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Storage Temperature of Botanical Insecticide Mixture Formulations and Its Activity Againsts *Crociodolomia pavonana* (F.) (Lepidoptera: Crambidae)

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ABSTRACT

The purpose of this research was to evaluate the safety of mixture formulations of *Tephrosia vogelii* and *Piper aduncum* at various storage temperatures and their insecticidal activity against *C. pavonana* larvae. Formulations were made from *T. vogelii* and *P. aduncum* (1:5) in emulsifiable concentrate (EC) and wettable powder (WP) form. Both formulations have strong insecticidal activity against *C. pavonana*. This research used a randomized completed design in different temperature treatments. Insecticidal activity of formulations after storage was tested against *C. pavonana*. The treatments were done using deep leaf method in 5 concentrations and 5 replications. The results showed that LC_{95} value of EC formulation after storage at condition: below 4°C, room temperature, and 40°C were 0.19%, 0.34% and 0.21% respectively. Based on LC_{95} value of EC formulation from each treatment, indicated that EC formulation after storage could hold insecticidal activity as good as insecticidal activity before storage due to LC_{95} value after storage relatively lower than LC_{95} value before storage (0.35%). In the contrary, WP formulation activity was significantly decreased in all treatments compared to WP formulation activity before storage based on LC_{95} value.

INTRODUCTION

Storing insecticide including botanical insecticide formulations becomes critical control points in pesticide distribution and pesticide storage. The quality of formulation, safety, and performance should be stable during a storage process. Additionally, pesticide containers have an important effect on storage and shelf life. The stability data of pesticide formulation during storage is required for pesticidal products registration (CIPAC Handbook, 1980). One indicator of pesticide formulation performance is insecticidal activity against targeted pest.

Tephrosia vogelii and *Piper aduncum* mixture formulation in form of emulsifiable concentrate (EC) and wettable powder (WP) have a strong insecticidal activity against *C. pavonana* with LC_{50} and LC_{95} of EC formulation were 0.15% and 0.13% and WP formulation were 0.35% and 0.31%. Besides causing the mortality of *C. pavonana*, EC and WP

formulations inhibited the development of treatment larvae (Lina, Manuwoto, Syahbirin, & Dadang, 2017).

T. vogelii leaves contain isoflavonoids compounds known as rotenon, elliptone and other rotenoid compounds namely deguelin and tefrosin (Mkenda & Ndakidemi, 2014; Mkenda et al., 2015). The bioactive compounds from *T. vogelii* offer great potential of developing botanical pesticides against post harvest insects in storing (Mkenda & Ndakidemi, 2014). Abizar & Prijono (2010) reported ethyl acetate leaf extracts of *T. vogelii* toxic to *C. pavonana* larvae and at LC_{95} level a mixture of leaf extract of purple-flowered *T. vogelii* and fruit extract of *P. cubeba* (5:9, w/w) was more toxic to *C. pavonana* larvae than each extract tested separately. Dilapiol from *P. aduncum* can inhibit the activity of cytochrome P450 enzymes in microsomes of gastrointestinal cells of corn borer larvae *Ostrinia nubilalis*. The synergistic character of dilapiol is very beneficial for the development of botanical insecticides as an

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alternative control in the future. Essential oil from *P. aduncum* showed bioactive potential to use as repellent against mosquito *Aedes aegypti* and caused mortality of *Ceratomyxa tingomarianus* beetle almost 100% by contact method (Arnason, Sims, & Scott, 2012; Guerrini et al., 2009; Misni, Sulaiman, & Othman, 2008).

Author were conducted a comprehensive research of mixture extract formulations of *T. vogelii* and *P. aduncum* (1:5). The results showed that the mixture extract formulation have a higher insecticidal activity compared to their single extracts. Based on the index combination value, emulsifiable concentrate (EC) and wettable powder (WP) formulations have strong synergistic actions without causing phytotoxic on broccoli leaf. The mixture extract of *T. vogelii* and *P. aduncum* (1: 5) influences a physiological function of *C. pavonana* through antifeedant effect, food assimilation, and increases the activity of cytochrome b5 and cytochrome P450 enzyme of *C. pavonana*. EC and WP formulations can kill and inhibit the growth and development of *C. pavonana* larvae besides having a low persistence and safe against natural enemy *Eriborus argenteopilosus*. EC and WP mixture formulations effectively suppress *C. pavonana* populations in field with similar effectiveness score of commercial insecticide *Bacillus thuringiensis* (BT). Overall, the EC and WP formulations are qualified and proper to use for field control of *C. pavonana*.

There are several factors causing a low application of botanical insecticides as an alternative pest control in field. The lack of ready-formulations, availability in quantity and quality, and safety storability are problems. The formulations technology will solve problems on safety storing, easy application, and increase the activity of botanical insecticide and in the contrary, incorrect storing of formulations will change an extract bioactivity becoming less active against target pest (Mediana & Prijono, 2014). The objective of this research was to evaluate the safety of mixture formulations of *T. vogelii* and *P. aduncum* at various storage temperatures and their insecticidal activity against *C. pavonana* larvae.

MATERIALS AND METHODS

This research was carried out in laboratory of Insect Bioecology, Plant Protection Department, Faculty of Agriculture, Andalas University, from June to October 2016.

Extraction of *T. vogelii* and *P. aduncum*

Extraction was done with maceration method using ethyl acetate and crude extract obtained using rotary evaporator. Crude extract can be directly used to make formulations and can also be stored in the refrigerator at temperature of $\pm 4^{\circ}\text{C}$ until 2 years.

Production of EC and WP Formulation

T. vogelii and *P. aduncum* extract were formed into a liquid formulation (EC) and powder formulation (WP). The reasons for the selection of EC and WP formulations because these two types of formulations are widely used in agriculture. They also have a low persistence and are relatively safe for the environment. The mixture extract with the best result in toxicity test was used as base composition for formulations. Each formulation contain 20% of active fraction (20 EC and 20 WP). Then they were added with 10% emulsifiers (Tween 80) and 70% carrier material (methanol and kaolin). Methanol is a carrier material for the EC formulations, while kaolin is a carrier material for WP formulations. The procedures for making formulations following Lina, Manuwoto, Syahbirin, & Dadang (2017).

Storage Formulations

EC and WP formulations were stored in sealed plastic bottles for 90 days in different temperatures. Storage conditions were at the temperature below 4°C (in refrigerator), room temperature, and 40°C (in oven). After storing for 90 days, each formulation was tested against *C. pavonana* using leaf residual method.

Preparation of Broccoli Plant and Rearing of *C. pavonana*

Broccoli (*Brassica oleracea* L.) was used as *C. pavonana* feed and bioassay media treatment in laboratory. Broccoli plants propagated in polybag plastic with composition of soil: compost (2:1). NPK fertilizer was given at dosage of ± 1 g per polybag. Moreover, maintenance was performed by watering, disposal of weeds, and pest control mechanically. Broccoli leaves were 2 months old and used to feed *C. pavonana* larvae and bioassay in the laboratory.

C. pavonana larvae were collected from cabbage planting area in Bukittinggi. The larvae were carried to laboratory with gauze plastic container (diameter 30 cm and height 35 cm). The larvae from field was maintained in laboratory using gauze plastic container and the broccoli leaves were put inside to feed *C. pavonana* larvae until the fourth

instar. Pupae were collected and put into a gauze cage (length 50 cm, width 50 cm, height 50 cm). Three days after imago emerges, the broccoli leaf in a small tube was put into cage for *C. pavonana* egg laying place. *C. pavonana* egg was collected every day and put in to petri dish until it hatched. The larvae were transferred into plastic containers (34 cm x 26 cm x 7 cm) containing broccoli leaf and ready to use for bioassay and rearing.

Implementation of the Experiment

The treatments were done using 6 concentrations and 5 replications. The toxicity test of formulations was conducted by dipping the leaves into the formulation that has been diluted with aquadest after storing for 90 days. The Broccoli leaves (4 x 4 cm) dipped on each dosage formulation and control, and then air dried. After the leaf dry, it was placed in petri dish (9 cm diameter) and covered with a tissue, two pieces of leaves were put to control and one piece of leaf for each treatment. Furthermore, in each petri dish was put 15 seconds instar larvae of *C. pavonana*. The Larvae were fed with the treatment leaves for 48 hours, after that with untreated leaves.

Observation

The observation variables were mortality and development of *C. pavonana* larvae from the second instar to the fourth. The mortality of *C. pavonana* larvae were calculated using formula:

$$P = \frac{a}{b} \times 100\%$$

Where: P = mortality (%); a = number of dead larvae; b = number of tested larvae

Data Analysis

The larval mortality data was processed by probit analysis using the POLO-PC program (LeOra Software, 1987). The larval development data was expressed as the mean value \pm standard deviation. In addition, Statistics 8 was used to analyze the mortality data of larvae and duration of development of *C. pavonana* larvae and then continued with Least Significant test.

RESULTS AND DISCUSSION

Formulation Toxicity Againsts *C. pavonana*

This study focused on determining the insecticidal activity of mixture formulation against *C. pavonana* after the formula was stored on different

temperature. Bioassay of EC formulations after storing for 90 days showed a high activity against *C. pavonana*. Storing the formulation at different temperatures: room temperature, below 4°C, and 40°C did not influence the activity of EC formulations against *C. pavonana*. The mortality of *C. pavonana* larvae treated with EC formulations stored for 90 days at room temperature, below 4°C, and 40°C were 81.3%, 96%, and 100% respectively. The result showed that the EC formulations activity was stable in different storage conditions during 90 days. The formula stability was characterized by formula activity which was still high and following pattern of mortality of *C. pavonana* before storage. Pattern of mortality of *C. pavonana* larvae treated with EC formulations after 90 days of storage can be seen in Fig. 1.

Mortality of larvae began on the first day of treatment and significantly increased on the second day treatment. Larvae mortality was low on the third day and the days after because the treatment leaves replaced with fresh leaves without treatment on the third days and usually the survival larvae will grow well depend on the amount of toxin residue inside the larvae body. Mortality of *C. pavonana* was caused by stomach poison from EC and WP formulations. The main toxin comes from *T. vogelii* extract which is called rotenone. Rotenone has strong insecticidal activity against a variety of insects as a stomach poison and contact poison (Perry, Ishaaya, & Perry, 1998). *T. vogelii* extract was found also a significant antifeedant, toxic and repellent effect (Mkenda & Ndakidemi, 2014).

The activity of WP formulation was stored for 90 days at various temperatures significantly decreased against *C. pavonana* larvae. The mortality of *C. pavonana* treated with WP formulation after 90 days stored at room temperature, temperatures below 4°C, and 40°C temperature were 24%, 10.67% and 17%, respectively. The mortality of *C. pavonana* was below 24% compared to activity of WP formulation before storage treatments. The result showed that the activity of WP formulation was unstable marked by the amount and patterns of *C. pavonana* larvae mortality. The mortality patterns of *C. pavonana* treated with WP formulation after storing can be seen in Fig. 2

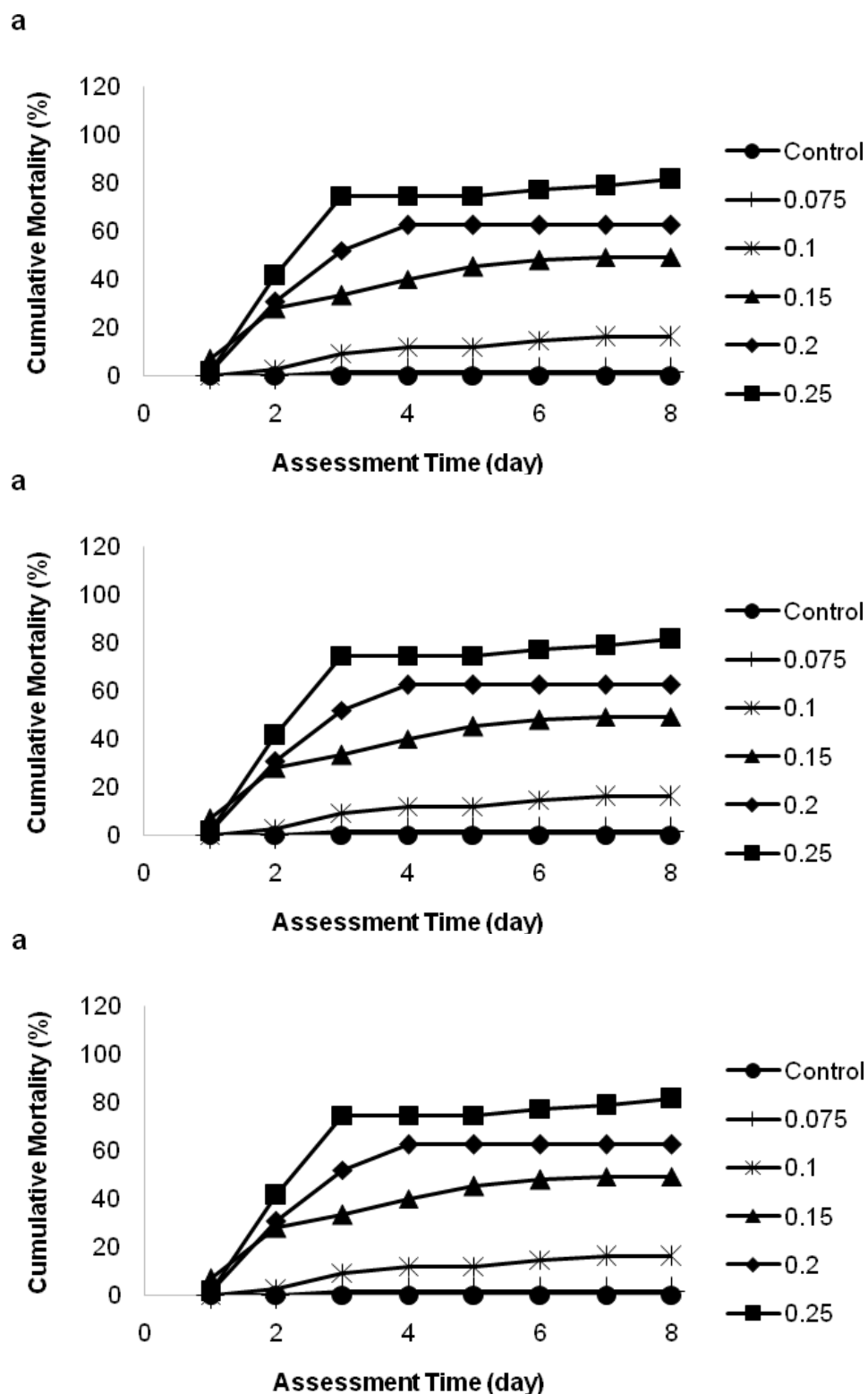


Fig. 1. Time-course mortality of *C. pavonana* larvae caused by EC formulation mixture of *T. vogelii* and *P. aduncum* (1:5) after storing for 90 days in conditions: a) Room temperature, b) Temperature under 4°C, and c) 40°C temperature.

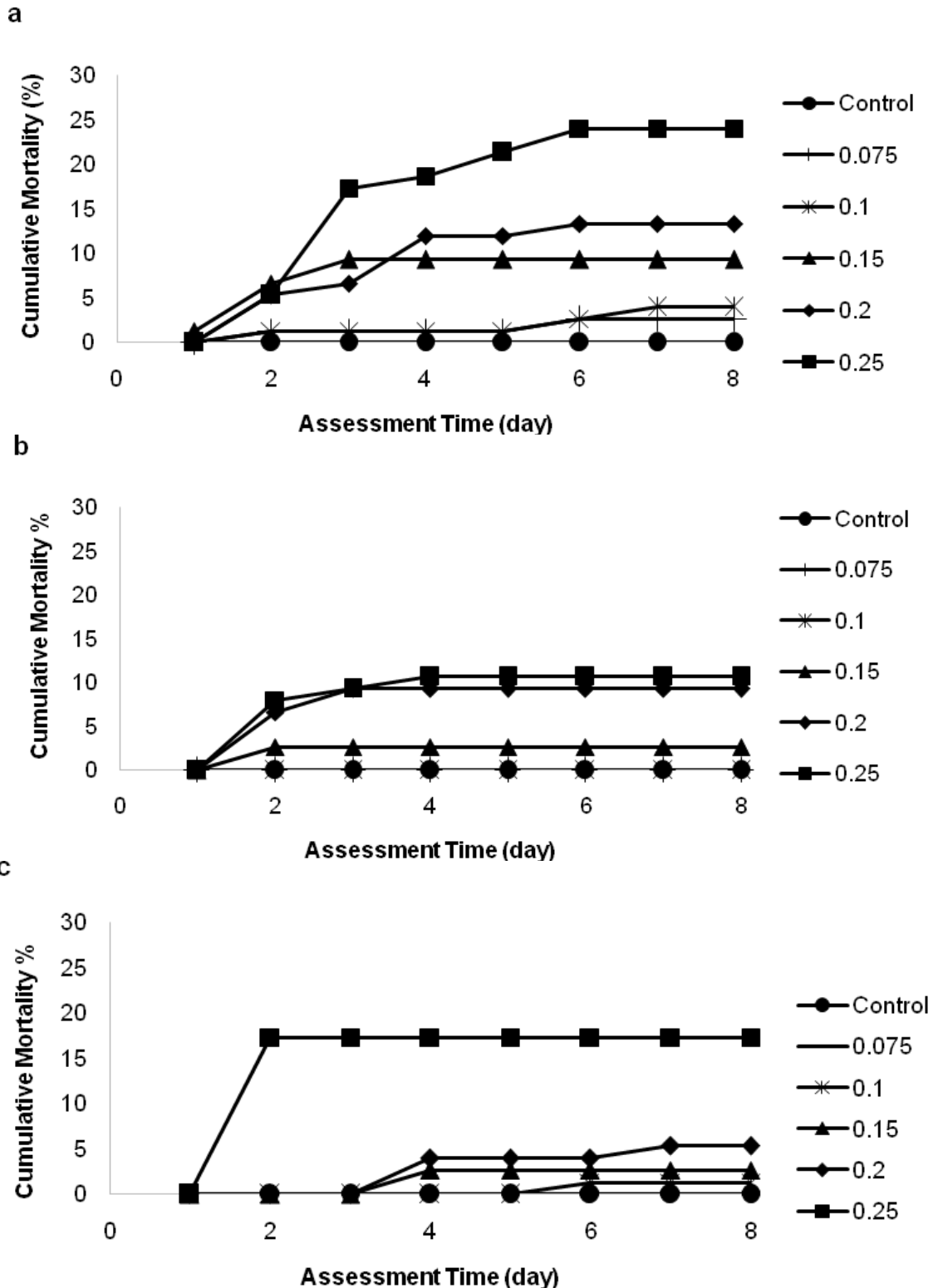


Fig. 2. Time-course mortality of *C. pavonana* larvae caused by WP formulation mixture of *T. vogelii* and *P. aduncum* (1:5) after storing for 90 days in conditions: a) Room temperature, b) Temperature under 4°C, and c) 40°C temperature.

The active compounds contained in *T. vogelii* and *P. aduncum* extracts formed EC and WP formulations causing mortality of *C. pavonana* larvae. As well as being toxic, the mixture *T. vogelii*: *P. aduncum* (1:5) also works as facilitators; active ingredients of *P. aduncum* that inhibit activity of cytochrome P450 enzyme to decompose toxic compounds in insect body. The Inhibition of cytochrome P450 enzyme allowed active ingredients of *T. vogelii* toward target site without cytochrome P450 enzyme decomposition. Lignans compound containing methylenedioxypheonil on *P. aduncum* extract can inhibit the activity of cytochrome P450 enzymes, and decreased toxicity of foreign compounds. Therefore, extracts of *P. aduncum* containing dilapiol showed potential synergistic action when mixed with other plant extracts. Inhibition of enzymes involved in detoxifying xenobiotic components in *C. pavonana* provide flexibility for *T. vogelii* active ingredients namely rotenon and other rotenoid compounds such as deguelin and tefrosin toward target site (Mkenda & Ndakidemi, 2014).

Storing was carried out on unused formulations resulted a change in the physicochemical properties of the formulation, including pH of the formulation. When EC formulations are stored at high temperature they will be a decrease in pH, while the opposite occurs in WP formulations, which are experienced a rise in pH after storage at elevated temperatures (Smyth, Hoffmann, & Shelton, 2003).

Inhibition of Growth and Development of *C. pavonana* Larvae

EC and WP formulations were toxic against *C. pavonana* and interfere the growth and development of treated *C. pavonana* larvae. EC and WP formulations at high concentrations could inhibit the change of larvae from instar 2 to 3 and instar 2 to 4. The control takes 2 days to change from instar 2 to 3, while EC and WP formulation take 2-4 days and 3-4 days respectively. While the larvae changes from instar 2 to 4 take about 3 days in control, 4-5 days and 5-6 days on EC and WP formulations treatment respectively.

Inhibition of growth and development of *C. pavonana* larvae during 90 days of storage treatment of EC and WP formulations can be seen in Table 1. The inhibition of growth and development due to the residues of active ingredients of mixture formulation *T. vogelii* and *P. aduncum* (1:5) remain inside the *C. pavonana* body and disrupt insect physiology function of *C. pavonana*. Based on Table 1, the growth and development of *C. pavonana* treated with EC formulation is longer than those of treated with WP formulation. This suggests that EC formulation after storage for 90 days at various temperatures have higher activity compared to WP formulations after storage in inhibiting growth and development of instar larvae. *T. vogelii* extracts showed deleterious effects on the growth and development of insects (Mkenda & Ndakidemi, 2014).

Table 1. Duration of *C. pavonana* larval development cause by 20 EC and 20 WP *T. vogelii* and *P. aduncum* (1: 5) Formulations after 90 days storing in various temperatures.

Storage Condition	Formulation Concentration (%)	Mean duration \pm SD (days)			
		EC Formulation		WP Formulation	
		Instar 2-3	Instar 2-4	Instar 2-3	Instar 2-4
Below 4°C Temperature	Control (0)	2 \pm 0	3.93 \pm 0.55	2 \pm 0	3.48 \pm 0.55
	0.075	3.11 \pm 0.59	4.87 \pm 0.42	2.49 \pm 0.81	4.35 \pm 0.48
	0.1	3.37 \pm 0.86	5.42 \pm 1.18	2.67 \pm 0.76	4.33 \pm 0.48
	0.15	4.5 \pm 1	6.75 \pm 0.5	2.95 \pm 0.79	4.65 \pm 0.56
	0.2	5 \pm 1	6.67 \pm 0.57	2.88 \pm 0.72	4.78 \pm 0.73
	0.25	4 \pm 0	6 \pm 0	3.42 \pm 0.67	5.26 \pm 0.69
Room Temperature	Control (0)	2.09 \pm 0.37	3.93 \pm 0.55	2 \pm 0	3.48 \pm 0.55
	0.075	3.19 \pm 0.43	5.15 \pm 0.56	2.76 \pm 0.45	3.66 \pm 0.77
	0.1	3.52 \pm 0.59	5.22 \pm 0.45	2.37 \pm 0.64	4.32 \pm 0.49
	0.15	4.37 \pm 0.63	5.79 \pm 0.77	2.39 \pm 0.67	4.26 \pm 0.56
	0.2	4.11 \pm 0.78	5.82 \pm 0.67	3.05 \pm 0.78	4.84 \pm 0.64
	0.25	4.78 \pm 0.80	6.36 \pm 0.84	2.61 \pm 0.77	4.33 \pm 0.57
40°C Temperature	Control (0)	2.01 \pm 0.11	3.91 \pm 0.29	1.92 \pm 0.27	3.83 \pm 0.99
	0.075	3.03 \pm 0.58	5.11 \pm 0.66	2.13 \pm 0.34	4.12 \pm 0.33
	0.1	3.47 \pm 0.69	5.79 \pm 0.84	2.41 \pm 0.59	4.64 \pm 0.81
	0.15	3.88 \pm 0.75	6.52 \pm 0.94	2.40 \pm 0.59	4.65 \pm 0.81
	0.2	-	-	3.04 \pm 0.80	4.66 \pm 0.84
	0.25	-	-	4.03 \pm 1.11	5.89 \pm 1.09

Table 2. Parameter of probit regression for relationship 20 EC and 20 WP of *T. vogelii*: *P. aduncum* (1:5) formulations and mortality of *C. pavonana* larvae.

Treatment	Storage Temperature	b ±SE ^a	LC ₅₀ (CI 95%)	LC ₉₅ (CI 95%)
EC Formulations	Below 4°C	6.38 ± 0.54	0.11 (0.08-0.14)	0.19 (0.15-0.44)
	Room temperature	4.92 ± 0.46	0.16 (0.14-0.18)	0.34 (0.28-0.50)
	40°C	6.32 ± 0.54	0.11 (0.08-0.14)	0.21 (0.16-0.42)
WP formulations	Below 4°C	3.81 ± 1.13	0.49 (0.35-1.72)	1.33 (0.66-18.26)
	Room temperature	2.38 ± 0.55	0.53 (0.34-2.80)	2.59 (1.10-24.08)
	40°C	3.80 ± 0.99	0.47 (0.34-1.14)	1.27 (0.67-8.92)

Remarks: ^ab = slope, SE= standard error, CI= Confidence Interval

Probit Analysis of EC and WP Formulation After Storage

Probit analysis result (LC₅₀) of EC formulations after storage in various temperature conditions below 4°C, room temperature, and 40°C temperature were 0.11%, 0.16% and 0.11% respectively. The LC₅₀ value after storage was not significantly different from LC₅₀ value of EC formulations before storage treatment (0.15%) (Lina, Manuwoto, Syahbirin, & Dadang, 2017). LC₉₅ value of EC formulations after storage at a temperature under 4°C, room temperature, and 40°C temperature, were 0.19%, 0.34% and 0.21%, respectively. While, the LC₉₅ value of EC formulations without storage treatment was 0.35% (Lina, Manuwoto, Syahbirin, & Dadang, 2017). The EC formulation showed a high activity although it has been stored for 90 days in various temperatures. The results of probit analysis for WP formulation was stored in a temperature under 4°C, room temperature and 40°C temperature, the LC₅₀ values were 0.49%, 0.53% and 0.47%, respectively which showed significantly different results with the LC50 value of WP formulations without storage (0.13%). Likewise, the LC₉₅ value of WP formulations after storage in various temperatures were higher than 0.31% of the LC₉₅ value of WP formulation without storage (Lina, Manuwoto, Syahbirin, & Dadang, 2017). It showed a decline in activity of WP formulation after storage for 90 days in various temperatures against *C. pavonana* larvae. A slope regression of EC formulation after storage treatment was higher than WP formulation after storage, inversely proportional to the regression value of formulation without storage. The result mean that increasing of concentration of EC formulation in multiples particular, will kill tested larvae higher than increasing concentration of WP formulation (Table 2). It was caused by different carrier materials of each formulation. In WP formulation, the carrier material used is kaolin (Al₂O₃·2SiO₂·2H₂O) which

is a hygroscopic materials (easier to bind water). Hygroscopic materials were unstable and cannot be stored for a long time. While the carrier of EC formulation is methanol (alcohol) which is more resistant and stable in storage conditions.

CONCLUSION

EC formulations showed a relatively consistence in maintaining their activity and stability after being stored for 90 days. While WP formulation was less stable in the storage, WP formulation was effectively used immediately without storage. A suggestion for further research is to try a carrier other than kaolin for WP formulation in order to survive longer in storage.

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