticide and fungicide that can be used in agriculture. Thus, the liquid smoke coming from hard wood waste such as palm shells and coconut shells can be used to support “go organic” agricultural programs. However, up until now, deep study on the effectiveness of the liquid smoke as fungicides has not been intensively conducted. Further research associated with liquid smoke and its application in agriculture is required in order to develop a sustainable agricultural industry. Utilization of waste and organic materials from agricultural by-products to produce liquid smoke will generate additional economic value and an increase in social and cultural values. Organic pest control can also maintain the balance of ecosystems and impact on health factors and the quality of agricultural products (Gani *et al.*, 2014).

Chili (*Capsicum annuum* L.) is an important crop in tropical and subtropical countries. Due to its use on daily basis, large areas of lands are devoted to cultivation of chili in many countries. However, anthracnose a fungal disease caused by *Colletotrichum* species (i.e., *C. capsici*) limits chili production (Nantawanit *et al.*, 2010). Anthracnose disease caused by *Colletotrichum* (Gloeosporium) of the Glomerella group is very common and destructive to numerous crops and

**Corresponding Author:** M. Faisal, Department of Chemical Engineering, Syiah Kuala University, Banda Aceh, Indonesia
ornamental plants worldwide (Munch et al., 2008; Cai et al., 2013), resulting in substantial economic damage due to fruit loss and reduced market value of the affected fruits (Manandhar et al., 1995; Petkovsek et al., 2013). When infected by C. capsici, small black circular spots appear on the surface of chili fruit and then, the spots become diffuse and black. After that, the typical anthracnose symptoms develop gradually from the infected areas and appear as expanded necrosis (Nantawanit et al., 2010). The disease mainly affects mature fruits but can also occur on developing fruits, leaves and stems. Several Colletotrichum species have been reported to cause pepper anthracnose (Petkovsek et al., 2013; Than et al., 2008; Dannm et al., 2012; Diao et al., 2013). Several studies have been conducted to manage the anthracnose on chili, mainly on the use of chemicals and biological agents, as well as microbial antagonists (Nantawanit et al., 2010).

This purpose of this study is to evaluate the effectiveness of the liquid smoke generated from the pyrolysis of palm kernel shell as a fungicide in the control of anthracnose fungus C. capsici in chilies.

**MATERIALS AND METHODS**

**Preparation of liquid smoke:** Pyrolysis reactor with a capacity of 5 kg day⁻¹ was designed using stainless steel material so that the resulting liquid smoke products were free from metals content. Procedures for liquid smoke production has been discussed elsewhere (Ginayati and Faisal, 2015). The pyrolysis temperature were divided into four treatment, i.e., T₁ = at temperature of 100-200°C; T₂ = at temperature of 200-250°C; T₃ = at temperature of 250-300°C; and T₄ = at temperature of 300-350°C. Compounds or chemical components contained in the liquid smoke were identified using GC-MS QP2010 (Shimadzu, Japan) based on the method developed and modified by Guillen and Ibargoitia (1999, 1998).

**Sampling of chili:** Samples were taken from the plantations in Aceh Besar, Aceh, Indonesia. The variety of chili used is the TM 999. Chilies used were 30 days old and in the same conditions. The medium used was a mica box with a size of 21×21 cm. Each box was filled with 10 chilies and labeled as in-treatment.

**Isolation and propagation of anthracnose fungus:** The pathogen of the fungus causing anthracnose, C. capsici inoculum was isolated from infected chilies as observed by Gautam (2014) that the causative agent of anthracnose, C. capsici is often found on chilies. The source of C. capsici inoculum was then cultured in a medium of moist rice paper and then removed repeatedly for further culture to a petri dish containing PDA medium, to obtain pure cultures of C. capsici. These cultures were inoculated into chilies that have been provided in the box, by wounding (one stitch) parts/layers of the epidermis on the chilies with the use of a pin on the surface of the chilies. In the sections, the ones which have been harmed are infected with an inoculum of C. capsici by using small size cock borer (0.5 mm). Chilies that have been inoculated were incubated for 24 h and then liquid smoke is applied in accordance with a predetermined concentration.

**Liquid smoke application as biofungicide on chili:** The application of liquid smoke was performed 1 day after inoculation of C. capsici by spraying the entire surface of the chilies.

A completely randomized design, with factorial pattern of 4×6 accompanied by 4 replications consisting of two factors was used for the data analyses. The first factor was Temperature (T) with four types (i.e., T₁ = 100-200°C, T₂ = 200-250°C, T₃ = 250-300°C, T₄ = 300-350°C). The second factor was the liquid smoke concentration (K) with six levels which include K₀ = control, K₁ = 2%, K₂ = 4%, K₃ = 6%, K₄ = 8%, K₅ = 10%.

**RESULTS AND DISCUSSION**

**Incubation period:** Observation of the incubation period of the disease aims to determine the effectiveness of the temperature and concentration of liquid smoke applied to the chilies, in delaying the emergence of the fungus that cause anthracnose disease (C. capsici).

Results of the analysis of variance showed that the temperature had significant effect on the incubation period of the fungus causing anthracnose disease, the treatment with varying concentration of liquid smoke concentration had no effect on the incubation period of the fungus C. capsici while the combination of temperature and concentration of the liquid smoke significantly affected the incubation period of the fungus C. capsici. From further test, it was known that T₂ (temperature 200-250°C) was significantly different from other temperatures (Table 1). The effect of the interaction between the pyrolysis temperature and concentration of liquid smoke to the incubation period can be seen in Fig. 1.

Based on Fig. 1, the symptoms of the disease on T,K₄ treatment (temperature of 200-250°C with the concentration of 8% liquid smoke) began to appear in the 4.5 DAI (days after incubation). The long incubation
Fig. 1: The interaction between temperature pyrolysis liquid smoke and various concentrations of liquid smoke (K0 = control, K1 = 2%, K2 = 4%, K3 = 6%, K4 = 8%, K5 = 10%).

Table 1: The incubation period of the fungus C. capsici due to the influence of pyrolysis various temperature. Values followed by the same letter indicates no significant difference in the HSD (Honestly Significant Different) test with the level of 5%, HSD2.47 = 0.65. Coefficient Variance (CV) = 27.01%.

<table>
<thead>
<tr>
<th>Treatment (Pyrolysis temperature, °C)</th>
<th>Spot diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 =100-200</td>
<td>3.68 ab</td>
</tr>
<tr>
<td>T2 =200-250</td>
<td>3.92 b</td>
</tr>
<tr>
<td>T3 =250-300</td>
<td>2.79 a</td>
</tr>
<tr>
<td>T4 =300-350</td>
<td>2.79 a</td>
</tr>
</tbody>
</table>

The incubation period in T3K4 treatment resulted in the resistance of the chilies to the disease; this also occurred in other T3 treatments (T3K1, T3K2, T3K3, and T3K4). The long incubation period might slow down the appearance of the disease symptoms by inhibiting the growth of the pathogen. It was suspected that the liquid smoke treatment could provide resistance to the chilies which can delay the symptoms of the fungus. Results showed that the treatment with the temperature of 200-250°C and the concentration of 8% of liquid smoke was effective in suppressing the growth of fungus which caused the anthracnose disease.

**Spot diameter on treatment with different liquid smoke pyrolysis temperature:** The observation of spot diameter was done on 7th day after the observation of the incubation period. Observations were carried out by measuring the spots on the longest side. Results of the analysis of variance showed that there was no interaction between temperature and concentration treatment.

The temperature treatment and various concentration treatments of liquid smoke had very significant effect on the spots diameter (Sig. value >t-table 0.05). The average value of the effect of temperature and concentration can be seen in Table 1.

Fig. 2: Average spot diameter due to the influence of the temperature of liquid smoke produced. (Values followed by the same letter indicates no significant difference in the level of 5% HSD test, HSD2.47 = 0.088, CV = 39.97%).

From Fig. 2, it can be seen that the temperature of the pyrolysis treatment had significant effect on the spot diameter of the fungus causing anthracnose disease. Based on the observations, the highest spot diameter was found in T1 treatment with the temperature of 250-300°C (i.e., 0.351 cm). It was not too different with the diameter found in the T4 treatment with the temperature of 300-350°C (i.e., 0.340 cm). While the lowest spot diameter was found in T2 treatment with the temperature of 200-250°C (i.e., 0.206 cm). The liquid smoke in T2 treatment contained 16.28, 14.40 and 14.26% of acetic acid, benzamine and phenol, respectively. The combination of phenol functional components and high organic acid content was known to cause damaging effect on fungal cell membrane and disrupt cell metabolism as well as inhibit cell growth of the fungus.

Wide and small spot diameter average that occurred at the temperature treatments might be caused by the various components phenolic compounds contained in the liquid smoke itself (because it was produced at different pyrolysis temperature). From the analysis of the contents of T1 liquid smoke, there was a difference in the chemical compounds being produced. The difference in temperature of the liquid smoke can lead to different chemical compounds and composition of the active ingredient. This statement is also consistent with previous studies conducted by Chien et al. (2008) who examined the liquid smoke from Moso bamboo (Phyllostachys heterocyla Milf) at various pyrolysis temperatures. Differences on phenol and acetic acid component in liquid smoke from Moso bamboo pyrolysis are influenced by various factors such as the wood type,
wood moisture and pyrolysis temperature used. According to Velmurugan et al. (2009), there are several components in the liquid smoke obtained from the pyrolysis results of Pinus densiflora var. Liau and Quercus serrata breviflora var and Ding tomentosa sawdust can be used as an anti-fungal agent, its constituents are 2.6 Dimethoxy Phenols, Phenol (Izal), 2-methyl phenol, 4-methyl phenol, 2-methoxy phenol, 2-methoxy-4 methyl phenol and 4-ethyl-2-methoxy phenol.

A very high concentration of phenol functional components and organic acid contents were known to damage the fungal cell membranes causing cell growth disruption. Regarding the mechanism of phenolic compounds, the activity of phenolic compounds includes reaction with cells that leads to increased permeability of the cell membrane and results in loss of nuclei, functional inactivation of genetic material and its essential enzymes (Karseno and Kapti, 2001). Phenol is an organic compound that is commonly used in anti-microbial preparations (Yunokawati et al., 2013). Oramah et al. (2011) also argues that the content of organic acids and phenols are responsible for the activity of the growth of the fungus, Aspergillus sp.

From the explanation above, it can be concluded that the constituent compounds in the liquid smoke are effective as anti-fungal agent. It was also suspected that 2-methoxy-4 methyl phenol plays a significant role in inhibiting the growth of fungus. From the results of the GCMS analysis, the concentration of 2-methoxy-4 methyl phenol at each pyrolysis temperature was different, that is T1 = 2.49%, T2 = 7.35% T3 = 1.96% and T4 = 0.94%.

The results of this research agree with the investigation of Kurniawati and Achrom (2014) that the in vitro use of liquid smoke obtained from palm kernel shell can inhibit the growth of the fungus Alternaria Porri, Botryodiplodia teobromae, Colletotrichum capsici and Sclerotium rolfsii. Liquid smoke of palm kernel shell also has potential as a contact fungicide to eradicate airborne seed of fungus pathogens.

The growth of the fungus, C. capsici, is characterized by the growth of mycelium which forms the dark brown spots or dry black dots that colonize and extend into soft rot. Severe attacks can cause fruits to shrivel and have color-like straw (Junadi, 2013).

**Spot diameter due to the varying concentration of liquid smoke**: Results of the analysis of variance showed that some degree of concentration of liquid smoke had effects on the size of the spot diameter formed by the fungus causing Anthracnose disease. The average spot diameter due to concentration treatment of liquid smoke can be seen in Fig. 3. It can be seen that the concentration of the treatment had a significant effect on the spot diameter of the fungus that causes the anthracnose disease. The highest value of spot diameter was found on K5 treatment, without liquid smoke with the value of 0.801 cm. While the lowest value of spot diameter was found on K5 treatment with concentration of 6% by value of 0.454 cm which is not different from the concentration of K3, K4 and K5 treatments.

The highest value of the spot diameter appeared on K5 treatment; it was caused by the absence of liquid smoke of any concentration. Although, statistically, the average value of the spot diameter shown on K5 treatments was no different from the other concentrations, the growth of fungus causing Anthracnose disease were able to be inhibited. The component activities of high organic acid content of phenolic compounds are known to damage the cell membrane and to disrupt the cell metabolism and also, inhibits the cell growth of the fungus. For the efficiency and effectiveness of the use of liquid smoke as fungicides, appropriate dosages and targets were put in place in an effort to control the disease by the use of liquid smoke, especially the ones derived from palm product.

**CONCLUSION**

Temperature and concentration had significant effect on the spot diameter of fungus that caused the anthracnose disease. Chemical compounds of liquid smoke in T1 treatment were able to inhibit the mycelial growth of C. capsici, causing anthracnose disease on chilies. The lowest spot diameter was found at K5 concentration with an average value of 0.454 cm and...
although, it was not significantly different from the treatment of $K_r = 0.504$ cm and $K_r = 0.519$ cm. The best treatment was obtained from the combination treatment of $T_2K_4$ (a pyrolysis temperature of 200-250°C and a concentration of 8% liquid smoke). The long incubation period (i.e., 4.5 DAI) resulted in the resistance of the chillies to the disease which could slow down the appearance of the disease symptoms and inhibit the growth of pathogens. Future research on the field test of the ability of liquid smoke as biofungicide is required to ensure its effectiveness.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Research, Technology and Higher Education of Indonesia for research grand of International Research Collaboration and Scientific Publication. The authors also thank Mr. Hendra Fahmi, Mr. Hariya Cahyo, and Ms. Afriyani who helped in the data analysis of this study.

REFERENCES


Jumadi, H., 2013. Application few vegetable fungicides with different concentrations to control anthracnose (Colletotrichum capsici) on hot pepper (Capsicum annuum L.). MSc Thesis, Syiah Kuala University, Banda Aceh, Indonesia.


