Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [12]: 95-104
[http://scholarsresearchlibrary.com/archive.html]

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

In-vitro Consistently Biopesticide Effects of *Piper aduncum* and *Cymbopogon flexuosus* Essential Oils against *Phytophthora palmivora* Colony Growth

Nasril Nasir[1], Vera Putria Murza[1], Anthoni Agustien[1*], Mairawita[1], Feskaharny Alamsjah[1] and Nurmansyah[2]

[1]*Faculty of Mathematics and Natural Sciences, Department of Biology, Andalas University, Padang 25163, West Sumatra Indonesia*

[2]*Laing Research Station of Bogor, Research Institute for Spices and Medicinal Plants in Solok, Indonesia*

***Corresponding author:** Anthoni A, Faculty of Mathematics and Natural Sciences, Department of Biology, Andalas, University, Padang, West Sumatra, Indonesia. E-mail: aagustien@gmail.com*

ABSTRACT

One of the major disadvantages of natural plant products as a biopesticide is due to its inconsistence pesticide effects. This in-vitro work aimed to observe the best dosage and consistency effect of essential oils of P. aduncum and C. flexuosus to P. palmivora. P. palmivora is a major obstacle of cocoa production in West Sumatra with disease percentage up to 40%. Activities were carried out at the laboratory of microbiology, Biology Department Andalas University in Padang and laboratory of plant protection of Bogor Research Institute for Spices and Medicinal Plants in Solok, both laboratories are in West Sumatra Indonesia. Research used completely randomized design (CRD) in a factorial with 10 treatments included control and three replications. The dosage of essential oils used were control, 250 ppm, 500 ppm and 1000 ppm. The best result was the mixture of these essential oils at the dosage of 1000 ppm due to its ability to control colony growth of P. palmivora to 100% and its fungicide consistency effect.

Keywords: Cocoa, *P. palmivora*, Production of cocoa, Essential oils, Fungicide consistency effect.

INTRODUCTION

Phytophthora palmivora is the most virulent pathogen on cacao and causes cacao black pod disease in the world [1,2], including in West Sumatra [3- 5]. The pathogen's attack causes rotten fruit, brownish-black with clear boundary between the healthy and diseased fruit, commonly starting from the base of the fruit and then cover almost the entire surface of the fruit [6]. In other parts of Indonesia, severe attacks of *P. palmivora* caused production down to 80% [7], even in Africa and Latin America the attack causes a total failure [8-10]. In some cocoa production centers in the Province of West Sumatra, production ranged only between 20-30% [11] of the potential production that can reach 2000 kg per ha [12]. Currently none of the cocoa cropping areas free of *P. palmivora* in the province [13]. Though Indonesia has a target to boost national cocoa bean production to 1 million tons per year by 2013 [14].

Chemical control of such diseases is expensive and unattractive from commercial and environmental points of view [15,16]. In West Sumatra, continuous research by mainly uses natural plant products (essential oil) as a source of biopesticides for controlling *P. palmivora* on cocoa has been started since the last 5 years [17-19]. The results of these studies succeeded to decrease *P. palmivora'*s colony growth, however fungicide activities of the essential oils used still inconsistence [20,21]. Basic considerations using *P. aduncum* and *C. flexuosus* in this study is to gain the best natural plant product as well as focus on the potential use of local West Sumatra's plants that can be used as a source of biopesticides. In fact, most biopesticides had the advantage of higher selectivity and non-target biological safety [22].

P. aduncum is a wild plant that is often found on the edge of the forest, riparian, shrub and hills in West Sumatra with the height can reach up to 6 m [23]. The main components of essential oils *P. aduncum* is phenylpropanoid dilapiol (32.9 to 61.8%) which is an effective antifungal [24]. While *C. flexousus* commonly known as lemongrass that has antifungal and antibacterial activity mainly because it contains geraniol and geranil acetate. These compounds are effectively control the pathogens *Aspergillus flavus, Aspergillus fumigatus* and *Staphylococcus aureus* [25,26].

MATERIALS AND METHODS

Activities carried out at the laboratory of microbiology, Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University in Padang Indonesia from September 2016 to April 2017. Research used a completely randomized design (CRD) in a factorial with 10 treatments included control and three replications.

The treatments are:

A1B1 Essential oils *P. aduncum* 250 ppm, A1B2 Essential oils *P. aduncum* 500 ppm, A1B3 Essential oils *P. aduncum* 1000 ppm, A2B1 Essential oils *C. flexuosus* 250 ppm, A2B2 Essential oils *C. flexuosus* 500 ppm, A2B3 Essential oils *C. flexuosus* 1000 ppm, A3B1 *P. aduncum* 1000 ppm + *C. flexuosus* 250 ppm, A3B2 *P. aduncum* 1000 ppm + *C. flexuosus* 500 ppm, A3B3 *P. aduncum* 1000 ppm + *C. flexuosus* 1000 ppm and A0B0 control.

Isolates and essential oils

Fruit with *P. palmivora* symptom as the source of inoculum was collected in a 2 ha cocoa plantation in the District of Padang Pariaman, West Sumatra. Up to 40% of the cocoa plants in this plantation are being infected by *P. palmivora* [27]. Fruit surface

sterilization was conducted with 70% alcohol and allowed for three minutes. The surface of the fruit that has been sterilized were taken (diameter and thickness of 5 mm) using a sterile cork borer, implanted onto PDA medium then incubated for 1 week . Later this isolate purified based on Tondok et al. [27] at the microbiology laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, Andalas University. The pure isolate of *P. palmivora* then was used as a source of inoculum in this study. Essential oils of *P. aduncum* and *C. flexuosus* that were used at concentration of 25% are the properties of Laing Research Station of Bogor Research Institute for Spice and Medicinal Plants in Solok, West Sumatra, Indonesia.

Equipment sterilization and PDA preparation

All tools such as petridishes, test tubes, erlenmeyer were cleaned and soaked, then wrapped with paper and sterilized in an autoclave at a temperature of 121°C with a pressure of 15 psi for 15 minutes. Tools such as needle loop, pipette, stirring rod were sterilized by using 70% alcohol. The medium used was PDA (Potato Dextrose Agar) that was prepared by dissolving 39 g instant PDA into 1 liter of distilled water. Then put in erlenmeyer and boiled. Before using, the medium re-sterilized with autoclave at a temperature of 121°C at a pressure of 15 lbs for 20 minutes [28].

Inhibition ability test

This test was performed according to Nasir [29]. Each treatment of factors A1 (*P. aduncum*), A2 (*C. flexuosus*) and A3 (mixture) with three different concentrations of B1=250 ppm, B2=500 ppm and B3=1000 ppm were poured into PDA medium (± 45^0 C) in several 250 ml erlenmeyers. Then the medium with the essential oils formula were homogenized and poured into 9 cm petridishes and let to hardened. Furthermore the pure inoculums of *P. palmivora* were planted in the middle of PDA medium by putting a 5 mm diameter of fungal mat by using sterile corkborer. Each treatment was replicated three times and incubated at room temperature. Observations were made on the first, third, fifth, and seventh day after inoculation by measuring the diameter growth of fungal colonies in each treatment. Observation was stopped when the diameter of fungal colonies already filled the entire surface of PDA in petridish in the control treatment. The inhibition ability of *P. aduncum*, *C. flexuosus* and the mixture of both essential oils against fungal colonies to *P. palmivora* was calculated based on Awang et al. [30]:

$$\text{Inhibition rate (\%)} = \text{C - T/T} \times 100\%$$

C = The diameter (mm) of untreated fungal colony (control).
T = The diameter (mm) of treated fungal colony.

Resistance test

Resistance tests carried out based on Nasir [31] on the inoculum of *P. palmivora* derived from the most high suppression growth caused by essential oil treatments. The inoculum was taken by using 2 mm sterilized cork borer then re-planted at the middle of PDA pure medium in 5 petridishes (replications). Observation was conducted up to 7 days after replanting. After 7 days, fungicide consistency effect was decided by 100% inhibition of colony growth which was corresponded to the absence of growth on the plate [32,33].

RESULTS AND DISCUSSION

Source of inoculum derived from the infected fruit by *P. palmivora* (Figure 1) in a cocoa plantation (farmer property) in the district of Padang Pariaman, West Sumatra Province. The percentage of *P. palmivora* attacked in this 2 ha plantation has been reported up to 40% [34].

Inhibition rate

All the essential oil treatments suppressed the growth of colonies of *P. palmivora* (*in-vitro*), but the best result in inhibiting the growth up to 89.05% was by the mixture of *P. aduncum* with *C. flexuosus* (Table 1). While the ability of inhibition rate of a single treatment of *P. aduncum* essential oil was 67.05% higher than *C. flexuosus* which was 59.95%.

Figure 1: Cocoa fruits attacked by *P. palmivora* in the District of Padang Pariaman West Sumatra. Healthy-looking fruit is also has a great potential to be attacked (*doc*. Nasril Nasir, 2016).

Table 1: Effect of essential oils *P. aduncum, C. flexuosus* and the mixture towards inhibition colonies growth of *P. palmivora* on the 7th day after inoculation (7DAI).

No	Treatment	Inhibition rate (%)
1	*P. aduncum*	67.05[b]
2	*C. flexuosus*	59.95[c]
3	Mixture	89.05[a]
4	Control	0.00[d]
Note: CV (%) 7.15 Numbers followed by the same small letter in each column of significantly different at 5% DMRT test (7 days after inoculation).		

Separately, both of these essential oils were also reportedly capable of controlling the pathogenic fungi such as *Sclerotium rofsii, Phytophthora capsici, Colletotrichum musae, Fusarium oxysporum, Aspergillus kongini, Alternaria solani, Curvularia lunata, Fusarium nivale, Pestalotia* sp, *Rhizoctonia solani, Trichophyton mentagrophytes, Aspergillus* sp *and Verticillum* sp [35-37]. In terms of concentration dosage using essential oils of *Elettariosis slahmong, Syzygium aromaticum* and *Cinnamomum burmanii*, the dose of 1000 ppm resulted the best suppression effect up to 100% to the colonies growth of *P. palmivora* on cocoa, *Rigidoporus macroporus* on rubber and *Colletotrichum gloeospoiroides* on red dragon fruit [38-40].

Increase in dose concentration of essential oils have also been reported significantly suppressed colony growth and pathogenicity of the pathogens [41-43]. In this study, dose of 1000 ppm of each type of essential oils and the mixture, suppressed the growth of colonies of *P. palmivora* up to 100% (Table 2).

Table 2: Effect of concentration levels of essential oils against fungal growth inhibition of *P. palmivora* on the 7th day after inoculation (7DAI).

No	Treatment	Inhibition rate (%)
1	250 ppm	40.68 c
2	500 ppm	76.14 b
3	1000 ppm	100.00 a
4	Control	0.00d
Note: CV (%) = 7.15 Numbers followed by the same small letter in each column of significantly different at 5% DMRT test (7 days after inoculation).		

P. aduncum contain antifungal compounds those are fenillpropanoid, isoeugenol, phenol, dilapiol, monoterpenes, sesquiterpenes, cineol, piperton, β- caryophyllen. The content of essential oil of *P. aduncum*, mainly terpenes compound, combined with sitronellal, geraniol, geranyl acetate and mono-olefins terpenes such as limonene [44] of *Cymbopogon* simultaneously turns their strength antifungal activities, as it was also found in this study (Table 3). Eventhough not all the mixture of essential oils are able to provide the best results in suppressing the growth of pathogens [45], however [46-49] found that mixture increased the effectiveness of essential oils by accelerating suppression time and fungicide effect capability against the pathogen. The strength antifungal activities due to the influence of dose and mixture of essential oils to decrease *P. palmivora* colony growth are presented in Table 3.

During this study, the shortening at the tip of hyphae occurs mainly in the mixture treatment with 1000 ppm. Knobloch et al. [50] stated that essential oil damages plasma membrane associated with proteins and enzymes and catalyzes cell membrane to penetrate cell walls of fungi. The chemical components are also accumulating fat globules in the cytoplasm, interferes cell metabolism, reduces mitochondrial and damages nuclear membrane resulting in fungal cell death. According to Fessenden [51], fungal cell membrane formed from the protein that is fused with a double layer of molecules phosphoglycerides with the hydrophobic tip facing into and the hydrophilic facing out. Essential oil compound with a high concentration of diffusing is

captured by hydrophilic sensor. Hydrophilic component that will bind the molecules of essential oil, causing lysis of the membrane lipoproteins and inhibits the growth of cell walls.

Table 3: Inhibition rate on the *P. palmivora* colonies growth caused by the level of concentrations and mixture of the essential oils.

No.	Treatment	InInhibition rate (%)
1.	A1B1 (*P. aduncum* 250 ppm)	23.20 [e]
2.	A1B2 (*P. aduncum* 500 ppm)	77.90 [c]
3.	A1B3 (*P. aduncum* 1000 ppm)	100.00 [a]
4.	A2B1 (*C. flexuosus* 250 ppm)	19.23[e]
5.	A2B2 (*C. flexuosus* 500 ppm)	60.63[d]
6.	A2B3 (*C. flexuosus* 1000 ppm)	100.00 [a]
7.	A3B1 (Mixture 250 ppm)	79.56 [c]
8.	A3B2 (Mixture 500 ppm)	89.90 [b]
9.	A3B3 (Mixture 1000 ppm)	100.00 [a]
10	Control	0.00 [f]
Note: CV: 7.15% Numbers followed by the same small letter in each column of significantly different at 5% DMRT test (7 days after inoculation).		

Damage to the cell wall which is a protector of the fungus resulting in cell death of the fungus. One or more of the capacities of the essential oils expressed by Chairgulprasert et al. [52] and Pino et al. [53] are found in this study, resulting in clumping, shortening or abnormal branching of hyphae as in Figure 2.

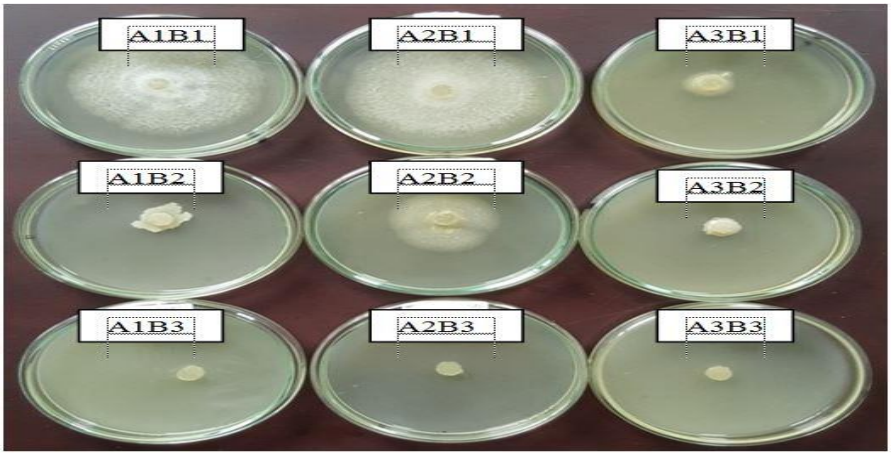

Figure 2: The difference size of the diameter of colonies of *P. palmivora* due to effect of essential oil treatments *P. aduncum*, *C. flexuosus* and the mixture of both at the end of the observation at seven days after inoculation (7 DAI). (A1B1 = *P. aduncum* 250 ppm; A1B2 = *P. aduncum* 500 ppm; A1B3 = *P. aduncum* 1000 ppm; A2B1 = *C. flexuosus* 250 ppm; A2B2 = *C. flexuosus* 500 ppm; A2B3 = *C. flexuosus* 1000 ppm; A3B1 = mixture of 250 ppm; A3B2 = mixture of 500 ppm; A3B3 = mixture of 1000 ppm).

Resistance test

Eventhough all dosages of 1000 ppm inhibited the growth of colonies of *P. palmivora* up to 100%, in term of the consistency, the best result was caused by the mixture of essential oils of *P. aduncum* with *C. flexuosus.* Observation of the consistency effect of this treatment was carried out for seven days. During the resistance test observation, there was no re-growth at all from the colony derived from the treatment of 1000 ppm of mixture of *P. aduncum* with *C. flexuosus*. The inconsistence biopesticide effects of natural plant products relate to the soil fertility level, agroclimatic conditions, geographic origin and the maturity at harvest which are expressed in their chemical contents [54-57], as well as which part of the plant [58-60] or species in a same genus is used as the source of the extract The methodology of extraction is also a very important procedure to find maximum chemical compound of the plant. In this study, consistency fungicide treatment was examined through resistance *in-vitro* test and showed a very significant result. The effectiveness of these results in the field are being carried out at a 4 hectare of eight years old cocoa plantation in West Sumatra.

CONCLUSION

Both of the essential oils of *P. aduncum* and *C. flexuosus* able to suppress the growth of colonies of *P. palmivora.* Increasing the dose and applying the mixture treatment of these essential oils, even improve suppression effect on colony growth. However, the best treatment in suppressing the growth of colonies of *P. palmivora* and has the consistency of fungicide character in this study is the mixture treatment of 1000 ppm essential oil of *P. aduncum* + 1000 ppm essential oil *C. flexuosus*.

ACKNOWLEDGEMENTS

The authors wish to thank to Director of Laing Research Station of Bogor Research Institute for Spices and Medicinal Plants in Solok West Sumatra Indonesia, on the permission given to use the essential oil supplies and laboratory facilities.

REFERENCES

1. Crouzillat, D., Quantitative trait loci analysis in *Theobroma cacao* using molecular markers. Inheritance of polygenic resistance to *Phytophthora palmivora* in two related cacao populations. *Euphytica*. **2000.** *114:* 25-36.
2. Evans, H.C., *Fall.* **2012.** 5(4): 29-35.
3. Dinas Perkebunan Tingkat I Propinsi Sumatera Barat. **2007**.
4. http//sumbar.litbang.deptan.go.id/ind/index.
5. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(4): 1756-1761.
6. Evans, H.C., *Fall.* **2012.** *5*(4): 29-35.
7. Wardoyo, S. The growth and development of the Indonesian economy, *Indonesian Agriculture Research Development J.* **1992.** 2 (1): 1-4.
8. Baah, F., Anchirinah, V. *A* A review of Cocoa Research Institute of Ghana extension activities and the management of cocoa pests and diseases in Ghana *American Journal of Social and Management Sciences.* **2011.** 2(1): 196-201.

9. Nyadanu, D., Efficacy of screening methods used in breeding for black pod disease resistance varieties in cocoa, *African Crop Science Journal*. **2009**. 17(4): 175-186.
10. Evans, H.C. *Fall.* **2012.** *5*(4): 29-35.
11. Nasir, N. *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(4): 1756-1761.
12. Hindayana. D. Direktorat Perlindungan Perkebunan Departemen Pertanian, Jakarta. **2002:** Edisi ke *2*, 98p.
13. Nasir, N. *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** 7(4): 1756-1761.
14. http://www.antara.co.id/en/. Accessed.
15. Gupta, S., and Dikshit, K., *Journal of Biopesticides*. **2010.** *3*(1): 186-188.
16. Leng, P., Zhang, Z., Pan, G. Zhao, M. *African Journal of Biotechnology*. **2011.** *10*(86): 19864-19873.
17. Mairawita, N., et al.. Prosiding BioETI ISBN 978-602-14989-0-3 *Universitas Andalas*, **2013.** 69-71.
18. Nurmansyah. *Jurnal Tambuo*. **2014**. 13(3): 296-302.
19. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.
20. Nasir, N., A Review: The prospects of wild Zingiberaceae Elettariopsis Slahmong to be developed as a green pesticide, *Der Pharmacia Lettre*. **2017**. 9(2): 21-28.
21. Leng, P., Applications and development trends in biopesticides, *African Journal of Biotechnology*. **2011.** 10(86): 19864-19873.
22. Nurmansyah. *Badan Penelitian dan Pengembangan Pertanian. Kementrian Pertanian.* **2012**. 121-127.
23. Cicclo, J.F.; Ballestero, C.M. *Biol. Trop*. **1997,** *45*, 783-790.
24. Ganjewala, D.; Ambika, K.; Khan, K.H. *Excel India Publishers*. **2008**, *17*, 82-92.
25. Kakarla, D. Vellore Institute of Technology University, India. **2009.**
26. Mairawita, E., et al. . Prosiding BioETI ISBN 978-602-14989-0-3 Universitas Andalas, **2013.** 69-71.
27. Tondok, E.T., et al. *J. Agron. Indonesia*. **2012.** 40(2): 146-152.
28. Schlegel, H.G., Gajah Mada University Press. Yogyakarta. **1994.** Edisi keenam.
29. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.
30. Awang, Y., et al. *African J Microbial. Res*. **2011.** 5(29): 5250-5259.
31. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.
32. Perez, JDL., et al. *Eur J Plant Pathol*. **2009.** 124: 595-601.
33. Mairawita, N., et al. Prosiding BioETI ISBN 978-602-14989-0-3 Universitas Andalas, **2013.** 69-71.
34. Nurmansyah. *Badan Penelitian dan Pengembangan Pertanian. Kementrian Pertanian.* **2012**. 121-127.
35. Mishra, AK., Dubey, NK., Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities, *Applied and Enviromental Microbiology*. **1994.** *60* (4): 1101-1105.
36. Ella, M.U.K. Uji Efektivitas Konsentrasi Minyak Atsiri Sereh Dapur (Cymbopogon citratus (DC.) Stapf) Terhadap Pertumbuhan Jamur Aspergillus sp. Secara *invitro*. *Agroekoteknologi Tropika*. **2013.** *2* (1): 39-48.

37. Nasir, N., Nurmansyah. *Research J of Pharmaceutical, Biological and Chemical Sciences.* **2016.** *7*(5): 2463-2471.
38. Nasir, N. *Der Pharmacia Lettre*. **2017.** *9*(2) : 21-28.
39. Mishra, AK., Dubey, NK., *Applied and Enviromental Microbiology*. **1994.** 60 (4): 1101-1105.
40. Nurmansyah. *Badan Penelitian dan Pengembangan Pertanian. Kementrian Pertanian.* **2012**. 121-127.
41. Nasir, N. *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.
42. Hanna, ARM., Sallam, Y.I., El-Leithy, A.S. ; Aly, S.E. *Annals of Agriculture Science.* **2012.** 57(2): 113-116.
43. Cicclo, JF., Ballestero, C.M. *Biol. Trop*. **1997.** 45*:* 783-790.
44. Nurmansyah. *Badan Penelitian dan Pengembangan Pertanian. Kementrian Pertanian.* **2012**. 121-127.
45. Nasir, N., Nurmansyah, *Research J of Pharmaceutical, Biological and Chemical Sciences.* **2016.** 7(5): 2463-2471.
46. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.
47. Nasir, N., *Der Pharmacia Lettre*. **2017.** 9(2): 21-28.
48. Knobloch, KA., Antimicrobial agents from plants: Antibacterial activity of plant volatile oils, *Bioflavour*. **1988.** 5: 287-299
49. Fessenden, RJ., Fessenden, JSJ., Organic Chemistry (Fessenden, Ralph J.; Fessenden, Joan S.), *Chem. Educ*. **1995.** 72 (7): 151-157.
50. Knobloch, KA., Antibacterial and antifungal properties of essential oil components, Bioflavour. **1988.** 5: 287-299
51. Fessenden, RJ., Fessenden, JSJ., Organic Chemistry, Fifth Edition (Fessenden, Ralph J.; Fessenden, Joan S), Chem. Educ. **1995.** 72 (7): 151-157.
52. Chairgulprasert, V., Chemical constituents of the essential oil, antioxidant and antibacterial activities from *Elettariopsis curtisii* Baker, *J. Sci. Technol.* **2008.** 30(5). 591-596.
53. Pino, JA., et al. Effect of collection time on essential oil composition of *Lantana camara* Linn (Verbenaceae) Growing in Brazil Northeastern. *J. Essent. Oil Res*. **2004.** 16, 186-188.
54. Wong, K.C. ; Essential oils of *Zingiber officinale* var. rubrum Theilade and their antibacterial activities, *J. of Essen. Oil Res*. **2006.** 18(2): 203-205.
55. Picheansoonthon, C., Yupparach, P., *Journal of Thai Traditional & Alternative Medicine.* **2007**. 5(3): 29-40.
56. Perez, JDL., et al. *Eur J Plant Pathol*. **2009.** 124: 595-601.
57. Nasir, N., Nurmansyah. *Research J of Pharmaceutical, Biological and Chemical Sciences.* **2016.** *7*(5): 2463-2471.
58. Nasir, N., *Research J of Pharmaceutical, Biological and Chemical Sciences*. **2016.** *7*(6): 2164-2171.

59. Mailina, J., Noor Azah, MA., Sam, YY., Chua, S.L.L; Ibrahim, J. *Journal of Tropical Forest Science.* **2007.** *19*(4): 240-242.

60. Chairgulprasert, V., et al. *J. Sci. Technol.* **2008.** 30(5): 591-596.