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NANOFORMULATION OF MINT OIL: ITS EFFECTIVENESS AGAINST PHOSPHINE RESISTANT STRAINS of *Tribolium castaneum* (Herbst) (COLEOPTERA: TENEBRIONIDAE) A MAJOR STORED PRODUCT PEST

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ABSTRACT

Essential oils have good potency to be used as alternative for controlling stored product insects, so our dependency on synthetic fumigants can be avoided since synthetic fumigant like phosphine can cause resistance to stored product insects. The objectives of this research were to: (1) producing nanoemulsion and nanopowder of mint oil, (2) producing nanoformulation, (3) find out the best nanoformulation, (4) collecting stored product insects from food and feed warehouses in the Provinve of West Sumatera, South Sumatera, and North Sulawesi, and testing their resistance against phosphine in the laboratory, (5) producing database of phosphine resistant strains of stored product insects in Indonesia. The results showed that nanoemulsion of mint oil can be fomulated and stabilized with 10% of mint oil concentration by adding Tween and glycerol as emulsifier in 1:1 composition. Particle size of nanoemulsion formed was 98.57 nm with the value of *PolyDispersion Index* (PDI) and zeta potensial -16.3 and 0.510 respectively. Nanopowder of mint oil was formed through the process of spray drying with maltodextrin 40% as encapsulate. Nanopowder produced was in form of white powder with a rounded surface shape, experiencing shrinkage, and the shape tends to be not uniform. Nanoemulsion of mint oil formulated as tablets showing higher effectiveness compared to tablet formulation of n-hexane fraction of crude mint oil at the same level of concentration. Stored product insects collected from food and feed waerhouses in the Province of West Sumatera, South Sumatera, and North Sulawesi showed varied level of resistance (RF) against phosphine: *Tribolium castaneum* 0.83 – 23.30 times and *Rhyzopertha dominica* 0.83 – 52.65 times.

Key words: *nanoemulsion*, *nanotablet*, *nanopowder*, R. dominica, *resistance*, T. castaneum

1. INTRODUCTION

1.1 Background

Protecting stored food and feed from insect attack is a crucial thing to do in order to ensure our self sufficiency program for those commodities succeed, because insect pests are the biggest threat to our food security program. Stored food and feed can be attacked by more than 600 species of beetles, 70 species of moths and around 355 species of mites that will cause the decrease of its quality and quantity (Rajendran 2002). The magnitude of stored product losses during is depend on insect species that attacks, the length and technique applied of storage, and pest management strategy implemented.

Very common stored product pest management implemented is fumigation. Methyl bromide and phosphine are two fumigants that are commonly applied. However, methyl bromide which is broad spectrum fumigant is also ozon depleting substance, so that their use has been stopped under the 1992 Montreal Protocol, except for quarantine and pre-shipment purposes. Therefore, phosphine use is the only choice for years in managing stored product pests. Insect resistance to phosphine has now become a global issue and failure of control in the field has been reported in several countries (Taylor 1989, Collins *et al.* 2002). In addition, there are several arguments about genotoxicity of phosphine (Garry *et al.* 1989). That is why alternative fumigants to replace phosphine need to be discover. Essential oils showing a good potential to be explored.

The study of the toxicity of fumigants distilled from essential oils of plants and their constituents has been sharpened lately (Isman 2006). Plant essential oils have traditionally been used to kill or repel insects, such as insecticides and repellents, which are considered as alternatives to conventional pesticides for protecting the seeds because of their low toxicity to warm-blooded mammals and their high volatility and can also be biologically degraded (Isman 2006, Shaaya *et al.* 1997, Sukmar *et al.* 1991).

Many recent studies have shown that this volatile substance consists of complex compounds and can be used to kill various species of insect pests in stored products (Mahmoudvand *et al.* 2011, Manzoomi *et al.*, 2010, Rani 2012, Tunc *et al.*, 2000). Research conducted by Harahap *et al.* (2016) showed the value of LC_{95} of n-hexane of mint oils against *Tribolium castaneum* was 1,75% or equivalen with 0,088 mL/L fumigation chamber. Despite the fact that essential oils have the most promising properties, problems related to their volatility, poor water solubility, and oxidation

potential must be resolved before being used as an alternative fumigant for pest management purpose.

The overall goal of the controlled release formulation consists of protecting the reagent supply and allowing automatic transmission to the target at a controlled level to maintain its concentration in the optimum level for a long time. Controlled release technology can also help increase the protection of stored grains against insect and rodent pests (Kenawy *et al.* 1992). Alternative formulations such as nanoformulation are being developed to improve the persistence of bioactive plant essential oils by reducing volatilization and slowing the rate of degradation in the environment (Batish *et al.* 2008).

Essential oils in the form of nanoparticles such as nanoemulsion are emulsions which droplet sizes are uniform and very small with sizes ranging from 20 to 200 nm. The use of nanoparticles for fumigant formulations will be a contemporary measure for controlling pests and reducing the toxic effects of synthetic mass pesticides on the environment (Kumar *et al.* 2013).

The series of studies on the exploration and development of essential oils formulations as fumigants in controlling stored product insects is very necessary to obtain alternative fumigants that can reduce the level of resistance of insect pests to phosphine. In addition, the active compounds of essential oils which have been known to be effective against stored product insects can be models of new synthetic compounds that are more environmentally friendly and have low toxicity to mammals.

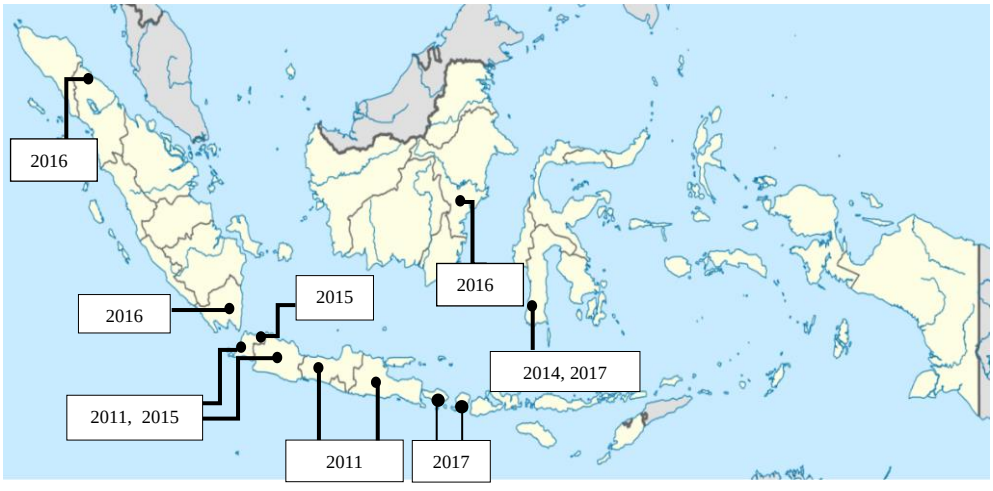

Figure 1 The location of survey during the 2011 to 2017 research period to collect stored product insect pests that were suspected of being resistant to phosphine

Our research program was carried out by implementing a road map (Table 1) combining between mapping of phosphine-resistant strains of stored product insects nation wide (Figure 1) and exploration of essential oils that are effective against those resistant

strains, especially *T. castaneum*. Crude essential oils that were known to be effective in previous studies were fractionated and tested for effectiveness in the following year.

Table 1 Road map, research plan that combining between mapping of phosphine resistant strains of stored product insects and exploring of essential oils effective for controlling those resistant strains

Year	Location and Resistance Factor	Essential oils tested and the results (LD_{95} at 72 hours fumigation; mL/L fumigation chamber	Next step of reserach
2014	Makassar; 1.2 – 15.1 Collected from cocoa beans	• Artemisia: 94% mortality at 0.7 ml/L fumigation chamber • Clove: 98% mortality at 0.1 ml/L fumigation chamber • Lemon peel: 97% mortality at 1.75 ml/L fumigation chamber • Peppermint: 100% mortality at 0.4 ml/L fumigation chamber • Patchouli: 98% mortality at 0.65 ml/L fumigation chamber	Fractionation of clove oil (2015) and mint oil (2016)
2015	Banten and West Java; 1.0 – 350.70 Collected from food and feed storages	• Cardamom: 0.195 on R- and 0.227 on Non R-strains • Cinnamon: 0.013 on R- and 0.010 on Non R-strains • Nutmeg: 1.845 on R- and 0.628 on Non R-strains • N-hexane fraction of clove oil: LD_{95} : 0.8 on R-strains	Fraksination of cardamom and cinnamon oils; and formulation of n-hexane of clove oil (2016)
2016	North Sumatera Lampung and East Kalimantan; 0.60 – 4; Collected from food and feed storages	• Callilawan oil: 0.437 on Non-R strain • Ginger oil: 0.436 on Non-R strain • Lemongrass oil: 1.065 on Non-R strain • N-hexane fraction of peppermint oil: 0.088 on Non-R strain • N-hexane fraction of cinnamon oil: 0.231 on Non R- and 0.375 on R-strain • Ethyl acetate fraction of cinnamon oil: 0.109 on Non R- dan 0.085 on R- strain • N-hexane fraction of cardamom oil: 0.213 on Non R- and 0.375 on R-strain • Formulation of clove oil in tablets: 54% mortality at 7 days fumigation using clove oil tablets, and 100% mortality at 7 hari fumigation using containing a mixture between clove oil and naphthalene	- Fraksination of cullilawan and ginger oils and tested their effectiveness against collected insect tests - Formulation of clove, mint, and cinnamon oils; and tested their effectiveness against collected insect tests keefektifannya
2017	Bali, South Sulawesi and West Nusa Tenggara (NTB); Collected from food and feed warehouses	• The most effective fumigant tablets containing fractionated essential oils in causing mortality on *T. castaneum* was the tablets contain a mixture of hexane fraction of cardamom oil with naphthalene and a mixture of hexane fraction of mint with naphthalene (1:1) with 7 day exposure time.	Filed survey and continuing to formulate the effective essential oil from the previous reserach: -Formulation of effective essential

		• Fumigant gel showing the highest reppelance level was hexane fraction of cardamom that contain 2 ml of essential oil in 30 g gel with the level of reppelance around 65%	oils - Formulation of essential oil mixture and comparing it with single oil formulation
2018	West Sumatera, South Sumatera, and North Sulawesi	Objectives: - Creating database of phosphine resistant strains of stored product insects in Indonesia - Exploring the best formulation for mint oil, and its application methods	- Efficacy tests of nanoformulations of mint oils at the bigger size of fumigation chamber

1.2 Objectives

The objectives of this research were: (1) to produce nanoemulsion and nanopowder of mint oil, (2) to produce nanoformulation of mint oils, (3) to find out the best nanoformulation, (4) to collect suspected phosphine resistance strains of stored product insects from food and feed warehouses in the Provinces of West Sumatra, South Sumatra, and North Sulawesi and test their resistance status, (5) produce a database of phosphine resistance starins of stored product insects pests in Indonesia.

1.3 Expected Output

The expected outputs of this research were the availability nanoformulation of mint oil to be used as an alternative fumigant for stored product insects management and the availability of a database about distribution of phosphine-resistant strains of stored product insect pests in Indonesia.

2. BENEFIT AND IMPORTANCE OF THIS RESEARCH

Database about distribution of phosphine resistant strains of stored product insects in Indoneisa is needed to determine the special management strategy, especially fumigation, to overcome the problems posed by those strains in certain province in Indonesia. In addition, by knowing the distribution map of this resistant strains, the effort of preventing them to spread to other location in Indonesia could be carried out.

More and more species of stored product insect pests detected to develop as resistant strains stimulate many researchers to search for alternative fumigants to stop this development. One of the alternatives is using essential oils. Essential oil is a secondary metabolite of plants consisting of various compounds that have different properties and characteristics. The use of essential oils as fumigants can be the right choice. This is due to the compound contained in the essential oil, so it is quite safe even if used for a long period of time.

The use of chemicals that consist of many compounds in controlling pests can slow the rate of insect resistance to these chemicals compared to those with single compound. In addition, essential oils are also relatively safer and environmentally friendly compared to the existing fumigants. Therefore, research on the potential exploration of essential oils as alternative fumigants from phosphine is very important to do.

3. METHODOLOGY

This research was conducted through five stages of activity: (1) preparing n-hexane fraction mint oil to become microparticles, (2) preparing nanoformulation mint tablets, (3) testing the effectiveness nanoformulation of mint tablet, (4) collecting insects suspected of being resistant from food and feed warehouses in the provinces of West Sumatra, South Sumatra and North Sulawesi, (5) detection of resistant strains from insect stored product insects collected from food and feed storage warehouses.

3.1 Preparing of Microparticles of Mint Oil

Microparticles of mint essential oil were made in two forms, namely nanoemulsion mint oil and mint nanopowder. The essential oil used to make mint essential oil microparticles is the n-hexane fraction of mint oil.

3.1.1 Preparing Nanoemulsion of Mint Oil

Preparing nanoemulsion of n-hexane fraction of mint oil was conducted by using magnetic stirrer and Ultra-Turrax homogenizer. The material used for this preparation were n-hexane fraction of mint oil, Tween 80 pro analysis (p.a), glycerol, and distilled water.

Nanoemulsion of n-hexane fraction was prepared by low energy spontaneous diffusion method. The emulsion system formed consisted of an oil phase in the form of mint oil n-hexane fraction and a water phase consisting of Tween 80, aquades, and glycerol. The n-hexane fraction of mint oil was obtained from simple fractionation using three types of solvents with different levels of polarity, namely methanol (polar), ethyl acetate (semi-polar), and n-hexane (non-polar). Mint oil from fractionation mixed in n-hexane solvent is then separated from the solvent using a rotary evaporator

The spontaneous emulsification method is carried out by adding the organic phase into the water phase through penetrating (drop by drop). When dripping the organic phase into the water phase, the water phase is stirred using a magnetic stirrer. The n-hexane fraction of mint oil was mixed with Tween 80, then stirred at a speed of 700 rpm for 10 minutes using a magnetic stirrer. Furthermore, the mixture of mint oil and Tween 80 was added dropwise to distilled water and/or glycerol (water phase) while still stirring at a speed of 700 rpm. Then constant stirring was carried out at 700 rpm for 60 minutes from the last drops of the oil phase (n-hexane mint fraction and Tween 80).

The concentration of essential oil in the emulsion are 3% and 10%, while the emulsion-forming material is made in several different concentrations (Table 2). The best nanoemulsion formulation was determined based on the analysis of physical characteristics, particle size, and potential zeta. The best nanoemulsion formulation was then used in the preparation of mint oil nanotablet formulations and tested for effectiveness against insect pests *T. castaneum*.

Table 2. Composition of nanoemulsion ingredients of mint oil

Ingredients	**Composition of ingredients in formulation**	
	FM-3	**FM-7**
n-hexane fraction of mint oil	10%	10%
Tween 80 p.a	10%	20%
Glycerol	10%	10%
Distilled water	70%	60%

3.1.2 Preparation of Mint Oil Nanopowder

Preparation of mint oil nanopowder was conducted based on modified of Artika *et al.* (2011) methods. Maltodextrin is used as a coating material to protect the active ingredients of mint oil from various conditions such as changes in temperature and humidity during the process of nanoparticle formation. The use of maltodextrin as a thin layer also allows essential oils to dissolve in water.

The ingredients used in the preparation of mint oil nanopowder was 10% mint nanoemulsions 3% and 10% with the code formulations FM-3 and FM-10 and maltodextrin as coating material. The composition of maltodextrin used is 40%. The preparation of nanopowder is conducted by spray drying process at 170^{o}C inlet temperature. Nanopowder formed was then observed for surface morphology using Scanning Electron Microscopy (SEM).

3.2 Preparation of Mint Oil Nanoformulation

3.2.1 Nanotablet Formulation

The ingredients used for the preparation of nanotablet formulations consisted of nanoemulsion of mint oil and pure talc (odorless). The tools used were manual tablet printers, glass jars, and plasticine (natural paraffin).

Preparing a nanotablet for mint oil formulation was carried out by adding mint nanoemulsion 10% into 10 g pure talc. The composition of mint nanoemulsion added to the pure talk can be seen in Table 3. The nanoemulsion of mint oil and talc mixture is then stirred evenly, then printed using a manual tablet printing device

Table 3 Composition of Nanotablet Formulation

Treatment codes	Composition		Number of tablets applied	Total amount of n-hexane fraction applied (ml)
	Pure talc (g)	10% mint oil nanoemulsion (ml)		
I	10	1	2	0.2
II	10	2	3	0.6
III	10	2.5	4	1
IV	10	3	3	0.9

[a]Each treatments was repalicated 5 times

3.3 Testing the Effectiveness of Mint Oil Nanotablet

Testing the effectiveness of mint oil nanotablets was conducted on insect pests *T. castaneum* using a 3.5-liter glass jar as fumigation chamber, as much as 40 g of rice were put into the jars. Furthermore, *T. castaneum* were infested into 20 glass jars. Each nanotablet is then put into a different jar that has been filled with rice and test insects. The jars are then closed and the gap between the jar and the lid is glued using plasticine to prevent gas leakage.

This bioassay consisted of 2 factors: (1) the effectiveness of each nanotablet, and (2) the effectiveness of nanotablet at different time tested; 3, 5, and 7 days. Variable observed in this bioassay was insect test mortality, which was checked at 3, 5, and 7 days.

3.3.1 Design of Experiment and Data Analysis

The design of experimen used for this bioassay was a factorial in completely randomized design for fumigant tablets. The first factor is the type of nanoformulation and the second factor is the length of time for exposure to fumigants. Mortality data were analyzed using Microsoft Excel 2007 and all data processed using SAS 9.2 software then Duncan's multiple comparison test ($\alpha = 0.5$).

3.4 Collection of Phosphine Resistant Strains from Food and Feed Warehouses

Field surveys were carried out in food and feed warehouses in North Sulawesi, West Sumatra and South Sumatra Provinces. Survey activities are carried out in food warehouses located in Palembang (South Sumatra), Padang (West Sumatra), and Manado (North Sulawesi).

The materials and tools needed during the survey are insect collection equipment, such as insect bottles, plastic bags, labels, permanent markers, and small brushes. For the multiplication of pest insect collections from the field in the laboratory, glass jars, flour, rice and bran are needed as feed.

Insect collection is done by visiting food and feed warehouses in North Sulawesi, West Sumatra and South Sumatra Provinces. During each visit, pest insects found in each warehouse are then collected directly using a small brush and aspirator. The insect samples obtained were then taken to the Entomology laboratory, BIOTROP SEAMEO in Bogor and propagated to be used as test insects in resistance testing.

3.5 Resistance Status of Stored Product Insects Collected from Food and Feed Warehouses

Resistance testing for stored product pest insects collected from field surveys was carried out at the Entomology Laboratory, SEAMEO BIOTROP, Bogor. All serial tests are carried out for five months.

The test insects used were the first offspring (F1) of collected insects. Phosphine gas used in testing is pure phosphine extracted using 10% H2SO4 from aluminum phosphide (AlP) in the form of pellets. Other materials used are feed for breeding test insects, namely dried corn, rice, and rice bran.

The equipments used were a set of fumigation testing instruments in the laboratory that consisted of jars with a volume of 2 liters with wire mesh hung in the middle of the jar. This jar is a modification of the desiccator used in the FAO method (Busvine 1980). The PVC pipe rings that was covered by gauze were used as container for test insects, a syringe to extract and inject phosphine gas, and a magnetic stirrer, as well as other supporting devices. For phosphine extraction a phosphine gas generator is used (apparatus for generating phosphine) based on the FAO method (Busvine 1980) (Figure 2).

Figure 2 Equipment for extracting phosphine gas from an aluminum phosphide formulation (Busvine 1980).

3.5.1 Preparation for Resistance Testing

Test insects collected from the field were propagated in the laboratory with appropriate feed. The insect offspring (F1) was then used as a test insect to assess its resistance to phosphine. As a comparison, a laboratory strain has been maintained in the laboratory for at least 10 generations.

3.5.2 Method for Resistance Test

Fifty test insects were inserted into PVC ring (2.5 cm in diameter, 2.5 cm in height) with base and top part covered by a fine gauze. The PVC ring contain insect tests then placed on a wire mesh set in the center of the glass jar. In each treatment unit (one glass jar is one treatment unit) 2 pieces of PVC ring containing 50 test insects are included (Figure 3).

Figure 3 Glass jars as a fumigation chambers in phosphine resistance testing

The jar containing the test insect was tightly closed with its lid and between the lid and the outer wall of the jar were glued using plasticine to prevent phosphine gas leakage. The lid of the jar is given a small hole, then the hole is clogged with rubber and on the edge of the rubber is also given plasticine to prevent phosphine gas leak. Rubber stopper as a place to inject phosphine gas into a jar. Gas phosphine extracted by the FAO method (Busvine 1980) with a concentration of 0.00, 0.005, 0.014, 0.023, 0.031 and 0.040 mg/L then injected into a jar using a syringe. The phosphine gas that has been put into the jar is then stirred for 2 minutes using a magnetic stirrer so that the gas is spread evenly throughout the inside of the jar (Figure 4).

Fumigation is carried out for 20 hours. After fumigation, the test insect is removed from the jar and transferred to another jar containing appropriate feed. The test insects were then kept in this jar for 14 days until the time to observe their mortality. If there is an indication of resistance, the test insect were still alive, then further testing is carried out

with a fumigation exposure for 48 hours. This advanced test aims to confirm the occurrence of resistance in the test insect.

Figure 4 Magnetic stirrer for homogenizing phosphine gas distribution in the fumigation chamber

3.5.3 Observation and Data Analysis

Test insect mortality was observed 14 days after the 20 hour period of fumigation was completed. Test insect mortality data were analyzed by Probit Analysis to obtain LC_{50} and $LC_{99.9}$ values from each test insect sample. The LC_{50} and $LC_{99.9}$ values are then compared with the value of the Discriminating Concentration (DC) listed in the FAO guidebook (Busvine 1980) to determine the resistance level of each sample of the test insect. Resistance factor (RF = resistance factor) is calculated using a formula:

$$RF = LC_{99.9} \text{ serangga uji}/\textit{Discriminating Concentration}$$

If the $LC_{99.9}$ value of the test insect sample obtained is greater than the value of the Discriminating Concentration (Busvine 1980) it is said that the test insect is resistant. For this reason, it is necessary to confirm the nature of the resistance by re-testing. Testing is done by extending the fumigation time to 48 hours. This is in accordance with the standard resistance testing methods listed in FAO Method No. 16 (Busvine 1980).

If the test insect tested for 20 hours is resistant and after 48 hours of further testing remains resistant (the RF value is > 1), it is confirmed that the sample of the test insect is indeed resistant to phosphine. However, if the confirmation test results show that the sample insect test is not resistant (the RF value is < 1), then the sample insect test cannot be ascertained the nature of the resistance and requires further testing.

4. RESULTS AND DISCUSSION

4.1 Nanoemulsion of n-Hexane Fraction of Mint Oil

Preparation of nanoemulsion of n-hexane fraction of mint oil was carried out with everal variations in the composition of emulsion-forming ingredients consisted of Tween 80, glycerol, and distilled water. Meanwhile, the concentration of n-hexane fraction of mint oil made in the nanoemulsion formulation in this study was 10%. The process of forming nanoemulsion in this study was carried out by spontaneous emulsification method. The formation of nanoemulsion by spontaneous emulsification method will occur if an emulsion is formed without the use of any outside stirrer (Lachman *et al.* 1994, Ben *et al.* 2013).

Tween 80 was chosen as the main emulsifier in forming the nanoemulsion formulation of n-hexane fraction of mint oil because Tween was known as anionic emulsifier. In addition, Tween is also a more stable emulsifier on the influence of changes in pH and changes in ionic strength and is safe for health because of its lower toxicity value compared to ionic emulsifiers (Azeem *et al.* 2009). Emulsifiers, like Tween 80 can form energy barriers between droplets or thin layers of interface that are coherent or thicken the continuous phase to inhibit the movement of droplets and cause the breakdown of the droplet fluid to become easier (Lachman *et al.* 1994). The results shown from the preparation of 3% mint oil (FM -3) and 10% mint oil (FM -10) nanoemulsions were milky white with different creaming levels (Figure 5).

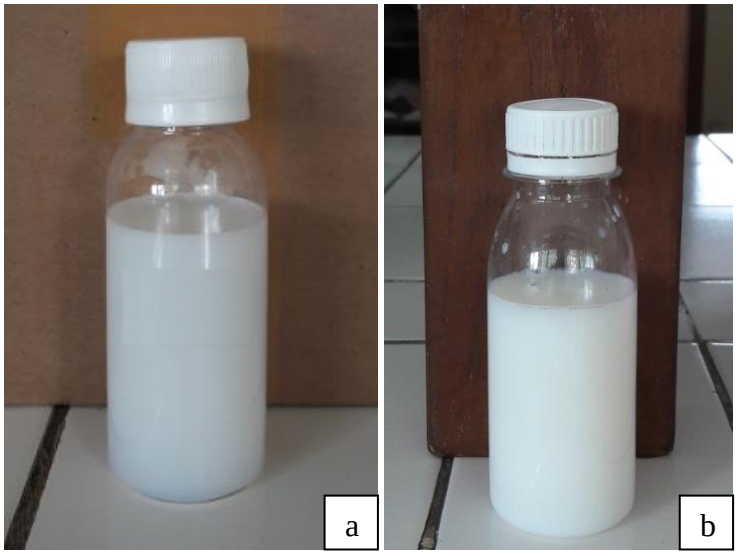

Figure 5 Comparison of the physical form of mint oil nanoemulsion formulation. FM -3 (a). FM -10 (b)

Creaming or the layer formed at the top of the nanoemulsion formulation can show the stability of a formulation. The thicker the layer is formed, the more unstable the formulation is. According to Sinko (2012), creaming is the separation of the emulsion into two layers, where one layer contains more droplets (dispersed phase) than the other layers. Data from the process of forming nanoemulsion formulations in each variation of the composition of emulsion-forming materials can be seen in Table 4.

Table 4 Physical form of mint oil nanoemulsion formulation with different composition of emulsion forming material

Formulation Code	Physical Characters
FM -3	The formulation was milky white, but more transparent than FM-10. Creaming is formed after 1 day.
FM -10	Milky white formulation. The formulation is quite stbale, but there is thinner creaming formed after 2 days.

These results were then supported by the results of particle size analysis and potential zeta values of each formulation code to determine the type of the best and most stable nanoemulsion formulation (Table 5). Particles referred to as nanoparticles, agreed as particles that have a size below 1 micron or 1000 nm (Buzea *et al.* 2007), but particles below 500 nm in size, generally will have better characteristics (Ningsih *et al.* 2017). FM -10 nanoemulsion has a smaller particle size compared to FM -3. The Tween concentration of FM -10 nanoemulsion was higher than FM -3 nanoemulsion. This shows that there is an association between Tween concentration as surfactants and the size of nanoemulsion particles produced. The greater concentration of Tween as surfactant in the nanoemulsion formulation, the particle size will be smaller. According to Affandi *et al.* (2011), the smaller particle size of nanoemulsion will cause the surface area greater, so it takes a lot of surfactants to fill the surface area.

Apart from observing the physical form, the stability of nanoemulsion can also be seen from the results of the measurement of PolyDispersion Index (PDI) and potential zeta values. The PDI indicates the homogeneity quality of a dispersion. The small PDI value shows a narrow particle size distribution, which means the particle size will be more homogeneous or or closer to the measured emulsion droplet distribution (Lovelyn & Attama 2011, Lemarchand *et al.* 2003). The PDI value on FM -10 nanoemulsion is smaller

than FM -3, which is equal to 0.510. This shows that, besides having a good characteristics as indicated by small particle size, FM -10 nanoemulsion also more homogeneous compared to FM -3. Although, according to Avadi *et al.* (2010) and Mao *et al.* (2009), nanoemulsion with a PDI value gretaer than 0.5 indicates a high heterogeneity and the ideal PDI value ranges from 0.09 – 0.40. The heterogeneity of particles can be caused by the tendency of particles to agglomerate to form larger particle aggregates (Ningsih *et al.* 2017).

Table 5 Particle size, potential zeta value, and PolyDispersion Index value of 3% and 10% mint oil nanoemulsion based on differences in the composition of the emulsion-forming material

No	Formulation Code	Particle size (nm)	*PolyDispersion Index*	Value of Zeta Potensial (mV)
1	FM -3	141.3	0.517	-13.8
2	FM -10	98.57	0.510	-16.8

Meanwhile, the zeta surface load has the potential to produce an electrical repulsion between oil droplets which can inhibit droplet combining. The potential zeta value of FM -3 was lower compared to FM -10 nanoemulsion. It means, FM -10 nanoemulsion had more stable formulations compared to FM -3 nanoemulsion. Thus, based on the data, FM -10 nanoemulsion shows better characteristics compared to FM -3, due to smaller particle size and PDI value, and more stable than FM -3 with -16.8 mV potential zeta value.

4.2 Nanopowder of n-hexane Fraction of Mint Oil

Preparing of mint oil nanopowder or mint oil encapsulated nanoemulsion in this study was carried out using a 40% maltodextrin coating. The selection of maltodextrin as coating material was because maltodextrin has the properties of fast dissolving in water and can be used singly as a coating material or combined with other coating materials. Maltodextrin itself is a polysaccharide that has good properties as an encapsulant material, is safe, non-toxic, and has a maximum usage limit of CPP (the amount needed is sufficient to produce the desired effect) (BPOM 2013).

The encapsulation of mint oil nanoemulsions produced from this process was white with a fairly fine powder (Figure 6). The observation of the surface morphology of mint oil nanopowder was carried out using SEM shows that the surface morphology of 3% mint

nanopowder has various forms (Figure 7). In Figure 7, its can be seen that particles of 3% mint nanopowder tend to be round with a smooth round surface and not porous, but there are some particles that have a shrinkage. Meanwhile, 10% mint nanopowder shows a more irregular and clustered particle shape (Figure 8).

Figure 6 Encapsulation of mint oil nanoemulsion with maltodextrin coating

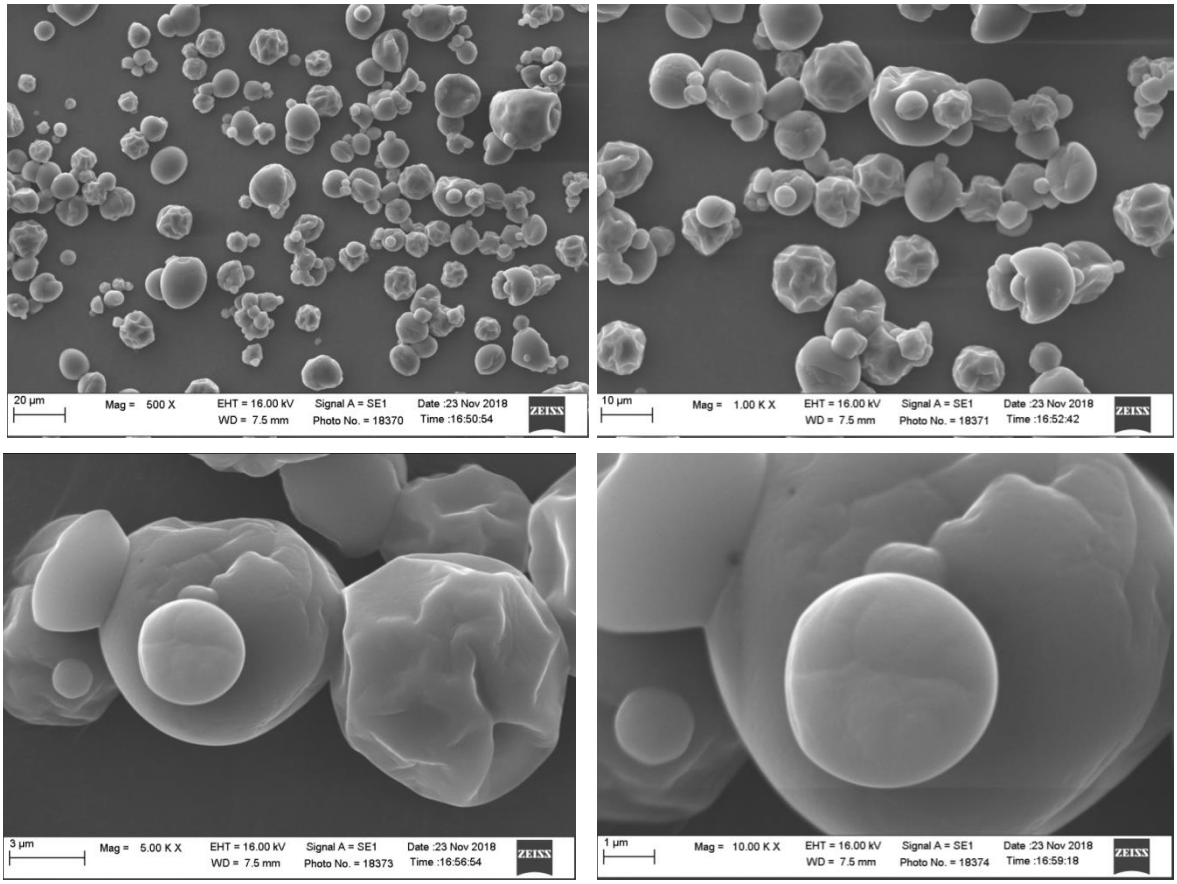

Figure 7 Results of SEM analysis of 3% mint oil nanoemulsion encapsulation (FM -3) with maltodextrin as coating material

In Figure 8, it can be seen that the small particles on the 10% mint nanopowder merge with each other to form particles of relatively larger size. The surface of 10% mint nanopowder particles are round and tend to be smooth and shrinkage in several parts. Therefore, based on the result, it is necessary to re-optimize the 10% mint nanopowder formulation to obtain powder with a more uniform shape and smoother surface. Thus, the effectiveness of 10% mint nanopowder cannot be able to tested against *T. castaneum*.

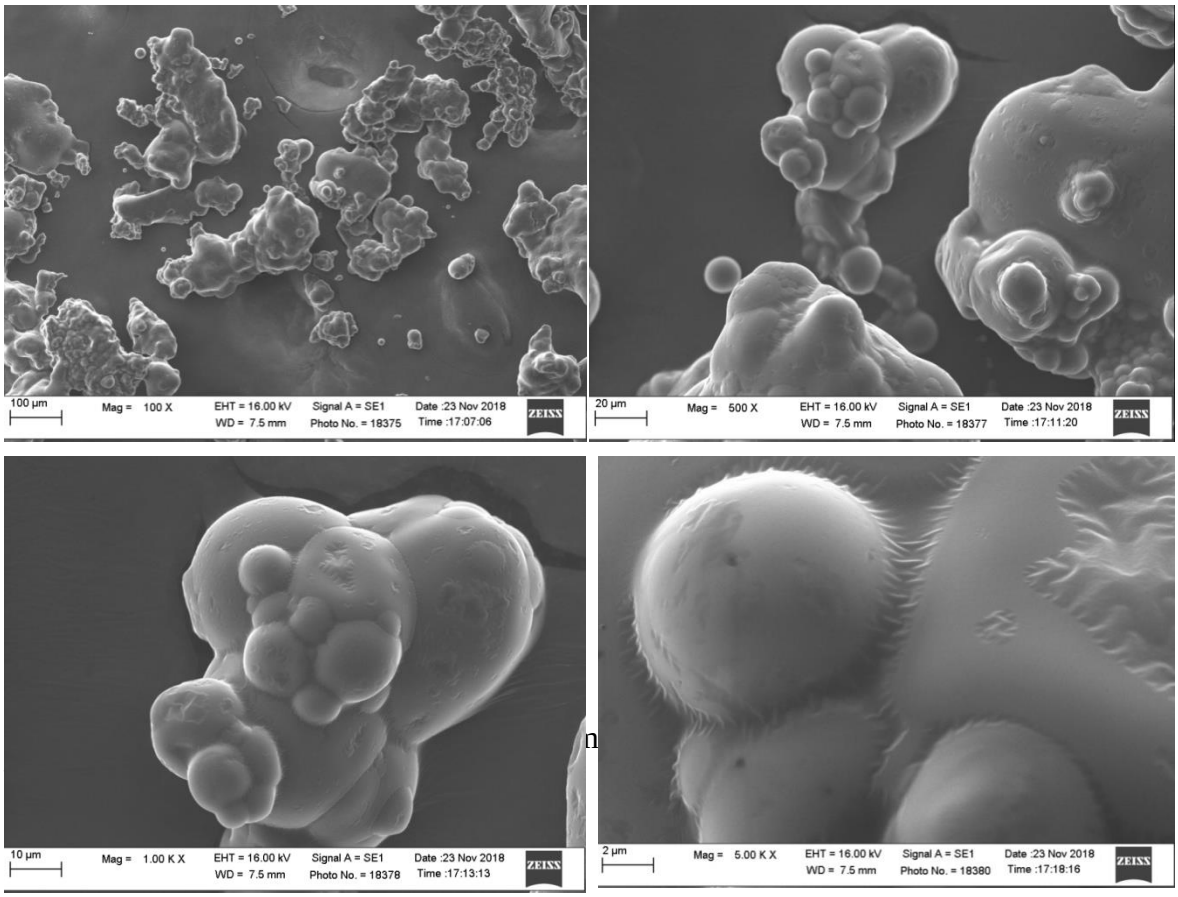

Figure 8 Results of SEM analysis of 10% mint oil nanoemulsion encapsulation (FM -10) with maltodextrin as coating material

According to Purnomo *et al.* (2014), encapsulation results that have a smooth surface and no cracks on the surface, will have low permeability to gases, and can protect core materials from oxidative processes and unwanted leaks. Meanwhile, shrinking the encapsulated surface can be caused by rapid evaporation of water during the spray drying process (Ali *et al.* 2014). The inlet temperature and solvent evaporation rate during the spray dring process can greatly influence the morphology of the nanopowder. In addition, the high heating temperature during the spray drying process can also cause the loss of

active compounds, so that the surface of the nanopowder produced will be more coarse, shrinking, and dense (Deladino *et al.* 2008).

4.3 Effectiveness of Mint Oil Nanotablets against *Tribolium castaneum*

The effectiveness of mint oil nanotablets was tested against *T. castaneum* with several different concentrations. Mortality of *T. castaneum* treated with mint oil nanotablets was strongly influenced by the concentration of nanoemulsion on nanotablets applied and the length of time for nanotablets exposure (fumigation time). In general, the higher the concentration of nanoemulsion contained in nanotablets and the longer the time for nanotablets exposure, the mortality of *T. castaneum* insects will increase (Table 6).

Table 6 Effect of mint nanotablets treatment on insect pests *T. castaneum* in fumigation treatment for 3, 5 and 7 days

Treatments[a]	Mortality at fumigation treatments (%)		
	3 days	5 days	7 days
Control	0 e	0 d	3 c
I	12 a	20 c	22 b
II	39 b	58 b	87 a
III	65 d	78 a	100 a
IV	52 c	61 b	96 a

[a] The percentage of mortality followed by the same letter on the same type of insect was not significantly different based on Duncan's multiple hose test at the 5% level.

Mortality of *T. castaneum* in treatment I, namely treatment of nanotablets containing 2 ml of 10% nanoemulsion or equivalent to 0.2 ml of n-hexane fraction of mint oil only reached 22% with a length of exposure time of 7 days. Meanwhile, the percentage of mortality in the other three treatments had reached more than 80% at 7 days, even up to 100% in treatment III, namely the treatment of nanotablets containing 2.5 ml of 10% nanoemulsion applied as many as 4 tablets or equivalent to 1 ml n-hexane fraction of mint oil. Results of analysis of variance were conducted through Duncan test with 95% confidence interval showed that treatment I was significantly different from the other three treatments, namely treatments II, III, and IV. Meanwhile, there is no significant difference between treatments II, III, and IV with an exposure time of 7 days.

The amount of mint oil n-hexane fraction contained in treatment III was the same as the amount of mint oil n-hexane fraction content in crude tablet formulations in the previous study, which was 1 ml (Harahap *et al.* 2017). However, the n-hexane fraction of mint oil that has been emulsified in the form of nanoemulsion has better toxicity compared

to the n-hexane fraction in the crude form (Figure 9). The mortality of *T. castaneum* due to the treatment of fumigant tablet formulations containing 1 ml of mint n-hexane fraction only reached 68% with a 7-day exposure period. This result is still lower when compared to the mortality that can be caused by the treatment of nanotablets II and III, namely nanotablets containing 0.6 (II) and 0.9 (IV) ml of n-hexane fraction of mint oil respectively.

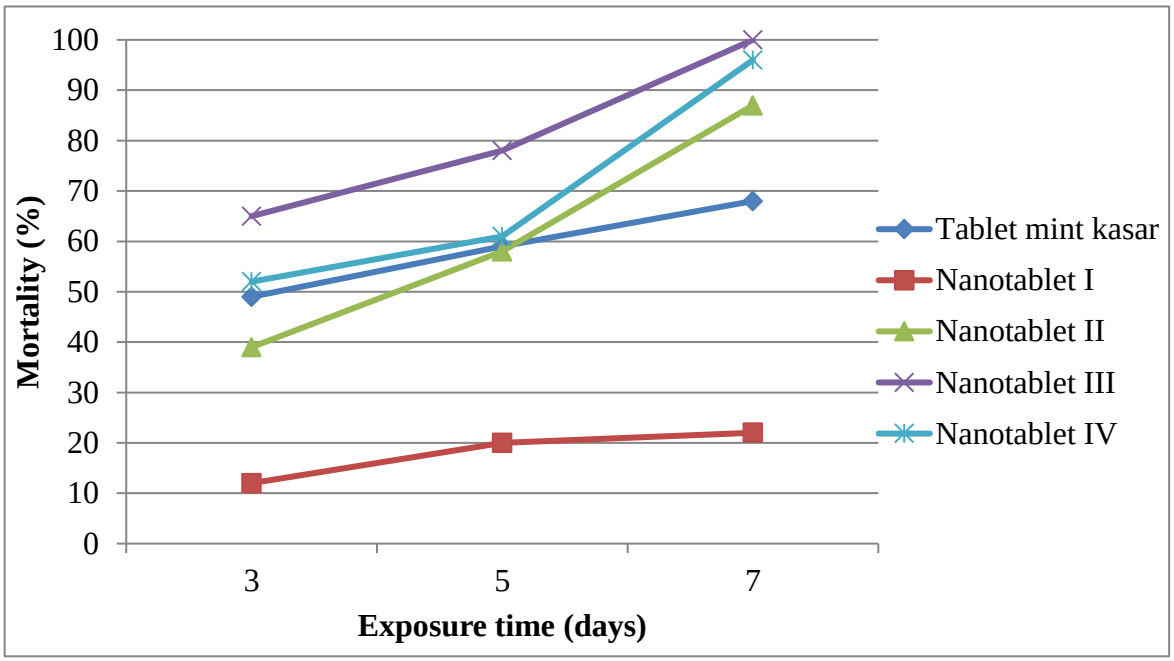

Figure 9. Comparison of mortality of *T. castaneum* due to treatment of crude tablet formulations of mint oil with mint nanotablets

Thus, based on these data it can be seen that changes in the particle shape of n-hexane fraction of mint oil into nanoparticles in the form of nanoemulsion can increase the toxicity of mint oil against *T. castaneum* test insects. The nanoscale in nanoemulsion particles causes the emulsion formed to have a higher surface area, thus enabling effective delivery of the active ingredients contained in the mint n-hexane fraction. In addition, according to Choupanian & Omar (2018), the presence of surfactants contained in mint oil nanoemulsion increases the chances of toxic substances in organic matter to work more efficiently and stably.

4.4 Collection of Suspected Phosphine Resistance Strains of Stored Product Insects from Food and Feed Warehouses

Collection of stored product insects suspected of having resistance to phosphine was carried out in food and feed warehouses in West Sumatra, South Sumatra, and North Sulawesi Provinces. Warehouse location in South Sumatra were one warehouse of rice in Palembang, namely Karang Sari warehouse, and three warehouses in Ogan Komering Ulu (OKU), Terukis Rahayu, Sukarame and Sukamaju warehouses (Table 7). Each insect pest found was collected in large quantities by directly collected using a small paint brush (Figure 10).

Table 7. List of locations and types of pest insect warehouse survey results in North Sulawesi, West Sumatra and South Sumatra Provinces

Origin of Insects		Insect Species
	WEST SUMATERA	
Kota Padang	Rawang Timur	*T. castaneum*
	Pampangan	*T. castaneum*
Pesisir Selatan	Pesisir Selatan	*T. castaneum*
Solok	Bukit Kili	*T. castaneum* *R. dominica*
	SOUTH SUMATERA	
Kota Palembang	Karang Sari	*T. castaneum* *R. dominica*
Ogan Komering Ulu (OKU) Timur	Terukis Rahayu	*T. castaneum* *R. dominica* *Cryptolestes* spp.
	Sukarame	*R. dominica*
	Sukamaju	*T. castaneum* *R. dominica* *Cryptolestes* spp.
	NORTH SULAWESI	
Gudang Tepung Terigu		*R. dominica*
Kotamobagu	Mogolaing	*T. castaneum* *R. dominica*
Bitung	Paceda	*Cryptolestes* sp. *T. castaneum* *Sitophilus* spp. *R. dominica*

Figure 10 Collecting insect samples in rice warehouses and wheat flour distributor warehouses is done by using the direct method using a brush (a-g)

In general, the most common insects found in rice warehouses located in South Sumatra Province were *Rhyzopertha dominica*. The *R. dominica* attack in the warehouse of the East OKU region was quite heavy. This can be seen from the amount of rice that has

decreased in quality due to the attack of *R. dominica* and the high insect population *R. dominica* in the warehouse. The high population of *R. dominica* in warehouses is also triggered by poor warehouse sanitation. Commodity stocks that are not cleaned in warehouses can be a source of insect infestation for new commodities entering the warehouse, because usually warehouse pest insects will survive and multiply in commodity spills that are in the warehouse when the warehouse is empty.

In addition to *R. dominica*, warehouse pest insects found in the Palembang region of South Sumatra are *T. castaneum* and *Cryptolestes* spp. Meanwhile, the insect pest collection in West Sumatra Province is carried out in the Padang area, namely two rice warehouses in the city of Padang, one rice warehouse in the South Coast, and one rice warehouse in Solok (Bukit Kili). Unlike the Palembang region, the most common insect found in the Padang region, West Sumatra is *T. castaneum*. *T. castaneum* insects are found in all warehouses that are the location of the survey. Meanwhile, in the Bukit Kili rice warehouse, Solok, in addition to *T. castaneum*, *R. dominica* is also found.

A survey in North Sulawesi Province was carried out in the Manado region. The survey was conducted at two rice warehouse locations, namely the Mogolaing rice warehouse at Kotamobagu and the Paceda rice warehouse in Bitung, as well as a distributor of wheat flour. Insect pests found in the rice warehouses consist of *T. castaneum*, *R. dominica*, and *Cryptolestes* spp. Meanwhile, the insect found in the distributor of wheat flour is *T. castaneum*. The number of samples of pest insects obtained from the three provinces was 21 insect samples. The insects were then taken to the laboratory to multiply their numbers to be sufficient for resistance testing. Propagation of these insects in the laboratory was carried out by placing insects from each location into different jar containers and given feed according to each type. Each container used for propagation is then labeled with information about the type, collector's name, and date of collection of insects.

4.5 Detection of Phosphine Resistance Status of Insects Collected from the Warehouses

Detection of the insect resistance status of warehouse pests was carried out on all collection insect pests in three provinces, namely South Sumatra, West Sumatra, and North Sulawesi. Warehouse pest insects used in the resistance test are warehouse pest insects which are sufficient to be used as test insects. Of the total of 21 samples of insects collected, there were seven samples of insects namely *Tribolium castaneum* from Terukis

warehouse (South Sumatra) and Bukit Kili (West Sumatra), *Cryptolestes* sp. from the Terukis and Sukamaju warehouses (South Sumatra), and *Rhyzopertha dominica* from Sukamaju (South Sumatra), Bukit Kili (West Sumatra), and Mogolaing (North Sulawesi) which cannot be tested for phosphine resistance. This is because the number of insects obtained from each warehouse is very little and cannot reproduce well in the laboratory, so the population is not sufficient to be used in testing. Test insects from each sampling location that was fumigated for 20 hours generally showed > 50% mortality at the highest test concentration of 0.040% and experienced an increase in the percentage of mortality after fumigation for 48 hours (Table 8).

Table 8 The mortality of *Tribolium castaneum* insects as a result of the survey on 14 days after fumigation for 20 and 48 hours

City/ Province	Warehouse location	Concentration (mg/L)	Mortality at the observation of 14 days after fumigation (%)	
			20 hours	48 hours
Palembang, South Sumatera	Karang Sari	Control	0	0
		0.005	35	75
		0.014	79	91
		0.023	91	97
		0.031	96	100
		0.040	100	100
	Sukamaju	Control	0	0
		0.005	10	53
		0.014	23	69
		0.023	49	76
		0.031	56	96
		0.040	60	100
Padang, West Sumatera	Rawang Timur	Control	0	0
		0.005	3	32
		0.014	30	59
		0.023	70	86
		0.031	81	96
		0.040	92	100
	Pampangan	Control	0	0
		0.005	22	51
		0.014	35	85
		0.023	57	95
		0.031	68	99
		0.040	76	100
	Pesisir Selatan	Control	0	3
		0.005	25	23
		0.014	28	65
		0.023	48	80
		0.031	55	85
		0.040	57	89

Table 8. Continuation

City/ Province	Warehouse location	Concentration (mg/L)	Mortality at the observation of 14 days after fumigation (%)	
			20 hours	48 hours
Manado, North Sulawesi	Wheat flour storage	Control	0	0
		0.005	15	32
		0.014	22	46
		0.023	30	54
		0.031	41	65
		0.040	60	80
	Paceda	Control	0	0
		0.005	44	61
		0.014	63	79
		0.023	80	93
		0.031	86	100
		0.040	100	100
	Mogolaing	Control	0	0
		0.005	18	31
		0.014	27	41
		0.023	29	55
		0.031	63	73
		0.040	82	92

The results of the estimation analysis of phosphine toxicity parameters on *T. castaneum* after 20 hours of fumigation showed that all *T. castaneum* insect samples in the provinces of South Sumatra, West Sumatra, and North Sulawesi were thought to have experienced phosphine resistance with RF values ranging from 1.18 - 111.50 times (Table 9). However, from a total of eight samples originating from the three provinces, there was one sample originating from the Karang Sari warehouse (South Sumatra) which was declared not experiencing phosphine resistance with an RF value of 0.83 times after confirmation testing through fumigation for 48 hours (Table 10). Meanwhile, another sample from the South Sumatra Province, namely the Sukamaju warehouse was stated to have experienced phosphine resistance with an RF value of 2.65 times.

In addition, *T. castaneum* insect samples originating from the other two provinces namely West Sumatra and North Sulawesi have all experienced phosphine resistance with RF values ranging from 1.00 - 23.30 times. The insect samples *T. castaneum* with the highest RF value of 23.30 times are *T. castaneum* insects originating from wheat flour warehouses. The wheat flour warehouse which is the location for insect sampling has poor sanitation conditions. On the warehouse floor there were many scattered flour and the

remnants of sweeping wheat flour that are not immediately cleaned in the corners of the warehouse walls so that it has the potential to become a pest breeding place.

Table 9. Estimator of phosphine toxicity parameters for insect mortality *Tribolium castaneum* survey results at 14 days after 20 hours fumigation and confirm the status of resistance

No	Location	DC[a] (mg/L)	20 hours fumigation		RF[b]	Resistance Status
			LC_{50}[a]	$LC_{99.9}$		
			.....mg/L.....			
South Sumatera						
1	Karang Sari	0.04	0.007	0.047	1.18	Suspected to be Resistant
2	Sukamaju	0.04	0.027	0.493	12.33	Suspected to be Resistant
West Sumatera						
3	Rawang Timur	0.04	0.018	0.073	1.83	Suspected to be Resistant
4	Pampangan	0.04	0.017	0.422	10.55	Suspected to be Resistant
5	Pesisir Selatan	0.04	0.030	4.460	111.50	Suspected to be Resistant
North Sulawesi						
6	Wheat flour storage	0.04	0.040	2.137	53.41	Suspected to be Resistant
7	Paceda	0.04	0.007	0.119	2.98	Suspected to be Resistant
8	Mogolaing	0.04	0.023	0.416	10.39	Suspected to be Resistant

[a]DC: *Discriminating dose*. LC: *Lethal concentration.* [b]RF: *Resistance factor*

The attack by many insect pests, especially *T. castaneum*, in this warehouse is quite a lot. *T. castaneum* insects are found in almost all sacks of flour in the warehouse. In addition, what is suspected to be the trigger for phosphine resistance of *T. castaneum* originating from this warehouse is the poor handling technique (fumigation) that is carried out, especially in maintaining fumigated staple tightness. The existence of a leak in the fumigation chamber causes the concentration of the applied dose to be reduced or not reached, so that insects will be exposed to sublethal doses which can trigger resistance in these insects.

According to Chaudhry (2000) and Lorini *et al.* (2007), less airtight fumigation space can increase the frequency of control failure, so it tends to increase application frequency or concentration of applications due to target insects not dying. Increasing the frequency of this application will certainly have an impact on the use of uncontrolled doses

and if it occurs continuously over a long period of time it can trigger the development of resistance of target insect pests to phosphine.

Table 10 Parameter estimation of phosphine toxicity for insect mortality *Tribolium castaneum* at 14 days after 48 hours fumigation and confirm the status of resistance

No	Location	DC[a] (mg/L)	48 hours fumigation		RF[b]	Resistance Status
			LC_{50}[a]	$LC_{99.9}$		
			.....mg/L.....			
South Sumatera						
1	Karang Sari	0.04	0.003	0.033	0.83	Not Resistant
2	Sukamaju	0.04	0.006	0.106	2.65	Resistant
West Sumatera						
3	Rawang Timur	0.04	0.009	0.062	1.55	Resistant
4	Pampangan	0.04	0.005	0.040	1.00	Resistant
5	Pesisir Selatan	0.04	0.011	0.112	2.80	Resistant
North Sulawesi						
6	Wheat flour warehouse	0.04	0.014	0.932	23.30	Resistant
7	Paceda	0.04	0.004	0.049	1.22	Resistant
8	Mogolaing	0.04	0.013	0.302	7.56	Resistant

[a]DC: *Discriminating dose*. LC: *Lethal concentration.* [b]RF: *Resistance factor*

Based on the results of previous studies, cases of insect resistance *T. castaneum* against phosphine have developed and occur in almost all regions of Indonesia. The resistance of *T. castaneum* to phosphine has been found in various regions in 10 provinces in Indonesia, namely DKI Jakarta, West Java, Central Java, East Java, North Sumatra, Banten, Bali, West Nusa Tenggara, East Kalimantan, and South Sulawesi . Meanwhile, based on the sampling carried out in the province of Lampung, there have not been any cases of resistance in the region. The resistance of *T. castaneum* to phosphine that occurs in Indonesia is in the range of RF values between 1.00 - 10 297.18 times (Harahap *et al.* 2015, Harahap *et al*. 2016, Harahap *et al.* 2017).

The highest RF value of *T. castaneum* against phosphine that occurs in Indonesia is a sample of insects originating from DKI Jakarta. This insect sample is obtained from one beverage factory with wheat raw material. Based on information obtained, the factory has

fumigated wheat using one type of active ingredient and the same fumigant trademark for 11 years and followed by improper fumigation techniques. Where fumigation has been carried out for 11 years on wheat silos is done without closing the gap or hole in the silo, so that the silo as a fumigation chamber is not maintained. The high value of insect resistance originating from this location is also proven by the fact that test insects are still alive after being dosed with appropriate doses and good fumigation techniques for 7 days of fumigant exposure, so a longer exposure time is needed to kill all the *T. castaneum* insects.

This is in accordance with Ling (1999) which states that cases of phosphine resistance can develop due to continuous use of phosphine for a long period of time without rotation of fumigants, then fumigation is carried out under unfit conditions (not in a tightened room), dosage use which is not well controlled with a short exposure time and the absence of an adequate method of checking fumigation success / failure, because the presence of dead imago found after fumigation does not guarantee the death of the egg, larvae and pupa phases. What's more, the egg and pupa phase is a phase that is quite difficult to control, generally requiring higher doses and longer exposure times.

In addition to *T. castaneum*, another warehouse pest insect that is quite common during the survey was *R. dominica*. The attack of *R. dominica* found in some rice warehouses has caused considerable damage, so the commodity has decreased in quality and cannot be distributed because most of the rice has become powder. Of the three provinces, the attack of *R. dominica* was only found in the provinces of South Sumatra and North Sulawesi. The most attacks occurred in South Sumatra Province, especially in Sukarame, Karang Sari, and Terukis warehouses. Meanwhile, in North Sulawesi Province, a relatively high attack of *R. dominica* was only found in Paceda's warehouse. The mortality of *R. dominica* which has been fumigated for 20 and 48 hours can be seen in Table 11. In general, *R. dominica* mortality reaches > 70% at the highest test concentration of 0.040% and has an increased percentage of mortality after fumigation for 48 hours to > 90 %.

The results of the estimator analysis of phosphine toxicity parameters for mortality of *R. dominica* which had been fumigated for 20 hours showed that the four insect samples from the provinces of South Sumatra and North Sulawesi were thought to have experienced phosphine resistance with RF values ranging from 1.35 - 371.25 times (Table 12). After being confirmed through 48 hours of fumigation, two of the three insect samples from South Sumatra Province were declared to have experienced phosphine resistance with

RF values of 4.78 (Karang Sari) and 52.65 (Terukis) times. Meanwhile, the sample from the Sukarame warehouse in South Sumatra was declared as having not experienced phosphine resistance because the results of the analysis of RF values were only 0.83 times (<1) (Table 13).

Table 11 Mortality of insect *Rhyzopertha dominica* survey results on observation of 14 days after fumigation for 20 and 48 hours

City/ Province	Warehouse Location	Concentration (mg/L)	Mortality at the observation of 14 days after fumigation (%)	
			20 hours	48 hours
Palembang, South Sumatera	Sukarame	Control	0	0
		0.005	13	64
		0.014	72	88
		0.023	86	97
		0.031	92	100
		0.040	97	100
	Karang Sari	Control	0	0
		0.005	48	56
		0.014	61	69
		0.023	68	81
		0.031	85	88
		0.040	88	96
	Terukis	Control	0	0
		0.005	41	68
		0.014	46	76
		0.023	51	78
		0.031	61	80
		0.040	70	92
Manado, North Sulawesi	Paceda	Control	0	0
		0.005	30	85
		0.014	50	93
		0.023	51	94
		0.031	49	99
		0.040	71	100

In addition to the provinces of South Sumatra and North Sulawesi, cases of phosphine resistance to *R. dominica* have also occurred in several other regions in Indonesia, such as South Sulawesi and Bali with a range of RF values ranging from 2.33 - 29.18 times (Harahap *et al.* 2017). In addition to insects *T. castaneum* and *R. dominica*, in the location of the survey in North Sulawesi Province there were also quite a number of other warehouse pest insects, *Sitophilus* sp. and *Cryptolestes* sp. found in Paceda's warehouse. Based on the results of the analysis of estimators of phosphine toxicity parameters against insect mortality *Sitophilus* sp. which has been fumigated for 20 hours, it

is known that the LC_{50} and $LC_{99.9}$ values are 0.003 and 0.045 mg / l respectively, so the RF value is 1.13 or is thought to have experienced phosphine resistance.

Table 12 Parameter estimation of phosphine toxicity for insect mortality *Rhyzopertha dominica* at 14 days after 20 hours fumigation and confirm the status of resistance

No	Location	DC[a] (mg/L)	20 hours fumigation		RF[b]	Resistance Status
			LC_{50}[a]	$LC_{99.9}$		
			.....mg/L.....			
South Sumatera						
1	Sukarame	0.04	0.010	0.054	1.35	Suspected to be Resistant
2	Karang Sari	0.04	0.007	0.428	10.71	Suspected to be Resistant
3	Terukis	0.04	0.014	14.850	371.25	Suspected to be Resistant
North Sulawesi						
4	Paceda	0.04	0.018	3.389	84.73	Suspected to be Resistant

[a]DC: *Discriminating dose*. LC: *Lethal concentration.* [b]RF: *Resistance factor*

Table 13 Parameter estimation of phosphine toxicity for insect mortality *Rhyzopertha dominica* at 14 days after 48 hours fumigation and confirm the status of resistance

No	Location	DC[a] (mg/L)	48 hours fumigation		RF[b]	Resistance Status
			LC_{50}[a]	$LC_{99.9}$		
			.....mg/L.....			
South Sumatera						
1	Sukarame	0.04	0.004	0.033	0.83	Not Resistant Resistant
2	Karang Sari	0.04	0.005	0.191	4.78	Resistant
3	Terukis	0.04	0.001	2.106	52.65	Resistant
North Sulawesi						
4	Paceda	0.04	0.001	0.051	1.29	Resistant

[a]DC: *Discriminating dose*. LC: *Lethal concentration.* [b]RF: *Resistance factor*

However, after being confirmed through 48-hour fumigation, *Sitophilus* sp. collected from Paceda warehouse, North Sulawesi was confirmed not resistant to

phosphine with an RF value of only 0.38 times. Unlike the case with *Sitophilus* sp., *Cryptolestes* sp. from Paceda's warehouse, North Sulawesi was declared to have experienced phosphine resistance with an RF value of 163.38 in 20-hour fumigation and 36.94 times after 48 hours of fumigation. *Cryptolestes* sp. is one type of insect pest that currently attacks rice commodities in Indonesia and is quite difficult to control.

This is because based on the facts in the field, there are still many insects found *Cryptolestes* sp. alive after fumigation using phosphine at the appropriate dose. Case of *Cryptolestes* sp. not only in Indonesia, but also in other countries such as Australia with a resistance level of 875 times. The results of testing the resistance status carried out using the FAO method standard with the upper limit of the concentration of resistance testing are 0.04 mg / L for insects *T. castaneum, R. dominica*, and *Sitophilus* sp. and 0.05 mg/L for insects *Cryptolestes* sp. is equivalent to 29 ppm (0.04 mg/L) and 36.5 ppm (0.05 mg/L) with a reference of 1 mg/L equivalent to 730 ppm (AFHB & ACIAR 1991).

The upper limit of this test concentration is the concentration limit for killing susceptible insects and this concentration is still very small compared to the concentration used for the application of phosphine fumigants in the field. Phosphine fumigation in the field was carried out using an application dose of 2 tablets/m^3 or with phosphine content of 2 g/m^3 which was equivalent to a phosphine concentration of 460 ppm. Thus, the application dosage used in the field should still be effectively used to control pest insects with a resistance level of up to 50 times for insects *T. castaneum, R. dominica*, and *Sitophilus* sp. and 40 times for insects *Cryptolestes* sp.

Meanwhile, for insects with an RF value of more than 50 times, it can be managed by increasing the fumigant exposure time. The addition of fumigant exposure time is known to be more effective in controlling pest insects that have been resistant to phosphine compared to the addition of doses or concentration of application. This has been proven in research conducted by Nayak *et al.* (2010). The study showed that one of the strategies to overcome *Cryptolestes ferrugineus* resistant in Australia which had a resistance factor of 875 times was to conduct eradication tests to destroy resistant insects in the laboratory and confirmed by testing in the field. The result is fumigation at a dose of 1 mg/L (720 ppm) with a 24-day fumigation period and a dose of 0.5 mg/L (360 ppm) with a 30-day fumigation period successfully eradicating *C. ferrugineus* resistant.

In addition, by not increasing concentration, the control carried out will remain economical and the level of security in control can also be more maintained. In addition to the use of concentration and the exact length of exposure, fumigation techniques carried

out in the field can also affect the level of incidence of resistance. Inadequate fumigation practices in the field, such as the failure to maintain the fumigation space due to the non-use of sand pads at the end of the plastic can cause a reduction in fumigant concentration which results in insect resistance due to exposure to sublethal doses. Fumigation practices in the field must be done in the right way to reduce resistance levels and maintain existing resistance levels so that they do not develop towards higher levels of resistance and achievement of control efficiency (Widayanti 2016).

5 CONCLUSION

The stability of the nanoemulsion formulation is very dependent on the composition of the emulsion-forming material used. Nanoemulsion of the n-hexane fraction of mint can be formed well and is quite stable with the concentration of mint n-hexane fraction in the nanoemulsion formulation of 10% and the addition of Tween and glycerol as emulsion-forming ingredients in the ratio 1 : 1. Mint oil nanopowder was formed through a spray drying process with maltodextrin 40% as a coating. Mint nanoemulsions formulated in tablet form have a higher effectiveness compared to tablet formulations which are formed from coarse mint n-hexane fraction at the same concentration. The survey results in the provinces of West Sumatra, South Sumatra, and North Sulawesi indicate that cases of insect resistance *Tribolium castaneum* have developed in all three regions with Resistance Factor (RF) values ranging from 0.83 - 23.30 times. Meanwhile, for *Rhyzopertha dominica* insects the RF value ranges from 0.83 - 52.65 times.

6 RESEARCH COORDINATOR AND TEAM MEMBERS

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APPENDICES

Appendix 1. Analysis of variance for mortality of *T. castaneum* after treated with mint nanotablet with exposure period of 3 days

The SAS System 09:58 Monday, December 3, 2018

The GLM Procedure
Class Level Information

Class	Levels	Values
PERL	5	K NI NII NIII NIV

Number of observations 25

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	589.8400000	147.4600000	49.82	<.0001
Error	20	59.2000000	2.9600000		
Corrected Total	24	649.0400000			

R-Square	Coeff Var	Root MSE	Y Mean
0.908788	25.60216	1.720465	6.720000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	4	589.8400000	147.4600000	49.82	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	4	589.8400000	147.4600000	49.82	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.96

Number of Means	2	3	4	5
Critical Range	2.270	2.382	2.454	2.504

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	13.000	5	NIV
B	10.400	5	NIII
C	7.800	5	NII
D	2.400	5	NI
E	0.000	5	K

Appendix 2. Analysis of variance for mortality of *T. castaneum* after treated with mint nanotablet with exposure period of 5 days

The SAS System 09:58 Monday, December 3, 2018

The GLM Procedure
Class Level Information

Class	Levels	Values
PERL	5	K NI NII NIII NIV

Number of observations 25

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	830.2400000	207.5600000	91.84	<.0001
Error	20	45.2000000	2.2600000		
Corrected Total	24	875.4400000			

R-Square	Coeff Var	Root MSE	Y Mean
0.948369	17.31947	1.503330	8.680000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	4	830.2400000	207.5600000	91.84	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	4	830.2400000	207.5600000	91.84	<.0001

Duncan's Multiple Range Test for Y
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.26

Number of Means	2	3	4	5
Critical Range	1.983	2.082	2.144	2.188

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	15.6000	5	NIV
B	12.2000	5	NIII
B			
B	11.6000	5	NII
C	4.0000	5	NI
D	0.0000	5	K

Appendix 3. Analysis of variance for mortality of *T. castaneum* after treated with mint nanotablet with exposure period of 7 days

The SAS System 09:58 Monday, December 3, 2018

The GLM Procedure
Class Level Information

Class	Levels	Values
PERL	5	K NI NII NIII NIV

Number of observations 25

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1778.640000	444.660000	56.14	<.0001
Error	20	158.400000	7.920000		
Corrected Total	24	1937.040000			

R-Square	Coeff Var	Root MSE	Y Mean
0.918226	22.12460	2.814249	12.72000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	4	1778.640000	444.660000	56.14	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	4	1778.640000	444.660000	56.14	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	7.92

Number of Means	2	3	4	5
Critical Range	3.713	3.897	4.014	4.096

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	20.000	5	NIV
A			
A	19.400	5	NII
A			
A	19.200	5	NIII
B	4.400	5	NI
C	0.600	5	K

Appendix 4. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Karang Sari after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Karangsari)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 35
input: 0.014 100 79
input: 0.023 100 91
input: 0.031 100 96
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        35.       .350
               .01400    -1.853872       100.        79.       .790
               .02300    -1.638272       100.        91.       .910
               .03100    -1.508638       100.        96.       .960
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -165.16214

            parameter       standard error      t ratio
insecta     6.0541828        .47290532        12.802104
SLOPE       2.8121147        .24373048        11.537805

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .2236394      .1137004
  SLOPE           .1137004  .5940455E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           35.          33.849          1.151
.338494
```

```
                100.             79.           79.979           -.979
.799793
                100.             91.           92.608          -1.608
.926076
                100.             96.           96.499           -.499
.964985
                100.            100.           98.312           1.688
.983124

chi-square   2.2867    degrees of freedom   3    heterogeneity   .76

Index of significance for potency estimation:
  g(.90)=.02032   g(.95)=.02886   g(.99)=.04984


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .00703  lower      .00609      .00590      .00553
                             upper      .00794      .00811      .00844
LD95  insecta        .02704  lower      .02350      .02294      .02193
                             upper      .03216      .03342      .03625
LD99  insecta        .04725  lower      .03886      .03760      .03540
                             upper      .06077      .06434      .07270


 uji resistensi phospine terhadap imago T. castaneum (Karangsari)
   insecta    subjects 500   controls 100
     log(L)=-165.2   slope=2.812+.244   nat.resp.=.000+.000
     heterogeneity=.76   g=.029
     LD50=.007   limits: .006 to .008
     LD95=.027   limits: .023 to .033
     LD99=.047   limits: .038 to .064

Stop - Program terminated.
```

Appendix 5. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Karang Sari after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Karangsari)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 75
input: 0.014 100 91
input: 0.023 100 97
input: 0.031 100 100
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        75.       .750
               .01400    -1.853872       100.        91.       .910
               .02300    -1.638272       100.        97.       .970
               .03100    -1.508638       100.       100.      1.000
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -102.61247

            parameter       standard error      t ratio
insecta     5.4334067        .62630764        8.6753000
SLOPE       2.0941692        .30718779        6.8172281

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .3922613      .1900903
  SLOPE           .1900903  .9436434E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           75.          73.061          1.939
.730611
```

```
               100.             91.           93.956          -2.956
.939559
               100.             97.           97.739           -.739
.977389
               100.            100.           98.852           1.148
.988519
               100.            100.           99.389            .611
.993893

chi-square 3.7526          degrees of freedom    3     heterogeneity
1.2509

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
  g(.90)=.14907   g(.95)=.27260   g(.99)=.91825

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .00254  lower      .00102      .00054
                             upper      .00396      .00443
LD95  insecta        .01552  lower      .01163      .01060
                             upper      .02444      .03239
LD99  insecta        .03283  lower      .02162      .01943
                             upper      .07652      .13871


 uji resistensi phospine terhadap imago T. castaneum (Karangsari)
   insecta    subjects 500   controls 100
     log(L)=-102.6   slope=2.094+.307   nat.resp.=.000+.000
     heterogeneity=1.25   g=.273
     LD50=.003   limits: .001 to .004
     LD95=.016   limits: .011 to .032
     LD99=.033   limits: .019 to .139

Stop - Program terminated.
```

Appendix 6. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Manado after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago T. castaneum (Gudang
Terigu)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 15
input: 0.014 100 22
input: 0.023 100 30
input: 0.031 100 41
input: 0.040 100 60

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.        0.       .000
               .00500    -2.301030       100.       15.       .150
               .01400    -1.853872       100.       22.       .220
               .02300    -1.638272       100.       30.       .300
               .03100    -1.508638       100.       41.       .410
               .04000    -1.397940       100.       60.       .600

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -295.73226

            parameter       standard error      t ratio
insecta     1.8813107        .35387685        5.3162864
SLOPE       1.3498221        .20677437        6.5279953

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1252288  .7211790E-01
  SLOPE       .7211790E-01  .4275564E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           15.          11.035          3.965
.110350
```

100.	22.	26.727	-4.727	.267271
100.	30.	37.068	-7.068	.370675
100.	41.	43.838	-2.838	.438378
100.	60.	49.774	10.226	.497742

chi-square 9.3935 degrees of freedom 3 heterogeneity 3.1312

A large chi-square indicates a poor fit of the data by the probit analysis model. Large deviations for expected probabilities near 0 or 1
are especially troublesome. A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
g(.90)=.40694 g(.95)=.74417 g(.99)=2.5067

"With almost all good sets of data, g will be substantially smaller than
1.0, and seldom greater than 0.4."
- D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5

Effective Doses

		dose	limits	0.90	0.95	0.99
LD50	insecta	.04039	lower	.02578		
			upper	.15971		
LD95	insecta	.66807	lower	.16539		
			upper	320.28165		
LD99	insecta	2.13649	lower	.33973		
			upper	7862.08757		

uji resistensi phospine terhadap imago *T. castaneum* (Gudang Terigu)
insecta subjects 500 controls 100
log(L)=-295.7 slope=1.350+.207 nat.resp.=.000+.000
heterogeneity=3.13 g=.744

Stop - Program terminated.

Appendix 7. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Manado after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago T. castaneum (Tepung
terigu)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 32
input: 0.014 100 46
input: 0.023 100 54
input: 0.031 100 65
input: 0.040 100 80

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.        0.       .000
               .00500    -2.301030       100.       32.       .320
               .01400    -1.853872       100.       46.       .460
               .02300    -1.638272       100.       54.       .540
               .03100    -1.508638       100.       65.       .650
               .04000    -1.397940       100.       80.       .800

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -318.85642

            parameter       standard error      t ratio
insecta     2.3654228        .32832422        7.2045333
SLOPE       1.2784925        .18550306        6.8920290

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1077968  .5994884E-01
  SLOPE       .5994884E-01  .3441139E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           32.          28.216          3.784
.282163
```

```
                100.             46.            49.811          -3.811
.498110
                100.             54.            60.677          -6.677
.606768
                100.             65.            66.881          -1.881
.668814
                100.             80.            71.842           8.158
.718424

chi-square 6.6055          degrees of freedom    3     heterogeneity
2.2018

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.25673            g(.95)=.46948            g(.99)=1.5814

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .01412  lower      .00822      .00536
                              upper      .02043      .02396
LD95  insecta         .27314  lower      .10456      .08494
                              upper     4.65113   107.02687
LD99  insecta         .93204  lower      .23961      .17951
                              upper    55.17470  5176.39531


 uji resistensi phospine terhadap imago T. castaneum (Tepung terigu)
   insecta    subjects 500   controls 100
     log(L)=-318.9   slope=1.278+.186   nat.resp.=.000+.000
     heterogeneity=2.20   g=.469
     LD50=.014   limits: .005 to .024
     LD95=.273   limits: .085 to 107.027
     LD99=.932   limits: .180 to 5176.395

Stop - Program terminated.
```

Appendix 8. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Mogolaing after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Mogolaing)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 18
input: 0.014 100 27
input: 0.023 100 29
input: 0.031 100 63
input: 0.040 100 82

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.        0.       .000
               .00500    -2.301030       100.       18.       .180
               .01400    -1.853872       100.       27.       .270
               .02300    -1.638272       100.       29.       .290
               .03100    -1.508638       100.       63.       .630
               .04000    -1.397940       100.       82.       .820

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -297.04269

            parameter       standard error      t ratio
insecta     3.0346679        .35697677        8.5010232
SLOPE       1.8571834        .20706514        8.9690782

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1274324  .7287512E-01
  SLOPE       .7287512E-01  .4287597E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           18.          10.772          7.228
.107716
```

```
               100.             27.           34.152          -7.152
.341522
               100.             29.           49.685         -20.685
.496847
               100.             63.           59.206           3.794
.592061
               100.             82.           66.947          15.053
.669465

chi-square 35.663          degrees of freedom    3     heterogeneity
11.888

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.81843            g(.95)=1.4966            g(.99)=5.0415

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .02323
LD95  insecta         .17851
LD99  insecta         .41553


 uji resistensi phospine terhadap imago T. castaneum (Mogolaing)
   insecta    subjects 500   controls 100
     log(L)=-297.0   slope=1.857+.207   nat.resp.=.000+.000
     heterogeneity=11.89   g=1.497

Stop - Program terminated.
```

Appendix 9. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Mogolaing after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Mogolaing)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 31
input: 0.014 100 41
input: 0.023 100 55
input: 0.031 100 73
input: 0.040 100 92

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        31.       .310
               .01400    -1.853872       100.        41.       .410
               .02300    -1.638272       100.        55.       .550
               .03100    -1.508638       100.        73.       .730
               .04000    -1.397940       100.        92.       .920

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -296.88728

            parameter       standard error      t ratio
insecta     3.2174533        .34307195        9.3783632
SLOPE       1.7155780        .19279368        8.8985178

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1176984  .6513376E-01
  SLOPE       .6513376E-01  .3716940E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           31.          23.265          7.735
.232651
```

```
                100.              41.             51.475         -10.475
.514754
                100.              55.             65.795         -10.795
.657948
                100.              73.            73.541           -.541
.735413
                100.              92.             79.366          12.634
.793658

chi-square 22.684          degrees of freedom    3     heterogeneity
7.5615

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.52888            g(.95)=.96715            g(.99)=3.2579

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.


Effective Doses
                       dose      limits           0.90             0.95
0.99
LD50  insecta         .01332
LD95  insecta         .12115
LD99  insecta         .30240


 uji resistensi phospine terhadap imago T. castaneum (Mogolaing)
   insecta    subjects 500   controls 100
     log(L)=-296.9   slope=1.716+.193   nat.resp.=.000+.000
     heterogeneity=7.56   g=.967

Stop - Program terminated.
```

Appendix 10. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Pampangan after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Tribolium castaneum
(Pampangan)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 22
input: 0.014 100 35
input: 0.023 100 57
input: 0.031 100 68
input: 0.040 100 76

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        22.       .220
               .01400    -1.853872       100.        35.       .350
               .02300    -1.638272       100.        57.       .570
               .03100    -1.508638       100.        68.       .680
               .04000    -1.397940       100.        76.       .760

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -305.95476

            parameter       standard error      t ratio
insecta     2.9560323        .34207887        8.6413766
SLOPE       1.6815506        .19548290        8.6020340

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1170180  .6587006E-01
  SLOPE       .6587006E-01  .3821357E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           22.          18.055          3.945
.180551
```

```
                100.             35.            43.591          -8.591
.435910
                100.             57.            57.973           -.973
.579727
                100.             68.            66.246           1.754
.662458
                100.             76.            72.752           3.248
.727519

chi-square 4.7620          degrees of freedom    3     heterogeneity
1.5873

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
  g(.90)=.11881   g(.95)=.21727   g(.99)=.73186

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose      limits           0.90             0.95
0.99
LD50  insecta         .01746  lower      .01336      .01181
                              upper      .02234      .02470
LD95  insecta         .16606  lower      .09163      .07909
                              upper      .54079     1.16533
LD99  insecta         .42221  lower      .18543      .15171
                              upper     2.22191     6.59322


 uji  resistensi  phospine  terhadap  imago  Tribolium  castaneum
(Pampangan)
   insecta    subjects 500   controls 100
     log(L)=-306.0   slope=1.682+.195   nat.resp.=.000+.000
     heterogeneity=1.59   g=.217
     LD50=.017   limits: .012 to .025
     LD95=.166   limits: .079 to 1.165
     LD99=.422   limits: .152 to 6.593

Stop - Program terminated.
```

Appendix 11. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Pampangan after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Pampangan)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 51
input: 0.014 100 83
input: 0.023 100 95
input: 0.031 100 99
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        51.       .510
               .01400    -1.853872       100.        83.       .830
               .02300    -1.638272       100.        95.       .950
               .03100    -1.508638       100.        99.       .990
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -142.55291

            parameter       standard error      t ratio
insecta     6.0123296        .52872397        11.371396
SLOPE       2.6295578        .26452879        9.9405354

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .2795490      .1381085
  SLOPE           .1381085  .6997548E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           51.          48.470          2.530
.484700
```

```
               100.             83.           87.233          -4.233
.872328
               100.             95.           95.585           -.585
.955847
               100.             99.           97.959           1.041
.979586
               100.            100.           99.026            .974
.990264

chi-square 3.4715          degrees of freedom    3     heterogeneity
1.1572

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
  g(.90)=.06486   g(.95)=.11860   g(.99)=.39952


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .00517  lower      .00374      .00319      .00122
                             upper      .00647      .00692      .00838
LD95  insecta        .02183  lower      .01745      .01632      .01347
                             upper      .03018      .03544      .09227
LD99  insecta        .03965  lower      .02893      .02652      .02109
                             upper      .06523      .08450      .43004


 uji resistensi phospine terhadap imago T. castaneum (Pampangan)
   insecta    subjects 500   controls 100
     log(L)=-142.6   slope=2.630+.265   nat.resp.=.000+.000
     heterogeneity=1.16   g=.119
     LD50=.005   limits: .003 to .007
     LD95=.022   limits: .016 to .035
     LD99=.040   limits: .027 to .085

Stop - Program terminated.
```

Appendix 12. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Paceda after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago T. castaneum (Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 44
input: 0.014 100 63
input: 0.023 100 80
input: 0.031 100 86
input: 0.040 100 100
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	44.	.440
	.01400	-1.853872	100.	63.	.630
	.02300	-1.638272	100.	80.	.800
	.03100	-1.508638	100.	86.	.860
	.04000	-1.397940	100.	100.	1.000

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -235.96393
```

	parameter	standard error	t ratio
insecta	4.0855949	.37746825	10.823678
SLOPE	1.9037124	.20358706	9.3508514

Variance-Covariance matrix

	insecta	SLOPE
insecta	.1424823	.7566772E-01
SLOPE	.7566772E-01	.4144769E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	44.	38.403	5.597	.384033
	100.	63.	71.102	-8.102	.711016
	100.	80.	83.318	-3.318	.833177
	100.	86.	88.755	-2.755	.887546
	100.	100.	92.282	7.718	.922823

chi-square 14.434 degrees of freedom 3 heterogeneity 4.8113

A large chi-square indicates a poor fit of the data by the probit analysis model. Large deviations for expected probabilities near 0 or 1
are especially troublesome. A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
g(.90)=.30475 g(.95)=.55729 g(.99)=1.8772

"With almost all good sets of data, g will be substantially smaller than
1.0, and seldom greater than 0.4."
- D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5

Effective Doses

		dose	limits	0.90	0.95	0.99
LD50	insecta	.00714	lower	.00240		
			upper	.01112		
LD95	insecta	.05223	lower	.02981		
			upper	.27415		
LD99	insecta	.11909	lower	.05295		
			upper	1.65288		

uji resistensi phospine terhadap imago *T. castaneum* (Paceda)
insecta subjects 500 controls 100
log(L)=-236.0 slope=1.904+.204 nat.resp.=.000+.000
heterogeneity=4.81 g=.557

Stop - Program terminated.

Appendix 13. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Paceda after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago T. castaneum (Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 61
input: 0.014 100 79
input: 0.023 100 93
input: 0.031 100 100
input: 0.040 100 100
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	61.	.610
	.01400	-1.853872	100.	79.	.790
	.02300	-1.638272	100.	93.	.930
	.03100	-1.508638	100.	100.	1.000
	.04000	-1.397940	100.	100.	1.000

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -151.55265
```

	parameter	standard error	t ratio
insecta	5.2053014	.49482412	10.519498
SLOPE	2.1953803	.25091364	8.7495456

Variance-Covariance matrix

	insecta	SLOPE
insecta	.2448509	.1224901
SLOPE	.1224901	.6295766E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	61.	56.106	4.894	.561063
	100.	79.	87.189	-8.189	.871885
	100.	93.	94.616	-1.616	.946156
	100.	100.	97.084	2.916	.970839
	100.	100.	98.367	1.633	.983672

chi-square 12.151 degrees of freedom 3 heterogeneity 4.0504

A large chi-square indicates a poor fit of the data by the probit analysis model. Large deviations for expected probabilities near 0 or 1
are especially troublesome. A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
g(.90)=.29303 g(.95)=.53585 g(.99)=1.8050

"With almost all good sets of data, g will be substantially smaller than
1.0, and seldom greater than 0.4."
- D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5

Effective Doses

		dose	limits	0.90	0.95	0.99
LD50	insecta	.00426	lower	.00117		
			upper	.00690		
LD95	insecta	.02389	lower	.01546		
			upper	.06850		
LD99	insecta	.04882	lower	.02651		
			upper	.30169		

uji resistensi phospine terhadap imago *T. castaneum* (Paceda)
insecta subjects 500 controls 100
log(L)=-151.6 slope=2.195+.251 nat.resp.=.000+.000
heterogeneity=4.05 g=.536

Stop - Program terminated.

Appendix 14. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Pesisir Selatan after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Tribolium castaneum
(Pesisir Selatan)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 25
input: 0.014 100 28
input: 0.023 100 48
input: 0.031 100 55
input: 0.040 100 57

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        25.       .250
               .01400    -1.853872       100.        28.       .280
               .02300    -1.638272       100.        48.       .480
               .03100    -1.508638       100.        55.       .550
               .04000    -1.397940       100.        57.       .570

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -324.53623

            parameter       standard error      t ratio
insecta     1.6394062        .32774658        5.0020543
SLOPE       1.0575971        .18789845        5.6285568

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1074178  .6062581E-01
  SLOPE       .6062581E-01  .3530583E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           25.          21.355          3.645
.213552
```

```
                100.             28.           37.401          -9.401
.374013
                100.             48.           46.286           1.714
.462862
                100.             55.           51.750           3.250
.517498
                100.             57.           56.393            .607
.563933

chi-square 5.1222          degrees of freedom    3     heterogeneity
1.7074

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.29849           g(.95)=.54584           g(.99)=1.8387

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                       dose      limits          0.90            0.95
0.99
LD50  insecta          .02818  lower      .01916
                               upper      .05746
LD95  insecta         1.01193  lower      .24090
                               upper   124.20220
LD99  insecta         4.46201  lower      .63467
                               upper  3239.89294


 uji resistensi phospine terhadap imago Tribolium castaneum (Pesisir
Selatan)
   insecta    subjects 500   controls 100
     log(L)=-324.5   slope=1.058+.188   nat.resp.=.000+.000
     heterogeneity=1.71   g=.546

Stop - Program terminated.
```

Appendix 15. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Pampangan after fumigation for 48 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Tribolium castaneum
(Pesisir Selatan)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 3
input: 0.005 100 23
input: 0.014 100 65
input: 0.023 100 80
input: 0.031 100 85
input: 0.040 100 89
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	3.	.030
	.00500	-2.301030	100.	23.	.230
	.01400	-1.853872	100.	65.	.650
	.02300	-1.638272	100.	80.	.800
	.03100	-1.508638	100.	85.	.850
	.04000	-1.397940	100.	89.	.890

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -259.72509
```

	parameter	standard error	t ratio
insecta	4.4849339	.38712734	11.585164
SLOPE	2.2742983	.21572638	10.542513

Variance-Covariance matrix

	insecta	SLOPE
insecta	.1498676	.8227745E-01
SLOPE	.8227745E-01	.4653787E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	23.	25.033	-2.033	.250327
	100.	65.	61.773	3.227	.617733
	100.	80.	78.280	1.720	.782796
	100.	85.	85.840	-.840	.858401

```
               100.        89.       90.703      -1.703       .907031

chi-square   1.2372    degrees of freedom   3    heterogeneity   .41

Index of significance for potency estimation:
  g(.90)=.02434   g(.95)=.03456   g(.99)=.05970


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .01067  lower      .00921      .00892      .00834
                             upper      .01206      .01233      .01285
LD95  insecta        .05639  lower      .04656      .04509      .04252
                             upper      .07251      .07682      .08705
LD99  insecta        .11243  lower      .08544      .08168      .07523
                             upper      .16255      .17712      .21346


 uji resistensi phospine terhadap imago Tribolium castaneum (Pesisir
Selatan)
   insecta    subjects 500   controls 100
     log(L)=-259.7   slope=2.274+.216   nat.resp.=.030+.000
     heterogeneity=.41   g=.035
     LD50=.011   limits: .009 to .012
     LD95=.056   limits: .045 to .077
     LD99=.112   limits: .082 to .177

Stop - Program terminated.
```

Appendix 16. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Rawang Timur after fumigation for 20 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Tribolium castaneum
(Rawang Timur)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 3
input: 0.014 100 30
input: 0.023 100 70
input: 0.031 100 81
input: 0.040 100 92
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	3.	.030
	.01400	-1.853872	100.	30.	.300
	.02300	-1.638272	100.	70.	.700
	.03100	-1.508638	100.	81.	.810
	.04000	-1.397940	100.	92.	.920

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -213.35657
```

	parameter	standard error	t ratio
insecta	6.6524671	.52115533	12.764845
SLOPE	3.7974250	.30875219	12.299265

Variance-Covariance matrix

	insecta	SLOPE
insecta	.2716029	.1594839
SLOPE	.1594839	.9532791E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	3.	1.851	1.149	.018511
	100.	30.	34.920	-4.920	.349203
	100.	70.	66.686	3.314	.666857
	100.	81.	82.213	-1.213	.822133

```
               100.        92.       91.051        .949       .910509

chi-square   2.4975    degrees of freedom   3    heterogeneity   .83

Index of significance for potency estimation:
  g(.90)=.01789   g(.95)=.02539   g(.99)=.04386


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .01771  lower      .01639      .01613      .01562
                              upper      .01900      .01925      .01975
LD95  insecta         .04801  lower      .04274      .04190      .04039
                              upper      .05555      .05739      .06149
LD99  insecta         .07257  lower      .06191      .06028      .05740
                              upper      .08897      .09314      .10272


 uji resistensi phospine terhadap imago Tribolium castaneum (Rawang
Timur)
   insecta    subjects 500   controls 100
     log(L)=-213.4   slope=3.797+.309   nat.resp.=.000+.000
     heterogeneity=.83   g=.025
     LD50=.018   limits: .016 to .019
     LD95=.048   limits: .042 to .057
     LD99=.073   limits: .060 to .093

Stop - Program terminated.
```

Appendix 17. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Rawang Timur after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago T. castaneum (Rawang
Timur)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 32
input: 0.014 100 59
input: 0.023 100 86
input: 0.031 100 96
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        32.       .320
               .01400    -1.853872       100.        59.       .590
               .02300    -1.638272       100.        86.       .860
               .03100    -1.508638       100.        96.       .960
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -196.51191

            parameter       standard error      t ratio
insecta     5.6347842        .43076174        13.080976
SLOPE       2.7329238        .22839197        11.965936

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1855557  .9701498E-01
  SLOPE       .9701498E-01  .5216289E-01


Chi-squared goodness of fit test

preparation   subjects   responses   expected    deviation   probability
insecta         100.        32.       25.663       6.337       .256635
                100.        59.       71.508     -12.508       .715082
                100.        86.       87.647      -1.647       .876468
                100.        96.       93.471       2.529       .934706
```

```
              100.       100.       96.519       3.481       .965186

chi-square 14.690          degrees of freedom    3     heterogeneity
4.8965

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.18940            g(.95)=.34635            g(.99)=1.1667

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .00867  lower      .00507      .00346
                              upper      .01195      .01327
LD95  insecta         .03468  lower      .02348      .02117
                              upper      .07875      .15072
LD99  insecta         .06158  lower      .03640      .03206
                              upper      .20950      .57669


 uji resistensi phospine terhadap imago T. castaneum (Rawang Timur)
   insecta    subjects 500   controls 100
     log(L)=-196.5   slope=2.733+.228   nat.resp.=.000+.000
     heterogeneity=4.90   g=.346
     LD50=.009   limits: .003 to .013
     LD95=.035   limits: .021 to .151
     LD99=.062   limits: .032 to .577

Stop - Program terminated.
```

Appendix 18. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Sukamaju after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Tribolium castaneum
(Sukamaju)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 10
input: 0.014 100 23
input: 0.023 100 49
input: 0.031 100 56
input: 0.040 100 60

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        10.       .100
               .01400    -1.853872       100.        23.       .230
               .02300    -1.638272       100.        49.       .490
               .03100    -1.508638       100.        56.       .560
               .04000    -1.397940       100.        60.       .600

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -293.39403

            parameter       standard error      t ratio
insecta     2.8964300        .37117127        7.8034864
SLOPE       1.8546837        .21866192        8.4819693

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1377681  .8008798E-01
  SLOPE       .8008798E-01  .4781303E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           10.           8.515          1.485
.085148
```

```
                100.             23.            29.394          -6.394
.293938
                100.             49.            44.352           4.648
.443522
                100.             56.            53.919           2.081
.539186
                100.             60.            61.932          -1.932
.619319

chi-square 3.4609          degrees of freedom    3     heterogeneity
1.1536

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
  g(.90)=.08881   g(.95)=.16240   g(.99)=.54706

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .02744  lower      .02269      .02125
                              upper      .03491      .03919
LD95  insecta         .21144  lower      .12147      .10541
                              upper      .57660     1.03599
LD99  insecta         .49275  lower      .23467      .19441
                              upper     1.91157     4.23527


 uji  resistensi  phospine  terhadap  imago  Tribolium  castaneum
(Sukamaju)
   insecta    subjects 500   controls 100
     log(L)=-293.4   slope=1.855+.219   nat.resp.=.000+.000
     heterogeneity=1.15   g=.162
     LD50=.027   limits: .021 to .039
     LD95=.211   limits: .105 to 1.036
     LD99=.493   limits: .194 to 4.235

Stop - Program terminated.
```

Appendix 19. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Sukamaju after fumigation for 48 hours 48 jam

```
POLO-PC
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Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  T.  castaneum
(Sukamaju)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 53
input: 0.014 100 69
input: 0.023 100 76
input: 0.031 100 96
input: 0.040 100 100
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	53.	.530
	.01400	-1.853872	100.	69.	.690
	.02300	-1.638272	100.	76.	.760
	.03100	-1.508638	100.	96.	.960
	.04000	-1.397940	100.	100.	1.000

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -217.51654
```

	parameter	standard error	t ratio
insecta	4.1014326	.39381819	10.414533
SLOPE	1.8224610	.20972724	8.6896723

Variance-Covariance matrix

	insecta	SLOPE
insecta	.1550928	.8134120E-01
SLOPE	.8134120E-01	.4398552E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	53.	46.331	6.669	.463307
	100.	69.	76.511	-7.511	.765106
	100.	76.	86.773	-10.773	.867734
	100.	96.	91.181	4.819	.911812

```
               100.       100.       93.988       6.012       .939877

chi-square 24.325        degrees of freedom   3    heterogeneity 8.1084

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.59472            g(.95)=1.0876            g(.99)=3.6634

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .00562
LD95  insecta        .04488
LD99  insecta        .10617


 uji resistensi phospine terhadap imago T. castaneum (Sukamaju)
   insecta    subjects 500   controls 100
     log(L)=-217.5   slope=1.822+.210   nat.resp.=.000+.000
     heterogeneity=8.11   g=1.088

Stop - Program terminated.
```

Appendix 20. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Karang Sari after fumigation for 20 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Rhyzopertha dominica
(Karangsari)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 48
input: 0.014 100 61
input: 0.023 100 68
input: 0.031 100 85
input: 0.040 100 88
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	48.	.480
	.01400	-1.853872	100.	61.	.610
	.02300	-1.638272	100.	68.	.680
	.03100	-1.508638	100.	85.	.850
	.04000	-1.397940	100.	88.	.880

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -281.45121
```

	parameter	standard error	t ratio
insecta	2.7996032	.34106467	8.2084233
SLOPE	1.2848526	.18817852	6.8278384

Variance-Covariance matrix

	insecta	SLOPE
insecta	.1163251	.6314525E-01
SLOPE	.6314525E-01	.3541116E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	48.	43.767	4.233	.437669
	100.	61.	66.190	-5.190	.661899
	100.	68.	75.637	-7.637	.756367
	100.	85.	80.544	4.456	.805443

```
               100.        88.       84.218       3.782       .842180

chi-square 7.4396        degrees of freedom   3    heterogeneity 2.4799

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.29461            g(.95)=.53874            g(.99)=1.8148

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .00662  lower      .00193
                              upper      .01060
LD95  insecta         .12626  lower      .05735
                              upper     1.52321
LD99  insecta         .42822  lower      .12999
                              upper    21.44237


 uji  resistensi  phospine  terhadap  imago  Rhyzopertha  dominica
(Karangsari)
   insecta    subjects 500   controls 100
     log(L)=-281.5   slope=1.285+.188   nat.resp.=.000+.000
     heterogeneity=2.48   g=.539

Stop - Program terminated.
```

Appendix 21. Results of parameter estimation analysis for toxicity of phosphine against *T. castaneum* collected from Karang Sari after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input:  =  uji  resistensi  phospine  terhadap  imago  R.  dominica
(Karangsari)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 56
input: 0.014 100 69
input: 0.023 100 81
input: 0.031 100 88
input: 0.040 100 96

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        56.       .560
               .01400    -1.853872       100.        69.       .690
               .02300    -1.638272       100.        81.       .810
               .03100    -1.508638       100.        88.       .880
               .04000    -1.397940       100.        96.       .960

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you  want  the  likelihood  function  to  be  maximized  [Y]  ?  LD's  to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The  parameters  are  to  be  estimated  by  maximizing  the  likelihood
function

Maximum log-likelihood  -236.22805

            parameter       standard error      t ratio
insecta     3.3641435        .36913270        9.1136424
SLOPE       1.4444189        .19950404        7.2400483

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1362590  .7246496E-01
  SLOPE       .7246496E-01  .3980186E-01


Chi-squared goodness of fit test

preparation   subjects   responses   expected    deviation   probability
insecta         100.        56.       51.615       4.385       .516150
                100.        69.       75.376      -6.376       .753762
                100.        81.       84.081      -3.081       .840810
                100.        88.       88.200       -.200       .881999
```

```
               100.        96.       91.068       4.932       .910676

chi-square 6.6642        degrees of freedom   3    heterogeneity 2.2214

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.23471            g(.95)=.42920            g(.99)=1.4458

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .00469  lower      .00140      .00044
                              upper      .00767      .00868
LD95  insecta         .06452  lower      .03666      .03205
                              upper      .27659     1.15240
LD99  insecta         .19121  lower      .07913      .06507
                              upper     2.19149    25.53792


 uji resistensi phospine terhadap imago R. dominica (Karangsari)
   insecta    subjects 500   controls 100
     log(L)=-236.2   slope=1.444+.200   nat.resp.=.000+.000
     heterogeneity=2.22   g=.429
     LD50=.005   limits: .000 to .009
     LD95=.065   limits: .032 to 1.152
     LD99=.191   limits: .065 to 25.538

Stop - Program terminated.
```

Appendix 22. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Paceda after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago R. dominica (Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 30
input: 0.014 100 45
input: 0.023 100 51
input: 0.031 100 54
input: 0.040 100 71
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	30.	.300
	.01400	-1.853872	100.	45.	.450
	.02300	-1.638272	100.	51.	.510
	.03100	-1.508638	100.	54.	.540
	.04000	-1.397940	100.	71.	.710

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -330.46128
```

	parameter	standard error	t ratio
insecta	1.7834645	.32288114	5.5235945
SLOPE	1.0240585	.18344288	5.5824380

Variance-Covariance matrix

	insecta	SLOPE
insecta	.1042522	.5829578E-01
SLOPE	.5829578E-01	.3365129E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	30.	28.335	1.665	.283348
	100.	45.	45.422	-.422	.454219
	100.	51.	54.212	-3.212	.542121
	100.	54.	59.427	-5.427	.594265
	100.	71.	63.754	7.246	.637540

```
chi-square 4.0528        degrees of freedom   3    heterogeneity 1.3509

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.24009            g(.95)=.43904            g(.99)=1.4789

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .01813  lower      .01203      .00945
                              upper      .02696      .03365
LD95  insecta         .73223  lower      .21120      .16150
                              upper    25.92356  1049.36133
LD99  insecta     3.3895      lower  .59655      .41143
                              upper  517.87      97157.


 uji resistensi phospine terhadap imago R. dominica (Paceda)
   insecta    subjects 500   controls 100
     log(L)=-330.5   slope=1.024+.183   nat.resp.=.000+.000
     heterogeneity=1.35   g=.439
     LD50=.018   limits: .009 to .034
     LD95=.732   limits: .162 to 1049.361
     LD99=3.389   limits: .411 to 97156.798

Stop - Program terminated.
```

Appendix 23. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Paceda after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago R. dominica (Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 85
input: 0.014 100 93
input: 0.023 100 94
input: 0.031 100 99
input: 0.040 100 100
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	85.	.850
	.01400	-1.853872	100.	93.	.930
	.02300	-1.638272	100.	94.	.940
	.03100	-1.508638	100.	99.	.990
	.04000	-1.397940	100.	100.	1.000

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -99.037292
```

	parameter	standard error	t ratio
insecta	4.0500161	.56018552	7.2297766
SLOPE	1.3368893	.28630163	4.6695134

Variance-Covariance matrix

	insecta	SLOPE
insecta	.3138078	.1579961
SLOPE	.1579961	.8196862E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	85.	83.492	1.508	.834921
	100.	93.	94.198	-1.198	.941978
	100.	94.	96.855	-2.855	.968545
	100.	99.	97.898	1.102	.978980
	100.	100.	98.541	1.459	.985413

```
chi-square 5.1724        degrees of freedom   3    heterogeneity 1.7241

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.43794            g(.95)=.80085            g(.99)=2.6977

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .00093  lower      .00001
                              upper      .00277
LD95  insecta         .01588  lower      .00929
                              upper      .04306
LD99  insecta         .05137  lower      .02512
                              upper     1.03723


 uji resistensi phospine terhadap imago R. dominica (Paceda)
   insecta    subjects 500   controls 100
     log(L)=-99.04   slope=1.337+.286   nat.resp.=.000+.000
     heterogeneity=1.72   g=.801

Stop - Program terminated.
```

Appendix 24. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Sukarame after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Rhyzopertha dominica
(Sukarame)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 13
input: 0.014 100 72
input: 0.023 100 86
input: 0.031 100 92
input: 0.040 100 97

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        13.       .130
               .01400    -1.853872       100.        72.       .720
               .02300    -1.638272       100.        86.       .860
               .03100    -1.508638       100.        92.       .920
               .04000    -1.397940       100.        97.       .970

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -181.01967

            parameter       standard error      t ratio
insecta     6.4279198        .45435423        14.147375
SLOPE       3.2414419        .24641354        13.154480

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .2064378      .1104693
  SLOPE           .1104693  .6071963E-01


Chi-squared goodness of fit test

preparation   subjects   responses   expected    deviation   probability
insecta         100.        13.       15.133      -2.133       .151332
                100.        72.       66.228       5.772       .662283
                100.        86.       86.812       -.812       .868122
                100.        92.       93.795      -1.795       .937946
```

```
               100.        97.       97.106       -.106       .971058

chi-square   2.4587    degrees of freedom   3    heterogeneity   .82

Index of significance for potency estimation:
  g(.90)=.01564   g(.95)=.02220   g(.99)=.03834


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .01040  lower      .00936      .00915      .00875
                              upper      .01142      .01161      .01199
LD95  insecta         .03345  lower      .02966      .02904      .02793
                              upper      .03869      .03994      .04268
LD99  insecta         .05428  lower      .04609      .04482      .04256
                              upper      .06658      .06966      .07664


 uji  resistensi  phospine  terhadap  imago  Rhyzopertha  dominica
(Sukarame)
   insecta    subjects 500   controls 100
     log(L)=-181.0   slope=3.241+.246   nat.resp.=.000+.000
     heterogeneity=.82   g=.022
     LD50=.010   limits: .009 to .012
     LD95=.033   limits: .029 to .040
     LD99=.054   limits: .045 to .070

Stop - Program terminated.
```

Appendix 25. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Sukarame after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Rhyzopertha dominica
(Sukarame)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 64
input: 0.014 100 88
input: 0.023 100 97
input: 0.031 100 100
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        64.       .640
               .01400    -1.853872       100.        88.       .880
               .02300    -1.638272       100.        97.       .970
               .03100    -1.508638       100.       100.      1.000
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -118.36282

            parameter       standard error      t ratio
insecta     5.9719412        .60290234        9.9053211
SLOPE       2.4665739        .29531522        8.3523426

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .3634912      .1759995
  SLOPE           .1759995  .8721108E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           64.          61.649          2.351
.616492
```

```
                100.             88.            91.913          -3.913
.919128
                100.             97.            97.326           -.326
.973260
                100.            100.            98.780           1.220
.987800
                100.            100.            99.420            .580
.994196

chi-square 4.1531          degrees of freedom    3     heterogeneity
1.3844

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
  g(.90)=.10991   g(.95)=.20098   g(.99)=.67701

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .00379  lower      .00221      .00160
                              upper      .00518      .00564
LD95  insecta         .01761  lower      .01355      .01253
                              upper      .02658      .03359
LD99  insecta         .03327  lower      .02292      .02078
                              upper      .06565      .09957


 uji  resistensi  phospine  terhadap  imago  Rhyzopertha  dominica
(Sukarame)
   insecta    subjects 500   controls 100
     log(L)=-118.4   slope=2.467+.295   nat.resp.=.000+.000
     heterogeneity=1.38   g=.201
     LD50=.004   limits: .002 to .006
     LD95=.018   limits: .013 to .034
     LD99=.033   limits: .021 to .100

Stop - Program terminated.
```

Appendix 26. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Terukis after fumigation for 20 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Rhyzopertha dominica
(Terukis)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 41
input: 0.014 100 46
input: 0.023 100 51
input: 0.031 100 62
input: 0.040 100 70

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        41.       .410
               .01400    -1.853872       100.        46.       .460
               .02300    -1.638272       100.        51.       .510
               .03100    -1.508638       100.        62.       .620
               .04000    -1.397940       100.        70.       .700

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -335.73543

            parameter       standard error      t ratio
insecta     1.4314365        .31695085        4.5162730
SLOPE       .76387923        .17896651        4.2682802

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .1004578  .5580653E-01
  SLOPE       .5580653E-01  .3202901E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           41.          37.211          3.789
.372109
```

	100.	46.	50.610	-4.610
.506104				
	100.	51.	57.142	-6.142
.571422				
	100.	62.	60.988	1.012
.609885				
	100.	70.	64.191	5.809
.641914				

chi-square 4.5162 degrees of freedom 3 heterogeneity 1.5054

A large chi-square indicates a poor fit of the data by the probit analysis model. Large deviations for expected probabilities near 0 or 1
are especially troublesome. A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
g(.90)=.45765 g(.95)=.83689 g(.99)=2.8191

"With almost all good sets of data, g will be substantially smaller than
1.0, and seldom greater than 0.4."
- D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5

Effective Doses

		dose	limits	0.90	0.95	0.99
LD50	insecta	.01337	lower	.00479		
			upper	.02236		
LD95	insecta	1.9028	lower	.27977		
			upper	33416.		
LD99	insecta	14.843	lower	.96397		
			upper	.18906E		

uji resistensi phospine terhadap imago *Rhyzopertha dominica* (Terukis)
insecta subjects 500 controls 100
log(L)=-335.7 slope=.764+.179 nat.resp.=.000+.000
heterogeneity=1.51 g=.837

Stop - Program terminated.

Appendix 27. Results of parameter estimation analysis for toxicity of phosphine against *R. dominica* collected from Terukis after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Rhyzopertha dominica
(Terukis)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 68
input: 0.014 100 76
input: 0.023 100 78
input: 0.031 100 80
input: 0.040 100 92

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        68.       .680
               .01400    -1.853872       100.        76.       .760
               .02300    -1.638272       100.        78.       .780
               .03100    -1.508638       100.        80.       .800
               .04000    -1.397940       100.        92.       .920

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -251.26652

            parameter       standard error      t ratio
insecta     2.0903935        .35271579        5.9265661
SLOPE       .72965131        .19453596        3.7507272

Variance-Covariance matrix
                  insecta       SLOPE
  insecta         .1244084  .6748102E-01
  SLOPE       .6748102E-01  .3784424E-01


Chi-squared goodness of fit test

preparation   subjects   responses   expected    deviation   probability
insecta         100.        68.       65.963       2.037       .659626
                100.        76.       76.966       -.966       .769656
                100.        78.       81.461      -3.461       .814613
```

```
               100.        80.       83.882      -3.882       .838818
               100.        92.       85.778       6.222       .857777

chi-square 5.3190        degrees of freedom   3    heterogeneity 1.7730

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.69801            g(.95)=1.2764            g(.99)=4.2997

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.


Effective Doses
                       dose      limits           0.90             0.95
0.99
LD50  insecta          .00136
LD95  insecta          .24512
LD99  insecta         2.10562


 uji  resistensi  phospine  terhadap  imago  Rhyzopertha  dominica
(Terukis)
   insecta    subjects 500   controls 100
     log(L)=-251.3   slope=.730+.195   nat.resp.=.000+.000
     heterogeneity=1.77   g=1.276

Stop - Program terminated.
```

Appendix 28. Results of parameter estimation analysis for toxicity of phosphine against *Cryptolestes* sp. collected from Paceda after fumigation for 20 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Cryptolestes spp.
(Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 15
input: 0.016 100 26
input: 0.027 100 30
input: 0.038 100 43
input: 0.050 100 53
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	15.	.150
	.01600	-1.795880	100.	26.	.260
	.02700	-1.568636	100.	30.	.300
	.03800	-1.420216	100.	43.	.430
	.05000	-1.301030	100.	53.	.530

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -299.90296
```

	parameter	standard error	t ratio
insecta	1.3434702	.30464906	4.4098944
SLOPE	1.0775209	.18392905	5.8583508

Variance-Covariance matrix

	insecta	SLOPE
insecta	.9281105E-01	.5495460E-01
SLOPE	.5495460E-01	.3382989E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	15.	12.799	2.201	.127991
	100.	26.	27.705	-1.705	.277050
	100.	30.	36.438	-6.438	.364383
	100.	43.	42.589	.411	.425892

```
               100.        53.       47.671       5.329       .476708

chi-square 3.5142        degrees of freedom   3    heterogeneity 1.1714

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.18903            g(.95)=.34569            g(.99)=1.1644

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                       dose      limits           0.90            0.95
0.99
LD50  insecta          .05665  lower      .03850      .03466
                               upper      .12500      .23202
LD95  insecta         1.90415  lower      .49069      .36191
                               upper    57.05539  1029.87037
LD99  insecta     8.1689       lower  1.3632      .91384
                               upper  745.30      34972.


 uji resistensi phospine terhadap imago Cryptolestes spp. (Paceda)
   insecta    subjects 500   controls 100
     log(L)=-299.9   slope=1.078+.184   nat.resp.=.000+.000
     heterogeneity=1.17   g=.346
     LD50=.057   limits: .035 to .232
     LD95=1.904   limits: .362 to 1029.870
     LD99=8.169   limits: .914 to 34972.009

Stop - Program terminated.
```

Appendix 29. Results of parameter estimation analysis for toxicity of phosphine against *Cryptolestes* sp. collected from Paceda after fumigation for 48 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Cryptolestes (PACEDA)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 32
input: 0.016 100 52
input: 0.027 100 61
input: 0.038 100 70
input: 0.050 100 73
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	32.	.320
	.01600	-1.795880	100.	52.	.520
	.02700	-1.568636	100.	61.	.610
	.03800	-1.420216	100.	70.	.700
	.05000	-1.301030	100.	73.	.730

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -318.32995
```

	parameter	standard error	t ratio
insecta	2.0346513	.28596176	7.1151167
SLOPE	1.0944090	.16643524	6.5755842

Variance-Covariance matrix

	insecta	SLOPE
insecta	.8177413E-01	.4660530E-01
SLOPE	.4660530E-01	.2770069E-01

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	32.	31.433	.567	.314329
	100.	52.	52.759	-.759	.527594
	100.	61.	62.473	-1.473	.624728
	100.	70.	68.451	1.549	.684512
	100.	73.	72.933	.067	.729332

```
chi-square    .2419    degrees of freedom   3    heterogeneity   .08

Index of significance for potency estimation:
  g(.90)=.06257   g(.95)=.08884   g(.99)=.15345


Effective Doses
                      dose    limits       0.90        0.95        0.99
LD50  insecta         .01383  lower      .01065      .01001      .00870
                              upper      .01701      .01765      .01898
LD95  insecta         .44038  lower      .23291      .21176      .17900
                              upper     1.25180     1.66131     3.27812
LD99  insecta        1.84728  lower      .73930      .64533      .50797
                              upper     8.40301    12.68715    34.18192


 uji resistensi phospine terhadap imago Cryptolestes (PACEDA)
   insecta    subjects 500   controls 100
     log(L)=-318.3   slope=1.094+.166   nat.resp.=.000+.000
     heterogeneity=.08   g=.089
     LD50=.014   limits: .010 to .018
     LD95=.440   limits: .212 to 1.661
     LD99=1.847   limits: .645 to 12.687

Stop - Program terminated.
```

Appendix 30. Results of parameter estimation analysis for toxicity of phosphine against S. zeamais collected from Paceda after fumigation for 20 hours

```
POLO-PC
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Input file >
input: = uji resistensi phospine terhadap imago Sitophilus spp.
(Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 20 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 72
input: 0.014 100 81
input: 0.023 100 98
input: 0.031 100 100
input: 0.040 100 100

preparation     dose      log-dose     subjects  responses  resp/subj
insecta        .00000      .000000       100.         0.       .000
               .00500    -2.301030       100.        72.       .720
               .01400    -1.853872       100.        81.       .810
               .02300    -1.638272       100.        98.       .980
               .03100    -1.508638       100.       100.      1.000
               .04000    -1.397940       100.       100.      1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -126.89878

            parameter       standard error      t ratio
insecta     5.1545550        .55033693        9.3661804
SLOPE       2.0519651        .27507866        7.4595576

Variance-Covariance matrix
                 insecta       SLOPE
  insecta         .3028707      .1494550
  SLOPE           .1494550  .7566827E-01


Chi-squared goodness of fit test

preparation      subjects      responses      expected        deviation
probability
insecta             100.           72.          66.746          5.254
.667464
```

```
               100.             81.            91.157        -10.157
.911568
               100.             98.            96.350          1.650
.963504
               100.            100.            98.025          1.975
.980247
               100.            100.            98.887          1.113
.988874

chi-square 17.955          degrees of freedom    3     heterogeneity
5.9849

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near 0
or 1
are especially troublesome.  A plot of the data should be consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.59569           g(.95)=1.0893           g(.99)=3.6694

"With almost all good sets of data, g will be substantially smaller
than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.


Effective Doses
                      dose      limits          0.90            0.95
0.99
LD50  insecta         .00308
LD95  insecta         .01948
LD99  insecta         .04185


 uji resistensi phospine terhadap imago Sitophilus spp. (Paceda)
   insecta    subjects 500   controls 100
     log(L)=-126.9   slope=2.052+.275   nat.resp.=.000+.000
     heterogeneity=5.98   g=1.089

Stop - Program terminated.
```

Appendix 31. Results of parameter estimation analysis for toxicity of phosphine against S. zeamais collected from Paceda after fumigation for 48 hours

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji resistensi phospine terhadap imago Sitophilus spp.
(Paceda)
input: = lima taraf konsentrasi plus kontrol
input: = dua ulangan per perlakuan, 50 larva per perlakuan
input: = data mortalitas 14 hari setelah fumigasi, fumigasi 48 jam
input: = konsentrasi (%), jumlah serangga uji, jumlah serangga mati
input: *insecta
input: 0.000 100 0
input: 0.005 100 82
input: 0.014 100 98
input: 0.023 100 100
input: 0.031 100 100
input: 0.040 100 100
```

preparation	dose	log-dose	subjects	responses	resp/subj
insecta	.00000	.000000	100.	0.	.000
	.00500	-2.301030	100.	82.	.820
	.01400	-1.853872	100.	98.	.980
	.02300	-1.638272	100.	100.	1.000
	.03100	-1.508638	100.	100.	1.000
	.04000	-1.397940	100.	100.	1.000

```
Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
you want the likelihood function to be maximized [Y] ? LD's to
calculate [10 50 90] > Do you want to specify starting values of the
parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
function

Maximum log-likelihood  -57.392174
```

	parameter	standard error	t ratio
insecta	7.6085061	1.3164516	5.7795564
SLOPE	2.9143670	.59758161	4.8769356

Variance-Covariance matrix

	insecta	SLOPE
insecta	1.733045	.7828691
SLOPE	.7828691	.3571038

Chi-squared goodness of fit test

preparation	subjects	responses	expected	deviation	probability
insecta	100.	82.	81.659	.341	.816594
	100.	98.	98.630	-.630	.986295
	100.	100.	99.770	.230	.997701
	100.	100.	99.934	.066	.999340

```
               100.       100.       99.980        .020       .999796

chi-square    .6178    degrees of freedom   3    heterogeneity   .21

Index of significance for potency estimation:
  g(.90)=.11375   g(.95)=.16151   g(.99)=.27896


Effective Doses
                     dose    limits       0.90        0.95        0.99
LD50  insecta        .00245  lower      .00144      .00122      .00078
                             upper      .00326      .00339      .00365
LD95  insecta        .00899  lower      .00753      .00731      .00689
                             upper      .01169      .01259      .01522
LD99  insecta        .01540  lower      .01181      .01136      .01060
                             upper      .02514      .02925      .04402


 uji resistensi phospine terhadap imago Sitophilus spp. (Paceda)
   insecta    subjects 500   controls 100
     log(L)=-57.39   slope=2.914+.598   nat.resp.=.000+.000
     heterogeneity=.21   g=.162
     LD50=.002   limits: .001 to .003
     LD95=.009   limits: .007 to .013
     LD99=.015   limits: .011 to .029

Stop - Program terminated.
```