International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-2, Issue-9,September 2016
ISSN: 2395-3470
www.ijseas.com

Efficacy and persistence of neem in emulsion-in-water (EW) formulation against the golden apple snail

Rosdiyani Massaguni[1], Ismail Ab Raman[2] and Siti Noor Hajjar Md. Latip[1]
[1]Faculty of Plantation and Agrotechnology,
Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Advanced Oleochemicals Technology Division,
Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

Abstract

This study was conducted to evaluate the efficacy of EW-neem formulations against golden apple snail and determine their persistency under field condition. The EW-neem formulations were prepared with various types of oleochemicals consisting of palm oil methyl ester, RBD palm olein and soybean oil. The results show that the EW-neem formulations have a potent molluscicidal activity on golden apple snail. The formulation of neem with RBD palm olein (96h LC_{50}, 24.61 mg/l) induced the best molluscicidal efficacy against golden apple snail in laboratory trials, whereas, neem formulated with palm oil methyl ester (96h LC_{50}, 45.30 mg/l) showed better efficacies under field condition and have longer persistence ($t_{1/2}$=1.85; r^2 = 97.75) on paddy leaves. The foliar residual toxicity have suggesting that the EW-neem formulations would have the best efficacy against golden apple snail up to three days after spraying. Overall, the present finding suggested that EW-neem formulation is very promising and can serve as a biological pesticide for golden apple snail.

Keywords: *Golden apple snail, Emulsion-in-water, Palm oil methyl ester, Persistence, Neem.*

1. Introduction

Plants are well recognized as alternatives to replace the synthetic pesticide in which they are rich with a variety of bioactive organic chemicals such as alkaloids, limonoid, terpenoids, phenolic, saponin, tannin and flavanoid (Phuagphong *et al.*, 2015). Neem has been known as an important source for compounds with pesticidal properties against over 300 pest species includes rice insect pests (Rimpi *et al.,* 2010). Azadirachtin ($C_{35}H_{44}O_{16}$), a tetranortriterpenoid of the limonoid is the most important active compound in neem which has antifeedant, growth inhibitory, oviposition deterrent, antihormonal, antifertility and repellent properties (Sundaram *et al.,* 1995). Azadirachtin is considered safe to the environment, since it narrow-spectrum, non mutagenic, does not bioaccumulate and harmless to mammalians and birds (Caboni *et al.,* 2006). Moreover, it has relatively short persistence in the environment which persists about four to seven days on plant after application (Pinheiro and Quintela, 2010).

Some research has been shown the potential molluscicidal activity of neem against land and freshwater snail. Alam *et al.,* (2010) have reported the efficacy of the neem extract on freshwater snail, *Lymnaea auricularia* and expected that azadirachtin in extract is responsible for the molluscicidal effect. Antifeedant effect of commercial neem-based insecticide, Nimbecidine® (containing 0.03% azadirachtin) was also documented on terrestrial snail, *Monacha obstructa* (Shoaib *et al.*, 2010). Meanwhile, the studies performed under field conditions have indicates that 1% azadirachtin in NeemAzal-T/S is proven as effective repellent for the land snail, *Arianta arbustorum* (Ploomi *et al.,* 2009).

Several neem-based pesticides are now available for commercial used. However, these commercial formulations of neem are commonly formulated as an emulsifiable concentrate (EC). The EC formulation is a solvent-rich (typically 10 parts to 90 parts of solvent in the formulation) and typically use aromatic hydrocarbon such as toluene and dimethyl benzene as a solvent which are harmful to environment and leaving residues in ecosystem, food and final products (Zhao *et al.,* 2009; Chin *et al.,* 2012). Moreover, the products were reportedly varied in their formula composition, which have different level of toxicity and may not perform similarly to different pest. Nevertheless, there is still no recommended rate for these products to be used in the management of freshwater snail, to be exact for golden apple snail (GAS). Development of neem-based products in oil-in-water (o/w) emulsion appears to solve this problem, as well as enhancing bioavailability and resulting in stable formulations without utilization of toxic hydrocarbon solvents. The ecofriendly formulation approach, with emulsion containing vegetable oils, non-ionic surfactants and water should also degrade rapidly with residue levels below the regulatory criteria in environment. Therefore, the new approach of formulated neem seed crude extract with oleochemical has widened the spectrum of pesticidal values of neem and improves their bioactivity. The current studies aimed to evaluate the efficacy of emulsion-in-water (EW)-neem formulation against GAS and determine their persistency under field condition. Three different EW-neem formulations consisting of palm oil methyl ester, refined, bleached and deodorized (RBD) palm olein and soybean oil were compared with the commercial neem formulation (Neemix® 4.5 EC) and niclosamide (Bayluscide 70 WP).

2. Materials and Methods

2.1 Test organism and plant compound

The GAS was collected from the paddy fields in FELCRA Seberang Perak, Perak. Afterwards, they were left to acclimatize in laboratory condition prior to the commencement of the experiment. Meanwhile, the methanolic extract of neem seed was obtained using the extraction procedure of Jadeja *et al.* (2011), with some modifications. The liquid-liquid extraction method was adopted for the isolation of azadirachtin from crude extract.

2.2 Preparation of emulsion

The EW-neem formulation was prepared using the emulsification procedure of Cho *et al.*, (2008) with some modifications. Three types of oleochemicals; palm oil methyl ester (PME), RBD palm olein (PO) and soybean oil (SO) were used as the oil phase, Tween 80 and Span 80 as emulsifiers, and xanthan gum as stabilizer in the formulations. Required amount of xanthan gum and Tween 80 were dissolved in aqueous phase, while neem seed crude extract and Span 80 in the oily phase. The oil phase was dispersed in aqueous phase by gradually added drop by drop with constant homogenization using a homogenizer (Ultra-Turrax model T25™, IKA Labortechnik, Staufen, Germany) at a speed of 13,500 rpm. The resulting formulations were further analyzed for stability and efficacy test.

2.3 Preliminary bioassay test of EW-neem formulation on golden apple snail

The bioassay test protocol was adapted and modified slightly from the Dai *et al.*, (2011) procedure. Five concentrations of EW-neem formulations ranging from 24.8 to 53.8 mg/l were evaluated under laboratory conditions. Water, Bayluscide 70 WP and Neemix® 4.5 EC at the recommended rate were used as control. Each treatment was replicated three times. Mortality of GAS was observed for 96 hours after treatment with 24 hours interval. The GAS was considered dead if it was remained immobile, floated upside down or hanged out of the shell

2.4 Field trial of the efficacy of EW-neem formulations

The field trials were conducted using $1m^2$ of glass aquariums in rainshelter at Faculty of Plantation and Agrotechnology Research Fields, UiTM Puncak Alam, according to the protocol of Kijprayoon *et al.*, (2014). The treatments consisted of EW-neem formulations were applied at 1x, 5x and 10x concentrations of their corresponding LC_{90} values (Table 1). Meanwhile, the aquariums containing water (no treatment), Bayluscide 70 WP and Neemix® 4.5 EC were used as control. The mortality of GAS was recorded after 24 hours up to 96 hours of treatment application.

Table 1: Concentrations of EW-neem formulations applied in field trials

Treatments	**Concentration (mg/l)**		
	1x	**5x**	**10x**
Palm oil methyl esters (PME)	33.58	167.90	335.80
RBD palm olein (PO)	38.96	194.80	389.60
Soybean oil (SO)	34.00	170.00	340.00
Bayluscide 70 WP (NS)	5.65	28.25	56.50
Neemix® 4.5 (NE)	0.5	2.5	5.0
Control (C)	-	-	-

Note: The LC_{90} values were found in preliminary test under laboratory condition

2.5 Persistence of the EW-neem formulations on paddy leaf surface

The persistency analysis of EW-neem formulations and commercial pesticides were carried out under small-scale field condition based on Wiwattanapatapee *et al.*, (2009) procedure, with some modifications. The paddy seedlings were sprayed with the developed formulations at the concentration of 335.8 mg/l (PME), 389.6 mg/l (PO) and 340.0 mg/l (SO), while the commercial pesticides were applied in accordance with the manufacture recommendation. The control plot was left untreated. The residues of applied pesticides on leaf surface were collected by rinsing the paddy leaves with acetone and concentrated to dryness. The residue was redissolved in 25 ml of methanol and sonicated for 10 minutes. The solutions were then filtered using 0.45µl syringe filter prior analysis in Uv-Vis spectrophotometer at 206 nm of wavelength. The amounts of residues were quantified by using external standard calibration technique. The dissipation rate of pesticide residues on paddy leaves were determined by plotting logarithm residue concentration against time. The half-life time ($t_{1/2}$) and decay constant (λ) values were determined from the fitted curve of $C_1=C_0*e^{-\lambda t}$ (Akbar *et al.*, 2010).

2.6 Effect of foliar residues of the pesticides on golden apple snail mortality

The foliar residues of EW-neem formulations as well as the Bayluscide 70 WP and Neemix® 4.5 EC were subjected for further toxicity evaluations on GAS. The samples were taken repetitively during the period of 0, 1, 3, 5, and 7 days, then supplied as food sources to the GAS in rectangular plastic aquarium. Mortality of the GAS was observed for 96 hours after exposure. Percent mortalities of GAS versus times in days were plotted graphically to determine the time at which mortality declined to 50% (T_{50}) and 20% (T_{20}) (Mansour and Abdel-Hamed, 2015).

2.7 Statistical analysis

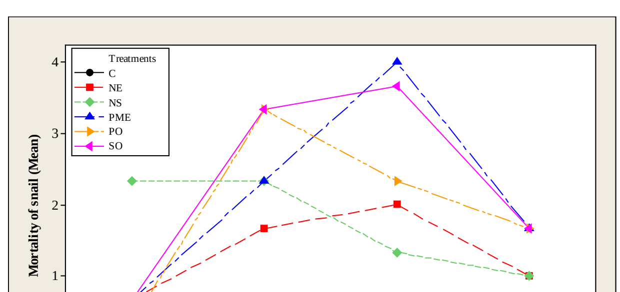
The statistical significance between treatment values was determined by analysis of variance (ANOVA) test followed by pairwise comparison using the statistical software package of Minitab® Release 14.1 (Minitab, Inc.). Meanwhile, the concentration-mortality regression was evaluated statistically by Probit analysis using the software package of POLO-Plus (LeOra Software).

3. Results and Discussions

3.1 Bioassay test of EW-neem formulation on golden apple snail

Fig.1 shows a significant interaction ($p<0.05$) between the mortality of GAS to the exposure time and treatment concentrations. In Fig. 1(a), it indicates that neem in PO formulation has induced the highest mortality of GAS within 48 hours of exposure, while neem formulated with PME and SO were required 72 hours. The commercial neem formulation (NE) showed relatively the similar trend of toxicity as neem formulated in PME and SO. This effect as mentioned by Mulla and Su (1999) is depends on the concentration of azadirachtin, type of formulation, as well as the target snail species. As illustrated in Fig. 1(b), it demonstrated that the molluscicidal efficacies were positively correlated with the treatment concentrations, as the rate of dead snail increased with increasing the concentration of formulations. On the other hand, Niclosamide (NS) having different mortality pattern which it had caused the highest mortality rate at 24 hours after exposure. Dela Cruz and Joshi (2001) and Kijprayoon *et al.*, (2014) also observed the same immediate ‘knockdown’ effect of Niclosamide 250 EC and Niclosamide 70% wettable powder (WP) on GAS. The quick knockdown of niclosamide was attributed to its double mode of lethal effect, which affecting the carbohydrate metabolism and the respiration of snails (Thammasiri *et al.*, 2009).

Comparatively, higher potency was observed in formulation of neem in PO (96h LC_{50}, 24.61 mg/l) with respect to neem formulated in PME (96h LC_{50}, 25.59 mg/l) and SO (96h LC_{50}, 25.05 mg/l) (Table 2). Singh *et al.*, (1996) reported that pure azadirachtin is toxic against *Lymnaea acuminata* (24h LC_{50}, 0.35 mg/l) and *Indoplanorbis exustus* (24h LC_{50}, 0.53 mg/l), but its maximum toxic effect only within 24 hours due to instability of azadirachtin in environment. Thus, the EW-neem formulations have improved its stability even up to 96 hours. The corresponding LC_{90} values were 33.58 mg/l (PME), 38.60 mg/l (PO), and 34.00 mg/l (SO) which was being considered feasible for field trials.



Parameters	Palm oil methyl esters (PME)	RBD palm olein (PO)	Soybean oil (SO)
LC_{50} (mg/l)	25.59	24.61	25.05
(95% CI)	(23.84-27.10)	(21.35-26.90)	(22.94-26.70)
LC_{90} (mg/l)	33.58	38.96	34.00
(95% CI)	(31.06-38.84)	(33.48-60.47)	(31.11-40.78)
Slope (β)	10.87±2.01	6.43±1.71	9.66±1.98
Chi-square (χ^2)	1.102	1.810	1.867
Heterogeneity	0.37	0.60	0.62

χ^2 values are significant at p<0.05

(a)

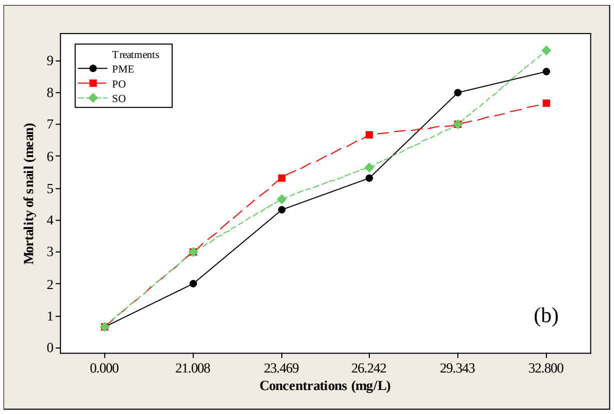


Fig.1 The interaction plot of snail mortality versus the exposure time (a) and snail mortality versus the concentrations (b) for each treatment; Control (C), Neemix® 4.5 EC (NE), Baylusicide 70 WP (NS), neem formulated in palm oil methyl esters (PME), RBD palm olein (PO) and soybean oil (SO)

The slope (β) of the concentration-mortality curve was higher in PME formulation, followed by SO and PO which suggesting that the GAS were susceptible to the neem formulated with PME (Table 2). The Chi-square (χ^2) test for goodness of fit values ranged from 1.102 to 1.867 of all treatments were significant at p<0.05 level, showing probably less heterogeneity of the test population. The heterogeneity factor values range from 0.37 to 0.62 which demonstrated that the data fit the model adequately, significant at heterogeneity <1.0 level. The findings demonstrated that the molluscicidal activity of EW-neem formulation was both time and concentration dependent. It is suggests that a formulation of neem with PO have a quick lethal effect and higher potency. The optimum lethal time is 48 hours which is better than neem seed crude extract tested in previous molluscicidal assay by Massaguni and Latip (2015). The efficacy was further tested in small-scale field to confirm their activity.

Table 2: Molluscicidal efficacies of EW-neem formulations against GAS
Note: CI: Confidence Interval; Slope±SE

3.2 Efficacy of the EW-neem formulations under small-scale field condition

In field trials, the formulation of neem with PME induced the best molluscicidal efficacy since PME has an excellent wetting behavior and good thermal stability that improving their efficacy (Baroutian *et al.*, 2009; Salim *et al.*, 2011). Comparing the concentration adopted, it indicate that GAS was significantly (p<0.05) susceptible to the highest concentrations (10 folds of LC_{90} values) of all EW-neem formulations, while there are no significant difference on GAS mortality when treated with the concentration of 1 and 5 folds of LC_{90} values (Table 3). Amongst the treatments, the highest significant mortality was observed on GAS treated with 56.50 mg/l of niclosamide (NS). Table 3 also presented the LC_{50} values, its ratios and slope of all treatments. The least LC_{50} value was obtained by neem formulated in PME (96h LC_{50}, 45.30 mg/l), then followed by PO (96h LC_{50}, 69.75 mg/l) and SO (96h LC_{50}, 92.27 mg/l). The GAS was observed more susceptible in Neemix® 4.5 EC about 10.96 times than the EW-neem formulations. The hydrocarbons (mineral oil) used as carrier in Neemix® 4.5 EC formulation have enhancing the molluscicidal activity of the product (Salimon *et al.*, 2012). Meanwhile, the toxicity of niclosamide (NS) is 5.74 times higher as compared to the EW-neem formulations. This result was in contrast with Singh *et al.*, (1996), as they have mentioned that neem (24h LC_{50}, 0.35 mg/l) against snail *Lymnaea acuminate* is about 33 times higher than niclosamide (24 hours LC_{50}, 11.8 mg/l). Separate estimate of LC_{50} was found to be within 95% confidence limits and the slope values given in Table 3 were steep.

Table 3: Statistical analysis for mortality of GAS treated at different concentrations for each treatment under small-scale field condition

Treatments	Concentration (mg/l)	Mortality	ANOVA	LC_{50}	LC_{50}	Slope

				(mg/l) (CI)	ratios	(β)
Palm oil methyl esters (PME)	33.58	4.7±2.08^{b}	F = 21.44 p = 0.000	45.30 (12.20-79.80)	-	1.29±0.36
	167.90	7.3±1.53^{bc}				
	335.80	9.0±1.00^{c}				
RBD palm olein (PO)	38.96	4.3±2.08^{b}	F = 19.09 p = 0.001	69.75 (14.49-131.12)	0.65	1.06±0.35
	194.80	6.3±1.53^{bc}				
	389.60	8.3±0.58^{c}				
Soybean oil (SO)	34.00	3.7±0.58^{b}	F = 88.00 p = 0.000	92.27 (27.57-183.04)	0.49	1.03±0.35
	170.00	5.7±0. 58^{c}				
	340.00	7.7±0. 58^{d}				
Bayluscide 70 WP (NS)	5.65	4.3±1.53^{b}	F = 51.64 p = 0.000	7.90 (3.71-12.21)	5.74	1.80±0.40
	28.25	8.0±1.00^{c}				
	56.50	9.7±0.58^{c}				
Neemix® 4.5 (NE)	0.5	2.0±1.00^{ab}	F = 31.28 p = 0.000	4.13 (2.17-29.20)	10.96	0.97±0.35
	2.5	3.7±0.58^{b}				
	5.0	5.7±0.58^{c}				
Control (C)	Water	0.3±0.58^{a}	-	-		

Note: 1x ,5x,10x: 1, 5 and 10 folds of LC_{90} values; Mortality = mean±SD; CI: Confidence Intervals (95% probability); Slope±SE
F: Analysis of variance; p: Probability value
Means followed by the same letters indicate no significant difference using Tukey's test; p<0.05.

3.3 Persistence of the EW-neem formulations on paddy leaf surface

Persistence analysis provides information about the dissipation of pesticide residues from the sprayed plants. Table 4 shows that the residues of pesticide deposited on leaves were decreased with time. The initial residue deposits after an hour of application were 2.02, 2.07, and 2.28 µg/ml for neem formulated in PME, PO, and SO, while 2.44 and 2.39 µg/ml for Neemix® 4.5 EC and Bayluscide 70 WP, respectively. After seven days, the residue levels of neem in PME, PO, and SO formulations were decreased to 0.15, 0.07, and 0.06 µg/ml, respectively. The dissipation rate (λ) for neem in PME, PO, and SO formulations was 0.375, 0.491 and 0.521 ($days^{-1}$). Neemix® 4.5 EC was found to have shorter persistence (λ=1.118/day) than EW-neem formulations, which it almost not detected after seven days of treatment application with residue recovered is 0.001 µg/ml (Table 4). The result is in conformation with Akbar *et al.*, (2010), who reported the residue of Biosal 10 EC (containing 0.32% of azadirachtin) on cabbage leaf surface was not detected after seven days of treatment application.

The differences of dissipation between Neemix® 4.5 EC and EW-neem formulations were probably attributed to the different formulation and adjuvant used. Sutherland *et al.*, (2002) and Gahukar (2014) have reported that solvents and adjuvants in the formulation have been found useful in extending residual activity and shelf-life of neem formulation.

The calculated half-life times ($t_{1/2}$) have indicate that neem in PME formulation have longer persistence ($t_{1/2}$=1.85; r^2 = 97.75), while Neemix® 4.5 EC showed a half-life of 0.62 day (Table 4). The oleochemical in neem formulation have improved its persistency, as the dissipation half-life of pure azadirachtin is 13.65 hours (Tan and Song, 2006). However, it still has relatively short persistence in the environment than quoted by Pinheiro and Quintela (2010). The half-life time ($t_{1/2}$) of niclosamide found in this study was 3.00 days which it was higher from the past studies reported by Calumpang *et al.*, (1995).

Table 4: Residues and half-life times ($t_{1/2}$) of azadirachtin and niclosamide on paddy seedling leaves

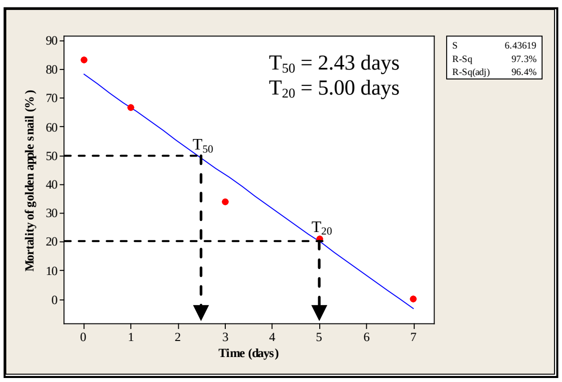
DAT	Residue recovered (µg/ml) ±SD				
	Palm oil methyl esters (PME)	RBD palm olein (PO)	Soybean oil (SO)	Bayluscide 70 WP (NS)	Neemix® 4.5 EC (NE)
0	2.02±0.06	2.07±0.02	2.28±0.08	2.39±0.19	2.44±0.09
1	1.55±0.11	1.27±0.03	1.50±0.35	1.80±0.01	1.28±0.03
3	0.92±0.10	0.64±0.05	0.54±0.23	1.06±0.03	0.10±0.01
5	0.27±0.01	0.22±0.09	0.42±0.01	0.52±0.05	0.04±0.01
7	0.15±0.01	0.07±0.01	0.06±0.00	0.47±0.09	0.001±0.00
λ^*	0.375	0.491	0.521	0.231	1.118
$t_{1/2}$ (days)	1.85	1.41	1.33	3.00	0.62

Note: DAT= Days after treatment; Residue level = mean±SD
λ^* = Decay constant ($days^{-1}$)

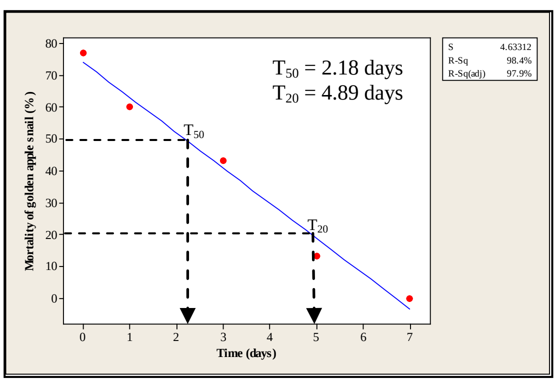
3.4 Effect of foliar residues of EW-neem formulation on golden apple snail

The residual toxicity presented in Fig. 2 shows that the GAS mortality was decreased by time. The T_{50} and T_{20}

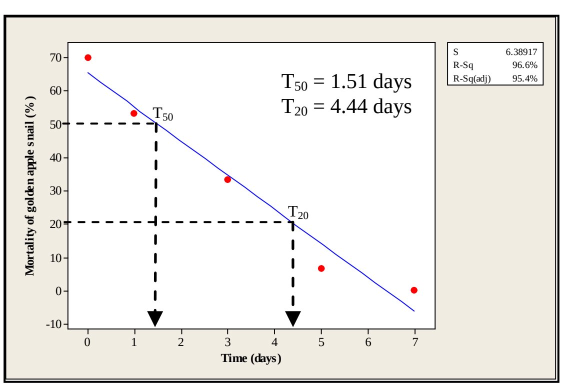
were estimated as illustrated in Fig. 2, in order to determine the time (in days) that elapse until mortality of snail declines to 50% (T_{50}) and 20% (T_{20}). These parameters were used in earlier work of Mansour and Abdel-Hamed (2015) to estimate residual toxicity of tested toxicants against the desert locust, *Schestocerca gregaria*. The T_{50} values for neem formulated with PME, PO and SO were 2.43, 2.18 and 1.51 days, while T_{20} was equaled to 5.00, 4.89 and 4.44 days, respectively (Figure 2a-c). It shows that neem in PME formulation was more persistence with longer T_{50} and T_{20} values as compared to other both formulations. The results also indicate that the persistence of EW-neem formulations last approximately three days which is consistent with Sutherland *et al.*, (2002). However, the persistence value is less than quoted by Mulla and Su (1999), who stated that the residual effect of neem pesticides lasts about four to eight days under field conditions.



(a) Mortality (%) = 78.44 - 11.68 Time (days)



(b) Mortality (%) = 74.15 - 11.07 Time (days)



(c) Mortality (%) = 65.45 - 10.24 Time (days)

Fig. 2 Dissipation pattern of the neem formulations in PME (a), PO (b) and SO (c) against golden apple snail.

Seljåsen and Meadow (2006) reported the effect of NeemAzal-T was last for one to two weeks against the cabbage pest, *Mamestra brassicae*. This is to be expected, as the azadirachtin in formulations has low persistency and high biodegradable in the environment. The presence of sensitive moieties such as p-electrons, ester linkages and epoxide ring has causes the azadirachtin has short environmental persistence (Tan and Song, 2006). These results of residual toxicity have implications for frequency of spraying and suggest that repeated applications might be necessary when the concentration drops to 50% or below (Wiwattanapatapee *et al.*, 2009). Indeed, the reasonable residual toxicity of the pesticide for a few days is needed, which it guarantees the safety of the crop.

4. Conclusions

The toxicity evaluations have demonstrated that EW-neem formulations have a significant molluscicidal activity against the GAS and neem formulated with PME found to be the best molluscicides for GAS. This study clarified that the molluscicidal effect of EW-neem formulations was almost equivalent to the commercial neem formulation and synthetic pesticide. The persistence analysis has indicated that EW-neem formulations have shorter half-life values and degraded rapidly than the conventional synthetic pesticides. The foliar residual toxicity suggesting that the EW-neem formulations would have the best efficacy against GAS up to three days after spraying. This suggests that no accumulation effect of the pesticide residue is caused by repeated treatment. Therefore, these formulations containing neem seed extracts and natural inert ingredients are very promising and this greener technology approach can serve as eco-friendly and economically viable means for GAS control in the near future.

Acknowledgments

The authors gratefully acknowledged the financial support from Faculty of Plantation and Agrotechnology, UiTM Shah Alam. Appreciation also goes to Advanced Oleochemical Technology Division, MPOB Bangi for their generosity in supplying Palm oil Methyl Esters and providing the facilities for the formulation studies.

References

[1] Akbar, M.F., Haq, M.A., Parveen, F., Yasmin, N., and Sayeed, S.A., (2010). Determination of synthetic and bio-insecticides residues during aphid (*Myzus persicae* sulzer) control on cabbage crop through high performance liquid chromatography. Pakistan Entomologist Vol.32 (2): p 155-162

[2] Alam, H.M., Kaur, A., Singh, N.K., Rather, M.A., and Rath S.S., (2011). Molluscicidal Effects of Acetone Extract of *Azadirachta Indica* (Neem) on Snails *Lymnaea auricularia* and *Indoplanorbis exustus*. Veterinary Practitioner Vol.12 (2): p 256-259

[3] Baroutian, S., Aroua, M.K., Raman, A.A., and Sulaiman, N.M., (2009). RBD palm olein-based methyl/ethyl esters. Journal of Oil Palm Research Vol.21: p 659-666

[4] Caboni, P., Sarais, G., Angioni, A., Garcia, A.J., Lai, F., Dedola, F., and Cabras, P., (2006). Residues and persistence of neem formulations on strawberry after field treatment. Journal of Agricultural and Food Chemistry 54 (26): p 10026-10032

[5] Calumpang, S.M.F., Medina, M.J.B., Tejada, A.W., and Medina, J.R., (1995). Environmental impact of two molluscicides: niclosamide and metaldehyde in a rice paddy ecosystem. Bull. Environ. Contam. Toxicol. (55): p 494-501

[6] Chin, C.P., Lan, C.W., and Wu, H.S., (2012). Application of biodiesel as carrier for insecticide emulsifiable concentrate formulation. Journal of the Taiwan Institute of Chemical Engineers 43 (4): p 578-584

[7] Cho, Y.H., Kim, S., Bae, E.K., Mok, C.K., and Park, J., (2008). Formulation of a cosurfactant-free O/W microemulsion using non-ionic surfactant mixtures. Journal of Food Science 73 (3): p E115-E121

[8] Dai, L., Wang, W., Dong, X., Hu, R., and Nan, X., (2011). Molluscicidal activity of cardiac glycosides from *Nerium indicum* against *Pomacea canaliculata* and its implications for the mechanisms of toxicity. Journal of Environmental Toxicology and Pharmacology 32; p 226–232

[9] Dela Cruz, M.S., and Joshi, R.C., (2011). Efficacy of commercial molluscicide formulations against the golden apple snail *Pomacea canaliculata* (Lamarck). The Philippine Agricultural Scientist Vol.84(1)

[10] Gahukar, R.T., (2014). Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: A review. Journal of Crop Protection 62: p 93-99

[11] Jadeja G.C., Maheshwari, R.C., and Naik, S.N., (2011). Extraction of natural insecticide azadirachtin from neem (*Azadirachta indica* A. Juss) seed kernels using pressurized hot solvent. Journal of Supercritical Fluids (56): p 253–258

[12] Kijprayoon, S., Tolieng, V., Petsom, A., and Chaicharoenpong, C., (2014). Molluscicidal activity of *Camellia oleifera* seed meal. ScienceAsia 40: p 393-399

[13] Mansour, S.A., and Abdel-Hamid, N.A., (2015). Residual toxicity of bait formulations containing plant essential oils and commercial insecticides against the desert locust, *Schestocerca gregaria* (Forskäl). Journal of Industrial Crops and Products 76: p 900-909

[14] Massaguni, R., and Md. Latip, S.N.H., (2015). Assessment the molluscicidal properties of azadirachtin against golden apple snail, *Pomacea canaliculata*. Malaysian Journal of Analytical Sciences Vol.19(4): p 781 – 789

[15] Mulla, M.S., and Su, T., (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. Journal of the American Mosquito Control Association 15 (2): p 133-152

[16] Phuagphong, P., Nawanopparatsakul, S., Kitcharoe, N., Chinpaisal, C., Nguanboonmak, J., and Ayukarn, N., (2015). Mechanisms of molluscicidal activity of *Citrus hystrix* against *Pomacea canaliculata*. A proceeding in The International Conference on Herbal and Traditional Medicine (HTM 2015): p 53-60

[17] Pinheiro, P.V., and Quintela, E.D., (2010). Neem oil antifeedant and insecticidal effects on *Oebalus*

poecilus (hemiptera: pentatomidae) males and females. Pesq. Agropec. Trop., Goiânia, Vol.40 (4): p 394-400

[18] Ploomi, A., Jõgar, K., Metspalu, L., Hiiesaar, K., Loorits, L., Sibul, I., Kivimägi, I., and Luik, A., (2009). The toxicity of neem to the snail *Arianta arbustorum*. Sodininkystė Ir Daržininkystė 28 (3): p153-158

[19] Rimpi, D, Chutia, B. C., Sarmah, M., and Rahman, A.,(2010). Effect of neem kernel aqueous extract (NKAE) on growth and development of red slug caterpillar, *Eterusia magnifica* butl in tea in North-East India, India. Journal of Biopesticides, 3(2): p 489 – 494

[20] Salim, N., Basri, M., Abd. Rahman, M.B., Abdullah, D.K., Basri, H., and Salleh, A.B., (2011). Phase behavior, formation and characterization of palm-based esters nanoemulsion formulation containing Ibuprofen. Journal of Nanomedic and Nanotechnology Vol.2:113

[21] Seljasen, R., and Meadow, R., (2006). Effects of neem on oviposition and egg and larval development of *Mamestra brassicae* L: Dose response, residual activity, repellent effect and systemic activity in cabbage plants. Journal of Crop Protection 25: p 338–345

[22] Shoaib, M.A., Mahmoud, M.F., • Loutfy, N., Tawfic, M.A., Barta, M., (2010). Effect of botanical insecticide Nimbecidine® on food consumption and egg hatchability of the terrestrial snail *Monacha obstructa*. J Pest Sci 83: p 27–32

[23] Singh, K., Singh, A., and Singh, D.K., (1996). Molluscicidal activity of neem (*Azadirachta indica* A.Juss). Journal of Ethnopharmacology 52: p 35-40

[24] Sundaram, K.M.S., Campbell, R., Sloane, L., and Studens, J., (1995). Uptake, translocation, persistence and fate of azadirachtin in aspen plants (*Populus tremuloides* Michx.) and its effect on pestiferous two-spotted spider mite (*Tetranychus urticae* Koch). Journal of Crop Protection Vol.14 (5): p 415-421

[25] Sutherland, J.P., Baharally, V., and Permaul, D., (2002). Use of the botanical insecticide, neem to control the small rice stinkbug *Oebalus poecilus* (Dallas, 1851) (Hemiptera: Pentatomidae) in Guyana. Entomotropica 17(1): p 97-101

[26] Tan, W., and Song, Z., (2006). Research on effect of four natural ultraviolet light absorbers on photostabilization of Azadirachtin A. Agricultural Science in China 5(11): p 855-858

[27] Thammasiri, C., Tesana, S., Suwannatrai, A., Piratae, S., Khampoosa, P., Kulsantiwong, J., Prasopdee, S., Ruangjirachuporn, W., Wongpanich, V., and Tarbsripair, P., (2009). Molluscicidal effects of Bayluscide (niclosamide) on *Bithynia siamensis* goniomphalos, first intermediate host of liver fluke, *Opisthorchis viverrini*. KKU Res J (GS) 9 (2): p 107-117

[28] Wiwattanapatapee, R., Sae-Yun, A., Petcharat, J., Ovatlarnporn, C., and Itharat, A., (2009). Development and evaluation of granule and emulsifiable concentrate formulations containing *Derris elliptica* extract for crop pest control. J. Agric. Food Chem. 57: p 11234–11241

[29] Zhao., F., Xia, H., and He, J., (2009). Formulation design of cyhalothrin pesticide microemulsion. Current Science Vol. 97(10): p 1458-1462

Rosdiyani Massaguni received the BSc. (Hons) in Plantation Technology and Management, and the Master degree in Plantation Industry Management from Universiti Teknologi MARA (UiTM) Shah Alam in 2009 and 2010, respectively. She is currently a Ph.D degree student under the supervision of Dr. Siti Noor Hajjar Md. Latip and Dr. Ismail Ab. Raman. Her research is centered on development of the biopesticide formulation.

Ismail Ab. Raman is a Principal Research Officer at Advanced Oleochemical Technology Division (AOTD), Malaysia Palm Oil Board (MPOB). He obtained his Ph.D degree in Surfactant and Colloid Chemistry from Universiti Malaysia Terengganu in 2006. He has coauthored over 30 publications and holds three patents. His current research interests on palm oil-based oleochemicals, surfactants, liquid crystals, emulsions, nanoemulsion and microemulsion applied in agricultural, industrial and household applications.

Siti Noor Hajjar Md. Latip earned her Ph.D degree in Agriculture Entomology from Universiti Putra Malaysia in 2006. She is a Senior Lecturer at Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Shah Alam. Her research interests cover the toxicity of plant based pesticide on golden apple snail, crop protection, entomology and insect genetic, with six awards won in Malaysian Exhibition.