The Journal of Applied Science **Vol. 18 No. 1: 31-38 [2019]**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2019.04.001
ISSN 1513-7805 Printed in Thailand ISSN 2586-9663 (Online)

Research Article

Larvicidal effects of *Paederia pilifera* Hook.f. leaf and *Cuscuta reflexa* Roxb. stem extracts against the dengue vector mosquito *Aedes aegypti* Linn.

Keerati Tanruean[1], Tiwtawat Napiroon[1], Sukanya Phusing[2], John Raymund D. Torres[3], Prescilita M. Villanueva[3] and Pisit Poolprasert[2]*

[1]Biotechnology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand
[2]Biology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand
[3]Biological and Physical Sciences Department, College of Arts and Sciences, Don Mariano Marcos Memorial State University, Agoo, La Union 2504, Philippines
***E-mail**: poolprasert_p@psru.ac.th
Received: 29/10/2018; Revised: 18/04/2019; Accepted: 22/04/2019

Abstract

Aedes aegypti is the carrier of dengue hemorrhagic fever that remains an important public health issue in various tropical countries, especially Thailand. The use of synthetic pesticides increases the mosquito resistance and leads to change the mosquito behavior. Recently, plant extracts and their products have become increasing popular for use as alternative natural agents in mosquito control. Hence, this research aimed to investigate the efficacy of the parasitic plant extracts from *Paederia pilifera* and *Cuscuta reflexa* for killing the early 3rd stage-larvae of *Ae. aegypti*. Both dry plants were separately extracted by maceration with 95% ethanol for 72 h, then filtered and evaporated using vacuum evaporator until dryness. The mosquito larvicidal activity was evaluated after 24 h exposure at different concentrations [0 (control), 25, 50, 100, 200, 400 and 800 ppm]. The results revealed that the *C. reflexa* extract gave the higher effectiveness to eliminate mosquito larvae than the *P. pilifera* extract with LC_{50} and LC_{90} of 80.55 and 297.94 ppm, respectively. Therefore, current investigation suggests that these plants extracts can be applied as alternative sources of mosquito larvicides. Further studies are needed for identification of the active compounds that can be used in broad spectrum for controlling mosquitoes and also for the determination of the mode of action of these compounds.

Keywords: *Aedes aegypti*, *Paederia pilifera*, *Cuscuta reflexa*, maceration, insecticidal activity

Introduction

Aedes aegypti (Diptera: Culicidae) is more likely to spread viruses like dengue, chikungunya, Zika, etc., which are serious problems in several tropical and subtropical countries. Sub-consequently, morbidity and mortality have been widely documented each year worldwide (Guzman & Kouri, 2002; El-Maghraby et al., 2012; Mohankumar et al., 2016). *Aedes* mosquitoes usually breed in household man-made water-storage container and preferentially feed indoors, especially in the morning times and in the late afternoon (Christophers, 1960;

Ponlawat & Harrington, 2005). Recently, a vaccine against dengue fever has been registered in the Thailand. However, it cannot be used for everyone because there are still some limitations in the prevention of the disease. Mosquito control is crucial in order to suppress proliferation of vector mosquitoes and to enhance quality of environment and public health. Herein, the prevention and control of mosquito-borne diseases relied on their eradication through two main measures larviciding and using synthetic chemical insecticides (Polsomboon et al., 2008; Thongpoon & Poolprasert, 2015). Nonetheless, controlling mosquitoes has led to other serious problems due to the unselective applications of chemical pesticides. Those insecticides are not only harmful to human being and other organisms in the environment but also are hardly degradable and spreading toxic effects. Besides, overuse of these insecticides for controlling *Aedes* mosquitoes, like other insects, may enter into the food web and may develop resistance, a capacity to survive contact with an insecticide (Ghosh, 1991; Govindarajan, 2010; Ghosh et al., 2012).

During that period, the utilization of natural products such as phytochemicals derived from plant sources for mosquito control has become popular. Some plant-derived insecticides including Chrysanthemum, Pyrethrum, Derris, Quassia, Nicotine, Hellebore, Anabasine, Azadirachtin, d-limonene, camphor and Turpentine have been extensively used in several countries. In the recent years, less than 2,000 recorded plant species especially in the families of Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae, Rutaceae and Solanaceae, that produce numerous chemical factors and metabolites of value have been previously documented in pest insect control program (Shaalan et al., 2005). These botanical insecticides can act as insect growth regulators, oviposition attractants, repellents as well as larvicides. They can also play a vital role in the interruption of the transmission of mosquito-borne illnesses at the individual as well as at the level of community. Moreover, a multitude of their active ingredients with distinguish modes of action which lessen the chance of resistance in mosquito population is included (Sharma et al., 2006; Ghosh et al., 2012; Govindarajan et al., 2012).

Cuscuta reflexa, a parasitic giant dodder belonging to the family Convolvulacea, is one of the commonly used in functional food and medicinal tonics. This parasitic plant is mainly confined to tropical Asia and had a wide host spectrum (Kaiser et al., 2015). Moreover, it is also used to prevent abortion as well as aging in clinical treatment and often in alcoholic beverages as nutrient (Anjum et al., 2013). This plant can grow on host by rapping itself around and absorb nutrient though the vascular system (Vaughn, 2003), which the organic substances from host plants are transported to Cuscuta plants via phloem connections (Ashwani et al., 2012). *Cuscuta reflexa* is one of the major distributed Cuscuta plant in Thailand. Many prior research studies have indicated that the stem of *C. reflexa* possessed antibacterial, antiviral, anticancer and antioxidant activities (Pal, 2006; Al-Fatimi et al., 2007; Perveen et al., 2013; Tanruean et al., 2017a, b).

Paederia pilifera is commonly known as sewer vine (family Rubiaceae) because of the strong fetid odor when it is crushed. This vine climbing plant is widely distributed throughout India and Southeast Asia (Saenphet et al., 2014; Nie et al., 2013). In this regard, the treatment of gastrointestinal disorders including diarrhea, gastritis, food poisoning, dyspepsia, jaundice and hyperbilirubinemia have been extensively applied as herbal pharmacopoeias (Sukkho, 2008). Additionally, some previous studies concerning antioxidant activities have also been documented. Recently, Saenphet et al. (2004) examined its gastroprotective effects and antioxidant activities based on scientific testes. It could be said that these plants are futile for villagers. Also, they grow so quickly and spread so they are very commonly seen in the areas.

As mentioned above, they were rich in secondary metabolites and appeared to have effective as many biological properties from prior studies; however, no such researches in terms of mosquito larvicial properties of these plant extracts has been documented. Thus, we attempted to evaluate the ethanolic extracts from *P. pilifera* and *C. reflexa* as insecticides against *Ae. aegypti* larvae in this current study. These precursory findings obtained could be applied as alternative sources and developed as ecofriendly larvicides in mosquito control program.

Materials and methods

Mosquito rearing

A laboratory reared colony of *Aedes aegypti* larvae was used for the larvicidal activity. The early 3rd instar larvae of F1 laboratory-reared *Ae. aegypti* were established in the insectarium of the Office of Disease Prevention and Control 2, Phitsanulok, Thailand and maintained under controlled insectary condition at 28 ± 2°C, 72 - 80% relative humidity, with a constant photoperiod of 12 h light: 12 h dark. *Aedes* mosquito was used as a test species because of its easy collection as well as expedience in rearing and maintaining the life cycle. Its sensitivity to larvicides makes *Ae. aegypti* larvae a good indicator of biocidal activity.

Plant materials

Fresh *Paederia pilifera* leaves and *Cuscuta reflexa* stems were collected from the riversides of Hua Suea sub-district, Maetha district, Lampang province, Thailand between June to December 2016. Plant specimens including *P. pilifera* (PSRU-Rubi-001) and *C. reflexa* (PSRU-Conv-001) were morphological identified by comparison with the main herbarium database of the Botanical Garden Organization, related literatures (Puff et al., 2005; Staples, 2010) and confirmed by specialist.

Plant extraction

Each plant material was cleaned in water and dried at 50ºC in hot air oven for 72 h; powder was made using an electric mixer. About 100 g of the powder were soaked in 500 ml of 95% ethanol as solvent at room temperature for 72 h. The fluids were then filtered using Whatman No. 1 filter paper. The crude plant extracts were evaporated to dryness in a rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of ethanol (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 25, 50, 100, 200, 400, and 800 ppm, respectively.

Larvicidal bioassy

The larval bioefficacy of the crude extracts was assessed by the procedure of World Health Organization (WHO) with some modifications (WHO, 1996; Thongpoon & Poolprasert, 2015). Twenty five healthy, early 3rd instar larvae of *Aedes aegypti* were placed into each 250 ml capacity plastic bowls containing 100 ml of each plant extract of desired concentration adjusted in water along with controls with ethanol at the same concentration as used for dissolution and preparation of extracts and water. Trials were conducted with a series of five different concentrations ranging from 25 to 800 ppm (25, 50, 100, 200, 400, and 800), each with four replicates of 25 *Aedes* mosquito larvae, with a final total number of 100 larvae. The larvae exposed to water with 2% ethanol served as control. All the experiments were carried out at room temperature of 27±2°C and relative humidity of 75-85%. Larval food was given for the test larvae. The larval mortality at different concentrations and in the control was counted after 24 h exposure.

Statistical analysis

The mortality data were subjected to log probit regression analysis (Finney, 1971) for calculation the median lethal concentrations (LC_{50}) and 90% lethal concentration (LC_{90}) and 95% confidence (fiducial) limits. The percentage of larval mortality was computed and when control mortality ranged from 5-20% it was corrected using formula of Abbott (1925). To determine the difference in larval mortality between concentrations, ANOVA followed by *Tukey's HSD* tests were performed by using SPSS for windows version 16.0 (SPSS Inc., Cary, NC, US). Results were expressed in terms of mean ± SD. A value of $p<0.05$ was also considered to be statistically significant.

Results and discussion

The consequence of the various treatments of the ethanolic extracts of *Paederia pilifera* and *Cuscuta reflexa* on the F1 larval progeny of *Aedes aegypti* was observed. The deleterious effect for both plant extract treatments on the F1 larval progeny of *Ae. agypti* resulted in larval mortality with LC_{50} (95% CI; lower-upper) and LC_{90} (95% CI; lower-upper) values of 281.96 ppm (176.51-552.90 ppm) and 1542.69 ppm (718.77-9832.76 ppm) for *P. pilifera* with slope of 5.827, while *C. reflexa* ethanolic extract treatments gave 80.55 ppm (70.43-91.59 ppm) and 297.74 ppm (245.79-372.17 ppm) for LC_{50} (95% CI; lower-upper) and LC_{90} (95% CI; lower-upper), respectively, and a slope of 28.102. The analysis suggests that having the lowest LC_{50} and LC_{90} values (80.55 and 297.94 ppm), the ethanolic extract from *C. reflexa* exhibits higher toxicity (Table 1).

Table 1. Larvicidal activity of ethanolic extracts of *P. pilifera* and *C. reflexa* against 3rd instar larvae of *Ae. agypti*. Means with the same letter were not significantly different.

Conc. (ppm)	% Mortality (Mean ± SD)	
	Paederia pilifera	*Cuscuta reflexa*
Control	0.00 (0.00 ± 0.00^{d})	0.00 (0.00 ± 0.00^{d})
25	5.00 (1.25 ± 1.50^{d})	17.00 (4.25 ± 2.22^{cd})
50	13.00 (3.25 ± 1.71^{cd})	27.00 (6.75 ± 2.36^{c})
100	22.00 (5.50 ± 2.52^{cd})	56.00 (14.00 ± 2.58^{b})
200	30.00 (7.50 ± 3.42^{bc})	82.00 (20.5 ± 2.65^{a})
400	51.00 (12.75 ± 4.99^{b})	94.00 (23.5 ± 1.73^{a})
800	90.00 (22.50 ± 1.00^{a})	100.00 (25.00 ± 0.00^{a})
LC_{50} (ppm) (95% CI)	281.96 (176.51 - 552.90)	80.55 (70.43 - 91.59)
LC_{90} (ppm) (95% CI)	1,542.69 (718.77 - 9,832.76)	297.74 (245.79 - 372.17)
F-test	35.17*	102.18*
$Tukey_{0.05}$	6.09	4.53
C.V. (%)	35.14	14.64
Regression#	Y = 5.827X + 0.108	Y = 28.102X + 0.114
r	0.992	0.824
R^2	0.984	0.679

# = Regression equation (Y): plant extract concentration (X) at 24 hours
r = Correlation Coefficient of mosquito larvae mortality and plant extract concentration
R^2 = Regression Coefficient
* = Significant difference ($p < 0.05$)
95% CI = the confidence interval for a group of data for 95% confidence level (lower-upper)

In this regard, no larval mortality was exhibited in the untreated groups; however, it was found that the concentration dependent larval mortalities were substantial in both extract treated groups. Increasing concentrations from 25 to 800 ppm were evident (Table 1). The highest mortality percentage of larval mosquito tested with *P. pilifera* extract was 90% whereas 100% larval mosquito mortality could be observed when they are tested with *C. reflexa* extract at 800 ppm. Based on the analysis of variance (ANOVA) F-test, there was a significant difference in means between the concentration groups (p<0.05). Although, the larvicidal activity slightly rose with increased dosage in all trials, differences between means using the *Tukey's HSD* tests were also considered significant at the 95% level of confidence. The results indicated that means with the same letter were not significantly different as demonstrated in Table 1.

Generally, several previous researches have been reported that the susceptibility of *Ae. aegypti* larvae to a graded series of plant extract under the laboratory conditions was dose dependent and mortality increased when exposed to higher concentrations (Kaushik & Saini, 2008; 2009). In addition, the toxic effect of these plant extracts were presumably on the neuromuscular system resulting in unnatural behavior of the treated larval mosquitoes including lifelessness, uneasiness and coiling motion that was consistent with many former observations such as the researches of Polsomboon et al. (2008); Kaushik & Saini (2008; 2009); Kumar et al. (2010); Ghosh et al. (2012) and Thongpoon & Poolprasert (2015). The biological properties of these plant extracts might be due to numerous chemical compositions including, phenolics, terpenoids, flavonoids and alkaloids. Such constituents might jointly or independently contribute to produce toxic activity against the mosquito species (Gohil et al., 2010; Kamaraj et al., 2011; Mohankumar et al., 2016). The present endeavor is of great medical importance. It could be useful in controlling a major vector of viral diseases like dengue fever, dengue hemorrhagic fever, Chikungunya fever and Zika virus which are serious health problems in Thailand and other developing countries (Guzman & Kouri, 2002; Jansen et al., 2008). Thus, the larvicidal property of both extracts from *P. pilifera* and *C. reflex* against *Ae. aegypti* could be explored further through studies on its chemical composition, mode of action and adulticidal property. Moreover, other aspects like finding the most effective concentration for application, evaluating the response of different strains (field or laboratory) and larval progeny of mosquitoes, maturity of the plant material, as well as method and solvent used during extraction should be further deliberated in the follow up studies. With all these, there is a greater chance of developing a new mosquito larvicide types from plant extracts for implantation in Integrated Mosquito Management (IMM) program. Also, other biological properties of *P. pilifera* and *C. reflexa* like arthropod control particularly controlling dengue and other mosquito-borne diseases can be elucidated.

Conclusion

Evaluation of the biological property of the ethanolic extracts of *Paederia pilifera* leaf and *Cuscuta reflexa* stem displayed a promising larvicidal effect against *Ae. aegypti*. The 24-hour exposure of *Ae. aegypti* larvae on the plant extracts resulted into a substantial larvicidal property with a LC_{50} and LC_{90} of 281.96 and 1542.69 ppm for *P. pilifera* and 80.55 and 297.74 ppm for *C. reflexa*, respectively. Furthermore, considering the mortality rate of mosquito larvae, 800 ppm showed the highest insecticidal efficacy in which a 90% mortality for *P. pilifera* and 100% mortality for *C. reflexa* was observed. Consequently, it is concluded that the two plant extracts could be are potential natural agents for controlling or preventing the growth of *Aedes* mosquito larvae under the laboratory condition. There are no available reports on the toxicity of *P. pilifera* and *C. reflexa* against mosquito larvae, therefore, this is considered as the first report

in this time. Meanwhile, findings could be further deliberated to elucidate the characterization to control mosquito vectors instead of using synthetic insecticides. In addition, results presented here will pave way for further evaluation of the efficacy of the extracts by considering the specific bioactive present and the most appropriate procedure to prepare the extracts to attain the best yield and quality of the natural products from both plants.

Acknowledgements

This research work was partly financially supported by the Research and Development Institute of Pibulsongkram Rajabhat University, Phitsanulok, Thailand. The authors would like to thank the Office of Disease Prevention and Control 2, Phitsanulok for providing mosquito larvae in this study. Appreciation is also extended to Ms. Nutnaree Panprom, a senior student, for her assistance in the laboratory experiment.

References

Abbott, M. S. (1925). A method of computing effectiveness of an insecticide. *Journal of Economic Entomology*, *18*, 265-267.

Al-Fatimi, M., Wurster, M., Schroder, G. & Lindequist, U. (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *The Journal of Ethnopharmacology*, *111*,657-666. doi: 10.1016/j.jep.2007.01.018.

Anjum, F., Bukhari, S. A., Shahid, M., Anwar, S., Afzal, M. & Akhter, N. (2013). Comparative evaluation of antioxidant potential of parasitic plant collected from different hosts. *Journal of Food Processing and Technology*, *4*, 1-6. doi: 10.4172/2157-7110.1000228.

Ashwani, K., Sapna, R., Somiya, S. & Niketa. (2012). Recent review on plant molecular biology, phytophysiology, phytochemistry and ethnopharmacology of *Cuscuta reflex*a Roxb. A wonderful parasitic plant. *International Research Journal of Pharmacy*, *3*, 30-38.

Christophers, S. R. (1960). *Aedes aegypti. The yellow fever mosquito. Its life history, bionomics and structure*. London: Cambridge University Press.

El-Maghraby, S., Nawwar, G. A., Bakr, R. F., Helmy, N. & Kamel, O.M. (2012). Toxicological studies for some agricultural waste extracts on mosquito larvae and experimental animals. *Asian Pacific Journal of Tropical Biomedicine*, *2*(7), 558-563.

Finney, D. J. (1971). *Probit Analysis* (3rd ed.). London: Cambridge University Press.

Ghosh, G. K. (1991). *Biopesticide and Integrated Pest Management*. New Delhi: A.P.H. Publishing Corporation.

Ghosh, A., Chowdhury, N. & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *The Indian Journal of Medical Research*, *135*(5), 581-598.

Gohil, M. V., Agrawal, S. K., Saxena, A. K., Garg, D., Gopimohan, C. & Bhutani, K. K. (2010). Synthesis, biological evaluation and molecular docking of aryl hydrazines and hydrazides for anticancer activity. *Indian Journal of Experimental Biology*, *48*, 265-268.

Govindarajan, M. (2010). Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. *Asian Pacific Journal of Tropical Medicine*, *3*, 874-877. https://doi.org/10.1016/S1995-7645(10)60210-6.

Govindarajan, M., Sivakumar, R., Amsath, A. & Niraimathi, S. (2012). Larvicidal efficacy of botanical extracts against two important vector mosquitoes. *European Review for Medical and Pharmacological Sciences*, *16*(3), 386-392.

Guzman, M. G. & Kouri, G. (2002). Dengue: an update. *Lancet Infectious Diseases*, *2*, 33-42.

Jansen, C. C., Webb, C. E., Northill, J. A., Ritchie, S. A., Russell, R. C. & Van Den Hurk, A. F. (2008). Vector competence of Australian mosquito species for a North American strain of West Nile virus. *Vector Borne and Zoonotic Diseases*, *8*(6), 805-812. https://doi.org/10.1089/vbz.2008.0037.

Kaiser, B., Vogg, G., Fürst, U. B. & Albert, M. (2015). Parasitic plants of the genus *Cuscuta* and their interaction with susceptible and resistant host plants. *Frontiers in Plant Science*, *6*(45), 1-9. https://doi.org/10.3389/fpls.2015.00045.

Kamaraj, C., Bagavan, A., Elango, G., Zahir, A. A., Rajakumar, G., Marimuthu, S. et al. (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. *The Indian Journal of Medical Research*, *134*(1), 101-106.

Kaushik, R. & Saini, P. (2008). Larvicidal activity of stem extract of *Cuscuta reflexa* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *Journal of Vector Borne Diseases*, *45*, 66-69.

Kaushik, R. and Saini, P. (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *Journal of Vector Borne Diseases*. *45*, 66-69.

Kaushik, R. & Saini, P. (2009). Screening of some semi-arid region plants for larvicidal activity against *Aedes aegypti* mosquitoes. *Journal of Vector Borne Diseases*, 46, 244-246.

Kumar, S., Warikoo, R. & Wahab, N. (2010). Larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different instars of an indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitology Research*, *107*(4), 901-907. doi: 10.1007/s00436-010-1948-1.

Mohankumar, T. K., Shivanna, K. S. & Achuttan, V. V. (2016). Screening of methanolic plant extracts against larvae of *Aedes aegypti* and *Anopheles stephensi* in Mysore. *Journal of Arthropod-Borne Diseases*, *10*(3), 303-314.

Nie, Z.-L., Deng, T., Meng, Y., Sun, H. & Wen, J. (2013). Post-Boreotropical dispersals explain the pantropical disjunction in *Paederia* (Rubiaceae). *Annals of Botany*, *111*, 873-886. doi: 10.1093/aob/mct053.

Pal, D. K., Mandal, M., Senthilkumar, G. P. & Padhiari, A. (2006). Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. *Fitoterapia*, *77*, 589-591. doi: 10.1016/j.fitote.2006.06.015.

Perveen, S., Bukhari, I. H., Ain, Q. U., Kousar, S. & Rehman, J. (2013). Antimicrobial, antioxidant and minerals evaluation of *Cuscuta europea* and *Cuscuta reflexa* collected from difference hosts and exploring their role as functional attribute. *Journal of Pharmaceutical and Applied Sciences*, *3*, 43-49.

Ponlawat, A. & Harrington, L. C. (2005). Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *Journal of Medical Entomology*, *42*, 844-849.

Polsomboon, S., Poolprasert, P., Bangs, M. J., Suwanakerd, W., Grieco, J. P., Achee, N. et al. (2008). Effects of physiological conditioning on behavioral avoidance by using a single age group of *Aedes aegypti* exposed to deltamethrin and DDT. *Journal of Medical Entomology*, *45*(2), 251-259.

Puff, C., Chayamarit, K. & Chamchumroon, V. (2005). *Rubiaceae of Thailand: A pictorial guide to indigenous and cultivated genera*. Bangkok, Thailand: The Forest Herbarium, National Park, Wildlife and Plant Conservation Department.

Saenphet, K., Saenphet, S. & Jirakittirat, K. (2004). Gastroprotective effects and antioxidant activities of *Paederia pilifera* Hook.f. Root extract. *Chiang Mai Journal of Science*, *41*(5.1), 1121-1131.

Shaalan, E. A.-S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H. & Mansour, A. H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment International*, *3*, 1149-1166. doi: 10.1016/j.envint.2005.03.003.

Sharma, P., Mohan, L. & Srivastava, C. N. (2006). Impact analysis of neem kernel extracts on the development profile of *Anopheles stephensi*. *Journal of Asia-Pacific Entomology*, *9*, 11-17. https://doi.org/10.1016/S1226-8615(08)60270-8.

Staples, G. (2010). Convolvulaceae. *Flora of Thailand*. *10*(3), 378-382.

Sukkho, T., (2008). *A survey of medicinal plants used by Karen people at Ban Chan and Chaem Luang Sub-districts, Mae Chaem District, Chiang Mai Province* (M.S. thesis), Department of biology, Faculty of Science, Chiang Mai University, Thailand. (in Thai)

Tanruean, K., Kaewnarin, K., Suwannarach, N. & Lumyong, S. (2017a). Comparative evaluation of phytochemicals, and antidiabetic and antioxidant activities of *Cuscuta reflexa* grown on different hosts in northern Thailand. *Natural Product Communications*, *12*(1), 51-54. https://doi.org/10.1080/14786419.2017.1392955.

Tanruean, K., Poolprasert, P., Kumla, J., Suwannarach, N. & Lumyong, S. (2017b). Bioactive compounds content and their biological properties of acetone extract of *Cuscuta reflexa* Roxb. grown on various host plants, *Natural Product Research*, *3*, 1-4. doi: 10.1080/14786419.2017.1392955.

Thongpoon C. & Poolprasert, P. (2015). Phytochemical screening and larvicidal activity of *Millingtonia hortensis* L.f. flower extract against *Aedes aegypti* Linn. *Kasetsart Journal (Natural Science)*, *49*(4), 597-605.

Vaughn, K. C. (2003). Dodder hyphae invade the host: a structure and immunocytochemical characterization. *Protoplasma*, *220*, 189-200. doi: 10.1007/s00709-002-0038-3.

WHO. (1996). *Report of the WHO Informal Consultation on the "Evaluation on the Testing of Insecticides*". Geneva: WHO Pesticides Evaluation Scheme, Division of Control of Tropical Diseases.