PRELIMINARY STUDY OF THE ACTIVITY OF SOME ESSENTIAL OILS AGAINST *Fusarium oxysporum* f. sp. *cubense*

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A B S T R A C T

Wilt disease, caused by *Fusarium oxysporum* f. sp. *Cubense* (*Foc*), is one of the most important diseases of bananas. An Integrated Pest Management (IPM) strategy is a promising concept for controlling this disease. This concept must be supported with all suitable control techniques that can be compatibly and effectively utilized. Essential oils, which have been long recognized as having good fungus-toxic compounds, are a recommend technology which may be used to complete previous control techniques. The aim of this experiment was to evaluate the antifungal activity of essential oils extracted from *Cymbopogon nardus, Eugenia aromatica*, *Pogostemon calbin*, and *Vitiveria zizanoides* against *Foc*. The experiment was conducted in the January-April time period of 2010, in the laboratory of the Indonesian Tropical Fruit Research Institute, at room temperature. The result showed that the essential oils which had been tested were able to suppress *Foc* mycelial growth. Essential oil extracted from *E. aromatica* provided the strongest suppression of *Foc* mycelial growth, mainly when used at a volume of 9 and 18 µl. This result indicated that essential oil of *E. aromatica* had good potency and may be developed as a control agent against wilt disease of banana.

Keywords: essential oils, *Eugenia aromatica*, *Cymbopogon nardus*, *Pogostemon calbin*, *Vitiveria zizanoides*, *Fusarium oxysporum* f. sp. *cubense*, antifungal activity

INTRODUCTION

Banana is an important fruit source of vitamins, minerals, and carbohydrate. Plants can grow in various environmental conditions. They are found in almost all areas of Indonesia. Planting areas in Indonesia reach 74,751 hectares and total production is 4,384,384 tons (Anonymous, 2003). The majority of banana production areas are located on the island of Java (54%). Java

contributes 68% of the national banana production, while large potential lands are available on the islands of Sumatera (over 1 million hectares), Kalimantan, Sulawesi and Papua (over 3 million hectares) (Djohar et al., 1999). In Indonesia, the production of banana is higher than that of other fruits. However, there are some problems in banana cultivation which decrease the production. Two of the problems are pests and diseases which attack nurseries until postharvest. The important pest and diseases are: banana weevil borer, fusarium wilt disease, blood disease, and bunchy top virus (Hasyim et al., 2005; Jumjunidang et al., 2005; Nasir et al., 2005a,b).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Cubense*, commonly known as Panama disease, is one of the most important diseases attacking the plants throughout the world, including Indonesia. In Lampung, fusarium wilt and blood diseases caused economic losses of about Rp 2.4 billion (approximately US $ 6.8 million) during the 1993-1994 period (Nurhadi et al., 1994). Since 1995, a commercial banana farm, located in Halmahera, predicted huge losses of Rp 30 billion (US $ 8.6 million) each harvest season. Around a thousand hectares of these plantations had already been affected by fusarium wilt.

The Integrated Pest Management (IPM) strategy is a promising concept for controlling this disease effectively, because all suitable pest/disease control techniques are utilized in as compatible a manner as possible. Some control techniques that have been known to decrease the intensity of a fusarium attack are resistant-plant varieties and biological control (Ploetz, 2004; Houbin et al., 2004). These techniques could support the arrangement of an IPM model in controlling fusarium wilt. In order to gain a more comprehensive technology for controlling fusarium wilt, other alternative techniques such as natural resources, consumer safety and environmental pollution should be used together with the technology.

One of the alternative techniques is to use essential oil extracted from plants as a control agent against pest and disease. Previous researches stated that essential oils were potent and could be developed as control agents against pest and disease (Isman 2000). Essential oils extracted from *Cymbopogon martini*, *Cinnamomum zeylanicum*, and *Eugenia caryophyllata* had antifungal activity against *Botrytis cinerea* (Wilson et al., 1997). Singh et al., (1980) evaluated antifungal activity of some essential oils against 22 species of fungi. Essential oils extracted from lemongrass, clove, and zeylanikum may reduce colony growth of *Fusarium oxysporum* attacking chili peppers (Dahlan et al., 1998).

Latent infections were especially difficult to be controlled because the pathogen resides in an inactive state. Non systemic and synthetic fungicides as well as biological control agents are generally ineffective in controlling such infection. However, natural fungicidal volatiles may be useful in latent infection control (Wilson et al., 1997).

Based on this information, more studies are needed to evaluate the potency of essential oils for controlling fusarium wilt disease race 4, which is known to be dominant and attacks almost all banana varieties in Indonesia. The findings are expected to be able to induce the use of natural resources in assembling pest and disease control technologies.

MATERIAL AND METHODS

The research was conducted at the plant protection laboratory of the Indonesian Tropical Fruit Research Institute, at room temperature, from January to April 2010. Preparation, isolation of *Foc*, extraction of plant materials and an evaluation of the effect of the essential oils on *Foc* was carried out.

Preparation

The Petri dishes, 9 cm (Petri dish A) and 5 cm (Petri dish B) in diameter, were washed using water and then dried. Sterilization was conducted using autoclave at 121 ^{o}C for 30 minutes. At the same time, a Potato Dextrose Agar (PDA) medium was made. After sterilization, Petri dish B was laid onto Petri dish A and then PDA was poured into Petri dish A. Small pieces of filter paper (0.3 cm in diameter) containing the essential oils were laid on Petri dish B. This process was conducted in laminar flow.

Isolation and identification of *F. oxysporum* f. sp. *cubense* race 4

The isolate of *Foc* was obtained from a vascular streak of 'Ambon Hijau' (AAA, giant cavendish) banana plant infected by Foc. The tissue was picked up between sick and healthy tissue using a scalpel and then it was laid into a Petri dish filled with PDA medium, and incubated for 3 days. To obtain a pure isolate of *Foc*, this fungus was picked up between other fungi and moved into new PDA medium in another Petri dish. After 3 days, the single hypha containing some spores was picked up and put into sterile water to make a suspension. One oze of the suspension was inoculated on water agar. After 24 hours, a selected germinated single spore was picked up and put into PDA medium.

To ensure that this isolate is *Foc* race 4, it has been cultured on sterile-steamed rice. The aldehyde flavour which was emitted from this culture in a 7-10 days period, indicated that the isolate is Foc race 4 (Nasir et al., 2005b).

Extraction of plant materials

Leaves of *Cymbopogon nardus* and *Pogostemon calbin*, clove of *Eugenia aromatica*, and root of *Vitiveria zizanoides* were obtained from the field. They were wind dried for 4 days and then blended. The obtained material was extracted using a steam distillation method (Guillet et al., 1998). The essential oils were poured into a brown bottle (volume 5 ml) and stored in a refrigerator.

Evaluation of the effect of the essential oils on *Fusarium oxysporum* f. sp. *cubense*

Potato Dextrose Agar in Petri dish A was inoculated with a pure isolate of

Foc. The diameter of this fungus colony was 0.5 cm. The essential oils tested in this treatment were pipetted onto the piece of paper (0.3 cm in diameter). The paper containing the essential oil was laid into Petri dish B that had been previously put inside Petri dish A. Then Petri dish A was covered with a cover and sealed with parafilm (Fig. 1). This was to ensure that the effect of the essential oils was only from the vapour of these oils.

The experiment was carried out in a completely randomized design. The treatments consisted of 3 µl, 9 µl, and 18 µl of essential oils, and the control (untreated). The tested essentials oils were extracted from *C. nardus*, *P. calbin*, *E. aromatica*, and *V. zizanoides*. The parameters observed were the daily *Fusarium* mycelial growth under two conditions: while the essential oil were present and when the essential oil was eliminated. The period in which the essential oil was present started from the first application of the essential oils until the mycelial growth of *Fusarium* in the control had filled the volume of Petri dish A. Afterward, pieces of papers containing essential oil in Petri dish B were removed, and this was referred to as the elimination of the essential oil period. In this period, the *Fusarium* mycelial growth which had not been treated with essential oil was not observed. There was no need to observe because the space of Petri dish A had been filled with the mycelium. Whereas, the treated remainder were observed continuously until 21 days after the first application of the essential oil or 7 days after the papers containing the essential oil were eliminated.

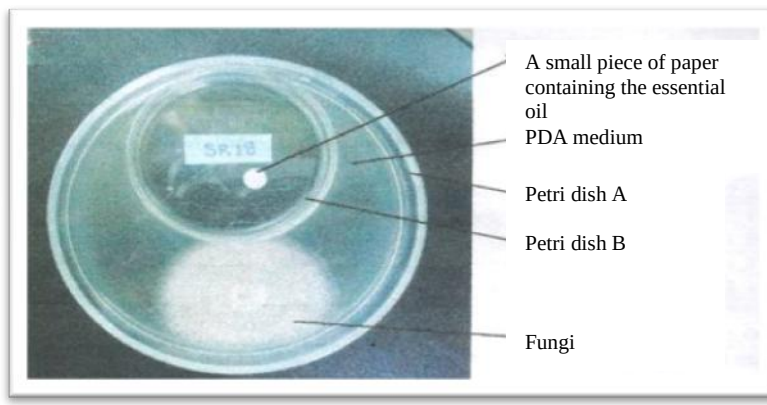


Figure 1. Treatment of the *Fusarium oxysporum* f. sp. *cubense* with essential oil

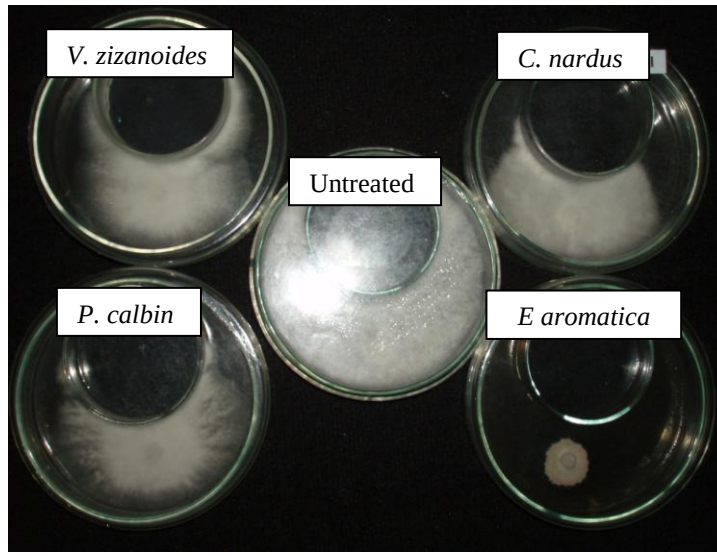


Figure 2. Mycelial growth of *F. oxysporum* f. sp. *cubense* treated with essential oil of *C. nadus, V. zizanoides, P. calbin, E. aromatica* at 9 µl, and the untreated control

RESULT AND DISCUSSION

The result showed that essential oils extracted from *C. nardus*, *E. aromatica*, *P. calbin* and *V. zizanoides* had a negative effect on *F. oxysporum* f. sp. *cubense* (Fig. 2). The growth of mycelium was significantly suppressed when it was treated with an essential oil compared to the untreated example. Suppression took place from the sixth day after treatment (Tab. 1).

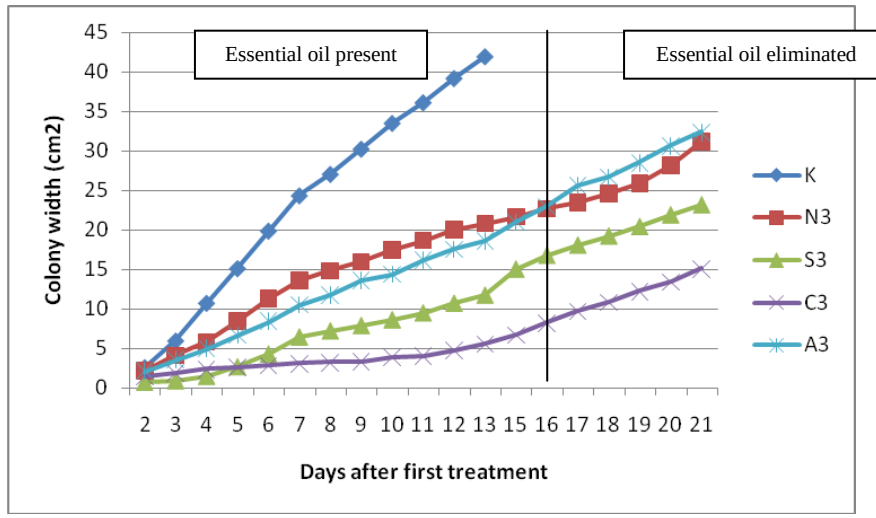
Each essential oil demonstrated different suppression to the mycelial growth of *Foc* during study period; either the influence of the essential oil existed or was eliminated (Tab. 1). Essential oil extracted from *E. aromatica* provided the highest suppression to *Fusarium* mycelial growth, mainly at 9 and 18 µl, and significantly differed from the other 3 essential oils tested. This effect took place in both treatment conditions: where the essential oil existed and where the essential oil was eliminated. The significant negative effect of essential oil extracted from *E. aromatica* at 9 and 18 µl, appeared from the 17th day after treatment (Tab. 1 and Fig. 4-5).

Essential oil extracted from *C. nardus* had a higher suppression effect on the growth of *Foc* mycelium and this oil was significantly different from the essential oil extracted from *V. zizanoides* and *P. calbin*, mainly at a volume of 18 µl. The suppression effect of this essential oil had been proven since the second day after treatment. However, essential oil from *C. nardus*

Table 1. The colony width of *Fusarium oxysporum* f. sp. *Cubense* when treated with essential oil extracted from *Cymbopogon nardus*, *Eugenia aromatica*, *Pogostemon calbin*, and *Vitiveria zizanoides* and when the oils were removed

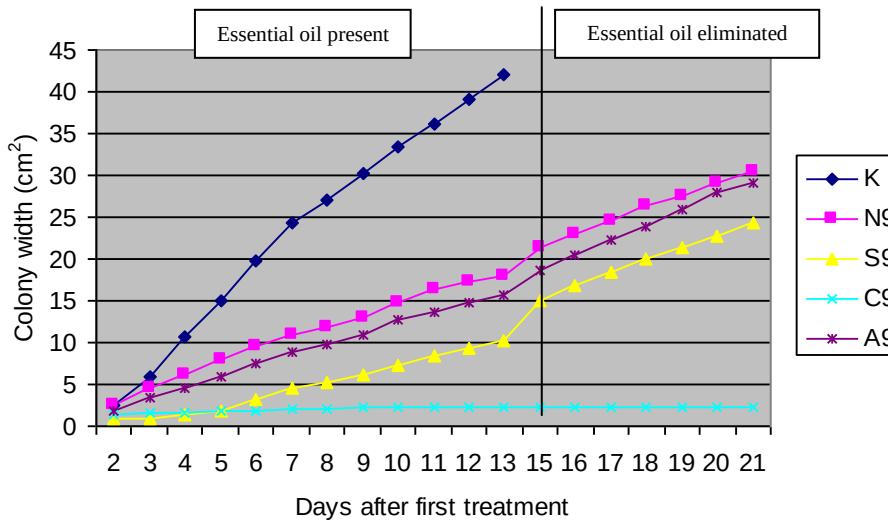
Source of the essential oil	Volume (µl)	The colony width (cm^2) at ….day after treatment							
		2nd	**6th**	**10th**	**13rd**	**15th**	**17th**	**19th**	**21st**
C. nardus	3	0.72 e*	4.30 ef	8.62 cd	11.73 cd	15.02 bc	18.04 bc	20.37 a -d	23.13 a-d
	9	0.80 de	3.19 f	7.18 d	10.32 de	14.99 bc	18.46 a-c	21.43 a-c	24.38 a-d
	18	0.80 de	1.32 f	2.01 e	3.06 f	6.51 de	10.09 d	14.29 cd	18.41 cd
E. aromatica	3	1.47 b-d	2.82 f	3.95 de	5.55 ef	6.69 de	9.71 d	12.2 d	15.1 d
	9	1.42 b-de	1.85 f	2.16 e	2.16 f	2.16 e	2.16 e	2.16 e	2.16 e
	18	0.95 cde	1.42 f	1.42 e	1.42 f	1.42 e	1.42 e	1.42 e	1.42 e
P. calbin	3	2.16 ab	11.33 b	17.43 b	20.81 b	21.63 a	23.42 ab	25.90 ab	31.17 ab
	9	2.50 a	9.53 bc	14.67 b	18.04 b	21.37 a	24.60 ab	27.56 a	30.54 ab
	18	1.54 bc	4.78 d-f	7.86 cd	10.75 de	11.76 cd	15.47 cd	18.15 bcd	21.16 b-d
V. zizanoides	3	2.08 ab	8.38 b-d	14.34 b	18.56 b	20.95 a	25.59 a	28.49 a	32.38 a
	9	1.91 ab	7.58 c-e	12.71 bc	15.58 b-d	18.55 ab	22.36 a-c	25.92 ab	29.18 ab
	18	1.77 b	7.51 c-e	12.68 bc	17.53 bc	19.39 ab	22.09 a-c	24.38 ab	26.92 a-c
Untreated		2.54 a	19.85 a	33.51 a	41.96 a				
		Essential oils present				**Essential oils removed**			

*Mean values in each column with the same letters are not significantly different at p = 0.05 based on Duncan Multiple Range Test (DMRT)



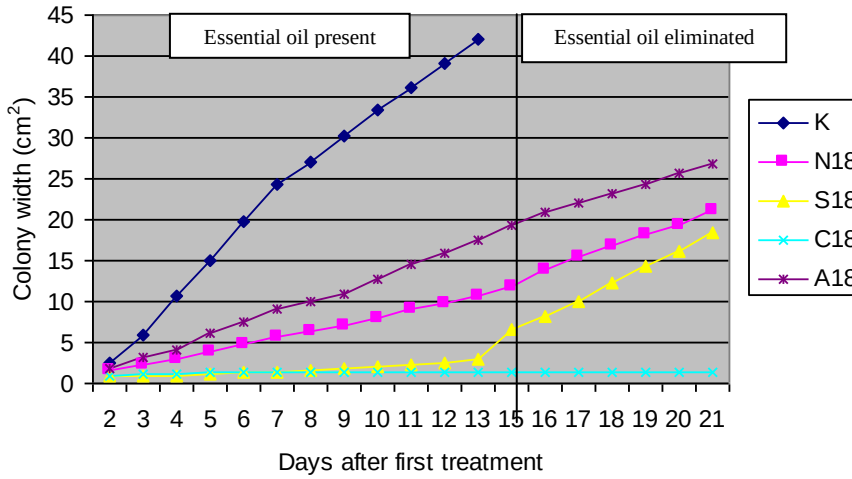
Note: K = untreated, N = *P. calbin*, S = *C. nardus*, C = *E. aromatica*, A = *V. zizanoides*

Figure 3. The growth of *Fusarium oxysporum* f. sp. *cubense* mycelium during the observation period, when 3 µl of essential oil was applied



Note: K = untreated, N = *P. calbin*, S = *C. nardus*, C = *E. aromatica*, A = *V. zizanoides*

Figure 4. The growth of *Fusarium oxysporum* f. sp. *cubense* mycelium during the observation period when 9 µl of essential oil was applied



Note: K = untreated, N = *P. calbin*, S = *C. nardus*, C = *E. aromatica*, A = *V. zizanoides*

Figure 5. The growth of *Fusarium oxysporum* f. sp. *cubense* mycelium during the observation period when an 18 µl of the essential oil was applied

could not stop the growth of the pathogen until the last observation. When the essential oil from *C. nardus* was eliminated, the growth of *Foc* mycelium which had been treated with this essential oil was slower than *Foc* mycelium treated with essential oil from *V. zizanoides* and *P. calbin* (Tab. 1 and Fig. 3-5).

Regardless of the condition, the suppression of *Foc* mycelia growth was lower on the essential oil of *P. calbin* than on the essential oil of *C. nardus*. An amount of 18 µl of *P. calbin* essential oil was more effective at suppressing the growth of this fungus than 3 and 9 µl. As with the *C. nardus* essential oil, the *P. calbin* essential oil also could not stop *Fusarium* mycelial growth until the last observation.

The essential oil extracted from *V. zizanoides* was the least effective in controlling the fungus. Treatment with *V. zizanoides* essential oil showed a wider colony size of *Fusarium* mycelial growth than in the treatments with the other 3 essential oils. The 3 different amounts of this essential oil showed no significant differences in suppressing the *Fusarium* mycelial growth. This result was different from the results of the other 3 essential oils tested, as the other 3 oils showed a different effectiveness at the 3 amount levels tested. The oil extracted from *C. nardus* and *P. calbin* showed effectiveness at 18 µl, while the oil extracted from *E. aromatica* showed effectiveness at 9 and 18 µl. However, the *Fusarium* mycelial growth treated with essential oil from *V. zi-*

zanoides was significantly slower than in the untreated example.

The *Fusarium* mycelial growth pattern during treatments using 3, 9 and 18 µl is presented in Figures 3-5. As mentioned above, mycelia treated with essential oils showed slower growth than the growth of the untreated mycelium. At 3 µl, none of the essential oils tested could stop mycelium growth during the study. At 9 and 18 µl, essential oil from *E. aromatica* was the most highly effective since it could stop *Fusarium* mycelial growth even when the influence of this essential oil was terminated. This study revealed that essential oil extracted from *E. aromatica* was the most effective in inhibiting *Fusarium* mycelial growth, hence it is promising as an alternative technology for controlling *Foc*.

The mechanism of these essential oils in inhibiting the growth of *Foc* mycelium had so far not been understood. However, in some previous research it was noted that essential oil caused some alteration of hyphae. Pina-Vaz et al. (2004) stated that essential oils caused morphological alteration. In general, hyphae appeared to collapse, was frequently flexuous, and showed surface alterations. In some cases, a fungicidal effect resulted primarily from an extensive lesion of the cell membrane. According to Chami et al. (2003) scanning electron microscopy analysis revealed that the surface of *Saccharomyces cerevisiae* cells that had been treated with oregano and clove oils were significantly damaged (Chami et al., 2003).

Based on the result of this research, essential oil showed good potency which can be developed further as an alternative control technology. Such technologies need to be developed for the safety of the environment and the consumer. These essential oils, if properly formulated and applied, can be used directly as a synthetic fungicide replacement. With the precipitous withdrawal of methyl bromide as a fumigant, it may be profitable to explore natural plant volatiles. Research into other effective essential oils having antifungal activity against *Foc* should be done. A dependence upon only one kind of essential oil can then be avoided. Furthermore, there must be evaluations of the essential oils in field conditions so that the tests can be put into practice.

CONCLUSIONS

1. Essential oils extracted from *C. nadus*, *V. zizanoides*, *P. calbin*, and *E. aromatica* were able to suppress the growth of *Fusarium oxysporum* f. sp. *cubense*.
2. Among the essential oils tested, oil of *E. aromatica* was the most effective in controlling mycelial growth of *Foc*. Mycelial growth could be stopped even when the influence of the essential oil of *E. aromatica* was eliminated.
3. The effective amounts of essential oil extracted from *E. aromatica* were 9 µl and 18 µl.

REFERENCES

Anonymous 2003. Information of Horticulture and Various Crops. Director-

ate General Production of Horticulture and Various Plants. Jakarta. Indonesia.

Chami F., Chami N., Bennis S., Bouchikhi T., Remmal A. 2003. Oregano and clove essential oils induce surface alteration of *Saccharomyces cerevisiae.* http://www3.interscience.wiley.com/cgi-bin/abstract/111080150/ABSTRACT?CRETRY=1&SRETRY=0.

Dahlan S., Nasrun, Erni S. 1998. Study of effects of essential oil of some crops to *Fusarium oxysporum* cause of wilt disease of chili (*Capsicum annum*) in vitro. Proc. Perhimpunan Fitopatologi Indonesia seminar, pp. 131-136.

Djohar H.H., Wahyunto, Suwandi V., Subagio H. 1999. The potency of area development for banana in Indonesia. INDONESIAN AGRIC. RES. DEVELOP. J. 18(2): 46-55.

Guillet G., Belanger A., Arnason J. 1998. Volatile monoterpenes in *Porophyllum gracile* and *P. ruderale* (Asteraceae): Identification, localization, and insecticidal synergism with α–terthienyl. PHYTOCHEMISTRY 49(2): 423-429.

Hasyim A., Yasir H., Azwana 2005. Seleksi substrat untuk perbanyakan *Beauveria bassiana* (Balsamo) vuillemin dan infektivitasnya terhadap hama penggerek bonggol pisang, *Cosmopolites sordidus* Germar. J. HORT. 15(2): 116-123.787.

Houbin C., Chunxiang X., Qirui F., Guibing H., Jianguo L., Zehuai W., Molina A.B. Jr. 2004. Screening of banana clones for resistance to Fusarium wilt in China. ADVANCING BANANA PLANTAIN R & D ASIA PACIFIC 13: 165-174.

Isman M.B. 2000. Plant essential oils for pest and disease management. CROP PROTECT 19: 603-608.

Jumjunidang, Nasir N., Riska, Handayani H. 2005. Teknik pengujian in vitro ketahanan pisang terhadap penyakit layu Fusarium menggunakan filtrat toksin dari kultur *Fusarium oxysporium* f. sp. *cubense*. J. HORT. 15(2): 135-139.

Nasir N., Jumjunidang, Riska. 2005a. Distribusi penyakit layu Fusarium dan layu bakteri Ralstonia pada lokasi sumber bibit dan sekolah lapang pengendalian hama terpadu pisang di Sumatera Barat. J. HORT. 5(3): 215-222.

Nasir N., Jumjunidang, Riska. 2005b. Deteksi dan pemetaan distribusi *Fusarium oxysporum* f. sp. *cubense* pada daerah potensial pengembangan agribisnis pisang di Indonesia. J. HORT. 5(1): 50-57.

Nurhadi, Rais M., Harlion 1994. The disease incidence of bacterial and Fusarium wilt disease in Lampung province. INDONESIAN INFO HORT. 2(1): 35-37.

Pina-Vaz C., Gonçalves Rodrigues A., Pinto E., Costa-de-Oliveira S., Tavares C., Salgueiro L., Cavaleiro C., Gonçalves M.J., Martinez-de-Oliveira J. 2004. Antifungal activity of Thymus oils and their major compounds. J. EUR. ACAD. DERMATOL. VENEREOL. 18(1): 73-78.

Ploetz R. 2004. Diseases and pests: A review of their importance and management. INFO MUSA, INTER. J. BANANA PLANTAIN 13(2): 11-16.

Singh J., Wan M.T., Isman M.B., Moul D.J. 1980. Fungitoxic activity of some essential oils. ECON. BOT. 34: 186-190.

Wilson C.I., Solar J.M., El Ghaouth A., Wisniewski M.E. 1997. Rapid evaluation of plant extracts and essential oil for antifungal activity against *Botrytis cinerea*. PLANT DIS. 81: 204-210.

WSTĘPNE BADANIA WPŁYWU NIEKTÓRYCH OLEJKÓW ETERYCZNYCH NA ZWALCZANIE *Fusarium oxysporum* f. sp. *cubense*

Mizu Istianto i Deni Emilda

S T R E S Z C Z E N I E

Choroba roślin spowodowana przez grzyb *Fusarium oxysporum* f. sp. *Cubense* (*Foc*) jest jedną z najczęstszych występujących u bananów. Zintegrowane metody ochrony przed szkodnikami (IPM) są obiecującą strategią kontrolowania tej choroby, jednak aby były one skuteczne, muszą być wsparte wszystkimi możliwymi technikami. Od dawna wiadomo, że olejki eteryczne zawierają składniki o działaniu toksycznym na grzyby. Dlatego też są one rekomendowane do ich zwalczania. Celem badań była ocena wpływu olejków eterycznych uzyskanych z *Cymbopogon nardus*, *Eugenia aromatica*, *Pogostemon calbin* i *Vitiveria zizanoides* przeciw grzybowi *Foc*. Badania przeprowadzono w temperaturze pokojowej od stycznia do kwietnia 2010 roku w laboratorium w Indonezyjskim Instytucie Owoców Tropikalnych. Wyniki wykazały, że badane olejki eteryczne mogą powstrzymać rozwój grzybni *Foc*. Najlepsze właściwości przeciwgrzybiczne wykazał olejek uzyskany z *E. aromatica* w stężeniach 9 µl i 18 µl. Olejek eteryczny z *E. aromatica* wydaje się mieć dobry wpływ na zwalczanie grzyba. Warto więc rozwinąć badania nad jego zastosowaniem do walki z chorobą grzybową bananów.

Słowa kluczowe: olejki eteryczne, *Eugenia aromatica*, *Cymbopogon nardus*, *Pogostemon calbin*, *Vitiveria zizanoides*, *Fusarium oxysporum* f. sp. *Cubense*, działanie przeciwgrzybiczne