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Development Of Saponin Based Wettable Powder Formulation from *Phaleria macrocarpa* To Control *Pomacea maculate*

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Abstract

Apple snail is one of the 100 invasive alien species of the world and saponin proved to be the most effective and promising bioactive compound to manage this pest. This study was conducted to develop a saponin based wettable powder formulation from *Phaleria macrocarpa* (Scheff.) Borel and to evaluate its efficacy against *Pomacea maculata* Perry undrer laboratory and glasshouse conditions. Results revealed that all prepared saponin based wettable formulations shown molluscicide effects towards *P. maculata.* However, F4 formulation consisted of Terwet® 157, Terspers® 2700 and Kaolin at proportion of 15:15:70 revealed significantly higher feeding deterrent activities among prepared formulations and it was not significantly different from positive controls niclosamide and tea seed cake. In terms of lethel effects, the shortest LT50 (154 minutes) was recorded in niclosamide followed by 702 minutes in F4 formulation and 728 minutes in tea seed cake. F4 formulation along with positive control tea seed cake was further assessed in glasshouse condition. F4 formulation illustrated shortest LT50 value (750 minutes) with lowest feeding consumption percentage (0.6%) compared to LT50 (784 minutes) and feeding consumption percentage (1.3%) in positive control tea seed cake. Feeding consumption percentage (40.6%) was recorded for negative control water. Results sugest that F4 saponin based wettable powder overall performed better and can be efficiently used to control the apple snails as an eco friendly botanical molluscicide.

Keywords: Saponin, wettable powder, WP, mahkota dewa, Phaleria macrocarpa, apple snails, Pomacea maculata.

1. Introduction

Pomacea spp., commonly known as apple snails are belong to Ampullariidae family. They are well known pests of rice crops in various countries of Asian contenent[1]. Historically, apple snails are native to South America and introduced as food source in Asia around 1980s, but due to mismanagement they

become serious pests of rice crop[2,3]. The apple snails are not only limited to Asia but now extended to Australia, USA and Spain as well (first European country)[2,4-6]. It has been listed in 100 invasive alien species of the world[1,6]. *Pomacea canaliculata* (Golden apple snail) and *Pomacea maculata* Perry (Black apple snail) are two species that infest rice crops all over the world[7-9]. Both species are different from each other in colour and suture structure of the shells and can be easily differentiate. *P. maculata* is black in colour with longer suture whereas, *P. canaliculata* is yellowish to golden in colour with short and deeply channelled suture[7]. In Malaysia, Black apple snails are most abundant and widely distributed comparing with golden apple snails[9]. Currently, synthetic molluscicides i.e., niclosamide are being used to manage these snails but unfortunately; these types of molluscicides are well known for their negative effects on humans and their environment[1,6]. Among the plant derived compounds, saponin is currently gaining a significant scientific attention as a most promising bioactive compound to manage apple snails[1,6,10,11]. *Phaleria macrocarpa* (Scheff.) Borel commonly known as God's crown and mahkota dewa, is a medicinal plant native to Malaysia and Indonesia. The plant is widely known for its medicinal properties and have been used in traditional medicines to treat various diseases. *P. macrocarpa* is reported to be rich in saponin[12]. Based on our previous study[6] both *P. macrocarpa* leaves and fruits contained high saponin content and showed almost similar effectiveness as a botanical molluscicide against the black apple snails. Nevertheless, the leaves of *P. macrocarpa* were chosen for formulation preparation as the leaves were easily available compared to the fruits. The leaves extract also displayed significantly higher mortality and antifeedant properties when compared to fruit extracts at the dosage of 500 mgL-1. Usually, active ingredients in unformulated form have stability issues, as the active ingredients do not mix well with water and become chemically unbalanced[1, 13]. So, the type of formulation applied is essential to enhance the delivery system of the molluscicide, and it also deals with issues concerning stability and handling process. Formulated products have been proven to demonstrate better delivery of active ingredient to the target pest. Wettable powder (WP) formulation is well known for its lower dermal hazard and lesser eye and skin absorption compared to liquid formulations. It also does not burn vegetations as readily as oil-based formulations. Furthermore, it is easy to handle, store and transport, and easily measurable[14,15]. This study, therefore, aimed to formulate saponin based WP formulation from leaves extract of *P. macrocarpa* to control black apple snails.

2. Materials and Methods

2.1 Development of saponin based wettable powder formulation

The leaves of *P. macrocarpa* were collected and saponin was isolated as described in our previous study[6] and used as an active ingredient in wettable powder (WP) formulation. However, additional ingredients for the development of WP formulation were selected by evaluating two wetting agents, three dispersing agents and a carrier. Terwet® 157 ethoxylated nonylphenol wetting agent (Huntsman, USA), Napthalenesulfonate anionic wetting agent (Sigma Aldrich, USA), Terspers® 2700 polymeric dispersing agent (Huntsman, USA), Lignosulfonic acid organic polymer dispersing agent (Sigma Aldrich, USA), Eltesol Sx anionic dispersing agent (Huntsman, USA), and Kaolin China clay (Sigma Aldrich, USA) as carrier were used to prepare WP formulation and hereafter, known as TT, N, TS, Lig, EL and K, respectively. The experiment was conducted in the Toxicology Laboratory, Department of Plant Protection, Universiti Putra Malaysia at 25 ± 1 °C.

2.2 Ternary phase diagrams

Selection of other ingredients was made using the ternary phase diagram system. Six ternary phase diagrams consisting of TT-Lig-K, TT-TS-K, TT-EL-K, N-Lig-K, N-TS-K, and N-EL-K were constructed for this purpose. The isotropic region was determined by proportionally selecting 21 points from each ternary phase diagram and preparing the formulations according to the composition in which the points

laid (Figure 1). All 21 formulations from each ternary phase diagram were tested for their wettability, suspensibility, and dispersibility. The isotropic regions were identified based on the established performance criteria, and ternary phase diagrams were plotted using Chemix version 3.5 phase diagram plotter (Chemix, UK). The performance criteria were established based on the international requirement of wettability, dispersibility, and suspensibility for WP formulation. Three ternary phase diagrams with high isotropic regions were selected. Within the isotropic region in each phase diagrams, 11 points were randomally chosen near to carrier axis and formulated with active ingredient and were further tested for their wettability, suspensibility, and dispersibility. The method used was modified from a method described in a manual by UNIQEMA[16]. Four formulations with the highest suspensibility, wettability, and dispersibility were selected, and tested for their efficacy against black apple snails.

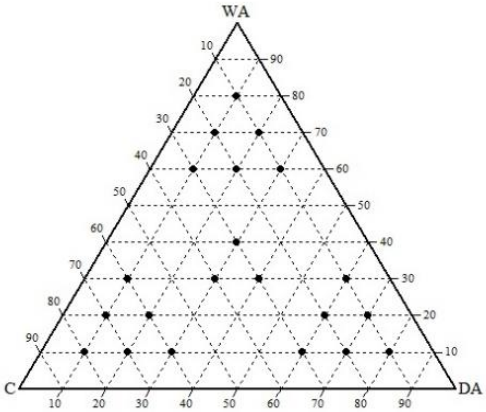

Figure 1: Selected points to determine isotropic region

2.3 Process in formulating wettable powder

The standard process for WP formulation described in previous study[17] was used with slight modifications. The formulations were processed by drying, blending, milling, and reblending. The isolate saponin was dried using a freeze dryer (Labconco) that was connected to a vacuum pump (Edwards). Prior to the blending process, all components of each respective formulation were weighed out and blending was performed using a laboratory blender (Waring®). Then blended materials were milled using lab-quality mortar and pestle to get the finest particles and finally the milled products were re-blended.

2.4 Wettability test

Wettability test was performed visually following the standard method of CIPAC MT 53.3 (MT 53.3, CIPAC F, 1995)[18]. One gram of WP was dropped into a beaker containing 100 mL of water from 20 cm. The test for wettability was repeated thrice for each formulation. Formulations were considered acceptable if complete wetting was observed within one minute without swirling.

2.5 Suspensibility test

Suspensibility test for WP formulations was performed using the method[19]. One gram of WP was dispersed into 100 mL of water with a hardness of 342 mgL-1, in a graduated cylinder and capped with polyethylene stopper. The dispersion was inverted 10 times and allowed to stand undisturbed for 5 min. The dispersion was then transferred to an Imhoff dispersion cone and allowed to stand undisturbed for an extended period of 30 min, and the top 90 ml was syphoned off. The remaining 10 mL of dispersion was passed through a pre-weighed Whatman No.1 filter paper, evaporated and dried for 24 hr at 60^{o}C. The test

for suspensibility was repeated thrice for each formulation. Suspensibility was determined in per centage; which reflected the final weight of sediment that remained on the filter paper as per the formula below.

$$Suspensibility\ (\%)\ =\ 111\ [1-(final\ weight\ -\ initial\ weight\ )]\ \%$$

Formulations were considered acceptable if the percent of suspensibility were between 60% to 105%.

2.6 Dispersibility test

Dispersibility test for WP was conducted using the method[20]. One gram of the WP was dropped carefully into 100 mL of water with a hardness of 342 mgL-1, in a graduated cylinder and was capped with a polyethene stopper without agitation. The gratitude cylinder was inverted slowly until the sediment at the bottom of the cylinder was not observed anymore. The number of inversions was recorded and the test for dispersibility was repeated twice for each formulation. Formulations were considered acceptable if the dispersion was completed in 20 or fewer cylinder inversions.

2.7 Snail rearing and rice planting

Before the experiment, apple snails were collected from Tanjung Karang paddy field, Selangor, Malaysia (3°25′27″ N 101°11′05″ E). The eggs, juveniles (hatchlings) and adults were collected by handpicking. The apple snails were reared in plastic aquariums (15 cm × 41 cm × 20 cm) in a glasshouse under natural environmental conditions. Throughout the rearing, the aquariums were washed and filled with a fresh supply of water every two days to avoid contamination. In the meantime, rice variety MR 219 was planted continuously to feed the apple snails and also be used in the bioassay experiments as described in our previous study[6]. Adult apple snails consumed rice leaves of up to 28 days old, whereas one to 20 days old hatchlings were fed with algae. The black apple snails were morphologically identified based on their shell morphology[21]. Apple snails with shell height of 4 cm were used in all experiments.

2.8 Laboratory bioassays

Toxicity of selected saponin based WP formulations under laboratory conditions was evaluated through lethal time response and feeding deterrent bioassays in the Laboratory of Ecology, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. The lethal time response bioassay of selected saponin based WP formulations against *P. maculata* was conducted using method described in previous study[1] with slight modifications. All selected formulations were assessed at five different concentrations (10,000, 7500, 5000, 2500 and 1000 mgL-1) against the positive control niclosamide at recommended rate (1.2 ml in 200 ml of water), tea seed cake (TSC) at recommended rate (0.5 g in 200 ml of water), and negative control water. Each respective concentrations of all treatments were prepared using distilled water. 200 ml of solution from the various prepared concentration of wettable formulations and controls were added to a plastic aquarium (13 cm × 18 cm), respectively. Five apple snails with shell height measuring 4 cm were released into each aquarium. The snails were starved for 24 hours before being subjected to the experiment. The apple snails were fed with 0.5 g of rice leaves throughout the experiment. The experiment was conducted under Completely Randomized Design (CRD) and replicated ten times. Mortality was assessed after every 30 minutes until all snails were dead. Apple snails were considered dead if they failed to show a coordinated movement when gently prodded with a small stick. The data obtained were analysed using POLO Plus Version 0.03 (LeOra Software)[22] to determine the LT_{50} of the treatments. The toxicity of the treatments was compared to 95% confidence limits of LT_{50}. The concentration of formulations that showed the lowest LT_{50} value when compared with the positive controls was selected for time response bioassay under glasshouse condition. While, feeding deterrent bioassay was conducted using the leaf dip method[23] with slight modifications. The same experimental

conditions were set for feeding deterrent bioassay as well. However, the leaf area and weight of rice leaves were measured before and after exposure using LI-3100 Leaf Area Meter (LI-COR, USA) and Sartorius BP221S analytical balance (Sartorius, Germany). The experimental design used was CRD with ten replications and the data were analysed by ANOVA for LSD test at 0.05 probability level using SAS 9.4 computer software (SAS Institute Inc. 2009)[24].

2.9 Glasshouse assessment

The glasshouse assessment was conducted in Field 2, University Putra Malaysia. Based on the laboratory bioassays, the best formulation with the most effective concentration was selected and evaluated on the black apple snails against the controls. The bioassay was conducted in plastic containers (50 cm × 70 cm × 13 cm). Rice variety MR219 was broadcasted at the rate of 7 g per container. The containers were covered with a net to protect the crop from birds and to also prevent the snails from escaping. Seven days after seed germination, 10 snails measuring 4 cm in shell height were released into each container, followed by the application of the selected treatment. The experiment was conducted in a Randomized Complete Block Design (RCBD) and replicated 10 times. Water was used as a negative control, and tea seed cake (TSC) was used as a positive control. The number of rice seedlings was counted and recorded before and after exposure to snails. The mortality of black apple snails was observed and recorded every hour until all snails died. The LT_{50} of the treatment was determined using POLO Plus Version 0.03 (LeOra Software)[22]. The toxicity of the treatment was compared to 95% confidence limits of LT_{50}. The number of seedlings consumed by snails was further analysed statistically by ANOVA using Least Significant Difference (LSD) test at 0.05 probability levels. The data were normalized with arcsine transformation and analysed using SAS 9.4 computer software (SAS Institute Inc. 2009)[24].

3. Results

There is no fixed method for selecting a formulation template, except through the trials and errors observed throughout the selection of ingredient[25]. However, the percentage of each component in the formulation can be determined by the ternary phase diagram[26]. The document by UNIQEMA[16] provided guidelines on using the ternary phase diagram in the solid formulation to identify the region in the triangle of three ingredients, where performance was satisfactory. Total six ternary phase diagrams were constructed using wetting agents, dispersing agents and a carrier Figure 2 (a to f). It was observed that maximum isotropic region 30% was observed in the ternary phase diagram system consisting of N-Lig-K (Figure 2 b), followed by 25% in TT-Lig-K system (Figure 2 a). However, the smallest isotropic region 5% was observed in ternary phase diagram system consisting of TT-EL-K (Figure 2 f), followed by 6% in N-TS-K system (Figure 2 d).

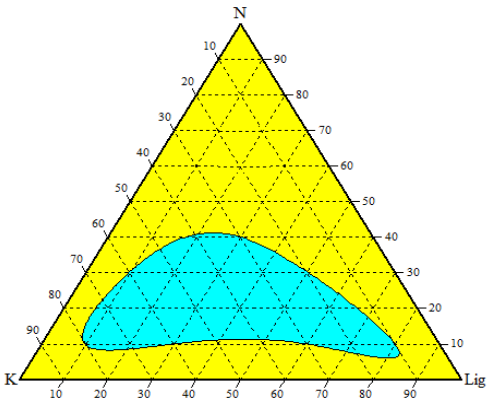

Figure 2: (a) TT: Lig: K

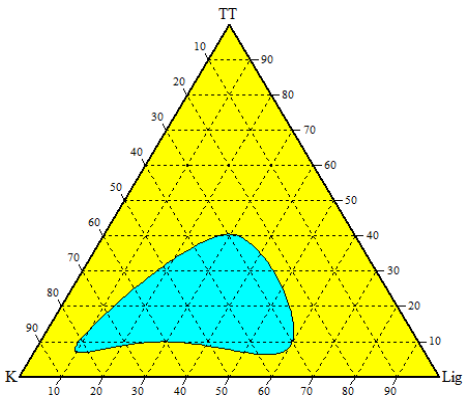

Figure 2: (b) N: Lig: K

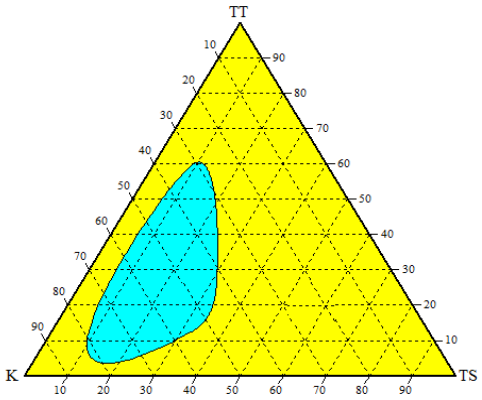

Figure 2: (c) TT: TS: K

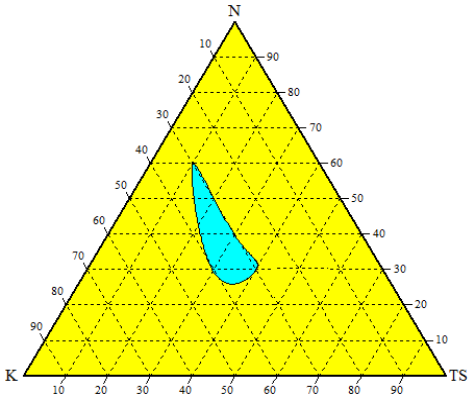

Figure 2: (d) N: TS: K

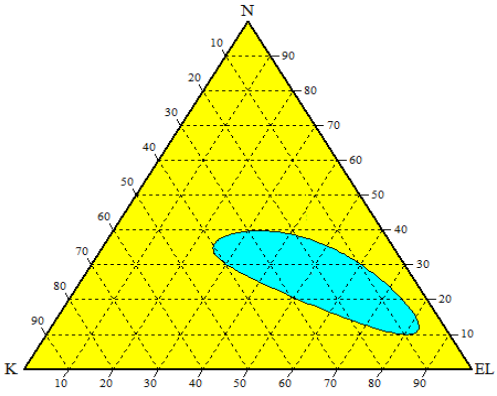

Figure 2: (e) N: EL: K

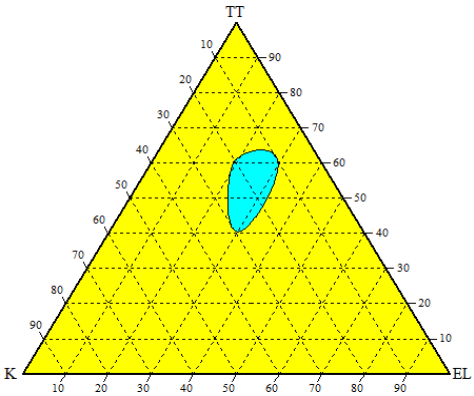

Figure 2: (f) TT: EL: K

Three ternary phase diagrams that revealed large isotropic/ acceptance region were selected and further processed with active ingredient. Eleven points were randamoly chosen within the isotropic region from each ternary phase diagram and formulated with isolated saponin from *P. macrocarpa*. The selection of points to formulate saponin based WP formulations was based on the low amount of wetting and dispersing agents with the higher amount of carrier to reduce the cost of the formulation. Table 1 shows the selected points of wetting agent: dispersing agent: carrier from each phase diagram system. The chosen points from TT: Lig: K were coded as P1 to P11 and from N: Lig: K were coded as P12 to P22, and the points selected from TT: TS: K were coded as P23 to P33. The selected points were tested for their performance with active ingredients through wettability, dispersibility, and suspensibility tests. Table 2 shows the performance of all the selected points. All the selected points passed the minimum requirements for performance. As this experiment aimed to select the best formulations, Therefore, four formulations i.e., P4, P8, P15 and P30 were selected based on their performance. These formulations revealed higher wettability, dispersibility, and suspensibility with a low amount of wetting and dispersing agents. The selected formulations showed wettability in 22, 12, 25 and 16 seconds, dispersibility in 14, 13, 12 and 10 cylindrical inversions and suspensibility at 103.2%, 104.6%, 104.0%, and 103.3% respectively. The selected formulations were coded as F1, F2, F3 and F4. Where, F1 and F2 consisted of TT: Lig: K at a composition of 15: 15: 70 and 11: 13: 76, respectively. Meanwhile, F3 consisted of N: Lig: K at the ratio of 11: 13: 76 and F4 consisted of TT: TS: K at the ratio of 15: 15: 70.

3.1 Laboratory bioassays

Table 3 shows the LT_{50} value of F1 (TT:Lig:K; 15:15:70), F2 (TT:Lig:K; 11:13:76), F3 (N:Lig:K; 11:13:76), and F4 (TT:TS:K; 15:15:70) wettable powder formulations which were tested against the positive control niclosamide and tea seed cake under laboratory condition. The shortest LT_{50} value was recorded in niclosamide (positive control) at 154 minutes (137-171). Meanwhile, the LT_{50} value for tea seed cake was 728 minutes (695-783). However, In the varying concentrations of F1, F2, F3, and F4 formulations, the shortest LT_{50} was recorded in 10,000 mgL-1 of the F4 formulation at 702 minutes (669-736) and the longest LT_{50} was in 1000 mgL-1 of the F3 formulation at 4473 minutes (4230-4779). Treatments containing a higher concentration of formulation had a shorter LT_{50} whereas, lower concentrated formulations revealed longer LT_{50}. The lowest concentration used in the experiment for all formulations was 1000 mgL-1, and the LT_{50} values observed were higher than 22 hours for all four formulations. Meanwhile, the highest concentration used for all formulation was 10,000 mgL-1, and the LT_{50} values at this concentration were shorter than 15 hours except for F3 formulation.

The lowest observed LT_{50} in F4 at the concentration of 10,000 mgL-1 was 26 minutes earlier than control tea seed cake followed by 10,000, 7500 and 10,000 mgL-1 concentrations of F2, F3 and F4 which were 79, 101 and 140 minutes slower than control tea seed cake respectively. Generally, higher concentrations were more effective in controlling black apple snails at the shortest LT_{50} values. However, the saponin based wettable formulations used in this study revealed that molluscicidal activities were present even in lower concentrations of formulation, but the LT_{50} values were the longest.

3.2 Feeding deterrent bioassay

Table 4 shows the mean weight and mean area of rice leaves consumed by *P. maculata* when exposed to 10,000, 7500, 5000, 2500 and 1000 mgL-1 of wettable powder formulations F1 (TT:Lig:K; 15:15:70) , F2 (TT:Lig:K; 11:13:76) , F3 (N:Lig:K; 11:13:76) and F4 (TT:TS:K; 15:15:70). After 24 hours, the mean leaf weight consumed by black apple snails when treated with niclosamide was 0.00g and 0.004g when treated with tea seed cake. The low leaf weight denotes the efficiency of the formulation in disrupting the feeding behaviour of *P. maculata.* The F4 formulation at 10,000 mgL-1 was not significantly different from positive control niclosamide and tea seed cake at $P < 0.05$. This explains that at 10,000 mgL-1, F4 showed antifeedant effects which were similar to niclosamide and tea seed cake. The lowest consumption of leaves in weight was 0.002 g in 10,000 mgL-1 of F4 followed by 0.01g , 0.012g, and 0.014g in 10,000 mgL-1, 7500 mgL-1 and 10,000 mgL-1 of F2, F4 and F1, respectively. Meanwhile, the highest consumption of leaf in weight was recorded as 0.416 g in 1000 mgL-1 of F3, followed by 0.372g, 0.333g and 0.294g in 2500 mgL-1, 5000 mgL-1, and 7500 mgL-1 of the F3, respectively. The weights of consumed leaves in all treatments were significantly different at $P < 0.05$ from the weight of leaves consumed in negative control water, which was 0.5g. After 24 hours, the lowest recorded mean leaf area was 0.018 cm^2 in treatment containing niclosamide. Meanwhile, in the treatment containing tea seed cake, the leaf area consumed by *P. maculata* was 0.042 cm^2. A low mean leaf area denotes the better ability of the formulation in disrupting the feeding behaviour of *P. maculata.* In terms of leaf area consumption, 10,000 mgL-1 of the F4 formulation was not significantly different from the positive control niclosamide and tea seed cake at $P < 0.05$. This proves that 10,000 mgL-1 of F4 has the same antifeedant effects as niclosamide. The lowest consumed mean area of leaves recorded was 0.026 cm^2 in 10,000 mgL-1 of F4 formulation followed by 0.344 cm^2, 0.398 cm^2, and 0.458 cm^2 in 7500 mgL-1, 1000 mgL-1 and 10,000 mgL-1 concentration of F4, F2, and F1, respectively. Meanwhile, the highest consumed mean leaf area was 10.196 cm^2 in 1000 mgL-1 of F3 followed by 9.934 cm^2, 9.142 cm^2, and 8.46 cm^2 in 7500 mgL-1, 5000 mgL-1, and 2500 mgL-1 of F3, respectively. The leaf areas consumed in all treatments were significantly different at $P < 0.05$ from the area consumed in negative control water, which was 16.036 cm^2.

Based on the results obtained from the feeding deterrent bioassays; it was concluded that the F4 (TT:TS:K; 15:15:70) formulation at 10,000 mgL-1 was not significantly different from niclosamide and

tea seed cake in antifeedant activities. Therefore, F4 formulation was selected for further test under glasshouse conditions.

3.3 Glasshouse assessment

Table 5 illustrates the LT_{50} values of F4 (TT:TS:K; 15:15:70) wettable powder formulation at the concentration of 10000 mgL-1 against black apple snails and positive control tea seed cake (TSC). Results revealed that the lethal time of F4 wettable powder was shorter as compared to control tea seed cake. The LT_{50} value of F4 wettable powder was 775 minutes which was 35 minutes earlier as compared to the LT_{50} value of 810 minutes for tea seed cake. Both formulations were overlapping at 95% confidence limits which indicates no significance difference between prepared F4 formulation and positive control TSC. However, the LT_{50} value of F4 wettable powder formulation was longer in the glasshouse experiment compared to the results obtained from laboratory experiment for the same formulation and same trend was observed in positive control TSC. Furthermore, table 6 shows the percentage of seedlings consumed by black apple snails throughout the glasshouse experiment. The lowest consumed percentage of seedlings was observed in F4 (TT:TS:K; 15:15:70) wettable powder formulation at 0.6 %; whereas the control tea seed cake displayed higher consumption at 1.3%. However, the results showed no significant difference between F4 wettable powder formulation and the control tea seed cake at $p < 0.05$. This indicated that the F4 formulation possesses the same antifeedant effects as tea seed cake. Meanwhile, the highest percentage of seedlings consumed (40.6%) was recorded in negative control water.

Table 1: Selected points from each ternary phase diagram consisting of TT, N, Lig, TS, and K.

Formulations			Wetting agent (%) TT/N	Dispersing Agent (%) Lig/TS	Carrier (%) K
TT: Lig: K	N: Lig: K	TT: TS: K			
P1	P12	P23	10	10	80
P2	P13	P24	10	15	75
P3	P14	P25	10	20	70
P4	P15	P26	11	13	76
P5	P16	P27	13	13	74
P6	P17	P28	13	15	72
P7	P18	P29	15	13	72
P8	P19	P30	15	15	70
P9	P20	P31	15	20	65
P10	P21	P32	20	15	65
P11	P22	P33	20	20	60

Table 2: The performance of selected points from each ternary phase diagram

Points	Wettability	Dispersibility	Suspensibility	Remarks
P1	47	18	105.3	Not selected
P2	36	19	104.8	Not selected
P3	34	19	103.2	Not selected
P4	22	14	103.2	Selected
P5	47	14	105.3	Not selected
P6	52	20	104.7	Not selected
P7	47	17	103.7	Not selected

P8	12	13	104.6	Selected
P9	58	14	104.7	Not selected
P10	54	12	104.2	Not selected
P11	52	18	104.2	Not selected
P12	55	17	104.7	Not selected
P13	48	14	104.9	Not selected
P14	46	16	104.2	Not selected
P15	25	12	104.0	Selected
P16	47	17	105.8	Not selected
P17	51	17	105.4	Not selected
P18	43	17	104.7	Not selected
P19	39	18	105.4	Not selected
P20	23	19	104.1	Not selected
P21	48	18	104.9	Not selected
P22	53	19	103.9	Not selected
P23	33	18	105.5	Not selected
P24	37	19	104.7	Not selected
P25	32	17	103.7	Not selected
P26	56	11	103.2	Not selected
P27	48	19	104.8	Not selected
P28	26	18	105.3	Not selected
P29	42	19	104.3	Not selected
P30	16	10	103.3	Selected
P31	57	14	105.0	Not selected
P32	47	13	104.3	Not selected
P33	53	17	104.4	Not selected

Table 3: LT_{50} values (minutes) of saponin-based wettable powder formulation developed from crude leaves extracts of P. macrocarpa against P. maculata.

Treatments	Concentration (mgL-1)	LT_{50}	95% confidence limits		Chi-Square
			Lower	Upper	
F1 (TT:Lig:K) (15:15:70)	1000	1888	1838	1938	19.95
	2500	1508	1470	1546	14.51
	5000	1277	1239	1316	19.30
	7500	1097	1062	1133	15.09
	10,000	868	835	901	14.31
F2 (TT:Lig:K) (11:13:76)	1000	1522	1485	1559	6.56
	2500	1345	1313	1378	15.51
	5000	1134	1098	1171	16.73
	7500	991	957	1025	19.09
	10,000	807	775	838	13.32
F3 (N:Lig:K) (11:13:76)	1000	4473	4230	4779	16.03
	2500	3246	3151	3352	17.04
	5000	2223	2177	2269	12.40
	7500	1789	1758	1819	13.83
	10,000	1661	1632	1690	19.03
F4	1000	1368	1335	1400	7.85

(TT:TS: K) (15:15:70)	2500	1203	1170	1237	17.93
	5000	971	933	1009	23.40
	7500	829	794	865	19.64
	10,000	702	669	736	18.09
Controls	Niclosamide	154	137	171	4.88
	Tea seed cake	728	695	763	22.38

Table 4: Mean leaf weight and area consumed by P. maculata at 24 hours when treated with saponin based wettable powder formulations.

Treatments	Concentration (mgL-1)	Mean Weight	Mean Area
F1 (TT:Lig:K) (15:15:70)	10,000	0.014ab	0.458ab
	7500	0.028b	0.97b
	5000	0.098c	2.558c
	2500	0.132e	4.342d
	1000	0.216g	7.034f
F2 (TT:Lig:K) (11:13:76)	10,000	0.01ab	0.398ab
	7500	0.022b	0.838b
	5000	0.092c	2.164c
	2500	0.126de	4.122d
	1000	0.21fg	6.646ef
F3 (N:Lig:K) (11:13:76)	10,000	0.228g	6.276e
	7500	0.294h	8.46g
	5000	0.33i	9.142gh
	2500	0.372j	9.934h
	1000	0.416k	10.196i
F4 (TT:TS:K) (15:15:70)	10,000	0.002a	0.026a
	7500	0.012ab	0.344ab
	5000	0.028b	0.76ab
	2500	0.108de	4.464d
	1000	0.192f	6.116e
Controls	Niclosamide	0.0a	0.018a
	Tea seed cake	0.004a	0.042a
	Untreated	0.5l	16.036j

Means with same letters within column are not significantly different at $P < 0.05$

Table 5: LT_{50} values (minutes) of saponin based wettable powder formulation and tea seed cake against P. maculata.

Concentration (mgL-1)	LT_{50}	95% confidence limits		Chi- Square
		Lower	Upper	
F4 (10000 mgL-1)	775	750	801	18.35
Control (TSC)	810	784	838	18.84

Table 6: Percentage of consumed seedlings by P. maculata when treated with saponin based powder formulation and tea seed cake

Concentration (mgL-1)	Percentage (%) of seedlings consumed
F4 (10000 mgL-1)	0.6 b
Control (TSC)	1.3 b
Control (Water)	40.6 a

Means with same letters within column are not significantly different at $P < 0.05$

4. Discussion

In this study it was observed that two regions were formed in each ternary phase diagram during the process of formulation. The regions are known as isotropic or acceptance regions and rejection region. In the isotropic region, all components formed a homogeneous mixture with uniform composition. Previous study[25] also defined isotropic or acceptance region in solid formulations as, a region where the mixture showed wettability, dispersibility, and suspensibility according to the established performance criteria. Meanwhile, the rejection region was region where the mixture was unable to pass the established performance criteria. WP formulations were successfully developed with minimum ratio of wetting and dispersing agents. In fact, considering a maximum amount of surfactant is relatively unnecessary in any formulation, as similar performance can often be achieved using the optimal amount of surfactant[1,27]. The results of study further revealed that the synthetic molluscicide (niclosamide EC), effectively killed apple snails within the shortest time after introduction. The positive control niclosamide was in liquid form, whereas the prepared F1, F2, F3 and F4 formulations were in solid form. Hence, the LT_{50} values of all formulations were compared with positive control tea seed cake which was in solid form to eliminate the influence of biases in physical properties of formulation and control. Farmers mostly preferred synthetic molluscicides due to their fast and rapid action against apple snails. But unfortunately, these synthetic molluscicides are also well known for their adverse effects on human and environment[1,6]. Increase the pest resistance due to the presence of residual in soil and plants have also been reported in previous studies[1,6,28,29]. Similarly, tea seed cake is also known to cause harmful effects on non-target organisms. Therefore, botanical molluscicides from new plants are the best alternative to synthetic molluscicides, as they are safe for human and environment, while effective against apple snails as observed in this study. Farmers should also not highlight duration as an issue to avoid using botanical molluscicides as this study has shown that LT_{50} was achieved at 750 minutes. Previous studies on botanical molluscicides tested against apple snails revealed that at least 24 hours was required to exhibit lethal effects[1,30-32]. These proved that it is normal for botanical molluscicides to take a longer time to show its effectiveness against apple snails. Similarly, some other studies also reported that high concentrations of saponin is required to function as an effective botanical molluscicide[33,34]. The formulated wettable powder used in this study contains only 20% active ingredient relative to other commercial botanical molluscicides that mostly contain a higher percentage of saponin compound[1,35,36]. Hence, the formulations prepared in this study require a higher concentration to become effective. Consequently, the concentrations tested to determine the LT_{50} value against *P. maculata* in this study ranged between 1000 to 10,000 mgL-1. All concentrations of the formulations tested in the experiment were toxic to the *P. maculata* at different times. Furthermore, our results are in line with previous study[1] in which saponin from from *Furcraea selloa* and *Entada spiralis* revealed molluscicidal effects against *P. maculata*. The toxicity of the formulation is dependent on the action of the active ingredient, and it also relies on the target organism[1].The relationship between the target organism and the active ingredient can be described in three phases; exposure, toxicokinetics and toxicodynamics[37-41]. The F4 (TT:TS:K; 15:15:70) wettable powder formulation displayed molluscicidal effect due to the presence of saponin bioactive compound which is proven toxic to apple snails[1,31,42-44]. Mortality occurs due to the haemolytic effects that are

associated with the disturbance in the surface tension of the apple snails gills when in contact with saponin bioactive compound[1]. The gills' membrane increase in permeability in the presence of saponin and this leads to physiological losses of electrolytes[45-47]. According to a previous study[48] when the apple snails' body surface is exposed to saponin, the respiratory system of the snail becomes affected. Meanwhile, previous studies[10,49] reported several responses displayed by the tested organisms when in direct contact with saponin. Snails that meet saponin had been seen to initiate the production and desecration of large volumes of mucus to reduce the contact of saponin with their body surface. This causes dehydration and as result snails eventually die[50]. Similarly, this was the reason for mortality in *P. maculata* when treated with saponin based wettable formulation.

Results also showed that the type and composition of wetting agent and dispersing agent in the formulation played a crucial role in the mortality of *P. maculata* as both adjuvants enhanced the performance of wettable powder formulation by increasing the rate of absorption into the target pest[1,51]. The LT_{50} value of F4 formulation (TT:TS:K; 15:15:70) was longer as compared to niclosamide because the niclosamide was in an emulsifiable concentrated form whereas the F4 formulation was in wettable powder form. The emulsions are in liquid state and usually have smaller particle size than any powder formulation. Therefore, they can readily mix and immediately become active, and on the other hand, powder formulations are in solid state and due to their greater particle size take time to mix and become effective. Therefore, 10,000 mgL-1 of F4 formulation displayed better efficiency when compared to tea seed cake which was also powder. Moreover, the results also revealed that higher concentrations of saponin-based wettable powder formulation reduced food consumption in *P. maculata*. This observation was supported by previous study[52] reporting that an increase in saponin content lowered the feeding activities, as saponin showed molluscicides properties against apple snails and it also produced a noxious odour that prevented snails from consuming the rice leaves. Besides saponin's insecticidal and molluscicide activities, it is also well-known for its antifeedant properties[53]. Another study[54] reported that saponin containing plant could function as an antifeedant to prevent molluscs from feeding on living plants. These observations were almost similar to the findings of previous study[1] that saponin-based nano-emulsion formulation developed from *F. selloa* could prevent the snails from consuming rice seedlings as low as zero percent. Additionally, saponin is well-known for its antifeedant activities in numerous insect pest and mites[13,55-57]. These past findings further strengthen the antifeedant characteristics observed in this study. Previous studies [1,10,45,58] suggested that novel molluscicide products from plants can be a new, safe, and eco-friendly option as compared to synthetic molluscicides. Therefore, based on the results obtained from toxicity study in the laboratory and glasshouse bioassays, the F4 (TT:TS:K; 15:15:70) wettable powder can be considered as an alternative eco-friendly botanical molluscicide that can be efficiently used to control the apple snails.

5. Conclusion

A wettable powder formulation from *p. macrocarpa* was successfully developed in this study and the results obtained from this study suggest that the application of F4 formulation (TT:TS:K; 15:15:70) at that specific concentration is advantageous in controlling *P. maculata*, considering its botanical origin and safety to the environment as compared to niclosamide.

Conflict of Interests

The Authors declares that there is no conflict of interests.

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