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AN EVALUATION OF MORTALITY OF SITOPHILUS ORYZAE TREATED WITH SELECTED INSECTICIDES VIA FILTER PAPER AND FOOD IMPREGNATED METHOD

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ABSTRACT

Laboratory bioassay were conducted at University Putra Malaysia in order to evaluate the toxicity and the antifeedant activity of five insecticides which is Cypermethrin, Malathion, Prevathon, Rotenone and also Spinosad against the adult of S.oryzae by using the filter paper impregnation and food impregnation method for 1, 3, 5 and 7 days. Rotenone found to be effectives in toxicity via filter paper impregnation method based on the LC_{50} values with 0.003% while the other was 0.04%, 0.017%, 0.106% and 1.109% were indicated Spinosad, Malathion, Cypermethrin and Prevathon. The less effective was Prevathon. Food impregnation method showed that the effective insecticides was Spinosad with LC_{50} values 0.001%. The lowest was Prevathon with LC_{50} values 5.776%. In addition, the other insecticides showed LC_{50} values was 0.003%, 0.05% and 0.875% which indicated Malathion, Cypermethrin and Rotenone. For the antifeedant activity via feeding deterrent index (FDI), it showed that, the lowest FDI was Spinosad with 0.535% while the highest was Rotenone with 1.756%. The other showed 0.63%, 0.869% and 0.949% indicated Malathion, Cypermethrin and Prevathon respectively. As conclusion, most effective insecticides against adult of S.oryzae was Spinosad because able to control the insect at the prepared concentration in both method.

Contribution/ Originality
The current study used the right amount of dosage of insecticides to prevent the losses to rice industries and able to reduce the infestation from the insect during storage. Hence, rice industries will able to control the insect pest and it will increase farmers profit in the rice industrial sector.

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1. INTRODUCTION

Majority people in the world regard rice as the second most widely grown cereal crop. In developing countries, most of the nation are counting on the rice for food calories and protein (IRRI, 2004). Hence, this rice (*Oryzae sativa* Linn.) is the most important crop and staple foods around the world's population. In Asia, world's rice is produced and consumed more than 90% every year (Spencer *et al*., 2009). For example, Thailand is one of the major rice exporter countries of the world, which is 70% of export good and values over 114,077 million baht a year (Agro-economic, 2007). The rice plant is vulnerable to many types of pests, from the seeds to the stored grains. The rice losses occur when milled grains are attacked by stored product insects and the most important one is the rice weevil (*Sitophilus oryzae* L.) (Coleoptera: Curculionidae). Damaging rice by the *S. oryzae* has seriously affected the availability of food for a large number of people worldwide (Adedire and Lajide, 2003). Without protection, the pests rapidly grow, develop, and damage the stored rice grains. The quality of rice grains is so poor that they do not meet the requirement for normal consumption, exportation, and industrial purposes (Campbell, 2008).

According to Sarker *et al*. (2006), qualitative and quantitative decline of stored agricultural and animal origin products resulted from invasions of around 70 moths' species, 355 species of mites as well as more than 600 species of beetle pests. Haque *et al*. (2000) infer that this damage might reduce about 20-30% in tropical zone and 5 to 10% in the temperate zone. To manage the pests during storing of rice, the usage of methyl phosphine (PH3) and bromide (MeBr) may lead to several problems (Negahban *et al*., 2006). Consequently, new strategies on how to manage the pests should be develop in crop industries. For instance, based on Huang and Ho (1998) an extract from higher plants can produced a lot of natural pesticides. These type of products will give a lower toxicity to mammals as well as keep environments safe and also give advantages to human such as medicinal properties (Negahban *et al*., 2006).

It is important to reduce the population of the insect pests such as *Sitophilus* species in order to maintain the quantity and quality of stored-product particularly cereals. Both *S. oryzae* (L.) and *S. granaries* (L.) are the most destructive and widespread insect pests of stored grains (Beckett *et al*., 1994). The quality as well as quantity of the rice grain may loss due to these type of pests which feed inside the rice grain (Kucerova *et al*., 2003). In order to control the insect pests of stored products the synthetic pesticide has been considered as more effective and accessible (Huang and Subramanyam, 2005).

2. MATERIAL AND METHODS

2.1. Rearing of the test insects

Colonies of *Sitophilus oryzae* were obtained from toxicology laboratory stock culture of Universiti Putra Malaysia (UPM) and reared on whole rice grains initially 13 to 14% moisture content in large aquarium container under the laboratory conditions (Rahman and Talukder, 2006). The subcultures and the tests were carried out under the same conditions. Before rearing process, the rice grains was sterilized by using oven at 50˚C for 20 to 30 minutes because it consider a normal temperature to sterile the rice grains (Chen, 2003).

For rearing process, each subcultures which is about 200 *S.oryzae* were placed for every 40 small aquarium covered with muslin cloth that contain rice grains in order to have a similar stage of age which is about 7 to 14 days old. For the feeding and oviposition process, the adults were kept for three days at the room temperature and humidity. Then, these 7 to 14 days old adults were removed from the jar after six days in order to have a new stage of age as mention above. At 27 ± 1°C and with 75 ± 5% of Relative Humidity (RH), these eggs were incubated for development and hatching process. These subcultures were kept safe inside a large tray that have been poured with water in order to prevent from any small ant and to prevent from any unwanted situation happened. Then, after appropriate 45 days old adult of *Sitophilus* oryzae were used in the experiment.

2.2. Procedure for mass rearing of *Sitophilus oryzae* from UPM's stock culture

5kg rice grains were used for stock culture. Rice grain was sterilized by using oven at 50°C in because consider as normal temperature for sterile the rice grains. Rice grain was placed in tray to let it dry for 10-15 minutes. After fully dried, 200 g rice grains was placed in small aquarium with 200 *S. oryzae* from stock culture UPM adult were used for rearing. Then, reared for about two months for adult emergence.

2.3. Procedures to prepare the test solution in this experiment for impregnated filter paper and food methods

In this experiment, the test solutions for each of tested insecticide were prepared. Five types of selected insecticides were used are cypermethrin, Malathion, prevathon, spinosad and also rotenone. By using electric weighting balance, all type of insecticides were measured based on the recommended rate that have been calculated in this study by using the equation $M_1V_1=M_2V_2$. Hence, the dilution for each tested insecticide have been identified. Solution for total six different concentrations of each insecticide were placed in conical flask, including control after serial dilution were done for eight replication. Then, solutions ready for experiment test.

2.4. Experimental design

The experimental design used for this experiment was Completely Randomised Design (CRD) because only two variables used in this experiment which is concentration and type of insecticides.

2.5. Determination range-finder of the chemicals

Recommended rate was identified from others previous studied that have working on the same insecticides. The recommended rate as stock solution used was 0.10% for the Rotenone, 12.19% for the Malathion, 1.00% for the Cypermethrin, Prevathon and Spinosad respectively in this experiment. Distilled water was used as a control. Hence, pre-test have been done based on each recommended rate and active ingredient for each selected insecticide. The active ingredient was identified based on the type of insecticide used.

By using dilution equation ($M_1V_1 = M_2V_2$), where (M_1) is referred as concentration in molarity (moles/litres) of the concentrated solution while (M_2) is the concentration in molarity of the dilute solution which is after solvent have been added. Besides that, (V_1) is the volume of the concentrated solution while (V_2) is referred to volume of the dilute solution. Hence, the volume of the concentrated for the pre-test was calculated. 100ml of distilled water were used for volume of the dilute solution (V_2). Hence, the difference concentrations of insecticides were tested for each method that has been used for contact toxicity and also for the antifeedant tests. The tables 1 and 2 below showed the different concentration between five insecticides.

Table 1: Five difference concentrations of insecticides were tested on the *Sitophilus oryzae* for contact toxicity

Insecticides	Recommended rate (%)	Concentrations (%)				
		C_1	C_2	C_3	C_4	C_5
Cypermethrin	1.00	0.500	0.250	0.125	0.062	0.031
Malathion	12.19	0.120	0.060	0.030	0.015	0.007
Prevathon	1.00	0.120	0.060	0.040	0.020	0.010
Rotenone	0.10	0.010	0.005	0.002	0.001	0.0006
Spinosad	1.00	0.500	0.250	0.125	0.062	0.0312

Note: $C_1 – C_5$ indicate the concentration of the insecticides

Table 2: Five difference concentrations of insecticides were tested on the *Sitophilus oryzae* for antifeedant test

Insecticides	Recommended rate (%)	Concentrations (%)				
		C_1	C_2	C_3	C_4	C_5
Cypermethrin	1.00	0.125	0.062	0.031	0.015	0.008
Malathion	12.19	0.012	0.001	0.0006	0.0003	0.0001
Prevathon	1.00	0.120	0.080	0.040	0.020	0.010
Rotenone	0.10	0.010	0.005	0.002	0.001	0.0006
Spinosad	1.00	0.100	0.050	0.020	0.012	0.006

Note: C_1 – C_5 indicate the concentration of the insecticides

2.6. Toxicity bioassays

2.6.1. Insecticide impregnated filter paper method

The contact toxicity for each of the insecticides which are Cypermethrin, Malathion, Prevathon, Rotenone and also Spinosad was determined in an impregnated filter paper dip technique for this bioassay. For each solution of the insecticides, 600 µl were applied by using pipette to Whatman No.1 filter paper which about 9 cm diameter. In this experiment, serial dilutions of the insecticides were prepared using distilled water. After that, the distilled water were used as a control purpose and then was allowed to evaporate for 10 minutes in order to make sure it fully dried. Then, the filter paper that have been cooperate with the insecticides were placed in the petri dish with 10 adult insect were released for this experiment. In order to prevent the insects from escaping, the petri dish were sealed by using the parafilm and then it were kept in laboratory condition at 28 ºC and with relative humidity is at 70 %. The mortality for each insect were recorded in excel software after 1, 3, 5, and 7 days at the same time while the LC_{50} which is lethal concentration values were analysed by using EPA Probit analysis.

2.6.2. Insecticide impregnated food method

The toxicity for each of insecticides which are Cypermethrin, Malathion, Prevathon, Rotenone and also Spinosad was determined by weighting 2.5g of rice grains on plastic weight boat by using electrical weighing balance. Then, it was soaked in the solution of insecticides that have been prepared through serial dilution with a different concentration and then it was allowed to evaporate until it fully dried which is about 15 to 20 minutes on the filter paper with 9cm in diameter because it easy to identified whether it fully dried or not. After fully dried, the rice grain that have been impregnated with insecticides were placed in vials of (30ml in volume) by using spatula. In order to prevent any incorrect results, the different spatula were used when transferring process into the vials have been made. Then, the same amounts of the rice grain also were soaked with distilled water for the control purpose and have been indicated as 0% concentration. After rice grains are fully dried, the 10 insects were placed into the vials and the data was collected after 1, 3, 5, and 7 days.

3. RESULTS

3.1. Impregnated filter paper method

3.1.1. Toxicity bioassay against adults of *sitophilus oryzae* via impregnated filter paper method

The results obtained from impregnated filter paper method showed a significant different between the five concentrations with distilled water. Cypermethrin showed the highest mortality which is 7% while Rotenone showed the lowest mortality which is 0% among the insecticides used against the adult of *S.oryzae* after one day exposure at concentration 0.500 and 0.010%. Cypermethrin was found to be most toxic insecticides with percentage mortality of 100% followed by Spinosad with a percentage mortality of 98.70% against adult of *S.oryzae* after 5 days of exposure at the same concentration were 0.500%. There is no significant difference between Rotenone and Malathion on the percentage mortality of 87.50% and 97.50%, respectively of insect after 5 days of exposure to the insecticides although they were comprised of the different concentrations which are at 0.010 and 0.120%. The lowest percentage of mortality was 40% obtained from Prevathon insecticides after 5 days of exposure.

Based from result obtained, all the concentrations are no significant difference against *Sitophilus oryzae* during the 7 days of exposure via filter paper impregnation method. However, the study revealed that the mortality of adult of *S.oryzae* was increased with the increasing of concentration after 7 days of exposure Table 3. All the insecticides showed no significantly difference at 7 days of exposure except for Cypermethrin at concentration 0.062, 0.031 and 0.125%, respectively, and also Spinosad at concentration 0.031%. Overall, the highest percentage is 100% of mortality were Cypermethrin while Prevathon showed the less toxic insecticide via filter paper impregnation method compared to other because showed the lowest percentage of mortality which is 96.30%.

Table 3: The toxicity of the tested insecticides against Adult of *Sitophilus oryzae* via impregnated filter paper method

Treatments	Concentration (%)	Mortality (%)			
		Day 1	Day 3	Day 5	Day 7
Cypermethrin	0.000	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d
	0.031	0.25 ± 0.16cd	1.00 ± 0.27cd	2.63 ± 0.26c	6.00 ± 0.80c
	0.062	0.75 ± 0.25cd	1.88 ± 0.29bc	2.88 ± 0.39c	7.40 ± 0.38cb
	0.125	1.50 ± 0.27c	3.13 ± 0.40a	4.63 ± 0.78b	8.50 ± 0.50ab
	0.250	5.37 ± 0.59b	7.00 ± 0.46a	8.50 ± 0.42a	9.88 ± 0.13a
	0.500	7.00 ± 0.46a	8.37 ± 0.38a	10.00 ± 0.87a	10.00 ± 0.00a
Malathion	0.000	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00d	0.00 ± 0.00b
	0.007	0.13 ± 0.13b	0.38 ± 0.18b	6.63 ± 0.57c	10.00 ± 0.00a
	0.015	0.38 ± 0.18b	1.13 ± 0.35b	7.50 ± 0.57bc	10.00 ± 0.00a
	0.030	0.88 ± 0.40b	1.50 ± 0.28b	8.50 ± 0.33ab	10.00 ± 0.00a
	0.060	1.25 ± 0.37b	5.25 ± 0.84a	9.0 ± 0.33ab	10.00 ± 0.00a
	0.120	4.38 ± 1.25a	7.13 ± 0.69a	9.75 ± 0.16a	10.00 ± 0.00a
Prevathon	0.000	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
	0.010	0.00 ± 0.00b	0.13 ± 0.13bc	3.13 ± 0.40a	8.38 ± 0.50a
	0.020	0.13 ± 0.13ab	0.38 ± 0.18abc	3.25 ± 0.41a	8.50 ± 0.42a
	0.040	0.25 ± 0.13b	0.63 ± 0.26abc	3.50 ± 0.42a	8.63 ± 0.60a
	0.060	0.38 ± 0.18ab	0.88 ± 0.23ab	3.88 ± 0.44a	9.25 ± 0.31a
	0.120	0.75 ± 0.16a	1.38 ± 0.26a	4.00 ± 0.38a	9.63 ± 0.18a
Rotenone	0.000	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
	0.0006	0.13 ± 0.13a	1.00 ± 0.42ab	7.63 ± 0.42a	9.63 ± 0.18a
	0.001	0.00 ± 0.00a	1.38 ± 0.26ab	7.88 ± 0.48a	9.75 ± 0.16a
	0.002	0.13 ± 0.13a	1.63 ± 0.42a	8.38 ± 0.32a	10.00 ± 0.00a
	0.005	0.13 ± 0.13a	1.88 ± 0.40a	8.50 ± 0.38a	10.00 ± 0.00a
	0.010	0.00 ± 0.00a	2.25 ± 0.31a	8.75 ± 0.37a	10.00 ± 0.00a
Spinosad	0.000	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00c
	0.031	0.38 ± 0.26c	2.63 ± 0.26b	7.00 ± 0.46c	7.88 ± 0.44b
	0.062	0.88 ± 0.30bc	3.75 ± 0.37b	8.50 ± 0.33b	9.25 ± 0.25a
	0.125	1.88 ± 0.40ab	5.25 ± 0.37a	9.00 ± 0.27ab	9.88 ± 0.13a
	0.250	2.50 ± 0.50a	5.63 ± 0.46a	9.25 ± 0.25ab	10.00 ± 0.00a
	0.500	3.25 ± 0.37a	6.63 ± 0.32a	9.88 ± 0.13a	10.00 ± 0.00a
Control	0	0	0	0	0

Values with the same letters in a column are not significantly different ($P<0.05$)

3.1.2. The LC_{50} values of tested insecticides against the *Sitophilus oryzae* adult via impregnated filter paper method

The LC_{50} values of each tested insecticides were used to evaluate the toxicity against the adult of *Sitophilus oryzae* shows in Table 4. Among the tested insecticides, Rotenone showed the highest toxic insecticides with the lowest LC_{50} values of (0.003 µL/100mL of H_2O) against the adult of *S.oryzae* via impregnated filter paper method. While, the Prevathon insecticides demonstrated the highest LC_{50} values of (1.109 µL/100mL of H_2O). Hence, it becomes the less toxic insecticides toward adult of *S.oryzae*. The other insecticides showed an average in toxicity against the insect were (0.040 to 0.106 µL/100mL of H_2O). Based on the mortality result, the Rotenone are able to kill half of the population although after 3 days of exposure. In this case, Rotenone showed the lowest value of LC_{50} because due to the highest percentage of mortality at 7 days of exposure compared to others with the lowest concentration used. As conclusion, based on the LC_{50} (0.003 µL/100mL of H_2O) values the toxicity of the insecticides tested after 7 days of exposure in increasing order were Prevathon ≥ Cypermethrin ≥ Spinosad ≥ Malathion ≥ Rotenone via impregnated filter paper method. The mortality of the adult of *S.oryzae* was affected by the concentration of the tested insecticides. The time exposure also might be the most important role in determining the level of toxicity of the insecticides.

Table 4: The LC_{50} values of the tested insecticides using impregnated filter paper method against the adult of *Sitophilus oryzae*

Treatment	LC_{50} (µL/100mL of H_2O)	Lower limit	Upper limit	Chi-square	Slope
Cypermethrin (5.5% w/w)	0.106	0.087	0.128	5.779	1.626
Malathion (84% w/w)	0.017	0.010	0.025	2.269	0.778
Prevathon (5% w/w)	1.109	<1.109	>1.109	0.135	0.282
Rotenone (0.1% w/w)	0.003	<0.003	>0.003	0.015	0.135
Spinosad (2.5% w/w)	0.040	0.013	0.068	0.498	0.646

3.2. Impregnated food method

3.2.1. Toxicity bioassay against adults of *Sitophilus oryzae* via impregnated food method

Table 5 shows the comparison between the percentage mortality of *S.oryzae* on different insecticide concentrations via impregnated food method. The results showed all the insecticides are significant different at 7 days after exposure against the adult. Spinosad showed the highest mortality which is 100% among the tested insecticides at lowest concentration (0.010%) excluding the control while Prevathon and Rotenone, both showed the lowest percentage of mortality against the adult of *S.oryzae* after 7 days of exposure. The results also showed a significant different at the highest concentration for each of the tested insecticides due the different in dosage that have been used after 7 days of exposure. Spinosad and Prevathon were applied with same concentration but Spinosad showed the highest percentage of mortality with 100% while Prevathon showed the lowest percentage of mortality in ranges 6.3 to 16.30% after 7 days of exposure. Besides that, based on the concentration used, percentages of mortality after 7 days of exposure in increasing order were starting with Rotenone ≥ Cypermethrin ≥ Malathion. Moreover, although Cypermethrin high in concentration used compared to Malathion and Rotenone but Malathion showed the highest percentage of mortality between both insecticides with in ranges of 16.30 to 100%, respectively after 7 days of exposure. Rotenone failed to kill half of the population of the adult of *S.oryzae* although after 7 days of exposure via food impregnation method.

Overall, mortality adult of *Sitophilus oryza*e increased with the increasing the concentration of the insecticides and all the concentration were differed significantly compared with the control at 7 days after exposure. As conclusion, based on the percentage of mortality, Spinosad insecticides was the most effectives insecticides via food impregnation method compared to other because it competent to kill half of the population of the adult as early one day after exposure.

Table 5: The Toxicity of the tested insecticides against *Sitophilus oryzae* by using the impregnated food method

Treatment	Dosage (%)	Mortality (%)			
		Day 1	Day 3	Day 5	Day 7
Cypermethrin	0.000	0.00 ± 0.00b	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d
	0.008	0.00 ± 0.00b	0.25 ± 0.16d	1.63 ± 0.46cd	2.25 ± 0.41c
	0.015	0.13 ± 0.13b	1.00 ± 0.33c	2.88 ± 0.35c	5.38 ± 0.65b
	0.031	0.00 ± 0.00b	2.13 ± 0.40c	7.13 ± 0.74b	8.50 ± 0.378a
	0.062	0.75 ± 0.31ab	4.75 ± 0.45b	8.50 ± 0.38ab	9.38 ± 0.26a
	0.125	1.75 ± 0.62a	6.38 ± 0.50a	9.13 ± 0.30a	9.38 ± 0.26a
Malathion	0.000	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00e
	0.00015	0.13 ± 0.13b	0.25 ± 0.16bc	0.63 ± 0.18cd	1.25 ± 0.37de
	0.0003	0.38 ± 0.18b	0.63 ± 0.26bc	1.13 ± 0.23cd	2.63 ± 0.57cd
	0.0006	0.50 ± 0.19b	0.88 ± 0.30bc	1.50 ± 0.50c	4.00 ± 0.42bc
	0.0012	0.75 ± 0.25b	1.88 ± 0.40b	4.25 ± 0.49b	5.00 ± 0.54b
	0.012	5.75 ± 0.88a	6.25 ± 0.81a	9.38 ± 0.40a	10.00 ± 0.00a
Prevathon	0.000	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
	0.010	0.00 ± 0.00b	0.13 ± 0.13bc	0.38 ± 0.26b	0.63 ± 0.26ab
	0.020	0.13 ± 0.12ab	0.25 ± 0.16bc	0.50 ± 0.27b	0.63 ± 0.26ab
	0.040	0.25 ± 0.16ab	0.50 ± 0.20abc	0.63 ± 0.18ab	0.88 ± 0.23ab
	0.060	0.38 ± 0.18ab	0.75 ± 0.16ab	0.88 ± 0.23ab	1.13 ± 0.23a
	0.120	0.75 ± 0.25a	1.13 ± 0.23a	1.50 ± 0.33a	1.63 ± 0.38a
Rotenone	0.000	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00c
	0.00063	0.00 ± 0.00a	0.25 ± 0.16a	0.13 ± 0.13b	0.25 ± 0.16c
	0.00125	0.13 ± 0.13a	0.13 ± 0.13a	0.38 ± 0.18ab	0.50 ± 0.19bc
	0.0025	0.13 ± 0.13a	0.25 ± 0.16a	0.63 ± 0.18ab	0.88 ± 0.30abc
	0.005	0.13 ± 0.13a	0.25 ± 0.16a	0.75 ± 0.25ab	1.25 ± 0.25ab
	0.010	0.25 ± 0.25a	0.38 ± 0.26a	1.00 ± 0.28a	1.63 ± 0.26a
Spinosad	0.000	0.00 ± 0.00b	0.00 ± 0.00d	0.00 ± 0.00c	0.00 ± 0.00b
	0.010	1.00 ± 0.39b	6.75 ± 0.62c	9.63 ± 0.18b	10.00 ± 0.00a
	0.020	4.63 ± 1.21a	6.88 ± 0.61bc	10.00 ± 0.00a	10.00 ± 0.00a
	0.040	5.38 ± 0.53a	8.00 ± 0.42abc	10.00 ± 0.00a	10.00 ± 0.00a
	0.080	6.38 ± 0.63a	8.63 ± 0.26ab	10.00 ± 0.00a	10.00 ± 0.00a
	0.120	5.50 ± 0.50a	9.38 ± 0.324a	10.00 ± 0.00a	10.00 ± 0.00a
Control	0	0	0	0	0

Values with the same letters in a column are not significantly different ($P < 0.05$)

3.2.2. The LC_{50} values of tested insecticides against the *Sitophilus oryzae* adult via impregnated food method

The LC_{50} values of the tested insecticides at different concentration were used to evaluate the mortality of rice weevil as shown in Table 6. Among the tested insecticides it was observed that Spinosad was the most toxic insecticides with the lowest LC_{50} value of 0.001% compared to other insecticides. Hence, it could provide the strong toxicity on the insect compared to Prevathon were showed the lowest toxic against the adult of *S.oryzae* with LC_{50} values of 5.776%. The other insecticides showed a moderated toxicity against the adult of *S.oryzae* with LC_{50} values of 0.003, 0.050 and 0.875%, respectively. Overall, all the insecticides showed significant higher control against adult of *S.oryzae* via food impregnation method except for Prevathon insecticides. Based on the LC_{50} values, it can be conclude that the toxicity of the tested insecticides after 7 days of exposure in increasing order were Prevathon ≥ Rotenone ≥ Cypermethrin ≥ Malathion ≥ Spinosad.

Table 6: The LC_{50} values of the tested insecticides using food impregnation method against the adult of *Sitophilus oryzae*

Treatment	LC_{50} (µL/100mL of H_2O)	Lower limit	Upper limit	Chi-square	Slope
Cypermethrin (5.5% w/w)	0.050	0.040	0.064	3.220	1.402
Malathion (84% w/w)	0.003	0.002	0.004	0.661	1.238
Prevathon (5% w/w)	5.776	<0.719	>0.719	0.368	0.720
Rotenone (0.1% w/w)	0.875	<0.073	>0.073	0.242	0.683
Spinosad (2.5% w/w)	0.001	0.000	0.003	1.106	0.579

4. DISCUSSION

In this study, different toxicity level for five types of insecticides against the adults of *S.oryzae* was determined. Besides that, each of the insecticides was applied for six different concentration based on the dosage recommendation from previous study including control that have been done in the laboratory condition. Hence, all the toxicities for each of the tested insecticide which are Cypermethrin, Malathion, Prevathon, Rotenone and also Spinosad showed a various level of toxicities in this experiment after 7 days of exposure. So that, some of the insecticides are able to kill the adults of *S.oryzae* in a short time when tested in the laboratory bioassay while some of them showed a slower mortality rate within the time of exposure. Among of them, some of the insecticides that have been tested showed a less toxic to the adult of *S.oryzae.* Since, the purpose in this study is to identify the effectiveness of the insecticides against the adult of Sitophilus oryzae, hence we able to identify the purpose in this study.

In the present study, after one day of exposure to the insecticides by using the filter paper bioassay, it was found that the rotenone gave the lowest value of mortality compared to the other four insecticides tested against adult of *S. oryzae* while the highest mortality for day one was cypermethrin. Since rotenone showed the lowest mortality for day one, hence it become less toxic to the *S. oryzae.* Next, the result for days three and five showed prevathon was the lowest mortality compared to others four insecticides while the highest still cypermethrin. Second highest in toxicity was rotenone. This was similarly with other plant study auch as Nukenine *et al*. (2011) reported that maximum mortality of 98.8 % was achieved to adult *S. zeamais* with neemazal at a concentration of 12 g/kg seed after 14 days of treatment and greatly reduced early progeny emergence. However, after days seven of exposure to insecticides the highest mortality was Malathion and the lowest was prevathon. For instance, study by Chintzoglou *et al*., (2008) for *L. bostrychophila*, chlorantraniliprole which is active ingredient for prevathon showed less effective in maize and whole rice than in barley, oats, peeled rice and wheat. This might due to the mode of action which is stomach and contact.

The mode of action also played an important role in increasing the mortality of the adult of *S. oryzae* in order to give a faster knock down when the insect fed the rice grains (Adeyemi, 2010). The concentrations used and the mode of action of insects played an important role in this experiment as indicate the toxicity of the insecticides (Kavallieratos *et al*., 2011). This can be proved by Iqbal *et al*., (2012), Malathion as a highly active insecticide to adult of *S. oryzae* that could be due to differences in the application of pesticide and environmental conditions. When organisms were treated with the insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase may in turn result sudden death of organisms. Generally, in this present study, all of the insecticides that have been tested able to control the adults of *S. oryzae* although their toxicities is different because some of insecticides might need a high dose in order to kill the adult at a fixed time. In order to get the same level of toxicities, the concentration of the insecticides need to be increase. So that it able to have a high level of toxicities in this experiment.

As conclusion, for the contact toxicity by using impregnated filter paper method showed the most effective's insecticides was Malathion insecticide compared to prevathon because able to kill 100% adult of *S. oryzae* after seven days of exposure and gave the highest percentage mortality of *S. oryzae*. However this concentration could be increase in order to get a better mortality of the insect. Classification of the insecticides also important in identify the toxicity of the insecticides. Besides that, concentration of the insecticides was played an important role in increasing the mortality of the rice weevils. The higher mortality might happened due to the classification and type of the insecticides used itself with different concentration also become an important factors in increasing the mortality of the insect (Kljajic *et al*., 2004).

Next, based on the second method in evaluating the toxicity of the tested insecticides against adult of *S. oryzae* was via impregnated food method. In present study, the result showed that the lowest mortality was rotenone after one day of exposure while the highest mortality was Malathion. However, after three, five and seven days of exposure to insecticides results showed the highest percentage of mortality was spinosad while rotenone remain the same which is the lowest percentage of mortality against adult of *S. oryzae*. Means, it showed that rotenone was the lowest toxicity against adult of *S. oryzae* compared to prevathon, cypermethrin, Malathion and spinosad. Hence, spinosad was the most toxic compared to rotenone and very effectives in controlling the adult of *S. oryzae*. It can kill the insect with a short period of time. The efficacy of spinosad in the present investigation was in tune with the findings of Subramanyam *et al*., (2006) who reported mortality of more than 98% within 12 days when seed was treated with spinosad 1 or 2 mg/kg. Based on Sparks *et al*., (2001), the Spinosad insecticides might need a minimum time in order to cause 50% mortality as it very toxic to insects because its action on the nervous system was at nicotine acetylcholine and also at gamma-aminobutric acid (GABA) receptor sites, so that it very effectives by ingestion and also contact.

In impregnated food method, the results showed that Rotenone less toxic compared to spinosad against adult of *S. oryzae* because lowest in percentage of mortality and the concentration used in this experiment were unable to kill half of the population adult of *S. oryzae* efficiently as in impregnated filter paper method. We can conclude that, the Rotenone was less effectives in controlling the adult of *S. oryzae* as in impregnated filter paper method because it easily evaporated and only effectives if the insecticides were prepared not more than one week (Norhayu, 2015). Rotenone was botanical insecticides and become more effective if the solutions was freshly prepared. Botanical insecticides have been designed to be more stable in the environment, safe to human being and also provide longer lasting control for the insect pests. A variety of responses have been reported from toxicological studies against *S.oryzae* by several toxicologist viz. essential oil, oil from eucalyptus, terpenes and benzaldehyde, neem extract RB-a in comparison with pyrethroids (Azmi *et al*., 1998). Similarly in this study, the results showed a different response for Rotenone and other four insecticides.

Besides that, Cypermethrin and Malathion insecticides also can be used for controlling the adult of *S. oryzae* efficiently because high in percentage of mortality after seven days of exposure. Although these insecticides are less toxic compared to the Spinosad, the results showed that both of them also intermediate in toxicity against the adult of *S. oryzae* via this method. Hence, based on the previous study, it state that the class of the Cypermethrin was in class II while the Malathion insecticides was in class III. The findings on mortality of adult of *S. oryzae* by Parimala and Maheswari (2011) increased marginally at 48 hours after exposure as increased in this present study. Besides that, their mode of action is similar which is stomach and contact. Mode of action is very important in order to know whether these species such as insect pests, fish, bird and mammals can be effects by the insecticides. Pesticide kills or inactive a pests can be identify through the mode of action. Insecticides can be classified based on this mode of action because each pest will have a different level of toxicity for each insecticide. In this study, the mode of action for contact dermal is through the skin of insects while for the stomach oral is through the mouth.

However, in this method, after spinosad, Malathion showed the second highest percentage of mortality compared to cypermethrin. Since, cypermethrin give more effective and show toxicity to wide range of insect's pest belonging to different orders other than coleopteran compared to other pyrethroids (Rani, 1997). (Ahsan *et al*., 2005) stated that the Cypermethrin were effectives against *S. oryzae* where LC_{90} found to be at 19 mug/cm^2. Thaung *et al*. (1986) reported that, the mortality of *S. oryzae* in wheat and maize when the temperature and insecticides where joint together and toxicity of cypermethrin decreased with increasing temperature. Although this present study was done in laboratory condition but the temperature was not the main factor. This can be prove in some studied, Rashid (2012) examined that temperature was controlled within a large incubator where aeration was also not a killing factor toward toxicity of cypermethrin and Malathion. Thus, the data observed solely the effects of the insecticides itself. Hence, Malathion and Spinosad insecticides much efficiently for controlling the populations of adult of *S. oryzae*.

Generally, the type chemical class of Malathion and Spinosad are from Organophosphorus and Spinosad respectively. In addition, the class of toxicity for both insecticides was located at class III and IV, their mode of action is similar which is by contact and stomach action but Spinosad insecticides revealed more unique in mode of action Srinivas *et al*. (2003), compared to Malathion insecticides against the stored-product pests compared to other known insecticides (Thompson *et al*., 2000). As conclusion, spinosad was the most effectiveness insecticide in controlling the adults of *S. oryzae* via impregnated food method because give the highest percentage of mortality as early five days of exposure.

In most studies, insecticides impact has been evaluated by exposure of the insect pest to a range pesticide concentration (Stark and Wennergren, 1995). Evaluation of insecticides toxicity may determine by the percentages of insect dead at the fixed time (LC_{50}). The different insecticides may show the different toxicity against insect pest. A highly toxic insecticides applied at a low concentration in the laboratory may cause high mortality compared to less toxic insecticides used at a higher concentration. In this case, it become more useful to compare the relative toxicity of insecticides against insect pest by measuring the LC_{50} while for the antifeedant activity of the insect that have been tested it become easier to identify by using the feeding deterrent index. In the present study, the toxicity of the tested insecticides was identified based on the values of LC_{50} against the adult of *S. oryzae.*

Starting with the impregnated filter paper method, prevathon showed the highest value of LC_{50} while the lowest was rotenone. In this case, prevathon gave highest value of LC_{50} it means that prevathon is the less toxic insecticide among the others because the highest values of LC_{50}, the less toxic insecticide and vice versa. Hence, rotenone with lowest value of LC_{50} become more toxic compared to Prevathon and it able to control and prevent the adult of *S.oryzae* from destruct the rice grain. Among the insecticides tested, Rotenone with the lowest value of LC_{50} able to reduce the F_1 progeny of *S.oryzae* might due to the types of the insecticides itself (McGovern, 1977). (Rajasekaran and Kumaraswami, 1985) reported that grains coated with plant extracts completely inhibited the development of insect like *S. oryzae*. Plant derivatives also reduce the survival rates of larvae and pupae and adult emergence (Koul *et al*., 2008). Development of eggs and immature stages inside grain kernel were also inhibited by plant derivatives (Boeke *et al*., 2004). The crude extract also retarded development and caused mortality of larvae, cuticle melanisation, and high mortality in adults (Jamil *et al*., 1984). Besides that, although Malathion showed the second highest toxic after rotenone based on the LC_{50} but Malathion still can be categorised as high in toxicity and they are able to control the adult population on *S.oryzae* due to higher concentration used compared to Rotenone. Rotenone become more toxic than malathion because Champ *et al*. (1969) established that, the Malathion insecticides was only gave effective results when applied to the larvae of the *S. oryzae* not to the adult stage. Lasota and Dybas (1996) stated that Malathion was the next best chemical in reducing *S. zeamais* population. Different with previous findings that, Malathion was less effective against *S. zeamais* by Pathak and Jha (1999) with LC_{50} value of 4.913 ppm. However, it highly active insecticide against adult could due to the differences in the application of pesticide and environment condition such as in air, malathion have been reported by Howard (1991) that sunlight able to broke down this type of insecticide easily approximately one and one-half days only because the solutions used is very quick to degrade. The sudden death of the insects occurs when it have been treated to the insecticides because the reaction with the continuous nerve impulse transmission due to inhibition of acetycholine esterase inside insect body. As conclusion, rotenone the most effectives against the adult of *S. oryzae* via impregnated filter paper method based on LC_{50} value in the laboratory condition.

As mentioned above for impregnated filter paper method, the highest values of LC_{50}, means the less toxic insecticides towards adult of *S. oryzae* while the lowest values of LC_{50}, the more toxic insecticides against insect. Similarly concept used in this present study via impregnated food method, the result showed lowest toxicity was prevathon while the highest toxicity was spinosad. The classification of insecticides played important role between this two insecticides because it showed a different in toxicity based on LC_{50} (Cordova *et al*., 2006). The class of toxicity for prevathon was located at class II and it a novel insecticide belonging to the chemical group of anthranilic diamides (Lahm *et al*., 2005) while spinosad was in class IV. The application of prevathon formulations reduced offspring emergence in comparison with the controls, however with adult suppression was not achieved, even at 10 mg chlorantraniliprole kg^{-1} grain, with the exception of maize (Kavallieratos *et al*., 2013). Fang *et al*. (2002) reported that mortality of *R. dominica* and *S. oryzae* in wheat treated with spinosad at 1mg spinosad kg^{-1} grain was 100% after 7 days of exposure, but at the same conditions, mortality was low for *T. castaneum*. Hence, spinosad showed the most effectives insecticide for controlling the adult of *S. oryzae* compared to prevathon via impregnated food method.

In this study, Malathion showed the second highest toxicity compared to spinosad based on the value of LC_{50} against adult of *S. oryzae* via impregnated food method. Malathion are less effectives because the wide used of this types of insecticides gave the resistance to the stored product pests including *S. oryzae* although the concentration used were higher than spinosad. However, Champ *et al*. (1969) established that malathion was effective only against newly hatched larvae of *S. oryzae*, but previous study represents by Iqbal *et al*. (2012) malathion as a highly active insecticide as similarly in this study against the adult that could be due to differences in the application of pesticide and environmental conditions. When organisms were treated with the insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase may in turn result sudden death of organisms.

On the other hand, it was found that Rotenone showed a less toxic against the adult of *S. oryzae* via impregnated food method compared to spinosad. This have been concluded by Rani *et al*. (1997) pyrethroids which is synthetic product give more mortality towards insect rather than plant extract due to the dilution of the crude extract of plant only produced a very little its actual active ingredient as compared to the pure synthetic compounds. As reported in previous studies by Dyck *et al*. (1985) synthetic compounds such as pyrethroids and Cypermethrin gave the differences in biocidal activities between the plant extract due to the immediate neurotoxic effect of synthetic products on insect's pests. The toxicity have been identified as small values of LC_{50}, the more toxic insecticides toward the adult of *S. oryzae* and it becomes more effective to control the insects storage-pest. Overall, for both methods, Spinosad and Malathion is more toxic than prevathon as showed in this present study.

As conclusion, Spinosad were found to have a strong repellence and feeding effect on the adult of *S. oryzae*. According to McGovern (1977) stated that all the compounds will considered as repellents if the compounds was in the class IV. In this study, repellency and deterrency decrease with increase in concentration and period of time and the result showed a decreasing in relative growth rate and also weight loss in this experiment. Among the tested insecticides, Spinosad exhibited more repellency and deterrency followed by Malathion, Cypermethrin, Prevathon, and last is Rotenone which showed the least effective toward adult of *S. oryzae* in this experiment. In this case, Spinosad can be identified as the most effective insecticides in controlling the insect pests on a several crops (Thompson *et al.*, 1997) because it has metabolite in the soil which is actinomycete bacterium, *Sacchoropolyspore spinosa*. Besides that, Spinosad have been proved that it provides good services in the crop protection because able to control the adult of *S. oryzae* in both method especially when they were works together with synthetic products. Moreover, Spinosad gave much effective among the others insecticides because it have a novel molecular structure and it mode of action is very suitable in this study as they acting on the insect nervous system at the nicotic acetylcholine and gamma-aminobutyric acid (GABA) receptor sites. Hence, is become very toxic to the insects by ingestion or contact bioassays (Sparks *et al.*, 2001). Generally, most of the worldwide studies have verified that Spinosad have many advantages in controlling the crops such as ornamentals, vegetables, cotton and also turf to control the pests in liquid state and become very effective insecticides (Bret *et al.*, 1997). This have been supported by Saunders and Bret (1997) by stated that it meets requirements for less human risk and eco-friendly.

Besides that, Spinosad also very good for insecticide resistance management (Salgado, 1997). In order for controlling the stored products Spinosad is very considerable efficacy against this types of pests (Toews and Subramanyam, 2003). In this study, Spinosad are able to control the adult of *S. oryzae* at dosage of concentration as 0.12000% are suitable dosage in controlling this insect. In our study showed that at all concentrations, Spinosad was able to kill half of adult of *S. oryzae* as early as one day after the exposure and killed all the adults after seven days of exposure. However, Athanassiou *et al*. (2008) and Vayias *et al*. (2009) found that Spinosad insecticides gave a lower mortality and less effective toward maize grain compared to the rice grain and barley. Similar observations have been reported by studies elsewhere (Nayak *et al*., 2005; Athanassiou *et al*., 2008) on the *T.castaneum* showed that their findings is highly tolerant to Spinosad and that high survival rate was observed at double dose of 1.4 ppm. As conclusion, the Spinosad is very effective towards adult of *S. oryzae* in both bioassays which is in impregnated filter paper and food method that have been study in this experiment.

5. CONCLUSION

The conclusion in this study from the toxicity bioassay and the antifeedant activity against adult of the *Sitophilus oryzae* was observed. In contact toxicity by using the filter paper impregnation method showed that Rotenone gave the highest value of LC_{50} which is 0.003 µL/100mL of H_2O, hence it gave the highest mortality rate compared to other insecticides in this experiment. The effective concentration for this insecticides was at 0.010% because it able to kill the insect within 7 days of exposure. Hence, it can be suggested the most effective dosage for controlling the adult of *S. oryzae* in the laboratory condition. Besides that, it showed a significant different among all the insecticides compared to the control concentration which is by using the distilled water. In this case, can conclude that, the distilled water will not affected the adult of the *S. oryzae* because after the observation that have been made, there was no mortality have been recorded within 7 days of exposure.

Besides that, for the toxicity by using the food impregnation method, the result showed that the lowest value of LC_{50} was Spinosad and Malathion insecticides. Means that, both of the insecticides gave the highest mortality rates compared to other insecticides in this experiment. The effective dosage for both insecticides was at concentration 0.12%. Then, results for the LC_{50} values from food impregnation method were carried out for further study of antifeedant activity in order to evaluate the feeding deterrence index against adult of the *S.oryzae*. The result showed that feeding deterrence index were significantly different among all the insecticides tested. Therefore, Spinosad showed the most effective at concentration 0.12% for controlling adult of *S.oryzae* and the advantages of using this type of insecticides was it give low environmental and human risk. Hence, all the rice industries could prevent their stored products against the adult of *S.oryzae* and able to reduce the qualitative and quantitative losses.

6. RECOMMENDATIONS

By knowing the best dosage and the antifeedant activity it may increase the level of effectiveness of the insecticides use. However, further research should be encouraged to standardise the dosage and the modes of action for the type of insect uses. Besides that, it also could possibly be produced in the industries for easier accessibility to farmer and store keepers. These will help to encourage wider adoption and use, avoid wastage and encourage greater economy of use. However, the plant-based bio-pesticides also recommended in the future study because it will keep our environment safe and gives less harmful to human, also it will avoid resistant of the insects towards pesticides. Thus, rice industry able to reduce the losses caused by the storage pests especially *S. oryzae.* Lastly, in the future study, preparation and handling of the insecticides should be more caution and precise in order to prevent from any error or inaccurate data collection. Safety rules also need to be applied while undergo the experiment.

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