Journal of Engineering and Applied Sciences 11 (12): 2583-2587, 2016
ISSN: 1816-949X

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

[1]M. Faisal, [1]Asri Gani, [2]Husni, [3]Akhmad Baihaqi and [4]Hiroyuki Daimon
[1]Department of Chemical Engineering,
[2]Department of Agro Technology,
[3]Department of Agri Business, Syiah Kuala University, Banda Aceh, Aceh, Indonesia
[4]Department 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 T_2 was able to impair a growth of the fungal mycelium of *C. capsici*. The smallest spot diameter was found at concentration K_3 with an average value of 0.454 cm; although it was not significantly different from the sizes of treatment K_2 = 0.504 cm and K_5 = 0.519 cm. However, the best treatment was obtained from a combination of treatment T_2K_4 (Pyrolysis temperature of 200-250°C and liquid smoke concentration of 8%). A long period of incubation in treatment of T_2K_4 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; Damm *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., T_1= at temperature of 100-200°C; T_2 = at temperature of 200-250°C; T_3 = at temperature of 250-300°C; and T_4= 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 cork 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., T_1 = 100-200°C, T_2 = 200-250°C, T_3 = 250-300°C, T_4 = 300-350°C). The second factor was the liquid smoke concentration (K) with six levels which include K_0= control, K_1= 2%, K_2= 4%, K_3= 6%, K_4= 8%, K_5= 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 T_2 (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_2K_4 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

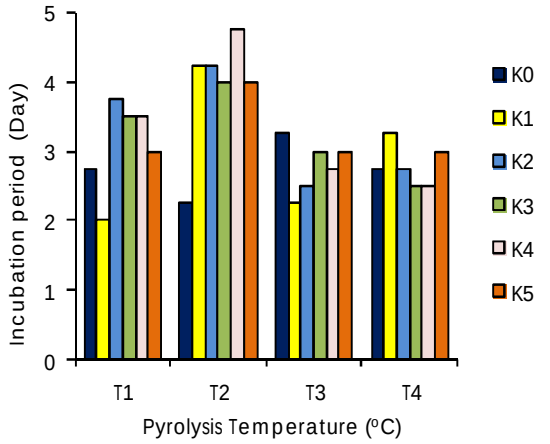

Fig. 1: The interaction between temperature pyrolysis liquid smoke and various concentrations of liquid smoke (K0 = control, K1 = 2% , K2 = 4%, K3 = 6%, K4 = 8%, K5 = 10%)

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 (Honesty Significant Different) test with the level of 5%, $HSD_{0.05}$ = 0.65, Coeficient Variance (CV) = 27.01%

Treatment (Pyrolysis temperature, °C)	Spot diameter (cm)
T1 =100-200	3.08 ab
T2 =200-250	3.92 b
T3 =250-300	2.79 a
T4 =300-350	2.79 a

period in T_2K_4 treatment resulted in the resistance of the chilies to the disease; this also occurred in other T_2 treatments (T_2K_1, T_2K_2, T_2K_3 and T_2K_5). 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 >f-table 0.05). The average value of the effect of temperature and concentration can be seen in Table 1.

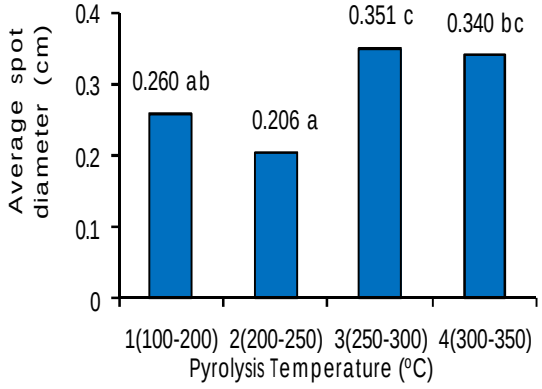

Fig. 2: Average spot diameter due to the influence of the temperature of liquid smoke produced. (Values followed by the same letter indicates no significant difference in the level of 5% HSD test, $HSD_{0.05}$ = 0.088, CV = 39.97%)

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 T_3 treatment with the temperature of 250-300°C (i.e., 0.351 cm). It was not too different with the diameter found in the T_4 treatment with the temperature of 300-350°C (i.e., 0.340 cm). While the lowest spot diameter was found in T_2 treatment with the temperature of 200-250°C (i.e., 0.206 cm). The liquid smoke in T_2 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 T_{1-4} 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 heterocycla 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, Liou* 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 (Yunikawati *et al*., 2013). Oramahi *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 T_1 = 2.49%, T_2 = 7.35% T_3 = 1.96% and T_4 = 0.94%.

The results of this research agree with the investigation of Kurniasih 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 (Jumadi, 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 K_0 treatment; without liquid smoke with the value of 0.801 cm. While the lowest value of spot diameter was found on K_3 treatment with concentration of 6% by value of 0.454 cm which is not different from the concentration of K_1, K_2, K_4 and K_5 treatments.

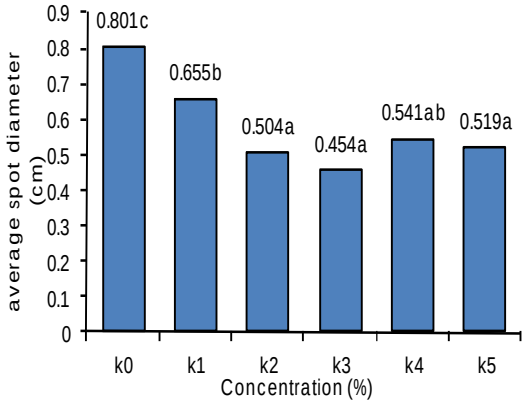

Fig. 3: Average spot diameter formed due to the treatment with various concentrations of liquid smoke. (Values followed by the same letter indicates no significant difference in the level of 5% HSD test, $HSD_{0.05}$ = 0.12, CV = 39.97%)

The highest value of the spot diameter appeared on K_0 treatment; it was caused by the absence of liquid smoke of any concentration. Although, statistically, the average value of the spot diameter shown on K_3 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 T_2 treatment were able to inhibit the mycelial growth of *C. capsici*, causing anthracnose disease on chilies. The lowest spot diameter was found at K_3 concentrations with an average value of 0.454 cm and

although, it was not significantly different from the treatment of K_2 = 0.504 cm and K_5= 0.519 cm. The best treatment was obtained from the combination treatment of T_2K_4 (a pyrolisis 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 chilies 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. Harita Cahyo and Ms. Afriyani who helped in the data analysis of this study.

REFERENCES

Cai, Z., G. Li, C. Lin, T. Shi and L. Zhai *et al*., 2013. Identifying pathogenicity genes in the rubber tree anthracnose fungus *Colletotrichum gloeosporioides* through random insertional mutagenesis. Microbiol. Res., 168: 340-350.

Chien, L.I.N.H., Y. Murase, T.C. Shiah, G.S. Hwang and P.K. Chen *et al*., 2008. Application of moso bamboo vinegar with different collection temperatures to evaluate fungi resistance of moso bamboo materials. J. Fac. Agr., Kyushu. Univ., 53: 107-113.

Damm, U., P.F. Cannon, J.H.C. Woudenberg and P.W. Crous, 2012. *Colletotrichum acutatum* species complex. Stud. Mycol., 73: 37-113.

Diao, Y.Z., J.R. Fan, Z.W. Wang and X.L. Liu, 2013. First report of *Colletotrichum boninense* causing anthracnose on pepper in China. Physiol. Mol. Plant Pathol., 84: 138-145.

Gani, A., H. Husni, A. Baihaqi and M. Faisal, 2014. Potential development of liquid smoke from oil palm solid waste as biofungicides. Intl. J. Sci. Eng., 7: 65-69.

Gautam, A.K., 2014. The genera colletotrichum: An incitant of numerous new plant diseases in India. J. New Biolog. Rep., 3: 9-21.

Ginayati, L. and S.M. Faisal, 2015. Form join pyrolysis liquid smoke from oil palm shells as a natural preservative know. J. Chem. Eng. Believe., 4: 7-11.

Guillen, M.D. and M.L. Ibargoitia, 1998. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. J. Agric. Food Chem., 46: 1276-1285.

Guillen, M.D. and M.L. Ibargoitia, 1999. Influence of the moisture content on the composition of the liquid smoke produced in the pyrolysis process of *Fagus sylvatica L.* wood. J. Agric. Food Chem., 47: 4126-4136.

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.

Karseno, D.P. and R. Kapti, 2001. Inhibitory power liquid smoke wood rubber latex pengkontaminan against bacteria and ribbed smoke sheet. Agritect, 21: 10-15.

Manandhar, J.B., G.L. Hartman and T.C. Wang, 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeoporioides*. Plant Dis., 79: 380-383.

Munch, S., U. Lingner, D.S. Floss, N. Ludwig and N. Sauer *et al*., 2008. The hemibiotrophic lifestyle of Colletotrichum species. J. Plant Physiol., 165: \-51.

Nantawanit, N., A. Chanchaichaovivat, B. Panijpan and P. Ruenwongsa, 2010. Induction of defense response against Colletotrichum capsici in chili fruit by the yeast Pichia guilliermondii strain R13. Biol. Control, 52: 145-152.

Oramahi, H.A., F. Diba and Wahdina, 2011. Anti-fungal activity of liquid smoke from wood sawdust acacia (*Acacia mangium* Willd) and Wood Laban (*Vitex Pubescens* Vahl). Bionatura J. Biol. Sci. Fisik, 13: 79-84.

Petkovsek, M.M., V. Schmitzer, J. Jakopic, V. Cunja and R. Veberic *et al*., 2013. Phenolic compounds as defence response of pepper fruits to Colletotrichum coccodes. Physiol. Mol. Plant Pathol., 84: 138-145.

Soldera, S., N. Sebastianutto and R. Bortolomeazzi, 2008. Composition of phenolic compounds and antioxidant activity of commercial aqueous smoke flavorings. J. Agric. Food Chem., 56: 2727-2734.

Than, P.P., R. Jeewon, K.D. Hyde, S. Pongsupasamit and O. Mongkolporn *et al*., 2008. Characterization and pathogenicity of colletotrichum species associated with anthracnose on chilli (Capsicum spp.) in Thailand. Plant Pathol., 57: 562-572.

Velmurugan, N., S.S. Chun, S.S. Han and Y.S. Lee, 2009. Characterization of chikusaku-eki and mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory scale. Intl. J. Environ. Sci. Technol., 6: 13-22.

Yunikawati, M.P.A., I.N.K. Besung and H. Mahatmi, 2013. Effectiveness against srikaya leaf juice power growth inhibition of *Escherichia coli*. Indonesia Medicus Veterinus, 2: 170-179.