

Antifeedant Activities of Neem Seed Extracts for Controlling Golden Apple Snail, *Pomacea canaliculata*

¹Siti Noor Hajjar Md. Latip, ^{1,2}Mohd Fahmi Keni and ¹Rosawanis Rosli
¹Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA,
40450 Shah Alam, Selangor, Malaysia

²Biology Research Division, Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi,
Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

Abstract: The golden apple snail, *Pomacea canaliculata* is an invasive alien species that seriously affects rice cultivation in many Asian countries. Due to the toxic hazards of the synthetic insecticides, biological control through botanical pesticides is the best alternative for reducing the golden apple snail damage in paddy fields. Neem or also known as *Azadirachta indica* was proven to have the molluscicidal potential for controlling golden apple snails. The purposes of this study were to quantify the Azadirachtin content and antifeedants activity of different neem extract for controlling golden apple snail. Azadirachtin content was quantified using UV-visible spectrophotometer. Antifeedant test was conducted with five different concentration of neem seed extract. Methanol extraction with fresh neem seed showed high antifeedant activities compared with other treatments with 81% Feeding Deterrent Index (FDI). Azadirachtin contents in the neem seed extract showed range from 53.36-54.08 ppm for methanol extraction and 52.95-53.97 ppm for water extraction. Based on the finding, the neem seed has the potential to be formulated as biopesticide for management of golden apple snail in the field.

Key words: Biopesticide, azadirachtin content, golden apple snail, neem seed extract, antifeedant activity

INTRODUCTION

Rice is the most important crop in Malaysia (Vengedasalam *et al.*, 2011). It has been put as a top priority by the government based on the strategic importance of rice as a staple food commodity. The government is implementing a food security policy for the rice industry towards self-sufficiency by 2020 by encouraging paddy farmers to increase their yield (Shamsudin *et al.*, 2015). The production of rice has increased to fulfill the demand due to the continuous growth of population number in Malaysia. However, the demand of paddy is impossible to fulfill because of several problems in paddy. The Golden Apple Snail, *Pomacea canaliculata* (GAS) is an invasive alien pest that seriously affects rice cultivation in many Asian countries. Golden apple snails devour young rice seedlings, causing extensive damage to both transplanted and direct seed (Latip and Massaguni, 2012). GAS had a very fast growth rate led to its rapid multiplication and widespread distribution throughout the country (Teo, 1999). It causes 100% destruction of the rice seedling in the germination stage and at least 20% in transplanted

seedling stage (Ranee, 2005). GAS is a global cause of concern in rice field which resulted in huge economic loss of \$1.47 billion per annum in rice production (Nghiem *et al.*, 2013). The biological control such as duck pasturing in paddy field is one of the controls for golden apple snail but at the same time were damaging to young rice seedlings (Nghiem *et al.*, 2013). Unfortunately, behind all those control measures, golden apple snail population still exceeds the economic injury level. Farmers begin to concentrate on using the chemical pesticide when other control methods were not effective. However, some of the compounds in chemical pesticides have shown to be extremely toxic to non-target organisms and cause health risks to workers in the field (Wu *et al.*, 2005). Therefore, for controlling the golden apple snail, many farmers rely mostly on chemical and synthetic pesticides for the immediate control of snails. The constant use of chemicals reduced the biological control efficacy, resulting in a resurgence, insecticide resistance and environmental hazard.

The increasing amount of research on insect-plant chemical intersections has unveiled the potential of utilizing botanical insecticides in the form of secondary

plant metabolites or allelochemicals (Nathan *et al.*, 2009). Normally, farmers will use pesticide for immediate control of the golden apple snail in paddy field and it increase the cost production of rice. The cost-effective, nontoxic, biodegradable, eco-friendly and botanical soft pesticide's are the need of present day agriculture as an alternative to the hazardous and recalcitrant synthetic pesticides (Nathan *et al.*, 2009). Neems top the list 2.400 plant species that are reported to have pesticide properties and is regarded as the most reliable source of eco-friendly biopesticidal property. Neem based pesticides are systemic in nature which have no ill effects on humans and animals and have no residual effect on agricultural produce. Besides, it also easy to prepare, cheap also highly effective and thus constitute an important source of pesticide for the economically poor farmers (Nathan *et al.*, 2009; Girish and Bhat, 2008).

Neem, *Azadirachta indica* is one of the potential plants reported as botanical pesticides. Neem leaves and seed product can be recommended for many Integrated Pest Management (IPM) programs (Schmutterer, 1990). The most important compound was identified in the neem tree is the azadirachtin which has been recognized for its insecticidal properties. It has a low risk of pest resistance due to a different mode of action and specific effect on pests. This plant affects insect pest by various mode of action such as the impact of growth disorders and antifeedant activities (Seljasena and Meadow, 2006). The toxic effect of pure azadirachtin against the golden apple snails is greater than the synthetic molluscides (Singh *et al.*, 1996).

Neem based pesticides are easy to prepare, cheap and highly effective and thus constitute an important source of pesticide for economically poor third world country farmers (Nathan *et al.*, 2009). Neem contains several biologically active chemicals called limonoids such as azadirachtin, nimbin, selenium, azadiractol, nimbidin and gedunin. These compounds are responsible for diverse activities such as insect antifeedant, insect growth disrupting, insecticidal, nematocidal, fungicidal, bactericidal, etc. Azadirachtin is the most potent and the most abundant (0.2-0.6% w/w) chemical found in seed kernels of neem. Azadirachtin is the most important active compound in neem compared with Nimbin, Nimbinin, oleic stearic and Palmitic acids. Azadirachtin was considered as main agent for controlling insect and pest from neem (Dubhashi *et al.*, 2013). Azadirachtin seem to be selective, non-mutagenic and readily degradable with low toxicity to non-target and beneficial organisms and cause minimal disruption to the ecosystem (Sundaram, 1996). All parts of the neem plant such bark, flower, leaves, fruits, leaves and seed can be used as medical treatment of agricultural

product such as biopesticides (Liauw *et al.*, 2008). Thus, the objective 9 of this study are to quantify the azadirachtin content and antifeedant activity of fresh and dried neem extract using different solvents for controlling golden apple snail.

MATERIALS AND METHODS

Golden apple snail: Golden apple snails were collected at FELCRA Seberang Perak. These golden apple snails were collected by using fishing nets and had been placed in a large box. Then, the golden apple snails were left for 24 h. Only active snails were selected to undergo the experiment. Ten golden apple snails were chosen randomly for antifeedant activity test.

Neem seed: Neem seeds were collected from FELCRA Seberang Perak near the paddy fields. Authentication of the plant materials was carried out at the FRIM Kepong, Selangor by a taxonomist and voucher specimens were deposited at the Postgraduate Laboratory of Plant Protection at Faculty of Plantation and Agrotechnology, UiTM Puncak Alam (Specimen code: SBID 026/12). In this experiment, neem seed extraction was carried out using fresh and dried neem seeds. The extraction methods for fresh neem seed were done 24 h after collected from the field. Meanwhile, the extract of dried neem seeds was done after the process neem seed drying was done under the shade.

Paddy: The MR219 variety of paddy was sown in the laboratory. About 2 weeks old seedlings were used as the food for the snails. Germination of rice was done according to the procedures recommended by the Department of Agriculture, Malaysia.

Quantification of Azadirachtin content: Quantity of azadirachtin in the neem seed extracts were analyzed by following the procedures by Dubashi *et al.* (2013) with some modifications by using UV-visible spectro photometer. The calibration curve from standard azadirachtin (95%) purity was prepared by following the protocol by Dubashi *et al.* (2013) with some modification. The wavelength 220 nm was selected for azadirachtin, where it shows maximum absorbance (emax). Five different concentration of standard azadirachtin which are 10-50 $\mu\text{g m}^{-1}$ was prepared. The calibration curve was prepared by plotted the absorbance value versus respective concentration. Then, 1.0 mL of each sample extracts were dissolved in HPLC grade acetonitrile prior to analysis. The value of Azadirachtin content in each neem crude extract was calculated based on external standard calibration curve.

Methanol extraction: Five different concentrations of 100,000-500,000 ppm for fresh and dried neem seed with additional methods from the protocol (Prakeh *et al.*, 2005) with some modification was used in this experiment. The solution was filtered using filter paper and concentrated using rotary evaporator. The same process was repeated for fresh neem seed. Then, the neem extract was diluted using distilled water according to different concentration. Tween 20 was added to 100 mL solution.

Water extraction: Protocol by Polaquini *et al.* (2016) with some modification was used for water extraction of neem seed in this experiment. The dried neem seed was grinded by using mortar. The dried neem was weighed and prepared for different concentration. Five different concentrations for each dried neem seed extracts (100,000-500,000 ppm) were added with 1 mL detergent and distilled water to get 100 mL of final volume. Then, the different concentration prepared was finally blended and filtered. The same process was repeated for fresh neem seed.

Antifeedant test: Antifeedant test was performed by following the procedure by Prakash (2015) with some modification. Every plastic aquarium was filled with 10 golden apple snails with average weight of 3.0 g for each snail. Then, 30 g of newly sprouted rice seedlings were dip into treatments and were put in the aquarium. The weights of rice seedling were taken before and after 4 day of treatment application. The efficacy of neem seed extract was determined on the basis of percent weight loss of treated and untreated (control) after 4 day. The weight loss (%) and Feeding Deterrent Index (FDI, %) of the sample was calculated on the paddy.

RESULTS AND DISCUSSION

Quantification of Azadirachtin content: A UV spectrophotometric was chosen for quantify the azadirachtin content in the neem seed extract in this experiment. The maximum absorbance (emax) 220 nm of the standard Azadirachtin was determined by using spectrophotometer by scanning the absorbance spectrum of standard Azadirachtin. Therefore, the absorbance at wave length 220 nm was selected for the measurement of azadirachtin content in the extraction of neem seed. The calibration curves for standard azadirachtin at 220 nm were obtained in the concentration range 10-50.0 $\mu\text{g mL}^{-1}$ as in Fig. 1. The linear regression analysis of the standard azadirachtin calibration curves yielded the following equations; $y = 0.0273x - 0.0136$. Correlation coefficient (R^2) was found to be 0.9994 and its >0.999 .

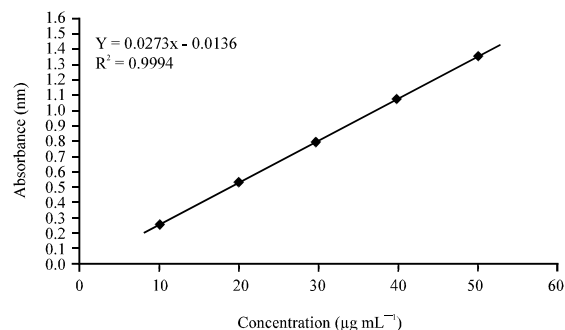


Fig. 1: Calibration curves for standard azadirachtin

Table 1: Quantity of azadirachtin in neem seed extract

	Methanol extraction		Water extraction		p-value	F-test
	Fresh	Dried	Fresh	Dried		
Azadirachtin concentration (ppm)	53.36±0.015	54.08±0.007	53.97±0.011	52.95±0.004	2710.63	0.000

F-test, Analysis of Variance (ANOVA); values significant at $p < 0.05$; \pm SE

The result recorded for concentration of azadirachtin were 53.36 ppm (0.005336%) for methanol extraction with fresh neem seed, 54.08 ppm (0.005408%) for methanol extraction with dried neem seed, 53.97 ppm (0.005397%) for water extraction with fresh neem seed and 52.95 ppm (0.005397%) for water extraction with dried neem seed in 100 g defatted neem seed (Table 1). Other finding was reported the azadirachtin compound in methanol extraction was 0.0512% and in water extraction was 0.0508% (Massaguni and Latip, 2015). Meanwhile, other report also stated that the aqueous extract of neem contained 0.001-0.02% of azadirachtin compound (Dubhashi *et al.*, 2013). Analysis of Variance (ANOVA) result showed ($F = 2710.63$; $p = 0.000$) that there were significant differences ($p > 0.05$) between quantity of azadirachtin in four different extraction of neem seed (Table 1).

Antifeedant activities: Based on Fig. 2, the weight loss of paddy is decreasing in the increasing of the concentration for neem seed extraction. The lines chart showed the control was recorded at 100% loss weight of paddy after 4 day treatment application. The weight loss in paddy seedling was higher in low concentration of neem seed extract. 100,000 ppm of methanol extraction fresh neem seed caused high weight loss of paddy (81%). Meanwhile, at 500,000 ppm, methanol extraction with fresh neem seed recorded 39.11% weight loss of paddy after 4 day of treatment. 500,000 ppm of methanol extraction with dried neem seed was recorded as the lowest weight loss of paddy with 18.56%. Water extraction with fresh neem seed showed lower (21.11%) in weight loss in paddy compared

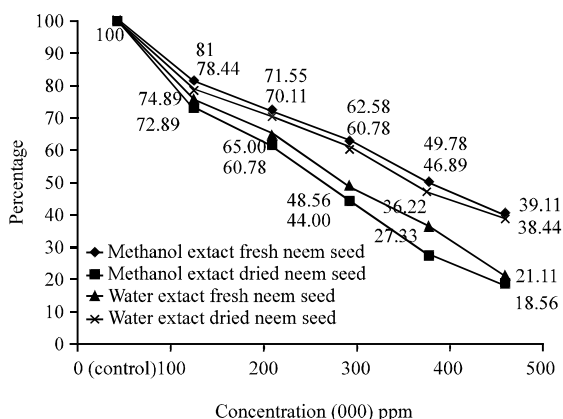


Fig. 2: Weight loss of paddy treated with different extraction

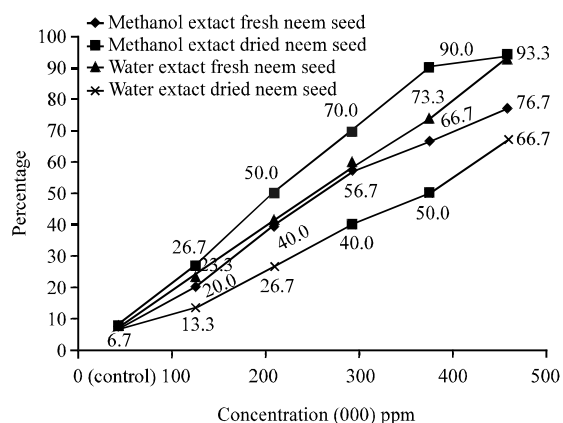


Fig. 3: Mortality of golden apple snail treated with different extraction

Table 2: Antifeedant Activity of Neem Seed Extraction on Golden Apple Snail

Test sample	Weight loss (Paddy %)	FDI (%)	F-test	p-value
Control	100.0	0 ^(a)	1295.71	0.000
Methanol extract Fresh neem seed	39.1-81.0	19.0-60.9 ^(b)		
Methanol extract Dried neem seed	18.6-72.9	27.1-81.4 ^(c)		
Water extract Fresh neem seed	21.1-74.9	25.1-78.9 ^(d)		
Water extract Dried neem seed	38.4-78.4	21.6-61.6 ^(b)		

^{a-d}FDI = Feeding Deterrent Index; values are the mean (n = 3); F-test, Analysis of Variance (ANOVA); values significant at p<0.05

to water extraction with dried neem seed (38.44%). Based on Fig. 3, the mortality of the golden apple snail was increasing with the increasing concentration of neem seed extraction. The lines chart showed the control was recorded 6.7% mortality for golden apple snail after 4 day treatment application. About 93.3% mortality of the

golden apple snail was recorded at 500,000 ppm of methanol extraction with dried neem seed and water extraction with fresh neem seed was recorded with the same mortality after 4 day of treatment application.

Result Analysis of Variance (ANOVA) in Table 2 showed $p < 0.001$ for Feeding Deterrent Index (FDI) on different treatments. It showed that there were significant different ($p > 0.05$) between four different extraction of neem seed and control on antifeedant activities. Methanol extraction with dried neem seed were no significant different with water extraction with fresh neem seed. This finding showed similar report from (Massaguni and Latip, 2015) that LC_{50} doses was significantly affecting the antifeedant activity compared to control. The higher Feeding Deterrent Index (FDI) range was recorded from treatment methanol extraction with dried neem seed which was in range of 27.1-81.4% compared to control treatment. Then, followed with water extraction fresh neem seed which showed the Feeding Deterrent Index (FDI) in range 25.1-78.9% compared to control treatment.

Finding by Parekh *et al.* (2005) reported Gaultheria procumbens Essential Oil (EO) and methyl salicylate (MS) cause 100% Feeding Deterrent Index (FDI) at LC_{50} doses on Sitophilus oryzae and Rhyzopertha domonica. Finding from antifeedant test showed there was high Feeding Deterrent Index (FDI) of extraction neem seed on golden apple snail. This showed that extraction neem seed caused antifeedant activities to golden apple snail. Report from Isman *et al.* (1990) also stated that the azadirachtin in neem is largely responsible for antifeedant activities.

CONCLUSION

The result recorded, concentration of azadirachtin were 53.36 ppm (0.005336%) for methanol extraction fresh neem seed, 54.08 ppm (0.005408%) for methanol extraction dried neem seed, 53.97 ppm (0.005397%) for water extraction fresh neem seed and 52.95 ppm (0.005397%) for water extraction with dried neem seed. Antifeedant test showed the methanol extraction with dried neem seed caused high of Feeding Deterrent Index (FDI) (81.4%), followed by water extraction with fresh neem seed (78.9%), water extraction with dried neem seed at 61.6% and methanol extraction with fresh neem seed at 60.9%. The higher the Feeding Deterrent Index (FDI) showed higher antifeedant activities in neem seed extract on the golden apple snail. The result showed higher antifeedant activities on the methanol extraction with dried neem seed, therefore there was high mortality on the golden apple snails. However, the field test should be carried out to rectify the result from laboratory study. Researchers can

use these results to formulate the botanical pesticide using neem tree plant based and produced a molluscicidal pesticide product for commercialization. Otherwise, the neem seed is recommended for farmers using direct application in the fields for controlling the golden apple snail.

ACKNOWLEDGEMENTS

Ministry of Higher Education (MOHE), Malaysia through Fundamental Research Grant Scheme (FRGS) 600-RMI/ST/FRGS5/3/Fst (270/2010) headed by Dr. Siti Noor Hajjar Md Latip. We also gratefully acknowledge to all FELCRA (Seberang Perak) staffs and postgraduate students under Dr. Siti Noor Hajjar Md Latip supervision which are really helpful in this study.

REFERENCES

- Dubhashi, S., V. Pranay, M. Singaiah and J. Satwik, 2013. Studies on extraction and HPLC analysis of azadirachtin from kernels of neem seeds. J. Adv. Pharm. Educ. Res., 3: 27-30.
- Girish, K. and S.S. Bhat, 2008. Neem-a green treasure. J. Biol., 4: 102-111.
- Isman, M.B., O. Koul, A. Luczynski and J. Kaminski, 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Agric. Food Chem., 38: 1406-1411.
- Latip, S.N.H.M. and R. Massaguni, 2012. Neem crude extract as potential biopesticide for controlling golden apple snail, *Pomacea canaliculata*. Pesticides Adv. Chem. Bot. Pesticides, 10: 233-254.
- Liauw, M.Y., F.A. Natan, P. Widiyanti, D. Iksari, N. Indraswati and F.E. Soetaredjo, 2008. Extraction of Neem oil (*Azadirachta Indica* A. Juss) using n-hexane and ethanol: Studies of oil quality, kinetic and thermodynamic. ARPN J. Eng. Applied Sci., 3: 49-54.
- Massaguni, R. and S.N.H.M. Latip, 2015. Assessment the molluscicidal properties of azadirachtin against golden apple snail, *Pomacea canaliculata*. Malaysian J. Anal. Sci., 19: 781-789.
- Nathan, S.S., M.Y. Choi, C.H. Paik, H.Y. Seo and K. Kalaivani, 2009. Toxicity and physiological effects of neem pesticides applied to rice on the *Nilaparvata lugens* Stal, the brown planthopper. Ecotoxicol. Environ. Saf., 72: 1707-1713.
- Nghiem, L.T., T. Soliman, D.C. Yeo, H.T. Tan and T.A. Evans *et al.*, 2013. Economic and environmental impacts of harmful non-indigenous species in Southeast Asia. PLoS. One, 8: 1-9.
- Parekh, J., D. Jadeja and S. Chanda, 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk. J. Biol., 29: 203-210.
- Polaquini, S.R., T.I. Svidzinski, C. Kemmelmeier and A. Gasparetto, 2006. Effect of aqueous extract from Neem (*Azadirachta indica* A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by *Candida albicans*. Arch. Oral Biol., 51: 482-490.
- Prakash, B., 2015. Assessment of toxicity, antifeedant activity and biochemical responses in stored-grain insects exposed to lethal and sublethal doses of *Gaultheria procumbens* L essential oil. J. Agric. Food Chem., 63: 10518-10524.
- Ranee, E.J., 2005. Off-Season mortality of golden apple snail, *Pomacea canaliculata* (Lamarck) and its management implications. Master Thesis, Central Luzon State University, Munoz Nueva Ecija, Philippines.
- Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol., 35: 271-297.
- Seljasen, R. and R. Meadow, 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. Crop Protec., 25: 338-345.
- Shamsudin, M.N., N.N. Ramli, A. Radam and Z.A. Mohamed, 2015. The impact of fertiliser subsidy and new variety of paddy on Malaysian paddy-rice industry. Pertanika J. Soc. Sci. Humanities, 23: 1-10.
- Singh, K., A. Singh and D.K. Singh, 1996. Molluscicidal activity of neem (*Azadirachta indica* A. Juss). J. Ethnopharmacol., 52: 35-40.
- Sundaram, K.M., 1996. Azadirachtin biopesticide: A review of studies conducted on its analytical chemistry, environmental behaviour and biological effects. J. Environ. Sci. Health Part B., 31: 913-948.
- Teo, S.S., 1999. Control of the golden apple snail in irrigated rice by integrated approach in Sabah. Proceedings of the 5th International Conference on Plant Protection in the Tropics, March 15-18, 1999, MARDI, Kuala Lumpur, Malaysia, pp: 1-18.
- Vengedasalam, D., M. Harris and G. MacAulay, 2011. Malaysian rice trade and government interventions. Proceedings of the 55th Annual Conference on Australian Agricultural and Resource Economics Society Melbourne, February 8-11, 2011, The University of Sydney, Australia, pp: 1-19.
- Wu, D.C., J.Z. Yu, B.H. Chen, C.Y. Lin and W.H. Ko, 2005. Inhibition of egg hatching with apple wax solvent as a novel method for controlling golden apple snail (*Pomacea canaliculata*). Crop Protec., 24: 483-486.