Laboratory-Based Toxicological Assessments: Toxicity Efficacy of *TinosporaCrispa*(Family: Menispermaceae) Against *MacrotermesGilvus*(Family: Termitidae) in Oil Palm Plantation

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Abstract.*Macrotermesgilvus*is a termite that is one of the most important pest species in wooden structures, rubber and oil palm plantation. A study was done to test the toxicity effect of the plant *Tinosporacrispa*against *M. gilvus. M. gilvus*were collected and subjected to seven treatments of 1000 ppm, 5000 ppm, 10000 ppm, 20000 ppm, 50000 ppm, 60000 ppm, 70000 ppm and control. The results revealed that 60000 ppm is the most effective dosage to cause 50% mortality within 12 hours for worker termites. 100% mortality was reached within 31 hours. The soldier termites was stronger with 50% mortality was within 18 hours while 100% mortality was reached faster within 42 hours after treatment. The LD50 value for worker and soldier termite at 24 hours are 47 mg/termite and 74.56 mg/termite respectively. GCMS analysis showed that 12-Pentadecanone, 6,10,14-trimethyl, 1-(+)- Ascorbic acid 2,6-dihexadecanoate, Phytol, 1,2-Benzenedicarboxylic acid, n-Hexadecanoic acid, Butylatedhydroxytoluene, 1-Heptacosanol and Dotriacontane was major compound in *T. crispa*extract. This study shows that stems extract of *Tinosporacrispa*has insecticidal property on *Macrotermesgilvus*.

Keywords: Toxicity, *Tinosporacrispa,* Termite, *Macrotermesgilvus,* Oil Palm Plantation

1 Introduction

Termite is one of the most dangerous wood destroying insects and life crop plantations (Supriadi and Ismanto, 2010). They have their own colonies below the surface of the ground and these has been made possible by the covering of root, tree stumps and waste timber. They are responsible for reducing soil fertility by removing both plant and animal debris and locking them in their underground nests thus making them unavailable for plant growth (Rajagopal, 2002). Since, also known as eusocial insect and mostly feed on dead plant material and wood due to presence of symbiotic cellulose decomposing bacteria in the gut of many species (Hojo et al., 2005). Termite queens can produce from a few hundred to more than 10 million eggs per year in some species, resulting in very large colonies. In *Macrotermesgilvus*castes, they have major and minor soldier as well as the worker, the king which is adult dealated male and the female which is the physiogastricqueen.

Termites are serious economic threats to agriculture and urban structures in most subtropical and tropical countries including Malaysia (Lee et al., 1999; Lee et al., 2003; Su andScheffrahn, 2000). Termites attack a wide range of crops at all stages of the growth cycle. Crop losses are estimated at between 3 – 30% while stand losses of up to 90% have been

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recorded (Mitchell, 2002). Lee and Sajap (2000) reports *Macrotermesgilvus*also present in oil palm and other cropplantation. Chemical that used in some termiticide products to prevent of termites relied heavily on the use of insecticides such as deltamethrin, bifenthrin, permethrin, chlorpyrifos, chlorfluazuron, hexaflumuron, triflumuron, imidacloprid, fipronil, and arsenic trioxide but also harzaderous to human being (Department of Health and Ageing office of Chemical safety, Australian Government,2007).

Insect Pest management (IPM) has to face up to the economic and ecological consequences of the use of pest control measures. Development of naturally produced plant compounds as alternative to synthetic insecticides has been a growing interest (Maistrello et al. 2001) because chemicals that produced naturally have less impact on environmental and human health (Mao et al. 2007). Zhu et al. (2001) study of evaluation of vetiver oil and seven insect-active essential oil aginst Formosan Subterranean termite concluded that clove bud was the most toxic, killing 100% termite in two days at 50 µg/ cm2 compared to the other 7 essential oil of *vetiver grass, cassia leaf, cedarwood, Eucalyptus globules, Eucalyptus citrodora,* lemongrass and geranium.

Tinosporacrispa(Family: Menispermaceae) is a climber found widely distributed in Indonesia, Malaysia, Thailand and Vietnam. It has been used as traditional medicine for treat fever, cholera, snake bites, rheumatism and fever due to malaria (Dweck and Calvin, 2006) and in moden medicine as antihyperglycemia effect by augmenting the release of insulin (Noor and Ashcroft, 1998), antimalarial activity (Rahman et al., 1999), antibacterial (Zakaria et al, 2006), anti-inflammatory (Sulaiman et al, 2008) and anti-oxidant properties (Dweck and Calvin, 2006) are also recorded. Cavin et al. (1998) reported the whole plant contains a bitter principle, colombine, 2.22%; traces of an alkaloid; and a glucoside. Therefore, in this study, *Tinosporacrispa*was chosen in toxicity test on termites to test the potential insecticidal bioactive compounds in this plant.

2 Material and Methods

The study was conducted in Laboratory Toxicology, Institute of Post Graduate Studies, University of Malaya, Malaysia.

2.1 Termite

*Macrotermesgilvus*species were collected from plantation field located in Kampung Sungai Besar, Pontian, Johor, Malaysia (1° 38' 0" North, 103° 16' 0"). This termite were collected manually by using forceps and separated according their caste. The species identification was made by termite specialist, MrQuah. The morphology of the collected termites was observed under microscope (Leica ZoomM 2000 with model number Z30V).

2.2 Extraction of the Plant Material

Fresh stems of *Tinosporacrispa*were collected from KampungParitAbdRahman, Pontian, Johor (1° 41' 0" North, 103° 9' 0" East). The fresh stems were cut obliquely (1 cm long) and sun-dried for three days. Dried stems of *Tinosporacrispa*were placed in a 2000 ml conical flask. Chloroform (SYSTERM®) which act as solvent, was added to the plant samples in a ratio 1:100 and soaked for 24 hours. Extract of *Tinosporacrispa*was filtrated using Whatmann filter paper and concentrated using rotary evaporator at 40°c until semi solid to be used as the

crudeextract.

2.3 Filter Paper Bioassay

Both soldiers and workers termite of *Macrotermesgilvus*(Family :Termitidae) were collected and subjected to bioassay with extract. The extract was prepared in 7 treatments which were 1000ppm, 5000ppm, 10000ppm, 20000ppm, 50000ppm, 60000ppm and 70000ppm respectively. Each acetone-sterilized glass petri dish werelined by filter paper. 10µl of each treatment was applied to the filter paper and let dry in 10 minutes. Ten termites worker were placed in each petri dish containing treated filter paper and distilled water as control with 10 replications per treatments. Under laboratory control tempature at 27°c and darkness, the behavior and mortality of the termites was observed and recorded. Similarity procedure wereapplied usingsoldier.

2.4 Purified of Extract

The crude extract was separated using simple flash column chromatography. A 23 cm long Pasteur pipette was plugged with glass wool at bottom narrow portion. The stationary phase which is dry silica gel 60 (0.040-0.063 mm) (weighed 6.3714 g) was packed with Pasteur pipette. This silica gelwas then washed and stabilized by 10 ml of 100% n-Hexane solution. After that, 1 ml of crude extract was then loaded to the surface of the packed gel. The fractions from the crude extract were eluted with 10 ml of stepwise gradient of hexane : diethyl ether respectively and ended with 100% ethylacetate.Fractionsofthesolutionwerecollectedin24separatedvialbottlesaseveryfractions were divide into 5 ml each and labeled as E1 to E24. The solutions were then used for bioassay purposes to determine the active fractions and the procedur similar with bioassay filter paper.

2.5 Isolation of Chemical Compound

Thin layer chromatography (TLC) is a solid-liquid form of chromatography used to determine the number of components in a mixture and analyzed the most effective fractions from the flash column chromatography. Aluminium – silica TLC plates (20 cm x 20 cm) (60 F254) was cut into 20 pieces of small strips with 10 cm x 2 cm. 1 cm starting line from the bottom end and 1cm solvent front from the upper end was marked on each of the TLC strips with pencil. The mobile phase comprised of hexane and diethyl ether. 10µl of sample was spotted on the plate 3 times and then placed into tank, containing n-Hexane and diethyl ether. The ratio of the solvents was prepared just as the ratio used in column chromatography fractions. After the solvent had run to the solvent front, the plate was removed using forceps and let dried for a moment. The plate was then visualized by using UV lamp and obvious blue – greenish spots were marked by pencil. The spots were then scraped off the plate along with the absorbent and dissolved in the n-hexane. The dissolved spots were used for bioassay purpose, same as the bioassay filterpaper.

2.6 Chemical Analysis Gas Chromatography Mass Spectrometry (GCMS)

The components in the crude extract, the fractions and the bioactive spot were isolated and identified by using GCMS. The sample for identification is injected into oven of GC-MS (Qp5050A) and the temperature was kept at 40ºC for 5 minutes and then increased to 280ºC at 10ºC minute. This step was maintained at 280ºC at 10ºC minute. Helium gas was used as the

carrier gas of Gas Chromatography. The helium gas was adjusted to 1.3 ml per minute for flow rate in mobile phase. The stationary phase was the BP5 capillary column. The BP5 column has the length of 29.0 m and diameter of 0.25 mm. the mass range at 80 and above was done for scanning. The combination of Gas Chromatography and Mass Spectrometer can further identified the compounds by checking the retention time of each detected compound and run again in Mass Spectrometer to determine the massspectra.

2.7 Statistical analysis

The results of this bioassay were subjected with Analysis of Variance (ANOVA) and Probitanalysis to determine values of LD50. This analysis has been done by StatPlus 2009 software.

3 Result and Discussion

The activity of *Tinosporacrispa*extractionapplied on filter paper at verious concentration and distilled water as control is expressed as mortality (mean) (Table 1 and 2). Initially after the treatments were applied, both soldiers and workers of *Macrotermesgilvus*were walking fast around the petri dish for few hours. The soldiers walked faster than the workers. After 12 hours observations, termites became weaker and started to grouping together. The soldiers started to attack the filter paper with mendible, while some were attacking each other.

The mortality increased through time with increase in concentration. For control, 50% mortality was achieved after 48 hours for worker (mean = 5), but only 4.7 mean mortality recorded at the end of the observation which is on 72 hours after treatment applied for soldier. Meanwhile other treatments, 50% mortality of worker termite at 1000 ppm (mean = 6.5), 5000 ppm (mean = 6.5) and 10000 ppm (mean = 6.6) for 36 hours, 20000 ppm (mean = 5.4) and 50000 ppm (mean = 8.5)needed 24 hours and 12 hours for 60000 ppm (mean = 4.9) and 70000 ppm (mean = 5.7) (Table 1). For soldier,1000ppm(mean=4.8),5000ppm(mean=5)and10000ppm(mean=6.3)at60hours.

Other concentration, 20000 ppm (mean = 6.5) at 48 hours, 50000 ppm (mean = 4.8) and 60000 ppm (mean = 7.8) at 24 hour and 70000 ppm at 12 hours (Table 2). With increasing concentrations of the treatments the alarm behaviour was exhibited mush earlier or faster.

Table 1. Mortality (mean) value of *Tinosporacrispa*extractionat different concentration (ppm) against workers *M. gilvus*(N = 100).

Concentration on (ppm)	**Mortality (mean)**					
Control	12 h	24 h	36 h	48 h	60 h	72 h
1000	0.1	0.7	2.4	5	7.8	10
5000	0.6	1.2	6.5	8.9	9.8	10
10000	0.3	1.7	6.5	9.3	10	10
20000	0.5	2.4	6.6	9.1	10	10
50000	0.8	5.4	9.1	10	10	10
60000	2.9	8.5	10	10	10	10
70000	4.9	8.8	10	10	10	10

Analyzed used LD50 (the dosage when 50% mortality), the lowest concentration needed

were after 33 hours (467.30 ppm) followed by 24 hours (25376.38 ppm) and 12 hours (64434.27 ppm) for worker. However, for soldier termites lowest concentration reading obtained until 61 hours (879.58 ppm) followed by 60 hours (1940.86 ppm) and 48 hours (15621.04 ppm) respectively (Table 3).

Table 2.LD50 value of *T. crispa*extraction at different concentration against *M. gilvus*soldiers and workers.

Time	**LD50 (ppm)**	
	Worker	**Soldier**
12	64434.27	74960.33
24	25376.38	45483.87
26	-	29599.25
48	-	15621.04
60	-	1940.86

The weight calculation is done when the total termite weigh (N = 10) were obtained and divide by the actual concentration unit which is one ppm is equal to 1000 ml. The average weight of 10 worker termite 5.4 mg. The value was divided by the readings obtained from the Probit analysis. For worker termite, total lethal dose needed to cause 50% mortality within 12 hours and 24 hours are 119.3 mg/ worker termite and 47.00 mg/ worker termite. While for soldier termite, the lethal dose needed to achieve 50% mortality within 12, 24, 36, 48 and 60 hours are 122.89 mg/ soldier termite, 74.56 mg/ soldier termite, 48.52 mg/ soldier termite, 25.61 mg/ soldier termite and 3.18 mg/ soldiertermite respectively. This indicate that the longer the time taken to achieve 50% mortality, the lower the LD50 value (Table 3).

Table 3.LD50 value of T. crispa extraction at different concentration against M. gilvus soldiers and workers.

Time	LD50 (mg/termite)	
	Worker	Soldier
12	119.32	122.89
24	47	74.56
36	-	48.52
48	-	25.61
60	-	3.18

3.1 Statistical Analysis (1-way ANOVA)

Analysis used ANOVA, at 24 hours the critical F value (3.501) is larger than calculated F (0.552) respectively for worker. Similarity in soldier critical F value (3.501) is larger than calculated F (0.552) respectively. As the critical F is greater than calculated F value, the null hypothesis (H0) is accepted. This indicates that, the mean mortality of *Macrotermesgilvus*with different treatment concentration is non significantly different. P-value indicates the probability of getting mean difference between groups is as high as what is observed by chance. The higher the p-value, the less the significant the different between the groups (Table 5).

Table 4. Analysis of variance (ANOVA) of *M. gilvus*at 24 hour.

Termite	Between Group	P-Value	F-Value	F-Calculated
Worker	6209.04	0.776	0.552	3.501
Soldier	6209.04	0.493	0.997	3.501

The toxic effect (100% mortality within 72 hours) showed in four fractions (90:10, 60:40, 70:30 and 100% ethyl acetate; hexane:diethyl ether) toward both workers and soldiers of *M. gilvus*. The total of 15 spots were obtained from isolated TLC with 24 fractions and labeled as S1 to S15. After further chromatography, 6 spots (S1, S3, S5, S9, S13 and S15) only showed most toxic effects on both soldier and worker *M. gilvus*(Table 5 and 6). Spot S13 showed strongest toxic effect toward both worker and soldier *M. gilvus*from the fraction of 60:40 followed by S5 (mean = 9.4) and S15 (mean = 9.7) both from the fraction 40:60 within 60 hours respectively (Table 5 and 6). Spot S9 (Rf = 0.56) and S10 (Rf = 0.78) showed more separation and long distance between other spots (Table 7).

Table 5. Mortaliy (mean) of worker *M. gilvus*with chemical compounds from spot in TLC.

Time (h)	Mortality (mean)														
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**
12	0.5	0.4	0.1	0.1	0.4	0.1	0.0	0.5	0.1	0.7	2.1	0.4	0.8	0.5	0.2
24	1.3	0.8	0.5	0.4	2.5	0.8	2.5	1.2	0.5	2.4	3.0	2.0	4.4	1.2	1.8
36	4.8	2.0	1.0	1.0	6.0	3.1	3.4	2.5	1.5	5.8	3.6	6.1	7.0	2.3	6.0
48	6.9	2.8	4.8	3.4	9.0	3.6	4.4	3.0	5.0	8.9	4.9	8.0	8.6	2.8	8.5
60	7.0	6.0	7.6	4.0	9.4	5.0	5.0	3.8	7.3	9.0	5.7	8.8	10.0	3.5	9.7
72	10.0	7.0	10.0	6.6	10.0	5.9	5.6	6.0	10.0	9.2	6.0	9.0	10.0	5.9	10.0

Table 6. Mortality (mean) of soldier *M. gilvus*with chemical compounds from spot in TLC.

Time (h)	Mortality (mean)														
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.0
24	1.1	0.0	0.0	0.1	0.0	0.3	0.4	0.0	0.1	0.0	0.5	1.0	3.2	3.5	0.0
36	4.1	0.1	0.4	0.5	0.5	1.0	1.0	0.0	0.8	0.3	1.0	2.6	4.6	5.0	4.5
48	6.3	2.8	4.0	3.0	7.8	3.4	2.5	1.0	4.0	4.1	1.8	4.9	5.1	5.6	6.6
60	8.0	5.1	6.9	3.8	8.6	4.5	4.3	4.1	6.2	8.1	4.6	6.6	9.4	10.0	8.4
72	10.0	5.8	10.0	6.2	10.0	5.5	5.3	6.0	10.0	8.9	5.0	7.5	10.0	10.0	10.0

Table 7. Calculation of retention factor (Rf) of extract *T. crispa*

Spot	Rentation factor (Rf)
S5	0.09
S6	0.13
S7	0.19
S8	0.31
S9	0.56
S10	0.78

3.2 Analysis *T. crispa*using Gas Chromatography Mass Spectrometry

By using GCMS with authentic standard, components were eventually recognized in the T. crispa extract. Comparing retention times with those compounds that coincided in retention checked the nature of the compounds. The crude, spot S5 and S13 from TLC and fraction 90:10, 70:30, 60:40, 100% ethyl acetate were analysed using GCMS. Based on the bioassay and GCMS analysis, only one spot were considered which is F (Table 9) spot from the TLC and the I (Table 10) spot from the column fraction which is the most toxic towards M. gilvus.

Table 8. Chemical compounds in *T. crispa*crude extract using GCMS

Peak	Rentention time	CAS number	CA index name	Formula
18	33.542	57-10-3	n-Hexadecanoic acid	C16H32O2
32	31.925	502-69-2	2-Pentadecanone, 6,10,14-trimethyl	C18H36O
49	40.35	544-85-4	Dotriacontane	C32H66
61	39.192	27554-26-3	1,2-Benzenedicarboxylic acid	C24H38O4
66	42.667	2004-39-9	1-Heptacosanol	C27H56O
68	35.258	150-86-7	Phytol	C20H40O
73	33.542	28474-90-0	1-(+)-Ascorbic acid 2,6-dihexadecanoate	C 38H68O8
85	39.467	630-0608	Hexatriacontane	C36H74

Table 9. Chemical compounds in *T. crispa*F spot using GCMS

Peak	Rentention time	CAS number	CA index name	Formula
15	41.358	5856-66-6	Tetrapentacontane	C54H110
33	36.967	112-95-8	Eicosane	C20H42
52	37.833	630-06-8	Hexatriacontane	C36H74

During the extraction, the drying process done using natural sun shine despite using oven to ensure the chemical compound in the stem remains natural. *Tinosporacrispa*stems were

dried totally to make sure no fungus could grow on the surface. Extraction used chloroform which is commonly used by scientists to carry out their study (Rahman et al., 1999). Since cloroform absorb polar and non-polar constituents present in the plant materials (Abdullah, 2004). Bisset and Nwaiwu (1984) reported extractions of stem and root of *Tinosporacrispa*contained quaternary alkaloids including berberine The variations of antioxidant compounds in plants obtained from several extraction processes could be explained by the different temperature and time prevailing in each case (Zulkhairi et al., 2008).

In this study, all bioassays have been done on both soldiers and worker of *Macrotermesgilvus*. Increased of concentration showed increasing in mortality of termites. Maximum mortality time for worker and soldier termites were achieved 72 hours respectively. The 60000 ppm is the mosteffective dosage that cause mortality within 24 hours, with 12.5 hours for worker and 17.5 hours for soldier. Soldier has higher degree of toleration than worker when exposed to toxic compounds. Production of lipophilic contact insecticides formally derived from fatty acids. Each of these defense substances possess a reactive electrophilic center (vinyl ketone, nitroalkene, or β-ketoaldehyde) responsible for toxicity in soldier from family Termitidae (Spanton and Prestwich, 1982).

Mortality of termites were increase with increasing duration of exposure. Within 36 hours after treatments applied, worker termites have achieved 2.4 mean of mortality for control, 6.5 mean for 1000 ppm and 5000 ppm, 6.6 and 9.1 mortality mean for 10000 ppm and 20000 ppm respectively. Whereas for 50000 ppm, 60000 ppm and 70000 ppm, they have achieved total mortality mean within 36 hours. During 72 hours after treatment, total 100% mortality achieved for every single treatment. For soldier termites, no mortality observed within 12 hours after treatment applied for control, 1000 ppm and 5000 ppm. But this situation happened adversely when 1.3, 3.9 and 5.7 mean of mortality achieved for treatment with 50000 ppm, 60000 ppm and 70000 ppm respectively. After 60 hours treatments applied, 50% until 100% mortality achieved for treatment range between 5000 ppm to 70000 ppm.

Based on LD50 calculation with Probit analysis, the lowest concentration to cause 50% mortality of worker of *Macrotermesgilvus*were 467.30 ppm which is during 35 hours while for the soldier termite were 879.58 ppm during 61 hours. From this analysis, the LD50, which is the dosage amount to cause 50% mortality of tested population obtained. Neoh et al. (2008) reported that 68.9 ± 13.4%, an equivalent of 841 ± 164 mg/termite of *Ocimum sanctum* within 36 hours. To kill 50% of the tested population, the total concentration for worker within 12 hours and 24 hours are 64434.27 ppm and 25376.38 ppm respectively. While for soldier termite was 74960.33 ppm, 45483.87 ppm, 29599.25 ppm, 15621.038ppm and 1940.859ppm for 12, 24, 36, 48, 60 and 72 hours respectively. The principle toword LD50, the lower the LD50 value, the more potential the toxicity effect of *Tinosporacrispa*on termites. LD50 for worker termites in 12 hours and 24 hours are 119.3 mg/ worker termite and 47.00 mg/ worker termite. While for soldier termite, the lethal dose needed to achieve 50% mortality within 12, 24, 36, 48 and 60 hours are 122.89 mg/ soldier termite, 74.56 mg/ soldier termite, 48.52 mg/ soldier termite, 25.61 mg/ soldier termite and 3.18 mg/soldier termite respectively. As the LD50 of *Tinosporacrispa*towards worker termites is lower than LD50 for soldier termites, this

indicates that the worker termites are more susceptible to the effect of *Tinosporacrispa*. In other words, soldier termites are more resistant to the effect of *Tinosporacrispa*and more concentrated treatment required to achieve the LD50. The LD50 is depends on the observation time after treatment application, different observation time will result in different LD50 value. Park and Shin (2005) reported that clove bud and garlic oils produce 100% mortality of Japanese termite (*Reticulitermesspera*tusKolbe) at 5.0 mμ L/L of air concentration. Garlic and clove bud oils produced 100% mortality at 0.5 μL/L of air, but this decreased to 42 and 67% after 3 days of treatment at 0.25 μL/L of air, respectively.

The active fraction ratio for soldier and worker from flash column chromatography was from 90:10, 60:40, 70:30 (hexane : diethyl ether) and 100% ethyl acetate respectively. Fraction number 6 and EA2 achieved 100% within 48 hours whereas fraction number 1 and 4 was within 60 hours. 72 hours taken to achieved 100% mortality in fraction 70:30. In another study, the active fractions from *Alpiniarafflesiana*extract was obtained from fraction 70:30 and 20:80 (Mohamad et al, 2004). The Rf value for positive spot are 0.09, 0.13, 0.19, 0.31, 0.56 and 0.78. TLC used to determine the polarity of compounds in the fraction. The mobile phase used in TLC is to separate the compound in fraction. By the TLC separation, there were four major compounds being separated (note that this compound appear strongly in the GCMS result with SI value is more than 90).

There are several compounds has been identified presenting in the crude extract of *Tinosporacrispa*by using GCMS. The compound being identified included 2-Pentadecanone, 6,10,14-trimethyl, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Phytol, 1,2-Benzenedicarboxylic acid, n-Hexadecanoic acid, Hexatriacontane, 1-Heptacosanol and Dotriacontane. The 18th peak in chromatogram with retention time 33.542 minutes and abundance of 7.51% was identified as n-Hexadecanoic acid with molecular weight 256 g/mol and chemical formula C16H32O2. It is also known as palmitic acid. The 32nd peak in the chromatogram with retention time 31.925 minutes and chemical formula C18H36O was 2- Pentadecanone, 6,10,14-trimethyl. The next peak which is the 49th with retention time 40.350 minutes and the chemical formula was C32H66 was Dotriacontane. Peak 61st with retention time 39.192 was 1,2-Benzenedicarboxylic acid with chemical formula as C24H38O4. Peak 66th and 68th with respective retention time 42.667 minutes and 35.258 minutes are 1-Heptacosanol (chemical formula C27H56O) and phytol (chemical formula C20H40O). Due to limited information of libraries of mass spectrometer to natural products, other peaks in the chromatogram were not identified.

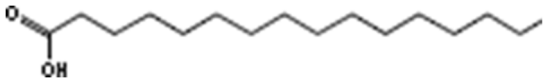

Fig.1. Chemical structure of n-Hexadecanoic acid (C16H32O2)

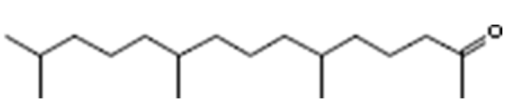

Fig.2. Chemical structure of 2-Pentadecanone, 6,10,14-trimethyl (C18H36O)

Fig.3. Chemical structure of Dotriacontane (C32H66)

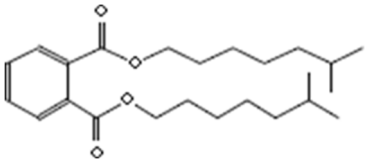

Fig.4. Chemical structure of 1,2-Benzenedicarboxylic acid (C24H38O4)

Fig.5. Chemical structure of 1-Heptacosanol (C27H56O)

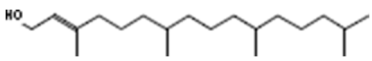

Fig.6. Chemical structure of phytol (C20H40O)

The compounds presented in spot F from the flash column chromatography fraction which is 60:40 was Eicosane which appear on the 33rd peak with retention time 36.967 minutes and the chemical formula was C20H42. On the peak 15th and 52nd, the retention time was 41.358 minutes and 37.883 minutes respectively. The compound name was Tetrapentacontane and Hexatriocontane. GCMS results from the TLC spot shows 4 different compound identified. The first was on the 3rd peak which on the retention time 25.183 minutes was Butylatedhydroxytoluene with chemical formula C15H24O. On peak 42, 65 and 79, the retention time was 36.950 minutes, 39.158 minutes and 37.817 minutes respectively was Eicosane, 1,2-Benzene-dicarboxylic acid and hexatriocontane. Previous study has found 5 active compounds: adenosine, uridine, salsolinol, higenamine and tyramine (Praman et al., 2012) in the study of Hypotensive and cardio-chronotropic constituents of *Tinosporacrispa*and mechanisms of action on the cardiovascular system in anesthetized rats. Pathak et al. (1995) reported that *T. crispa*contains borapetolA, borapetol B, borapetoside A, borapetoside B, tinocrisposide, N formylanondine, Nformylnornuciferine, N-acetyl nornuciferine, γ sitosterol, picrotein, tinotubride. In order to ascertain which compound is toxic to the termites, synthetic compound of both need to be further bioassayed with the termites. Nevertheless this study experimentally evidenced that *T. crispa*has a potential to be an alternative biopesticide against *M. gilvus*.

4 Conclusion

About 1320 workers and 1320 soldiers of *Macrotrmesgilvus*(Family: Termitidae) have been used throughout this study. From the preliminary bioassay on crude extract, it can be concluded that *Tinosporacrispa*(Family: Menispermaceae) have strong toxic effect towards *Macrotrmesgilvus*. 60000 ppm is the most effective dose to cause 50% mortality in 12 hours. LD50 for worker of *M.gilvus*was 47 mg/termite within 24 hours while soldier survived longer, who achieved LD50 74.56 mg/termite within 24 hours. In addition, the mortality of termite increases when the concentration of the treatment increases. From the bioassay conducted on fractions from column chromatography, fraction E3, E8, E9, E10 and E24 have been found to be most toxic fractions that cause mortality of the termites. From the Gas Chromatography Mass Spectrometry (GCMS) analysis, one of this 2- Pentadecanone, 6,10,14-trimethyl, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Phytol, 1,2- Benzenedicarboxylic acid, n-Hexadecanoic acid, Butylatedhydroxytoluene, 1-Heptacosanol andDotriacontane has been identified as the active compounds compounds present that leads to the mortality of the termites. In order to ascertain which compound is toxic to the termites, synthetic compound of all those need to be further bioassayed with the termites. Nevertheless this study experimentally evidenced that *T. crispa*has a potential to be an alternative biopesticide against termite.

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