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Liquid Smoke Toxicity Characteristic from raw Materials Variation Production with Different Temperature and Concentration Level

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This study aimed to determine the nature of the liquid smoke toxicity from raw materials treatment combination with different liquid smoke concentrationand temperature levels. This study is conducted experimentally by using complete random designon factorial pattern 3 x 4 x 6 with three repetitionsuntil 72 experimental units are obtained. Factor A is raw material type comprising of coconut fiber, coconut shell and cinnamon, factor B is the pyrolysis temperature level of 100 ± 10 ℃; 200 ± 10 ℃; 300 ± 10 ℃; and 400 ± 10 ℃ and factor C is the liquid smoke concentration level of 0 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm to 500 ppm and 1000 ppm. The observed parameterconsist of liquid smoke toxicity characteristicthat consist of salina leach artemiamortality percentage in the form of probit. The results of research shows significant interaction ($P < 0.01$) between the usage of raw materials type with pyrolysis temperature level to the liquid smoke toxicity characteristic. Based on these results, it can be concluded that: a). The best liquid smoke production quality can be found in cinnamon raw materials treatment at temperature level of 400 ±10 ℃ that shows mortality rate of artemiasalinaon 19.048% that is the smallest compared to the other two raw material, b) liquid smoke as the results of different raw materials treatment combination (coconutfiber, coconut shell, and cinnamon) with different pyrolysis temperature show toxic characteristic (LC50 <30 ppm) with LC values 50 respectively 14.9 ppm, 20.9 ppm and 20.5 ppm, c) liquid smoke as the results of treatment combination of raw materials (coconut fiber, coconut shell, and cinnamon) with different liquid smoke concentration show toxic characteristic (LC50 <30 ppm) with LC value 50 respectively 22.1 ppm, 19.6 ppm and 27 ppm. d) liquid smoke as the results of pyrolysis temperature treatment combination(100 ± 10 ℃, 200 ± 10 ℃, 300 ± 10 ℃ and 400 ± 10 ℃) at different liquid smoke concentration show toxic characteristic (LC50 <30 ppm) with LC value50 respectively 20.5 ppm, 22 ppm, 15.9 ppm and 17.9 ppm. e) liquid smoke as the results of different raw materials treatment combinationwith different pyrolysis temperature at concentration of 0 ppm,

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12.5 ppm, 100 ppm, 500 ppm respectively show toxic characteristic (LC50 <30 ppm) with LC value50 at 10.5 ppm, 11.6 ppm, 39.8 ppm, 18.6 ppm, 11.6 ppm while the concentration of 50 ppm and 1000 ppm at LC50 valuerespectively on 55 ppm and 48.4 ppm does not have toxic characteristic (LC50>30 ppm), next at the same different raw materials treatment combinationregression line with pyrolysis temperature on liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm have a weak relation to the value of probit with R^2value respectively at 0.1049, 0.2141 and 0.2308. While the other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm have stronger relation with the probit value as indicated by R^2value respectively at 0.7159, 0.8495, 0.807 and 0.8181.

Keywords: raw material type, temperature, liquid smoke, concentration, toxicity

Introduction

According to Meyer, *et al*., (1982), one of bioactivity test that is easy, fast, inexpensive and accurate is by using ArtemiasalinaLeach shrimp larvae. It is known as Brine Shrimp Lethality Test (BSLT). Shrimp larvae mortality test is one of bioactivity test method in natural materials compoundresearch. The use of shrimp larvae for the benefit of bioactivity studies has been conducted since 1956 and since then it has been used on lots of studies of environmental study, toxicity, and bioactive compounds screening from plant tissue. This test is a preliminary test in observing pharmacological activity of a compound, one of them is anti-cancer. The application for the bioactivity system by using the shrimp larvae, among others are to determine pesticide residues, local anesthetic, morphine derived compounds, mycotoxins, carcinogenicity of a compound and sea pollutants, it also become a cheap alternative method for cytotoxicity test (Astuti *el.al*, 2005). The active compound that has high bioactivity can be identified based on the value of Lethal Concentration 50% (LC50), which is the value that indicates the concentration of toxic substances that can cause death to the test animals up to 50%. Mortality data is obtained and then processed with probit analysis formulated by Finney (1971) for determining LC50 values at 95% validity degree. The chemical compound has the potential bioactive if it has LC50 values less than 1,000 µg/ml (Meyer, *et al.*, 1982).

BSLT test by using artemiasalinashrimp is performed with hatching those eggs in seawater assisted by aeration. Artemiasalina eggs will hatch perfectly into larvae within 24 hours. A. salina larvae to be used in BSLT test must be aged 48 hours because if more than 48 hours, it is feared that the death is not due to the extract toxicity but because of the limited supply of food (Meyer, *et al.*, 1982). The benefit of using shrimp larvae A. salina for this BSLT test is the larvae sensitive nature to the test material, faster life cycle time, easily bred and cheap price. A. salina sensitive nature is likely caused by circumstances of very thin skin membrane that allows the diffusion of substances from environment that affect the body metabolism. A. salina is found in almost all surface waters in the world that have salinity range of 10 - 20g/L, it is the cause that this larvae can be easily bred. Newly hatched larvae that are called naupliushas oval form and reddish color with length of 400 µmand weight of 15 µg. The body membersconsist pair of small antennae (anteluenaor antenna I) and pair of large antennae (antenna or antenna II). In the front of the two small antennae,there are red spots that has function as eyes (oselus). At the back of large antennae,

thereis a pair of small mandibular (jaw), while on the front belly (ventral), labrum can be found (Mudjiman, 1988).

Shrimp larvae toxicity test is one of toxicity testing that is fast, safe, practical and economical for screening, fractionation, and determination of natural materials compounds bioactivity. National Cancer Institute United State of America (USA NCI) has found significant relationship between toxicity testing on naupliusshrimp (Bhrine Shrimp Lethality Test) with human tumor cells inhibition in vitro.

Because there are lots of plantation waste like coconut fiber, coconut shell and cinnamon in the province of West Sumatra, and it has yet provided optimum surplus value, then it needs to be processed into liquid smoke. Research of liquid smoke toxicitycharacteristic from various raw materials typeand different temperature pyrolysis has not been done thoroughly, therefore in this research, it aims to find out the nature of the toxicity from different types raw materials treatment combination (coconut fiber, coconut shell and cinnamon) with different temperature pyrolysis.

Materials and methods

Tools and instruments used in this research consists of laboratory glassware, test tube rack, aluminum foil, evaporatorfilter paper, vortex, desiccator, hot plate, aerator, fluorescent lights, 65 mesh sieve, oven, analytical scale (AND GH-202), blender, label paper, rulers, pencil, aluminum foil, plastic, filter paper, cotton, erlenmeyer flask, becker glass, measuring cups, funnel, test tubes, spatulas, stirring rod, Pasteur pipette, glass bottles, incandescent lamps, weighing bottle, measuring cup, capillary tube, vial, micro pipette, magnifying glass, oven, and 1 set of liquid smoke laboratory-scale maker (Rodiah et.al., 2006 as seen in the Figure 1).

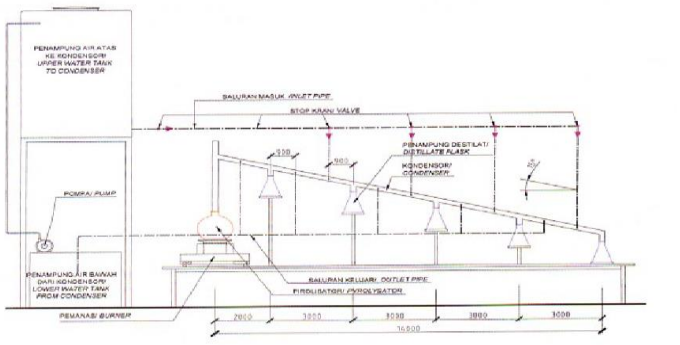
Materials and chemical reagents used in this study is coconut fibers and coconut shells waste from Padang central market and cinnamon without the outer skin from the cinnamon farmer in Tanah Datar, artemiasalina, DMSO 50%, ethanol 70%, pro analysis methanol (Merck), aquades, sea water.

Research Implementation

The stages in the implementation of this study consist of three phases:

Assembling liquid smoke pyrolysis tool

The circuitryof liquid smoke extraction tool is made at laboratory scale referring to the results of research and liquid smoke characteristic (Rodiahet.al., 2006). In this research, it uses the liquid smoke tool maker that consists of one condenser equipment unit with water drum capacity of 100 liters equipped with a water pump to circulate cooling water along 14 meter water hose for water circulation, liquid smoke container in form of 5 Erlenmeyer tube with capacity of 500 ml, stainless steel kiln with capacity of 3 kg and LPG fueled burner stove and at the end of pyrolysis pipe is a vacuum pump to draw the burning smoke in order to obtain the liquid smoke as seen in Figure 1 below.



Gambar 1. Alat penghasil asap cair skala laboratorium.

Figure 1. Laboratory-Scale Liquid Smoke Tool Maker

Liquid smoke pyrolysis (manufacture) process

Research on the making liquid smoke with pyrolysis method refers to the research activities above to provide input in redesigning laboratory scaleliquid smoke maker. After liquid smoke tool maker has been assembled then it is continued with producing liquid smoke. This process starts from raw material preparation by providing coconut fiber, coconut shell and dry cinnamon about 40 kg each with water content rangeson 4-10%, cleaned from dirt. Then raw materials are cut into small pieces with size ± 4-9 cm^2. The next activities is to put the raw materials into pyrolysis reactor for 5 (five) hours with 3 kg weigh for eachsample at the temperature of 100 ± 10 ℃; 200 ± 10 ℃; 300 ± 10 ℃; 400 ± 10 ℃, using LPG fueled burner stove. The water pump is used to channel water from the water source to the condenser. Burner and water pump are switched on simultaneously. Distillate container (liquid smoke) is kept by using glass bottles. The temperature is measured using thermometer and the measurements are performed every ½ hour measured at several places, namely in pyrolysator, distillatescontainer, water resources, as well as condenser inlet and outlet. After 5 hours, it will produce three fractions, which are solid fraction such as charcoal, in form of heavy fraction such as tar, and in form of light fraction such as smoke and methane gas.Then, light fraction is flowed to condensation pipe in order to produce liquid smoke while methane gas remains in gasform and is not condensed. Liquid smoke that is produced is kept for one week, after that the analysis can be performed. The purpose of 1 (one) week deposition is to precipitate impurities in liquid smoke. After 1 (one) week of deposition,then liquid smoke is tested with cytotoxic analysis.

Liquid smoke cytotoxic test

With Brine Shrimp Lethality Test (BSLT) method bioassay system (Meyer *et al*, 1982 and Harmita, *et al*., 2008) with phases of work as follows:

a. Selection of Artemiasalina Leach eggs

Shrimp eggs selection is performed by soaking the eggs in Aquadestfor one hour. The good eggwill sink while the bad egg will float.

b. Preparation of Artemia Salina Leachlarvae

Shrimp larvae preparation is performed by hatching shrimp eggs for 48 hours before testing. Eggs hatching is done by immersing those eggs in sea

water sufficiently and light the part of container that is not occupied by shrimp eggs with incandescent lights.

c. Dividing the treatment group

In this research, shrimp larvae are divided into five random treatment groups, which are:

a. Group K is 10 larvae shrimp fed liquid smoke with concentrations of 0 µg/ml.
b. Group P1 is 10 larvae shrimp fed liquid smoke with concentration of 12.5 µg/ml in the media.
c. Group P2 is 10 larvae shrimp fed liquid smoke with a concentration of 25 group µg/ml in the media.
d. Group P3 is 10 larvae shrimp fed liquid smoke with concentration of 50 µg/ml in the media.
e. Group P4 is 10 larvae shrimp fed liquid smoke with concentration of 100 µg/ml in the media.
f. Group P5 is 10 larvae shrimp fed liquid smoke with concentration of 500 µg/ml in the media.
g. Group P6 is 10 larvae shrimp fed liquid smoke with concentration of 1000 µg/ml in the media.

d. Implementation of toxicity tests

Test implementation is performed firstly by equalizing the final volume of liquid smoke results from treatment combination from the three materials (coconut fiber, coconut shell, cinnamon) with different pyrolysis temperatures, which are the temperature of 100 ± 10 ℃; 200 ± 10 ℃; 300 ± 10 ℃; and 400 ± 10 ℃ with a concentration ratio of treatment above is diluted by adding seawater in advance into each test tube until the liquid smoke above are mixed, then shrimp larvae that have been aged 48 hours can be put in the series of test tubes containing liquid smoke that have been prepared respectively about 10 larvae so that the volume for each tube becomes 5 ml. Test tubes then are placed under incandescent light illumination for 24 hours, then the number of dead shrimp larvae are counted. Standard criteria to assess mortality of shrimp larvae is when the shrimp larvae do not show movement for several seconds of observation.

e. Collection of data

The collected data are primary data obtained from the amount of shrimp larvae that died 24 hours after treatment in each combination of three (3) treatments, which are type of raw material, different pyrolysis temperature and different liquid smoke concentration.

f. HatchingArtemiasalinaeggs

Artemiaare soaked in fresh water for 15-30 minutes. Then soaked in 10 liters of seawater. Hatching temperature is 25-30 ℃ and pH ± 6-7. The eggs will hatch after 18-24 hours and the larvae are called nauplii. Naupliiare ready for BST test after these larvae are aged 48 hours.

g. Extract Toxicity Test with BST Method

Liquid smoke from results of raw materials type treatment combination with different pyrolysis temperature is taken 50 mg, each is dissolved in 5 ml of methanol solvent. Dilution is created at 1000, 500,100,50,25, 12.5 and 0 µg/ml. Testing is done by inserting 10 larvae of Artemiasalinaaged 48 hours into glass jars that already contain 1 ml liquid smoke solution and 4 ml of seawater. After 24 hours, the number of dead larvae are counted with the aid of magnifying glass.

Experimental design

The study is conducted by using factorial experimental design of 3 x 4 x 6 with 3 repetitionsso that 72 experimental units are obtained. Factor A is the type of raw material consists of coconut fiber, coconut shell and cinnamon, factor B is the level of pyrolysis temperature of 100 ± 10 ℃; 200 ± 10 ℃; 300 ± 10 ℃; and 400 ± 10 ℃ and factor C is the liquid smoke concentration level of 0 ppm, 12.5 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The observed parameters are the amount of dead Artemia 50% of the total test larvae. Then it is calculated with LC50 values by putting the probitvalue (50% test larvae death).

Data analysis

Toxicity effectis analyzed from the observations with death percentage.

$$\% \text{ Larvae} = \frac{\text{The amount of dead larvae}}{\text{Total test larvae}} \times 100\%$$

By knowing the mortality rate of larvae Artemiasalina, then Probitvalue is calculated through tables and line equation is made:

$Y = Bx + A$

where Y = log concentration, and X = probitvalue

Furthermore, probitvalue is calculated through tables and graphs is made with log concentration as the x-axis against mortality percentage in probit units as the y-axis. LC50 value is the concentration where the substance that causes 50% death of test animals is obtained by using the linear regression equation y = a + bx. A substance is said to be active or toxic when the LC50 value is less than 1000 ppm to extract and less than 30 ppm for a compound (Juniarti, et al., 2009).

Results and Discussion

Cytotoxic characteristic of liquid smoke

The Influence of raw material treatment combination with different pyrolysis temperature to mortality percentage, probitvalue and LC50

The analysis results of variance shows that different raw material treatment combinationon liquid smoke with different temperature pyrolysis provides significant effect on liquid smoke cytotoxic value (P<0:05), as well as in different raw materials treatment combination with different pyrolysis temperatures while for different pyrolysis temperature treatment combination with different concentrations shows no significant difference (P> 0.05) as well as on the interaction of raw materials, pyrolysis temperature and concentration treatments show no significant difference (P>0.05).

Toxicity test by using BSLT method is an acute toxicity test where the toxic effect of a compound is determined in a short time, which ranges up to 24 hours after the administration of test dose (Meyer et al, 1982). BSLT method chosen because it is one of bioactivity methods that is easy, fast, cheap, and accurate. This method is often used to determine the toxicity of natural material/botanical extracts as well as for screening compounds anticancer screening because there is positive correlation between BSLT methods with cytotoxic test by using cancer cell cultures (Carballo, et al., 2002). The average observation of liquid smoke toxicity test results that are raw materials given

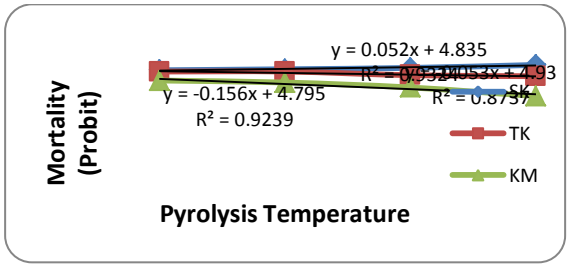
treatment with different pyrolysis temperature against (%) mortality of Artemia, probitvalue and LC50 can be seen from the following Tabel 1.

Table 1. The resultof average observation of liquid smoke toxicity test result that is given raw materials treatment with different pyrolysis temperature against (%) mortality of artemia, probitvalue, and LC50.

Sample code	(%) mortality	Probit	LC50 (ppm)
SKT1 (Coconut fiber, temperature100 ±10°C)	46.19 ± 31.69 ab	4,90	14,9
SKT2 (Coconut fiber, temperature 200 ±10°C)	47.143 ± 32.88 ab	4,93	
SKT3 (Coconut fiber, temperature 300 ±10°C)	49.048 ± 33.01 ab	4,97	
SKT4 (Coconut fiber, temperature 400 ±10°C)	52.381 ± 32.54 a	5,06	
TKT1 (Coconut shell, temperature100 ±10°C)	44.286 ± 31.08 bcd	4,86	20,9
TKT2 (Coconut shell, temperature200 ±10°C)	44.286 ± 31.24 bcd	4,86	
TKT3 (Coconut shell, temperature300 ±10°C)	40 ± 29.83 cde	4,75	
TKT4 (Coconut shell, temperature400 ±10°C)	38.971 ± 28.34 de	4,72	
KMT1(Cinnamon, temperature100 ±10°C)	34.286 ± 30.75 ef	4,59	20,5
KMT2 (Cinnamon, temperature200 ±10°C)	31.905 ± 28.04 fg	4,53	
KMT3 (Cinnamon, temperature300 ±10°C)	26.667 ± 22.21 g	4,38	
KMT4 (Cinnamon, temperature400 ±10°C)	19.048 ± 16.71 h	4,12	

* Different superscript letter on average column shows significant difference (P<0,05)

Table 1 shows the average% mortality of Artemiasalina on cinnamon raw material decreases along with the increase in pyrolysis temperature. The mortality percentage average of cinnamon raw materials on pyrolysis temperature of 400 ± 10 ℃ shows the lowest value of 19.048%, and the highest percentage level on artemiasalina deaths occurs in coconut fiber raw material on pyrolysis temperature of 400 ± 10 ℃ at 52.531%. This means that cinnamon liquid smoke has mortality characteristic lower than coconut shells and coconut fiberraw materials. Low mortality characteristic means that the toxiceffect in cinnamon smoke liquid such as the compounds of aldehyde, ketones, phenols provide lower toxic effect, it is indicated by the decline in the average mortality rate of artemiasalina. An increase in the pyrolysis temperature showsthe decrease in the artemia death percentagesupposedly caused by the fact that higher pyrolysis temperatures will produce more compounds, besides lots of compounds in liquid smoke will be lost so that it will cause less toxic effect. BSLT toxicity test is carried out by determining the LC50 value from the activity of plant's active components against the larvae of Artemiasalina Leach. An extract is said to be toxic by BSLT methods if the extract can kill 50% of test animals at concentrations less than 1000 ppm (Meyer et al, 1982). The picture of regression line equation onrelationship of liquid smoke raw material type with differentpyrolysis temperature against probitvaluemortality as in figure 2 below.



Information : 1= temp. 100 ±10°C; 2= temp. 200 ±10°C; 3=temp. 300 ±10°C;4= temp. 100 ±10°C

Figure 2. Probitvaluemortality average from several raw materials type with different Pyrolysis Temperature Level

Toxicity testthat is conducted on coconut fibercombination at different pyrolysis temperature show LC 50 value of 14.9 ppm, then coconut shell at different pyrolysis temperature of 20.9 ppm, and cinnamon in different pyrolysis temperature of 20.5 ppm. This indicates that liquid smoke the compound of the three different raw material has acute toxicity potential according to BSLT method and can be developed as anticancer because LC50<30 ppm (Juniarti et.al, 2005). Based on regression analysis that different raw materials combination (coconut fiber, coconut shell and cinnamon) shows close relationship to probitvaluewith R^2 respectively at 0.9324, 0.8737 and 0.9239.

Acute toxicity potential that is possessed by liquid smoke is influenced by the secondary metabolites content from the extract. The presence of the flavonoid extract in the cell environment causes the OH- groups in flavonoids to bind the cell membrane integral proteins. This causes the blocking of Na+ - K+active transport. Active transport that has stoppedcauses the insertion of uncontrolled Na+ ions into the cells, causing the rupture of cell membrane (Scheuer, 1994). This rupture of cell membrane becomes the death causeof Artemiasalinalarvae.

The influence of raw material treatment combination with different liquid smoke concentration against mortality percentage, probitvalue and LC50

The average observation result on liquid smoke toxicity test that is given raw material treatment with different liquid smoke concentration to (%)artemiamortality, probitvalueand LC50 value can be seen in table 2 below.

Table 2. Average observations result on liquid smoke toxicity test that are given raw material treatment with different liquid smoke concentration to (%)artemiamortality, probitvalueand LC50.

Sample code	(%) mortality	Probit	LC50 (ppm)
SKK0 (Coconut fiber, liquid smoke concentration 0 ppm)	3.33 ±4.92 i	3,16	22,1
SKK1 (Coconut fiber, liquid smoke concentration 12,5 ppm)	22.5 ±7.54 f	4,24	
SKK2 (Coconut fiber, liquid smoke concentration 25 ppm)	26.67 ±7.79 f	4,38	
SKK3 (Coconut fiber, liquid smoke concentration 50 ppm)	49.17 ±9.96 de	4,98	

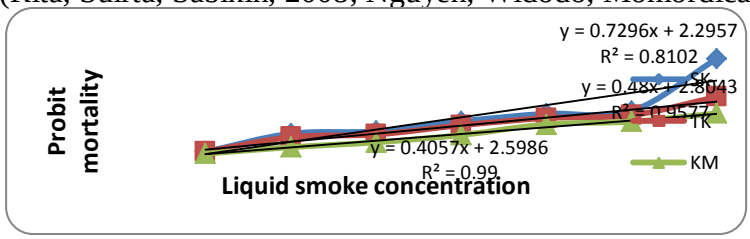
SKK4 (Coconut fiber, liquid smoke concentration 100 ppm)	66.67 ± 9.85 bc	5,43	
SKK5 (Coconut fiber, liquid smoke concentration 500 ppm)	72.5 ± 6.22 b	5,59	
SKK6 (Coconut fiber, liquid smoke concentration 1000 ppm)	100 ± 0.00 a	8,72	
TKK0 (Coconut shell, liquid smoke concentration 0 ppm)	3.33 ± 3.49 i	3,16	19,6
TKK1(Coconut shell, liquid smoke concentration 12,5 ppm)	16.67 ± 1.42 fgh	4,03	
TKK2(Coconut shell, liquid smoke concentration 25 ppm)	20.83 ± 1.42 fg	4,19	
TKK3(Coconut shell, liquid smoke concentration 50 ppm)	39.17 ± 0.83 e	4,74	
TKK4(Coconut shell, liquid smoke concentration 100 ppm)	56.67 ± 2.56 cd	5,17	
TKK5(Coconut shell, liquid smoke concentration 500 ppm)	63.33 ± 2.24 bc	5,34	
TKK6 (Coconut shell, liquid smoke concentration 1000 ppm)	92.5 ± 1.31 a	6,44	
KMK0 (Cinnamon, liquid smoke concentration 0 ppm)	2.5 ± 1.31 i	3,04	27
KMK1 (Cinnamon, liquid smoke concentration 12,5 ppm)	5.83 ± 1.49 hi	3,43	
KMK2(Cinnamon, liquid smoke concentration 25 ppm)	10 ± 1.23 ghi	3,72	
KMK3 (Cinnamon, liquid smoke concentration 50 ppm)	20 ± 2.13 fg	4,16	
KMK4(Cinnamon, liquid smoke concentration 100 ppm)	41.67 ± 3.22 e	4,79	
KMK5(Cinnamon, liquid smoke concentration 500 ppm)	48.33 ± 3.86 de	4,96	
KMK6(Cinnamon, liquid smoke concentration 1000 ppm)	67.5 ± 5.52 bc	5,45	

* Different superscript letter on average column shows significant difference (P<0,05)

Table 2 shows the phenomenon that when the concentration of liquid smoke on the three raw materials used in making liquid smoke is higher, then there is the tendency of an increase in the deaths percentage of artemiasalina. This means that the higher the concentration of liquid smoke that is used, then the concentration of liquid smoke will become more concentrated, so that the mortality percentage of artemiasalina will also increase. For the three raw materialsthat are used, it turns out that the liquid smoke with cinnamon raw material shows the lowest mortality percentage of artemiasalina compared to coconut fiber and coconut shellmaterials. The picture of regression equation of relationship between liquid smoke raw material typeswith different liquid smoke concentration on probitmortality values as in figure 3 below.

Toxicity tests conducted on liquid smoke from coconut fiber raw materials with different liquid smoke concentration indicates LC50 value of 22.1 ppm, coconut shell liquid smoke with different liquid smoke concentration shows LC50 value of 19.6 ppm, cinnamon liquid smoke with different liquid smoke concentration shows LC50 value of 27 ppm. This shows that liquid

smoke from the three raw materials have acute toxicity potential according to BSLT method and it can be developed as the anticancer of LC50<30 ppm value (Juniarti *el.al*., 2005). Based on regression line equation that treatment combinationfrom raw materialtypes with different concentration shows strong relationship to the value of R^2respectively at 0.8102, 0.9577 and 0.99. The image appearance of regression line equation shows the increase in probitvalue when liquid smoke concentration is raised. It is considered that when the liquid smoke concentration is high, then there will be more compounds such as aldehydes, ketones and phenols, so that it causes the probitvalue has tendency to rise. Besides flavonoids, there are secondary metabolites compound can be found in 70% ethanol extract. Those secondary metabolites compounds are saponins and glycosides. Such compounds can act as stomach poisoning. Therefore, when these compounds enter into the larvae body, larvae digestive system will be disrupted. In addition, these compounds inhibit the taste receptors in larvaemouth. This has caused the larvae fail to get taste stimulus that it cannot recognize the food anymore. As the result, the larvae will die of starvation (Rita, Suirta, Sabikin, 2008; Nguyen, Widodo, Momordica, 1999)



Information: 1=0ppm,2=12.5ppm,3=25ppm,4=50ppm,5=100ppm,6=500ppm,7=1000ppm

Figure 3. Probitmortality value average from several raw material typeswith different liquid smoke concentration levels

The influence of liquid smoke concentration treatment combination with different pyrolysis temperature against the mortalitypercentage, probit value and LC50

The observation result of the average liquid smoke toxicity test results that is given pyrolysis temperature treatment and different liquid smoke concentrationagainst (%)artemiamortality, probitvalue and LC50 can be seen in Table 3 below.

Table 3. Observations result on liquid smoke toxicity tests average resultthat is given liquid smoke concentration treatment and different pyrolysis temperature against (%)artemiamortality, probitvalues and LC50.

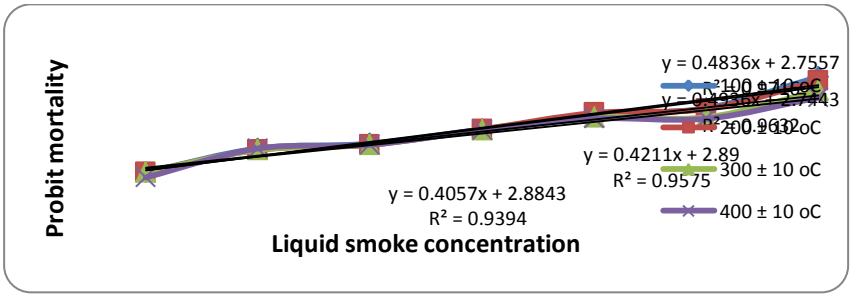
Treatment	(%) Mortality	Probit	LC 50 (ppm)
K0T1(liquid smoke concentration 0 ppm, temperature 100 ±10^{o}C)	3.333 ±5.0 hi	3,16	20,5
K0T2 (liquid smoke concentration 0 ppm, temperature 200 ±10^{o}C)	3.333 ±5.0 hi	3,16	22
K0T3 (liquid smoke concentration 0 ppm, temperature 300 ±10^{o}C)	3.333 ±5.0 hi	3,16	15,9
K0T4 (liquid smoke concentration 0 ppm, temperature 400 ±10^{o}C)	2.222 ±4.41 i	2,98	17,9
K1T1 (liquid smoke concentration 12.5 ppm, temperature 100 ±10^{o}C)	15.556 ±8.82 g	3,99	20,5

K1T2 (liquid smoke concentration 12.5 ppm, temperature 200 ±10°C)	14.444 ± 8.82 gh	3,94	22
K1T3 (liquid smoke concentration 12.5 ppm, temperature 300 ±10°C)	14.444 ± 8.82 gh	3,94	15,9
K1T4 (liquid smoke concentration 12.5 ppm, temperature 400 ±10°C)	15.556 ± 11.3 g	3,99	17,9
K2T1 (liquid smoke concentration 25 ppm, temperature 100 ±10°C)	20 ± 7.07 g	4,16	20,5
K2T2 (liquid smoke concentration 25 ppm, temperature 200 ±10°C)	18.889 ± 9.28 g	4,12	22
K2T3 (liquid smoke concentration 25 ppm, temperature 300 ±10°C)	18.889 ± 7.81 g	4,12	15,9
K2T4 (liquid smoke concentration 25 ppm, temperature 400 ±10°C)	18.889 ± 15.89 g	4,12	17,9
K3T1 (liquid smoke concentration 50 ppm, temperature100 ±10°C)	36.667 ± 15.81 f	4,66	20,5
K3T2 (liquid smoke concentration 50 ppm, temperature 200 ±10°C)	36.667 ± 13.22 f	4,66	22
K3T3 (liquid smoke concentration 50 ppm, temperature 300 ±10°C)	35.556 ± 15.89 f	4,63	15,9
K3T4 (liquid smoke concentration 50 ppm, temperature 400 ±10°C)	35.556 ± 18.11f	4,63	17,9
K4T1 (liquid smoke concentration100 ppm, temperature 100 ±10°C)	56.667 ± 10.0 cde	5,17	20,5
K4T2 (liquid smoke concentration 100 ppm, temperature 200 ±10°C)	58.889 ± 10.54 cde	5,23	22
K4T3 (liquid smoke concentration 100 ppm, temperature 300 ±10°C)	53.333 ± 15.0 de	5,08	15,9
K4T4 (liquid smoke concentration 100 ppm, temperature 400 ±10°C)	51.111 ± 20.76 e	5,03	17,9
K5T1 (liquid smoke concentration 500 ppm, temperature 100 ±10°C)	65.556 ± 8.82 c	5,40	20,5
K5T2 (liquid smoke concentration 500 ppm, temperature 200 ±10°C)	64.444 ± 10.14 cd	5,37	22
K5T3 (liquid smoke concentration 500 ppm, temperature 300 ±10°C)	53.333 ± 15 de	5,08	15,9
K5T4 (liquid smoke concentration 500 ppm, temperature 400 ±10°C)	51.111 ± 20.76 e	5,03	17,9
K6T1 (liquid smoke concentration 1000 ppm, temperature 100 ±10°C)	93.333 ± 7.07 a	6,49	20,5
K6T2 (liquid smoke concentration 1000 ppm, temperature 200 ±10°C)	91.111 ± 10.54 a	6,35	22
K6T3 (liquid smoke concentration 1000 ppm, temperature 300 ±10°C)	84.444 ± 19.44 ab	6,01	15,9
K6T4 (liquid smoke concentration 1000 ppm, temperature 400 ±10°C)	77.778 ± 26.35 b	5,77	17,9

* Different superscript letter on average column shows significant difference (P<0,05)

Based on the average data of artemiasalinadeathpercentage in Table 3 shows an increase in artemiadeath percentage with the increase in pyrolysis temperature and liquid smoke concentration that are used. The highest mortality percentage of artemiasalinacan be found in liquid smoke concentration treatment combination of 1000 ppm on pyrolysis temperature of 100±100 ℃ at

93.33% does not significant difference with liquid smoke concentration 1000 ppm on pyrolysis temperature of 200±100℃, while the lowest value of artemiasalina death occurs in the combination treatment without liquid smoke at temperature of 400±100℃.This is caused by the fact that when pyrolysis temperature is high and the use of liquid smoke concentration is high, the percentage of artemiadeath will also become high. This condition means that the toxicity effects from cinnamon liquid smoke will increase along with the liquid smoke concentration and pyrolysis temperature that are used. The picture of relationship between liquid smoke pyrolysis temperature with different liquid smoke concentration on mortality probit value as in figure 4 below.



Information: 1=0ppm,2=12,5ppm,3=25ppm,4=50ppm,5=100ppm,6=500ppm,7=1000ppm

Figure 4. Probitmortality average value from several pyrolysis temperature with different liquid smoke concentrationlevel.

Toxicity tests conducted on pyrolysis temperature treatment of 100±10℃, 200±10℃, 300±10℃ and 400±10℃ in different liquid smoke concentration shows that LC50 value respectively at 20.5 ppm, 22 ppm, 15.9 ppm, 17.9 ppm. This shows that liquid smoke on different pyrolysis temperatures has the acute toxicity potential according to BSLT method and it can be developed as anticancer because the value of LC50<30 (Juniarti *et.al.*, 2005). Based on the regression line equation that pyrolysis temperature combination of 100±10℃, 200±10℃, 300±10℃ and 400±10℃ with different liquid smoke concentration showsstrong relationship to the probitvalue of R^2 respectively at 0.9716, 0.9632, 0.9575 and 0.9394.

The influence of raw material treatment combination with different pyrolysis temperature and liquid smoke concentration against mortality percentage, probit value and LC50

The observation result of the average liquid smoke toxicity test results that is given different raw material, pyrolysis temperature, and liquid smoke concentration treatment combination against (%)artemia mortality, probit value and LC50 can be seen in Table 4 below.

Table 4. Average percentage of artemiasalinamortality, liquid smoke probit value and LC50 value that is given different raw materials, pyrolysis temperature and liquid smoke concentrationtreatment combination**.**

Raw Materials	Temperature	Liquid smoke concentration	Concentration log	(%) mortality	Probit	LC 50 (ppm)
Coconut fiber	100 + 10 oC	0 ppm	0	3.33±5.77 tu	3.162	10,5

		12.5 ppm	1.097	23.33 ±5.77 nopqrst	4.274	11,6
		25 ppm	1.398	26.67 ±5.77 nopqrst	4.375	39,8
		50 ppm	1.699	40 ±10.0 klmnop	4.747	55
		100 ppm	2	60 ±10.0 efghijk	5.253	18,6
		500 ppm	2.69	70 ±10.0 cdefgh	5.424	11,6
		1000 ppm	3	100 ±0.00 a	8.719	48,4
	200 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	20 ±10.0 pqrstu	4.158	11,6
		25 ppm	1.398	23.33 ±11.55 opqrst	4.915	39,8
		50 ppm	1.699	46.67 ±11.55 ijklmn	5.431	55
		100 ppm	2	66.67 ±5.77 defghi	5.431	18,6
		500 ppm	2.69	70 ±0.00 cdefgh	5.424	11,6
		1000 ppm	3	100 ±0.00 a	8.719	48,4
	300 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	20 ±10.0 pqrstu	4.158	11,6
		25 ppm	1.398	26.67 ±5.77 nopqrs	4.375	39,8
		50 ppm	1.699	53.33 ±5.77 ghijkl	5.082	55
		100 ppm	2	66.67 ±15.28 defghi	5.431	18,6
		500 ppm	2.69	73.33 ±8.77 bcdefg	5.622	11,6
		1000 ppm	3	100 ±0.00 a	8.719	48,4
	400 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	26.67 ±5.77 nopqrs	4.375	11,6
		25 ppm	1.398	30 ±10.0 mnopqr	4.476	39,8
		50 ppm	1.699	56.67 ±5.77 fghijk	5.168	55
		100 ppm	2	73.33 ±5.77 bcdefg	5.622	18,6
		500 ppm	2.69	76.67 ±5.77 bcdef	5.726	11,6
		1000 ppm	3	93.33 ±0.00 a	6.498	48,4
Coconut shell	100 + 10 oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	16.67 ±5.77 qrstu	4.034	11,6
		25 ppm	1.398	20 ±0.00 pqrstu	4.158	39,8
		50 ppm	1.699	50 ±10.0 hijklm	2	55
		100 ppm	2	60 ±10.0 efghijk	5.253	18,6
		500 ppm	2.69	66.67 ±5.77 defghi	5.431	11,6
		1000 ppm	3	93.33 ±5.77 ab	6.498	48,4

	200 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	16.67 ±5.77 qrstu	4.034	11,6
		25 ppm	1.398	23.33 ±5.77 opqrst	4.271	39,8
		50 ppm	1.699	40 ±10.0 klmnop	4.747	55
		100 ppm	2	63.33 ±5.77 efghij	5.082	18,6
		500 ppm	2.69	70 ±0.00 cdefgh	5.253	11,6
		1000 ppm	3	93.33 ±5.77 ab	6.498	48,4
	300 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	16.67 ±5.77 qrstu	4.034	11,6
		25 ppm	1.398	20 ±0.00 pqrstu	4.158	39,8
		50 ppm	1.699	33.33 ±5.77 lmnopq	4.568	55
		100 ppm	2	53.33 ±5.77 ghijkl	5.082	18,6
		500 ppm	2.69	60 ±10.0 efghijk	5.253	11,6
		1000 ppm	3	93.33 ±5.77 ab	6.498	48,4
	400 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	16.67 ±5.77 qrstu	4.034	11,6
		25 ppm	1.398	20 ±0.00 pqrstu	4.158	39,8
		50 ppm	1.699	33.33 ±5.77 lmnopq	4.568	55
		100 ppm	2	50 ±10.00 hijklm	2	18,6
		500 ppm	2.69	56.67 ±5.77 fghijk	5.168	11,6
		1000 ppm	3	90 ±0.00 abc	6.282	48,4
Cinnamon	100 + 10 oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	6.67 ±5.77 stu	3.501	11,6
		25 ppm	1.398	13.33 ±5.77 qrstu	3.888	39,8
		50 ppm	1.699	20 ±10.00 pqrstu	4.158	55
		100 ppm	2	50 ±10.00 hijklm	2	18,6
		500 ppm	2.69	60 ±10.00 efghijk	5.253	11,6
		1000 ppm	3	86.67 ±5.77 abcd	6.112	48,4
	200 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
		12.5 ppm	1.097	6.67 ±5.77 stu	3.501	11,6
		25 ppm	1.398	10 ±0.00 rstu	3.718	39,8
		50 ppm	1.699	23.33 ±5.77 opqrst	4.271	55
		100 ppm	2	46.67 ±5.77 ijklmn	4.917	18,6
		500 ppm	2.69	53.33 ±11.55	5.082	11,6

			ghijkl		
	1000 ppm	3	80 ±10.00 abcde	5.842	48,4
300 + 10oC	0 ppm	0	3.33 ±5.77 tu	3.162	10,5
	12.5 ppm	1.097	6.67 ±5.77 stu	3.501	11,6
	25 ppm	1.398	10 ±0.00 rstu	3.757	39,8
	50 ppm	1.699	20 ±10.00 pqrstu	4.158	55
	100 ppm	2	40 ±10.00 klmnop	4.748	18,6
	500 ppm	2.69	46.67 ±11.55 ijklmn	4.917	11,6
	1000 ppm	3	60 ±10.00 efghijk	5.253	48,4
400 + 10oC	0 ppm	0	0 ±0.00 u	0	10,5
	12.5 ppm	1.097	3.33 ±5.77 tu	3.162	11,6
	25 ppm	1.398	6.67 ±5.77 stu	3.501	39,8
	50 ppm	1.699	16.67 ±5.77 qrstu	4.034	55
	100 ppm	2	30 ±10.00 mnopqr	4.476	18,6
	500 ppm	2.69	33.33 ±5.77 lmnopq	4.568	11,6
	1000 ppm	3	43.33 ±5.77 jklmno	4.831	48,4

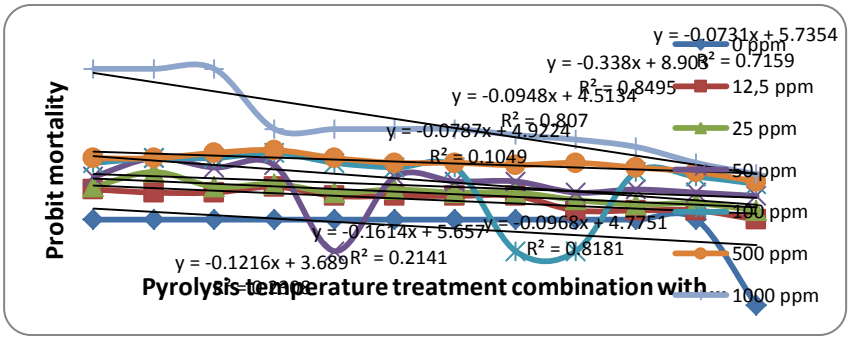
* Different superscript letter on average column shows significant difference (P<0,05)

Mortality percentage test results on artemiasalina on liquid smoke showsliquid smoke concentration in a medium can kill A. salina Leach larvae in a row with concentration of 1000, 500, 100, 50, 25 and 12.5 ppm and 0 ppm. The mortality amount of A. salina Leach larvaeon each test cup in various raw materials, pyrolysis temperature with different treatment concentrationcan be seen in table 4. From that table it can be seen that the combination of the raw material, pyrolysis temperature and different liquid smoke concentration in this experiment shows the insignificant effect on the death of A. salina Leachlarvae. The amount of larvae per test cup is 10 larvae and each concentration is performed in three repetitions. The total amount of A. salina Leach larvae that are used are 2520 larvae, those larvae are aged 48 hours, because at this age, larvae body members are already complete compared to when the larvae hatch. In observing the growth and development of larvae until the extract toxicity testing, magnifying glass are used to observe.

Based on Tabel 4 that there is a tendency that the higher liquid smoke concentration used on each raw materials, then the higher of response or impact caused by the death of test animals. Mortality and survival in a time period of exposure is a specific effect in acute toxicity tests with long-term exposure. The data from lethality test are quantal, which means the test animals are deador alive after the experiment.

Based on death percentage average data of artemiasalina on the combination of three treatments (raw material, pyrolysis temperature and different liquid smokeconcentration) shows an increase in deaths percentage of artemiaalong with the increase of liquid smoke concentration that is used. This is caused by the higher consumption of liquid smoke concentration that also

increase the death percentage of artemia. This condition means that mortality effects from cinnamon liquid smoke will increase along with liquid smoke concentration and temperature pyrolysis that are used. Furthermore, table 4 shows the higher concentration of liquid smoke that is given to the larvae, a tendency shows the mortality rate of larvae will increase. It happens to the three raw material of liquid smoke maker at different pyrolysis temperature. Coconut fiber liquid smoke with different pyrolysis temperature shows the highest mortality rate of larvae at each concentration after one hour, then it is followed by coconut shell and cinnamonliquid smoke. Acute toxicity potential that is possessed by liquid smoke is influenced by the content of secondary metabolites of that liquid smoke. The presence of the flavonoid extract in the cell environment has caused the OH- groups in flavonoids binds to cell membrane integral proteins. This causes active transport obstruction of Na+ - K+. Active transport that is stopped will cause the influx of uncontrolled Na+ ions into the cells, causing rupture of cell membrane (Scheuer, 1994). This Rupture of cell membranes has caused the death of artemiasalinalarvae. In order to find for a death percentage of artemia up of 50%, then it will use LC50 (lethal concentration), it means that this numbers will be useful in predicting the concentration of liquid smoke that is used until the death of artemiasalinaon 50%. The following is artemiaprobit mortality value to find LC 50 in Figure 5 below.



Information1=SKT1,2=SKT2,3=SKT3,4=SKT4,5=TKT1,6=TKT2,7=TKT3,8=TKT4,9=KMT1,10=KMT2,11=KMT3, 12=KMT4.

Figure 5. Mortality average on Probit value from several raw materials with different pyrolysis temperature andliquid smokeconcentration

LC50 values in different raw materials treatment combinationwith different pyrolysis temperature at concentration of 0 ppm, 12.5 ppm, 50 ppm, 100 ppm, 500 ppm and 1000 ppm respectively at 10.5 ppm, 11.6 ppm, 39.8 ppm, 55 ppm, 18.6 ppm, 11.6 ppm and 48.4 ppm. Based on the opinion of Juniarti *et al*, (2005) that mortality percentage data of Artemiasalina leach (Table 5) and LC50 value that the combination of raw materials with pyrolysis temperature at concentration of 0 ppm, 12.5 ppm, 50 ppm and 500 ppm are active because LC50<50 and concentration of 50 ppm, 100 ppm and 1000 ppm are not active. The data shows the tendency that the higher concentration of liquid smoke that is used, then the mortality percentage of artemia will increase. The large number of dead Artemiais equal with the increase of liquid smoke concentration that is used. It is caused by the higher liquid smoke concentration that is used, then there is an increase in the amount of aldehyde and phenol

compounds that are formed, it will further increase the amount of dead artemia. Then, figure 4 shows that toxicity tests on probit value that is indicated by different raw materials treatment combinationwith pyrolysis temperature on liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm has weak relation to probitvalue. It is indicated by the value of R^2respectively at 0.1049, 0.2141 and 0.2308. For other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm, it has a close relationship with probit value as indicated by the value of R^2respectively at 0.7159, 0.8495, 0.807 and 0.8181. Besides flavonoids, there are several secondary metabolites compounds in liquid smoke. Thosesecondary metabolites compoundsamong others are saponins and glycosides. Such compounds can act as stomach poisoning. Therefore, when these compounds enter into larvaebody, larvae digestive system will be disrupted. In addition, these compounds inhibit taste receptors in the mouth of larvae. This causes the larvae fail to get a taste stimulus that it cannot recognize the food anymore. As the result, the larvae will die of starvation (Rita *et al*, 2008; Nguyen *et al*, 1999)

Conclusion

1. The best liquid smoke production quality on cinnamon raw material treatment at temperature level of 400±10 ℃ that shows mortality rate against artemiasalinaat 19.048% that is the smallest compared to other two raw materials.
2. Liquid Smoke as the result of different raw materials treatment combination (coconut fiber, coconut shell and cinnamon) with different pyrolysis temperature show toxic characteristic (LC50<30 ppm) with LC50 value respectively at 14.9 ppm, 20.9 ppm and 20.5 ppm.
3. Liquid Smoke as the result of raw materials treatment combination (coconut fiber, coconut shell and cinnamon) with different liquid smoke concentration show toxic characteristic (LC50<30 ppm) with LC50 value respectively at 22.1 ppm, 19 6 ppm and 27 ppm.
4. Liquid Smoke as the result of pyrolysis temperature treatment combination (100±10 ℃, 200±10 ℃, 300±10 ℃ and 400±10 ℃) at different liquid smoke concentration show toxic characteristic (LC50<30 ppm) with LC50 values respectively at 20.5 ppm, 22 ppm, 15.9 ppm and 17.9 ppm.
5. Liquid Smoke as the result of different raw materials treatment combination with different pyrolysis temperature at concentration of 0 ppm, 12.5 ppm, 100 ppm, 500 ppm shows toxic characteristic (LC50<30 ppm) with LC50 valuerespectively at 10.5 ppm, 11.6 ppm, 39.8 ppm, 18.6 ppm, 11.6 ppm while the concentration of 50 ppm and 1000 ppm, LC50 values at 55 ppm and 48.4 ppm are not toxic (LC50> 30 ppm), then in regression line equation, different raw materials treatment combination with pyrolysis temperature on liquid smoke concentration of 50 ppm, 500 ppm and 1000 ppm have weak relation to the probitvalue with R^2 values respectively at 0.1049, 0, 2141 and 0.2308, while the other concentration of 0 ppm, 12.5 ppm, 50 ppm and 100 ppm have stronger relationship with probit value as indicated by the value of R^2respectively at 0.7159, 0.8495, 0.807 and 0.8181.

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