J Vector Borne Dis 50, March 2013, pp. 68–70

The larvicidal activity of brown algae *Padina minor* (Yamada 1925) and *Dicyota linearis* (Greville 1830) against the dengue vector, *Aedes aegypti* (Linn 1762) (Diptera: Culicidae)

Venus Bantoto[1, 2] & Danilo Dy[1]

[1]*Department of Biology, University of San Carlos, Talamban, Cebu City;* [2]*Biology Department, Negros Oriental State University, Dumaguete City, Negros Oriental, Philippines*

Key words Macroalgae; mortality; mosquito

Dengue is currently one of the emerging diseases challenging global public health, ranging from self-limiting dengue fever to more severe life-threatening form termed as dengue hemorrhagic fever. In the last 50 yr, its worldwide incidence increased by 30 fold and it was estimated that 1/3 to 1/2 of the world's population is at risk of becoming infected. Currently, epidemic dengue hemorrhagic fever is a leading cause of hospitalization and death among children in Southeast Asia. Worldwide, out of 50 million infections estimated by the World Health Organization (WHO) each year, 500,000 cases are dengue hemorrhagic fever and 22,000 deaths were mostly children[1]. In the Philippines, the Department of Health (DOH) surveillance report data in 2011 showed that there were 70,204 dengue cases mostly from 1–10 yr age groups and with 396 deaths.

It is already a common knowledge that *Aedes aegypti* (Diptera: Culicidae) is the vector of dengue hemorrhagic fever and is endemic in Southeast Asia, Pacific Islands, Africa and America. Because dengue virus is transmitted to human by the bite of infected *Aedes* mosquito, endemicity or hyper-endemicity of dengue virus requires existence of sufficient vectors[2]. The recrudescence of dengue hemorrhagic fever is due to the high number of breeding places and increasing resistance to insecticides[3]. In developing and underdeveloped countries, the habit of adding water to containers for drinking, cooking or bathing, water storage for ornamentation trigger dengue outbreaks even during the dry season[2]. The use of synthetic chemicals to kill the vector mosquito is not so effective because it is highly domesticated and many adults are found indoors[3]. So far, no available vaccine for dengue has been made or discovered. The only successful way to reduce mosquito densities is attacking the larval breeding places through sanitation activities.

The marine algae are rich sources of biologically active metabolites. These active metabolites are synthesized to protect themselves from herbivores and bacterial infections and maintain homeostasis in their environment[4, 5]. In the Philippines, there are 820 species of marine macroalgae recorded, mostly have economic importance as sources of food, industrial, bioactive and nutritional products and growth promoting substances[6]. The high diversity of algae and their metabolites represent a significant economic potential for the discovery of new drugs and organic chemicals that might have synergistic and antagonistic effects.

The idea of using algal extracts to kill mosquito larvae is not new. However, considering the biodiversity of macroalgae in the tropical regions, there is a need to study their larvicidal potential. The algae's metabolites have also been shown in several studies to have larvicidal activities[3, 5, 7, 8].

In this study, the crude extracts of Philippine *Padina minor* and *Dictyota linearis* were compared for their larvicidal activity and established their LC_{50} and LC_{90} so that we can further compare the values with other similar studies. If a potent larvicidal activity is detected, it may serve as a suitable alternative to expensive synthetic insecticides.

Dictyota linearis and *Padina minor* were collected from the shore of Agan-an, Sibulan, Negros Oriental (9° 20.29′N, 123° 18.51′E). The algae were both washed with unchlorinated water to remove the epiphytes, sand, coral rubbles and other detritus matter. The algae were then air- dried for a week. The dried sample was powdered by a laboratory blender and weighed using an analytical balance. The crude components of *D. linearis* and *P. minor* were extracted by bulk extraction. Briefly, pre-weighed powdered sample was poured to a 1000 ml erlenmeyer flask containing ethanol and soaked for a week. The ratio between powdered algae to 95% ethanol was 500 mg: 1 L.

After a week, the mixture was filtered using a filter

paper. The filtrate was placed in an oven and set at 60°C to let the ethanol evaporate and concentrate the crude extract. From this stock solution 20, 40, 60, 80 and 100 mg/ml of *D. linearis* and *P. minor* extracts were prepared. Unchlorinated water was used as a negative control and 2% ethanol was used as positive control. The response variable was mortality of the IV instar *Ae. aegypti* larvae collected from Dumaguete city, Negros Oriental. The larvae of *Ae. aegypti* were identified by the presence of comb teeth with well-defined denticles and less defined denticles in its pectin teeth. Fourth instar *Ae. aegypti* larvae were isolated, placed in a separate container and were maintained at room temperature (26–29°C), and 12 : 12 Light : Dark photoperiod.

To test the larvicidal activity of the algal extracts, *Ae. aegypti* larvae were collected using a dropper, placed in a filter paper to remove excess water and transferred in a petri dish containing 10 ml of different concentrations of algal extracts. Ten larvae were tested per concentration of *D. linearis* and *P. minor* extracts for 24 h at room temperature. The larva was considered dead when it was immobile after touching it with a dissecting needle. There were five replicates per extract concentration per algal species in a randomized complete block design (RCBD) with replicate as the blocking variable. Probit analysis was used to derive the LC_{50} and LC_{90}. A *t*-test for independent sample was used to compare the LC_{50} and LC_{90} between the two algae.

In both algal extracts, the mortality of *Ae. aegypti* larvae increased as the concentration of the extract increased (Fig. 1). At 20 mg/ml, no dead larvae were observed in *D. linearis* extract while in *P. minor*, 8% of the larvae died. From 40 to 80 mg/ml, the number of dead larvae increased with the *P. minor* extract having a consistently higher percentage of larval mortality than the *D. linearis* extract. At 100 mg/ml, both the extracts showed cent percent larval mortality. In the positive and negative

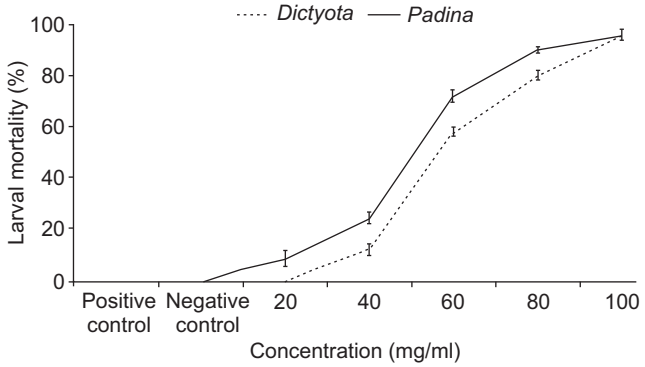

Fig. 1: Larval mortality (Mean ± SE) of *Aedes aegypti* at different concentrations of *Padina minor* and *Dictyota linearis* extracts after 24 h of exposure (Error bars are standard error).

controls, no dead larva was observed after 24 h of exposure. The LC_{50} of *D. linearis* was 60 mg/ml (95% LCL–UCL, 48.7–70.6 mg/ml). *Padina minor* has lower LC_{50} than *D. linearis* at 50.8 mg/ml (95% CI 39.7–48.8 mg/ml). Likewise, *P. minor* extract has a lower LC_{90} (84 mg/ml; 95% CI 68–140.5 mg/ml) than *D. linearis* (91.6 mg/ml; 95% CI 76.5–141 mg/ml). *Padina minor* has significantly lower LC_{50} than *D. linearis* but at $LC_{90,}$ their effectiveness was comparable (p = 0.11). Hence, the extract of *P. minor* is more potent than *D. linearis*.

Secondary metabolites produced by plants showed significant activity against *Ae. aegypti* larvae. For example, sesquiterpene isolated from seeds of *Apium graveolens* showed 100% mortality against IV instar of *Ae. aegypti* at a concentration of 0.5 mg/ml, while 75–77 diterpenes isolated from *Pterodon polygalaeflorus* exhibited siginificant larvicidal activity at LC_{50} values of 0.2 mg/ml[9]. Marine brown algae are prolific producers of secondary metabolites, i.e. sesquiterpenoids, diterpernoids and compounds of mixed biosynthesis origin. Dictyotaceae are rich sources of bioactive terpenes that could be an evolutionary response of the brown algae to herbivore. Species of *Dictyota* and *Padina* were found to produce terpenoids such as diterpene and sesquiterpene[4]. In the present study, *P. minor* and *D. linearis* showed a significant larvicidal activity against *Ae. aegypti* larvae suggesting the presence of larvicidal metabolites such as terpenoid in *Dictyota* and *Padina*. The larvicidal effect of brown algae could also be due to its phenolic or unsaturated fatty acids and phlorotannins. *Padina pavonica* significantly retards the larval growth rate of *Culex pipiens* due to its polyphenolic compounds[5].

Padina minor showed significantly greater larvicidal activity compared to *D. linearis* at LC_{50}. Greater larvicidal activity of *Padina* than *Dictyota* was also observed in *P. tetrastromatica* and *D. dichotoma* from southwest Coast of India[7]. The LD_{50} of *P. tetrastromatica* on the II instar larvae of *Cx. quinquefasciatus* is 0.96 mg/ml (96 µg/ml) while *D. dichotoma* is 0.25 mg/ml (226 µg/ml)[7]. Both *P. minor* and *D. linearis* showed greater potency compared to *Ulva fasciata* and *Grateloupia lithophila* collected from Kovalam, near Chennai[8]. The LC_{50} of *U. fasciata* in methanol extract was 431 mg/ml, 504.5 mg/ml in acetone extract and 478.7 mg/ml in benzene while for *G. lithophila,* the LC_{50} was 431.9, 349.7 and 425.4 mg/ml for methanol, acetone and benzene extracts, respectively. The wide difference between the effectivity of *P. minor* and *D. linearis* with other algal species could be due to geography and ecological parameters. It is believed that production of carbon-based secondary metabolites is highest in areas with high light intensity, low nu-

trition and high grazing[10]. There is a strong correlation between the unpalatability of brown algae in the tropics and the presence of secondary compounds such as terpenoids or prenylated phenolics[11].

A very interesting observation in this study was the prolonged larval stage for those which were not killed by the extracts. In the control groups, the larvae became pupae in 2–3 days, however, larvae of the same stage in *P. minor* and *D. linearis* extracts became pupae in 5–7 days. Prolonged larval stage was also observed in *Cx. quinquefasciatus* treated with 31.25 ppm of *Argenome mexicana* extract[12]. This was attributed to hormonal imbalance which delayed larval metamorphosis. This study showed that low concentration of *P. minor* and *D. linearis* extracts may have some effects on the *Aedes* larvae even if it did not kill it. In view of this finding, the need to identify the active ingredients responsible for the mortality of *Aedes* larvae will be pursued.

The present study showed that extracts of *P. minor* and *D. linearis* have larvicidal activity against IV instar *Ae. aegypti* larvae and could serve as a suitable alternative to synthetic insecticides.

ACKNOWLEDGEMENTS

We thank the University of San Carlos and Negros Oriental State University for laboratory facilities and logistic support. This is a science contribution of both the universities. We would also like to thank the anonymous reviewers for their comments and suggestions.

REFERENCES

1. Phillips M. Dengue reborn: Widespread resurgence of a resilient vector. *Environ Health Perspect* [serial on the internet] 2008. Available from: *http://www.nrdc.org/health dengue/files/fdengue.pdf*. (Accessed in August 2012).
2. Barrera R, Amador M, Mackay AJ. Population dynamics of *Aedes aegypti* and dengue as influenced by weather and human behavior in San Juan, Puerto Rico. *Rev Biomed* [serial on the Internet] 2012. Available from: *http://www.revbiomed.uady.mx/pdf/rbw 2137. pdf*. (Accessed in August 2012).
3. Ciccia C, Coussio J, Mongelli E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J Ethnopharmacol* 2000; *72:* 185–9.
4. De Paula JC, Vallim MA, Teixeira VL. What are and where are the bioactive terpenoids metabolites from Dictyotaceae (Phaephyceae). *Brazilian J Pharmacog* 2011; *21* (2): 216–28.
5. Elbanna SM, Hegazi MM. Screening of some seaweeds species from South Sinai, Red Sea as potential bioinsecticides against mosquito larvae, *Culex pipiens*. *Egyptian Acad J Biol Sci* 2011; *4*(2): 21–30.
6. Trono G. Diversity of the seaweed flora of the Philippines and its utilization. *Hydrobiol* [serial on the Internet] 1999. Available from: *http://www.link.springer.com* (Accessed in August 2012).
7. Manilal A, Sujith S, Kiran G, Selvin J, Shakir C, Gandhimathi R, Panikkar M. Biopotentials of seaweeds collected from southwest coast of India. *J Mar Sci Technol* 2009; *17*(1): 67–73.
8. Poonguzhali TV, Nisha LJ. Larvicidal activity of two seaweeds, *Ulva fasciata* and *Grateloupia lithophila* against mosquito vector, *Culex quinquefasciatus*. *Int J Curr Sci* 2012; *4:* 163–8.
9. Kishore N, Mishra BB, Tiwari VK, Tripathi V. A review on natural products with mosquitocidal potentials. In: Tiwari V, editor. *Opportunity, challenge and scope of natural products in medicinal chemistry*. Kerala, India: Research Signpost 2011; p. 335–65.
10. Coley P, Byrant J, Chapin F. Resource availability and plant antiherbivore defense. *Science* 1985; *230:* 895–9.
11. Steinberg PD, Paul VJ. Fish feeding and chemical defenses of tropical brown algae in western Australia. *Mar Ecol Prog Ser* 1990; *58:* 253–9.
12. Karmegam N, Sakthivadivel M, Anuradha V, Daniel T. Indigenous-plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. *Bioresource Technol* 1997; *59:* 137–40.

Correspondence to: Venus Bantoto, Department of Biology, University of San Carlos, Talamban, Cebu City 6000, Philippines.
E-mail: vdbbio@yahoo.com

Received: 20 October 2012 *Accepted in revised form:* 23 January 2013