The larvicidal effects of *PADINA MINOR, SARGASSUM CRISTAEFOLIUM*, AND *TURBINARIA ORNATA*, extracts on *ANOPHELES FLAVIROSTRIS*

Lizbeth V. Alzona
Ma. Natalia C. Orig

Submitted to the
Department of Biology
College of Arts and Sciences
University of the Philippines Manila
Padre Faura, Manila

In partial fulfillment of the requirements
For the degree of
Bachelor of Science in Biology

March 2000

Department of Biology
College of Arts and Sciences
University of the Philippines Manila
Padre Faura, Manila

Announcement of
Undergraduate Thesis Presentation

LIZBETH VILLEGAS ALZONA
MARIA NATALIA CUENCA ORIG

Entitled

THE LARVICIDAL EFFECTS OF *Padina minor, Sargassum cristaefolium* AND *Turbinaria ornata* ON *Anopheles flavirostris* MOSQUITO

For the degree of
Bachelor of Science in Biology

4 p.m., 17 March 2000
Rizal Hall, room 301

THESIS ADVISER
Natividad F. Lacdan
Assistant Professor
Department of Biology, CAS
UP Manila, P. Faura, Manila

THESIS READER
Arnold V. Hallare
Assistant Professor
Department of Biology, CAS
UP Manila, P. Faura, Manila

Endorsed by:

Rosario R. Rubite
Chairman
Thesis Committee
Department of Biology, CAS
UP Manila, P. Faura, Manila

Authorized by:

Sylvia A. Sustento
Acting Chairman
Department of Biology, CAS
UP Manila, P. Faura, Manila

ENDORSEMENT

This is to certify that this undergraduate thesis entitled "The Larvicidal Effects of *Padina minor, Sargassum cristaefolium* and *Turbinaria ornata* on *Anopheles flavirostris* mosquito" submitted by Lizbeth V. Alzona and Ma. Natalia C. Orig in partial fulfillment of the requirements for the degree of BS Biology was successfully defended on 17 March 2000.

Natividad F. Lacdan, M.S.
Adviser
17 March 2000

Endorsed by

Sylvia A. Sustento
Acting Chairman
Department of Biology
17 March 2000

Officially accepted by

Josefina G. Tayag, D.P.A.
Dean
College of Arts and Sciences
17 March 2000

CURRICULUM VITAE

Personal Background

Name: Lizbeth Villegas Alzona
Date of Birth: August 30, 1979
Age: 20
Place of Birth: Calamba, Laguna
Address: #159 Lazaro Street, Elepaño Subdivision, Calamba, Laguna
Father's Name: Rosenberg Domingo Alzona
Mother's Name: Alicia Nano Villegas

Educational Background

Elementary: Maquiling School Incorporated
High school: University of the Philippines Rural High School
College: University of the Philippines, Manila

Organization

Biological Majors Association
Lingkod e.r. Foundation, Incorporated
Ugnayan ng Pahinungod

CURRICULUM VITAE

Personal Background

Name: Maria Natalia Cuenca Orig
Date of Birth: September 25, 1979
Age: 20
Place of Birth: Manila
Address: #33 Cadiz Street, Alabang Hills, Muntinlupa
Father's Name: Ruben de Ungria Orig
Mother's Name: Lourdes Relova Cuenca

Educational Background

Elementary: PAREF Woodrose School, Inc.
High school: PAREF Woodrose School, Inc.
College: University of the Philippines, Manila

Organization

Lingkod e.r. Foundation, Incorporated
Ugnayan ng Pahinungod

ACKNOWLEDGEMENT

It is 20 March 2000, 2:00 in the morning.

This is the last all-nighter installment to our thesis. And *IT* is finally finished. Before we succumb to sleep, we would like to extend our gratitude to those who have helped us in this colossal task.

Our Creator, for giving us patience and strength.

Our parents, Rosenberg Domingo Alzona and Alice Villegas Alzona; Ruben de Ungria Orig and Lourdes Cuenca Orig, for understanding the long hours and distances that we had to put in.

Our adviser, Professor Natividad Lacdan, for her guidance and encouragement.

Our reader, Professor Arnold Hallare, for giving us his time and suggestions.

Sir Dennis Dantic for his important contributions and encouraging text messages.

Department of Biology for just being there.

Dr Violeta Villegas, Dr. Lito Magdalita, and the staff of the Institute for Plant Breeding for allowing us to use their facilities.

Dr Augusto Sumalde and Mang Ed for permitting us to enter the Hortorium of the Museum of Natural History.

Mayor Edward Hagedorn for his hospitality.

Our friends, Maan, Kneef, Ate Neens, Ate Ans, Pia, and Ro-c, for the most valuable contribution of all – keeping us sane during those hectic days.

With that, we formally declare our thesis "complete!"

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

LIST OF PLATES

Abstract

Three seaweeds, *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata* were tested for their potential larvicidal properties against the *Anopheles flavirostris* mosquito. Results showed that *Padina minor* and *Turbinaria ornata* were effective against the *Anopheles flavirostris* larvae at a concentration of 37.5% while *Sargassum cristaefolium* was effective at a lower concentration of 25%. However, Pearson Correlation Coefficient analysis showed that, unlike *Padina minor* and *Turbinaria ornata*, the extract concentration of *Sargassum cristaefolium* was not the sole cause for the resultant percent mortality. Extraneous factors could have contributed to the increased efficacy of *Sargassum cristaefolium*. It would be safe to conclude that all three seaweeds have identical larvicidal properties and are as effective as the water-based Baygon insect repellant from a concentration of 37.5% to 100%.

The larvicidal effects of *PADINA MINOR, SARGASSUM CRISTAEFOLIUM,* and *TURBINARIA ORNATA* extracts on *ANOPHELES FLAVIROSTRIS*

Lizbeth V. Alzona and Maria Natalia C. Orig

INTRODUCTION

Background of the Study

Malaria is one of the most debilitating diseases in the world. In fact, it is one of the six top priority diseases under the World Health Organization Special Programme for Research and Training in Tropical Diseases. The principal vector of malaria in the Philippines is *Anopheles flavirostris* which transmits the causative parasite, *Plasmodium sp.*, to man. This results in fever, chills, nausea, muscle pain, severe complications, and death. Understanding the man-vector-parasite relationship can lead to proper control strategies. Control of the malaria can be undertaken in two ways: treatment of the symptoms and prevention of the disease. Most efforts are focused on finding the cure, however, the biodiversity of the tropics could provide other solutions geared towards the prevention and control of the most important mosquito-borne disease in this region (Salazar, 1989).

As a tropical country, the Philippines is endowed with a rich biodiversity. Most land resources have been utilized, but the potential of the archipelago's marine assets have not been realized. The application of marine resources range from food and medicine to commercial and industrial uses. Algae are often overlooked but have as varied uses as other marine resources. They can be utilized as food in ice cream manufacture, as medicine in goiter treatment, and as an industrial product in fertilizer (Chennubhotla et al., 1987). Because of the components of brown seaweed, a type of

algae, it can also be used in food, medicine, commercial products, and industrial goods. Many brown seaweeds rank among the most vitamin-rich plants in the world. Their anti-berberic activity has been known for centuries in the Far East (Hoppe et al., 1979). For its pharmaceutical uses, its component laminaran in combination with sulfate has the potential to become an anti-coagulant, one-third as good as heparin. The mannitol found in brown algae can be used in the paint, plastic, leather and explosives industries (Chapman, 1980). Among the other industrial uses of brown seaweeds is organic insecticide (Moreland, 1979).

The larvicidal properties of representative species from divisions Phaeophyta, Rhodophyta and Chlorophyta were tested against *Aedes aegypti*. Among the three divisions tested, brown seaweeds exhibited the highest potential for larvicidal properties (Padla, 1994).

The larvicidal properties of brown algae exhibited on *Aedes aegypti* can further be extended to apply to *Anopheles flavirostris*. With this premise, extracts of *Padina minor, Sargassum cristaefolium and Turbinaria ornata* were selected to treat the test organism, *Anopheles flavirostris* larvae.

Statement of the Problem

The problem of the study is to determine the potential larvicidal property in each of the three species of brown algae: *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata* on the larvae of mosquito *Anopheles flavirostris*.

Objectives

General Objective:

To determine the potential larvicidal properties in the extracts of three seaweed species *Padina minor*, *Sargassum cristaefolium,* and *Turbinaria ornata.*

Specific Objectives:

(1) To determine the resulting mortality in *Anopheles flavirostris* larvae after the application of the extracts from *Padina minor*, *Sargassum cristaefolium,* and *Turbinaria ornata.*

(2) To ascertain the effective concentration of the three seaweed extracts against *Anopheles flavirostris* larvae.

(3) To establish which among the three seaweed extracts was the most effective against the *Anopheles flavirostris* larvae.

(4) To determine the efficacy of the larvicidal activities of *Padina minor*, *Sargassum cristaefolium,* and *Turbinaria ornata* on the basis of the positive control, water-based Baygon.

(5) To establish the association between the seaweed extract concentration and the resulting larval mortality.

Hypothesis

Null Hypotheses: *Padina minor, Sargassum cristaefolium* and *Turbinaria ornata* do not have larvicidal properties against *Anopheles flavirostris*.

Alternative Hypotheses: *Padina minor, Sargassum cristaefolium* and *Turbinaria ornata* have larvicidal properties against *Anopheles flavirostris*.

Significance of the Study

Few studies exist on the use of seaweeds for insect control. The results of the present study could, therefore, contribute to the information regarding the subject in addition to facilitating future studies.

Most researches regarding the control of malaria are geared towards finding a cure. The results could provide a new approach to medical science by presenting a novel means of preventing the disease. By eradicating the vector at its larval stage, it tackles the disease at its root and thereby terminating the further transmission of the disease. Unlike chemical insecticides that further compound the problem of malarial prevention and control by the occurrence of insecticide resistant mosquito strains, the potential of a biological larvicide eliminates this problem. The three brown seaweeds can therefore be used as environment-friendly biological controls of *Anopheles flavirostris* larvae.

The natural conservation movement in Palawan, the site of seaweed collection, can further be strengthened by the additional value of the marine resources, particularly brown algae. Incidentally, malaria is endemic in Palawan, and the results of the study would provide an inexpensive and novel of mosquito control.

Scope and Limitation

1. This study made a comparison on the larvicidal effects of the three seaweed extracts of the species *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata* against water-based Baygon insect repellant.
2. The extracts along with the water-based Baygon insect repellant were tested against the 4th instar larval stage of the mosquito, *Anopheles flavirostris*.

3. Larvicidal activity was evaluated through mortality counts, which include moribund and dead *Anopheles flavirostris* larvae after 24 hours.
4. The results were interpreted using a descriptive analysis, Pearson Correlation Coefficient, and Sample Coefficient of Determination.

REVIEW OF RELATED LITERATURE

Malaria is one of the most debilitating diseases in the developing world. Every minute, two children die from the effects of malaria in the tropics. Almost half of the world's population is living in endemic areas and at the risk of catching malaria. About 300 to 500 million people suffer from the disease each year and more than one million die of it (Salazar, 1989).

The principal vector of malaria in the Philippines is *Anopheles flavirostris*. It belongs to the Class Insecta of the Phylum Arthropoda. Like all other flies, it is included in the Order Diptera. *Anopheles flavirostris* shares its family, Culicidae, with *Aedes sp.* and *Culex sp.* (Roberts and Janovy, 1996). It belongs to the subgenus *Cellia* along with the majority of the most important vectors of malaria in the world (Salazar, 1989). Widely distributed in the Philippines, its habitat includes foothill or hilly zones and forest edges. It can survive in any climate provided residual aquatic habitats remain for larval breeding. It is most abundant in the summer, before and right after the rainy season. High mosquito densities often result in transmission peaks or malaria season (Salazar, 1989).

The entire life cycle of *Anopheles sp.* occurs within 8 to 14 days. Eggs hatch 2 days after laying, and the fourth instar larval stage is reached in seven days (Salazar, 1989).

Mosquitoes are frail insects and often subject to dehydration, therefore, they are found in areas of high humidity, low temperature and low degree of illumination. Their susceptible physiology determines their habitat, the most favorable ones situated near a freshwater source. In can be found in most parts of Luzon particularly in Laguna,

Mountain Province and Puerto Princesa. In addition, it can also be found in Bukidnon and Lake Lanao in Mindanao (Salazar, 1989). This habitat also makes a suitable breeding ground for adults and fosters the development of larvae. However, these breeding places are relatively unprotected from various kinds of parasites such as protozoa, nematode worms, and fungi. As a result, larvae often become hosts to these parasites particularly *Plasmodium sp.*, the causative agent of malaria (Goma, 1966).

Malaria is the most important mosquito-borne disease in the tropics and subtropics (Salazar, 1989). As a result, various efforts have been made to control the disease by treating it and preventing its spread. This was accomplished through appropriate drug treatment of host, destruction of breeding places, introduction of mosquito-eating fish *Gambusia affinis*, and use of insecticide (Roberts and Janovy, 1996). However, the disease has evolved through the drug-resistance in *Plasmodium falciparum* and insecticide resistance in vector mosquitoes. Chloroquine and sulfadoxine-pyrimethamine resistant *Plasmodium* were detected in the 1950's and 1970's, respectively. In 1980, fifty-one anopheline species were shown to be resistant to one or more insecticides, including DDT [1, 1, 1-trichloro-2, 2-di (p-chlorophenyl ethane)]. These efforts have only compounded the problem (Salazar, 1989).

Since it is a tropical disease, the biodiversity of the region may provide the solution to the problem. The Philippine archipelago is an appropriate example of the rich biodiversity of the tropics. Like every developing country, land resources have been exhausted, but marine assets are yet unexplored. At present, the use of marine wealth, particularly seaweeds, as sources of food, medicine, commercial products and industrial products is still undertaken in a small scale. Agar-agar is a gelatinous substance obtained

from red algae, which is used in food as filling, stabilizing, and thickening agents of jellies, ice creams, and sherbets. Because of its ability to keep substances in suspension, agar-agar is also a constituent of pills and capsule medications, a lubricant for drawing tungsten in electrical bulbs, and photographic film coats (Chennubhotla et al., 1987). Components of brown algae, such as laminaria, mannitol, and algin, can also be employed in the same applications. Because of its inability to gel or form a viscous solution, laminaran sulfate can be used as anti-coagulant and is said to be one-third as effective as heparin. Mannitol offers many promising technical possibilities, such as paints, lacquer preparation, and plastic products that are better than those obtained from glycerine. Mannitol can also be nitrated to form nitro-manite, a powerful explosive similar to nitro-glycerine. In the food industry, they are used to prepare jelly products, milk and dairy products, and meat and sausage products in order to prolong its shelf-life (Chapman, 1980). Algin is also used as thickening and dispersing agents in ointments, creams, jellies, liquid emulsions, lotions, toothpastes, compact powder, bathing preparations, and additives in hair dyes (Venkataraman et al., 1974).

Among the other industrial uses of brown algae is its role in insect control. According to Thangam and Kathiresan as cited by Padla (1994), *Dictyota dichotoma* extract induced 50% mortality among *Anopheles stephansi* and *Culex quinquefasciatus* larvae at low concentrations of 22 ppm and 34 ppm, respectively. This study showed that *Anopheles stephansi* larvae were more susceptible to the larvicidal activity of the seaweed. Padla (1994) conducted her own study involving 21 species of seaweeds and found *Hydroclathrus tenuis* extract to be the most effective, inducing 50% mortality among *Aedes aegypti* larvae at the lowest concentration (i.e. 692 +/- 286 ppm). *Padina*

sp., *Sargassum sp.* and *Turbinaria sp.* also resulted in 50% mortality but at higher concentrations.

Other studies have shown that seaweeds are effective pesticides as evidenced by the lesser incidence of insect infestation in plants sprayed with seaweed extract. Stephenson (1965) found that liquefied seaweed, although had no direct insecticidal effect, had shown to increase the resistance of crop plants to certain pests, including aphids, red spider mites, powdery mildew, and botrytis. Venkataraman and his colleagues (1974) noted that alginates, the major component of brown algae, were used in industries dealing with pesticides and insecticides. They observed that, in the United Kingdom, there was a decrease in the fecundity of black bean aphids when they fed on plants sprayed with seaweed extract. They also noted that seaweed sprays reduced the presence of aphids on strawberries and red spider mites on potatoes in the United States. While Venkataraman attributed this property to alginates, others suggested that agar was the active component in agricultural insecticides (Hoppe *et al.*, 1979).

Among the researches conducted in the Philippines regarding this subject, Moreland (1979) was one of the first to survey the use of brown seaweeds as pesticides in the local setting. He recorded an incidence wherein a resident of Barangay San Vicente, Sta. Ana, Cagayan used "aragan" (i.e. *Sargassum sp.* or *Turbinaria sp.*) hung on "upo" vines to keep away insects. Like Venkataraman and his colleagues, local research also attributed the pesticidal property to algin in seaweed extracts (Ronquillo and Llana, 1982). However, other studies credited the presence of phenols and terpenoidal compounds in seaweeds for this property (Aliño *et al.*, 1990). In an article of the SICEN Newsletter (1993), others attributed this property to the undesirable odor and taste

conferred by the seaweed extracts on plants in addition to the hydrogen sulfide and other sulfides produced by the seaweeds.

MATERIALS AND METHODOLOGY

Processing of Seaweeds

1. Collection and Processing of Specimens

Padina minor, Sargassum cristaefolium and *Turbinaria ornata were* obtained from the Central Park, Ulugan Bay, Puerto Princesa, Palawan (Appendix A, Figure 1). Five to ten kilograms of algae were harvested by wading or diving during low tide. The complete plant was removed from the substrate by use of plant pruners after which they were then placed in net bags. The specimens were washed thrice with water to remove debris. The collected algal samples were labeled and grouped accordingly. The specimens were submerged in a cooler filled with ice water during the transfer from the study site to the accommodations.

2. Identification of the Algal Species

Some mature algae with complete morphological structures were used in identification. They were sprayed with 5% ethanol and wrapped in sheets of newspapers. They were then placed between a wooden plant presser secured with nylon straw. The sheets of newspaper were occasionally replaced until the seaweed specimens were completely dry. The dried specimens were mounted on the white face of the illustration board and submitted to the Marine Science Institute for species identification. Photographs of the dried specimen were taken.

3. Preparation of the Extract

Approximately 100 grams of seaweed was placed in a mortar and extracted with 80% ethanol using a pestle 12 hours after collection. Eighty percent ethanol

was prepared by diluting 80mL ethanol with 20mL distilled water. The extracts were then filtered using a glass funnel lined with Whatman No.1 filter paper to remove solid particles. The filtrates were temporarily stored in amber bottles for 2 days. They were then poured in separate beakers and placed in a water bath at 50°C until the texture of the mixture was syrupy. The remaining residues were stored at 4°C +/- 2°C in rubber-capped vials.

Collection of *Anopheles flavirostris* larvae

Populations of *Anopheles flavirostris* larvae were collected from the breeding sites found in the Mulawin Creek at the Hortorium, College, Laguna (Appendix A, Figure 2). Larval rearing trays were set-up one week prior to the actual collection of larvae in order to obtain 4th larval instars. After the collection, the larvae were transported to a laboratory in the Institute of Plant Breeding (Plate 1) wherein the actual larval testing was done.

Anti-Larval Testing

Extracts of the three marine seaweeds and water based Baygon insect repellant were screened for larvicidal activities against the *Anopheles flavirostris* larvae.

1. Performance of the Test

The collected *Anopheles flavirostris* larvae were used in this experiment. Water-based Baygon insect repellant was used as the positive control. Groups of 25 4th instar larvae were scooped out using a porcelain spatula and transferred into separate compartments in the plastic ice trays. After the excess water was

drained, the 2 mL seaweed test solutions were poured into their respective compartments (Plate 2). Concentrations of 12.5%, 25%, 50%, 62.5%, 75%, 87.5%, and 100% were prepared adding parts of extract to parts of distilled water (Appendix B). The same number of larvae was also placed in the each of the control set-ups (Plate 3). All experimental and control solutions were maintained at 26°C +/- 2°C. Larvae that pupated during the test were discarded. In both the 25% concentrations of *Padina minor and Turbinaria ornata*, more than 10% of the larvae pupated, the test was discarded and consequently repeated (Padla, 1994).

2. Scoring for Mortality

Mortality count included the moribund and dead larvae, and was done after 24 hours. Moribund larvae were classified as those that could not rise to the surface, did not show characteristic diving motion when the water was disturbed, or showed discoloration, unnatural position, incoordination, rigors, and tremors. Those that could not be induced to move when probed with a needle in the siphon or cervical region were considered dead larvae.

3. Data Analysis

All tables and graphs were descriptively analyzed. Pearson Correlation Coefficient was used to determine the relationship between larval mortality and seaweed extract concentration.

RESULTS

Processing of Seaweeds

1. Collection and Processing of Specimens

Collection of the specimens was done at high tide from 10 a.m. to 12 noon. The specimens were submerged in calm water. The most abundant seaweed specimens present were *Sargassum cristaefolium*. They grew on sandy substratum approximately 30 meters from the shore. Interspersed between them were less dominant *Padina minor* specimens. They were found either attached to rocky or sandy substratum. The least predominant algal species was *Turbinaria ornata* that was rooted to rocks. Only two other species of *Sargassum* and *Padina* were found in the area.

2. Identification of Algal Species

Padina minor Yamada (Plate 4)

Padina minor belongs to the Class Phaeophyceae of Division Phaeophyta. It is among the Order Dictyotales and Family Dictytaceae (Trono, 1997).

Padina minor, commonly known as lap-lapayag in Ilocano, is a light brown or yellowish brown plant, about 5-8 cm. tall (Hurlado-Ponce *et. al.*, 1992). It is divided into several flabellate lobes that are about 1-3 cm. wide. The lower surface of the blade exhibits concentric zones demarcated by hair lines that are equidistant from each other. The habitat of this seaweed consists of solid substrates in inner and outer flats and tide pools. Its local distribution include Bulusan in Sorsogon, Calatagan in Batangas, Siquijor Island in Negros Oriental,

and Siasi in Sulu. This seaweed is employed in industrial use as human food and an algin source (Ganzon-Fortes and Trono, 1988).

Sargassum cristaefolium C. Agardh (Plate 5)

Sargassum cristaefolium belongs to the Class Cyclosporeae of Division Phaeophyta. It is among those in Family Fucaceae of the Order Fucales (Abbott and Hollenberg, 1966).

Sargassum cristaefolium is a yellowish-brown to dark brown seaweed with discoid, conical, or branching holdfasts. It is commonly known as samo, boto-boto, or lusay-lusay in Cebuano and aragan in Ilocano (Aliño *et. al.*, 1990). It is often attached to coarse sandy coralline and rocky substrates at the inner reef area or outer reef margins. It is commonly found in Bulwarte and Bulusan in Sorsogon; Puerto Galera in Oriental Mindoro; Currimao in Ilocos Norte; Hundred Islands, Tandoyong Island, and Bolinao in Pangasinan; Batan Island in Batanes; Balayan in Batangas; Borongan in East Samar; Batan and Tangalan in Aklan; Solong-on and Siquijor in Negros Oriental; Salong-salong, Manubul Island, and Siasi in Sulu; Samal Island in Davao; and Cagwit in Surigao. It can be used as human food, medicine for goiter, antibacterial, anti-tumor, animal feed, fertilizer, and control for heavy metal pollution. It is also considered as a good source of iodine, protein, vitamin C, minerals, algin, tannins, phenols, and growth regulators (Ganzon-Fortes and Trono, 1988).

Turbinaria ornata (Turner) C. Agardh (Plate 6)

Turbinaria ornata is very closely related to *Sargassum cristaefolium*. Like *Sargassum cristaefolium*, it also belongs to Division Phaeophyta, Class

Cyclosporeae, Order Fucales, and Family Fucaceae (Abbott and Hollenberg, 1966).

Turbinaria ornata is a yellowish-brown to dark brown seaweed with coarse, branched holdfasts. It commonly grows on coralline rocks in calm waters or in rock reef areas exposed to strong water turbulence. The local distribution of the seaweed include Nasugbu and Calatagan in Batangas; Currimao in Ilocos Norte; Sinait and Narvacan in Ilocos Sur; Hundred Islands and Bolinao in Pangasinan; Puerto Galera in Oriental Mindoro; Baler and Mauban in Quezon; Babuyan Islands in Batanes; Catanduanes; Liloan in Cebu; Dumaguete in Negros Oriental; Culion in Palawan; and Gnat Island and Turtle Island in Sulu. The industrial importance of *Turbinaria ornata* includes human food, fertilizer, and insect repellant. It can also serve as a source of alginates, tannins, and phenols (Ganzon-Fortes and Trono, 1988).

These seaweeds occur primarily in temperate and cold water regions, dominating the subtidal and intertidal zones. The cytological structure of Phaeophyta is basically of uni-nucleated cells containing chloroplasts, chromoplasts and numerous vesicles. The chloroplasts contain chlorophylls a and c, along with α and β carotene. The abundance of xanthophylls especially fucoxanthin gives the distinct brown coloration of this family. Their numerous vesicles contain polyphenolic by-products called physodes or fucosan granules that result from photosynthesis. These granules function to sieve excessive sunlight as protection from chloroplasts, to act as anti-foulants, and to form wood plugs during traumatic tissue excision. The cell walls of brown algae cells are primarily composed of cellulose (Taylor, 1967).

Anti-larval Testing

1. Scoring of the Mortality

For all three seaweed extracts, the negative control set-up, which consisted of distilled water, yielded a zero percent mortality. However, at a 12.5% concentration, *Padina minor* extract resulted in an increased 4% mortality compared to the to extracts of *Sargassum cristaefolium* and *Turbinaria ornata* which still exhibited a 0% mortality. At a concentration of 25%, the *Sargassum cristaefolium* extract rose to a 91.67% mortality while that of *Padina minor* and *Turbinaria ornata* were 20% and 4.17% respectively. Starting from 37.5% concentration, all three seaweed extracts induced 100% mortality (Table 2).

The positive control set-up, which consisted of varying solutions of water-based Baygon, was able to induce 100% mortality from a concentration of 12.5% to 100% (Table 3).

2. Data Analysis

The Pearson Correlation Coefficient (r) values of *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata* were 0.84, 0.75, and 0.83, respectively. When the Sample Coefficient of Determination was computed, the r^2 values of *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata* were 0.71, 0.56, and 0.69, respectively.

DISCUSSION

Processing of Seaweeds

1. Collection and Processing of Specimens

The predominance of a species in a particular area during collection period is indicative of the ecological conditions. In their location, the tall *Sargassum cristaefolium* specimens and the *Turbinaria ornata* situated on high rocks would have been exposed during low tide. The desiccating action of exposure to sunlight may account for the darker color observed in these specimens. It may also explain the low species diversity in the area. *Padina minor* only reaches a height of 5 to 8 centimeters. Therefore, it was not frequently exposed to light but survived in the area because of its large photosynthetic flabellate lobes.

Since most of the specimens were located in an unprotected area of the cove, strong holdfasts are required in order to be predominant. This explains why *Sargassum cristaefolium* with its discoid holdfasts were more abundant that *Padina minor*.

2. Preparation of the Extract

Placing the extracts in a hot water bath at 50°C until the texture of the mixture was syrupy ensured the complete removal of volatile ethanol through evaporation. In this manner, the mortality could be attributed as a result of pure extract rather than the contributing effect of ethanol.

Collection of *Anopheles flavirostris* larvae

Fourth instar larvae were selected as test organisms because, among the four larval stages, it was the least vulnerable.

Anti-larval Testing

1. Performance of the Test

Two mL of test solution was used in order to ensure that all the larval set-ups got an equal amount of treatment.

2. Data Analysis

Sargassum cristaefolium extract was effective at a concentration as low as 25%, almost inducing a hundred percent mortality. On the other hand, both *Padina minor* and *Turbinaria ornata* extracts caused 100 % mortality at a concentration of 37.5% (Table 4).

The results indicate that *Sargassum cristaefolium* is more effective than both *Padina minor* and *Turbinaria ornata* since it demonstrates an earlier increase in percent mortality at a lower concentration. Given the same concentration of 25%, *Sargassum cristaefolium* yielded 91.67 percent mortality while *Padina minor* and *Turbinaria ornata* only induced 20 percent mortality and 4.17 percent mortality, respectively (Graph 1).

In a comparison between the efficacy of the extracts and the positive control, *Padina minor* and *Turbinaria ornata* extracts are as effective as the water-based Baygon from concentrations 37.5 to 100% (Graphs 2 and 4). On the other hand,

Sargassum cristaefolium yielded a percent mortality almost equal to that of the positive control beginning at a concentration of 25% (Graph 3).

To further establish the efficacy of the extracts, statistical analysis using Pearson Correlation Coefficient and Sample Coefficient of Determination was adopted. Pearson Correlation Coefficient shows the relationship between the induced percent mortality and the extract concentration. Pearson Correlation Coefficient values range from negative one to positive one. A value close to positive one would indicate a strong positive linear relationship between extract concentration and percent mortality. A value close to negative one is interpreted as a strong negative linear relationship. A value of zero indicates that no relationship exists between the two variables. The Sample Coefficient of Determination value corresponds to the percentage of total variation attributed to the extract concentration.

With a Pearson Correlation Coefficient (r) value of 0.84, the resultant larval mortality is strongly related to the concentration of *Padina minor* extract (Table 5). A positive linear relationship exists between the two variables. Analysis of the Sample Coefficient of Determination (r^2) yielded a value of 0.71, indicating that 71% of the total variation in the values of the percent mortality can be accounted for by the linear relationship with the concentrations of the seaweed extract. The remaining 29% may be due to lack of food, crowding, or cannibalism.

An analysis of the Pearson Correlation Coefficient of *Turbinaria ornata* resulted in an r-value of 0.83 (Table 5). This suggests a strong, positive, linear relationship between larval percent mortality and the concentrations of *Turbinaria ornata* extract. The Sample Coefficient of Determination value of 0.68 denotes that 69% of the total

variation in the values of percent mortality can be explained by the linear relationship with the concentrations of *Turbinaria ornata* while 31% may be a result of the lack of food, crowding, or cannibalism.

Compared to the r-values of *Padina minor* and *Turbinaria ornata*, the Pearson Correlation Coefficient value of 0.75 for *Sargassum cristaefolium* exhibited a weaker relationship between larval mortality and extract concentration (Table 5). Like the two previous analyses, it also showed a positive linear relationship. The 0.56 value of the Sample Coefficient of Determination means that 56% of the total variation in the values of percent mortality can be accounted for by the linear relationship with seaweed extract concentrations. The rest, a percentage almost equal to that of the Sample Coefficient of Determination, may be caused by lack of food, crowding, or cannibalism.

Given the assumption that concentration is the sole cause for the percent mortality, the results show that *Sargassum cristaefolium* is the most effective seaweed extract against the *Anopheles flavirostris* larvae. However the Pearson Correlation Coefficient value for *Sargassum cristaefolium* contradicts this assumption. Through this analysis, it has been determined that extraneous factors also played a relatively large role in inducing larval percent mortality. Therefore, there is no sufficient proof to state that *Sargassum cristaefolium* is the most effective even though it rendered a high percent mortality at a low concentration.

From the data, it can state that all three seaweeds have identical larvicidal properties.

The results of the study are varied from that of Padla (1994). Both studies used *Turbinaria ornata*, but the concentration that induced 100 percent mortality were different. *Aedes aegypti* at had one hundred percent mortality at a concentration of 7.46% (Padla, 1994). However, the same extract yielded the same results on *Anopheles flavirostris* at a higher concentration of 37.5 %. Comparing the two, it would seem that *Anopheles flavirostris* is less vulnerable to *Turbinaria ornata* extract. These findings are the only comparable results between the two studies because of the difference in the other algal species used in larval testing.

SUMMARY

Malaria is one of the most debilitating diseases in the world. In the Philippines, its principal vector is *Anopheles flavirostris* which transmits the causative parasite, *Plasmodium sp.*, to man. Knowledge of the man-vector-parasite relationship can lead to proper control measures. Control of the malaria can be undertaken in two ways: treatment of the symptoms and prevention of the disease. Most efforts are focused on finding the cure, however, prevention and control of the most important mosquito-borne disease in the tropics may be found in the biodiversity of the region. (Salazar, 1989).

As a tropical country, the Philippines is endowed with a rich biodiversity, especially untapped marine resources. Brown algae are often overlooked but can be utilized as food, as medicine, and as industrial products (Chennubhotla et al., 1987). Among the other uses of brown algae is organic insecticide (Moreland, 1979).

The larvicidal properties of representative algal species were tested against *Aedes aegypti*. Compared to red and green algae, brown seaweeds exhibited the highest potential for larvicidal properties (Padla, 1994).

The larvicidal properties of brown algae exhibited on *Aedes aegypti* can further be extended to apply to *Anopheles flavirostris*. With this premise, extracts of *Padina minor, Sargassum cristaefolium and Turbinaria ornata* were selected to treat the test organism, *Anopheles flavirostris* larvae.

The results showed that *Padina minor* and *Turbinaria ornata* were as effective as water-based Baygon at a concentration of 37.5% against the *Anopheles flavirostris* larvae, while *Sargassum cristaefolium* was as effective as water-based Baygon at a lower concentration of 25%. Further analysis through Pearson Correlation Coefficient and the

Sample Coefficient of Determination showed that external factors played a greater role in inducing mortality among the larvae treated with *Sargassum cristaefolium* extract than among the larvae of *Padina minor* and *Turbinaria ornata.* These factors could have contributed to the increased efficacy of *Sargassum cristaefolium.*

CONCLUSION

The effective concentration for *Sargassum cristaefolium* was at 25% while both *Padina minor* and *Turbinaria ornata* were effective at 37.5%. The descriptive study of the efficacy of *Sargassum cristaefolium* cannot be supported by the results of Pearson Correlation Coefficient. The statistical analysis showed that 44% of the total variation in the percent mortality values could be attributed to extraneous factors. With all factors in consideration, all three seaweeds have identical larvicidal properties and are as effective as water-based Baygon only after a 37.5% concentration.

RECOMMENDATIONS

1. This paper has provided a preliminary data on the larvicidal effects of *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata*. An experimental study inclusive of statistical analysis consisting of several replicates may be done in the future.
2. Since this study has established that all the three seaweeds yielded 100% mortality starting at 37.5%, future studies can use concentrations between zero and this value.
3. Larval mortality was observed even before the 24-hour exposure to the treatment was completed. The efficacy of the seaweed extracts may be measured using the parameter of time increments.
4. Other studies can test the seaweed extracts on the different stages of the *Anopheles flavirostris* life cycle to determine at which stage would the test organism be most vulnerable.
5. Different literatures present different causes for the larvicidal property. A study could be conducted to determine the specific component of brown algae that would cause larval mortality.

BIBLIOGRAPHIC ENTRY

_____. (1993, April). Seaweeds for a bountiful agricultural harvest. *SICEN Newsletter*, p. 3.

Abbott, I.A , Hollenberg, G.J. (1966). *Supplement to Smith's marine algae of the Monterey Peninsula.* California: Stanford University Press.

Alio, P.M., Cajipe, G.J B., Ganzon-Fortes, E.T., Licuanan, W.R.Y., Montano, N.E., Tupas, L.M. (1990, February). The use of marine organisms in folk medicine and horticulture: a preliminary study. *SICEN Leaflet 1*, pp. 2, 6, 8.

Chapman, V.J., Chapman, D.J. (1980). *Seaweeds and their uses.* London: Chapman and Hall Ltd.

Chennubhotla, B.S.K., Kaliaperumal, N., Kalimuthu, S. (1987). *Seaweed research and utilization in India.* India: Central Marine Fisheries Research Institute.

Ganzon-Fortes, E.T., Trono, G.C. Jr. (1988). *Philippine seaweeds.* Manila: National Bookstore, Inc.

Goma, L.K.H. (1966). *The mosquito.* London: Hutchinson & Co., Ltd.

Hoppe, H., Ledzing, T., Tanaka, Y. (1979). *Marine algae in pharmaceutical science.* New York: Walter de Gaiyter.

Hurlando-Ponce, A.Q., Luhan, M.R.J., Guanzon, N.G. Jr. (1992). *Seaweeds of Panay.* Tigbauan, Iloilo: SEAFDEC Aquaculture Dept.

Moreland, P. (1979). Edible seaweeds of North Luzon, Philippines: market price, local taste preference, seaweed recipes, and other local uses. *Philippine Journal of Science, vol 108, 41-53.*

Padla, E.P. (1994). *Antibacterial, antifungal, and larvicidal properties of selected seaweeds in Bolinao, Pangasinan*. Unpublished MS Thesis, University of Santo Tomas, Manila.

Roberts, L.S., Janovy, J. Jr. (1996). *Foundations of parasitology* (5th ed.). Boston: Wm.C. Brown Publishers.

Ronquillo, I.A., Llana, M.E.G. (1982). Seaweeds, their uses. *Fisheries Newsletter*, p. 25.

Salazar, N. (1989). *Malaria situation in the Philippines*. Alabang, Muntinlupa: Research Institute for Tropical Medicine.

Stephenson, W.M. (1965). The effect of hydrolyzed seaweed on certain plant pests and disease. In E.G. Young and J.L. McLachlan (Ed.), *Proceedings of the 5th international seaweed symposium, Halifax, August 1965*. Oxford: Pergamon Press.

Taylor, W.R. (1967). *Marine algae of the eastern tropical and subtropical coasts of the Americas*. USA: University of Michigan Press.

Trono, G.C. Jr. (1997). *Field guide: atlas of the seaweed resources of the Philippines*. Makati: Bookmark, Inc.

Venkataraman, G.F., Goyal, S.K., Kaushik, B.D., Dhury, P.R.C. (1974). *Algae form and function*. New Delhi: Today and Tomorrow Publishers.

APPENDIX

Appendix A

Figure 1. Map of the Philippine Islands (A) and the location of seaweed collection in Palawan (B).

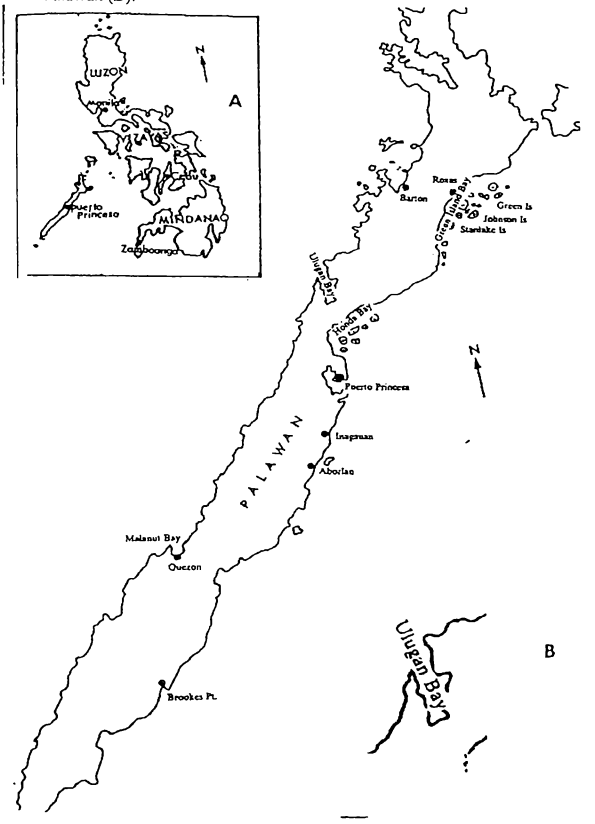
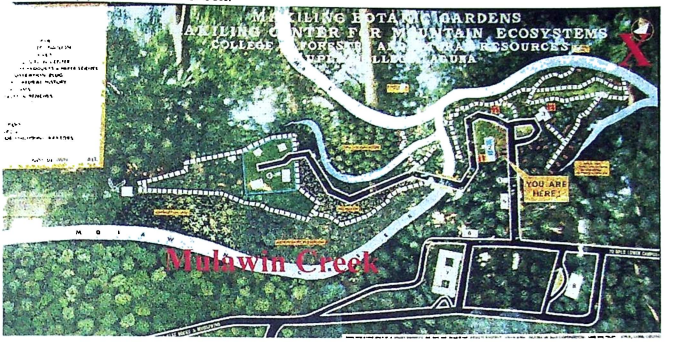


Figure 2. Map of the Makiling Forest Reserve showing the location of larval breeding site (x) in Mulawin Creek.



Appendix B

Table of the proportions of seaweed extract to distilled water to produce desired concentrations.

Concentration (%)	Amount of Extract (mL)	Amount of Distilled Water (mL)
12.5	0.25	1.75
25	0.50	1.5
37.5	0.75	1.25
50	1.0	1.0
62.5	1.25	0.75
75	1.5	0.5
87.5	1.75	0.25
100	2.0	0

Appendix C

Table 1. Master table of the pupal, survival, and mortality count of 25-4th instar *Anopheles flavirostris* larvae after 24 hour exposure to varying concentrations of *Padina minor, Sargassum cristaefolium, and Turbinaria ornata* solutions.

Concentration (%)	*Padina minor*			*Sargassum cristaefolium*			*Turbinaria ornata*		
	Pupal Count	Survival Count	Mortality Count	Pupal Count	Survival Count	Mortality Count	Pupal Count	Survival Count	Mortality Count
0	0	25	0	0	25	0	0	25	0
12.5	0	24	1	1	24	0	2	23	0
25	0	20	5	1	2	22	1	23	1
37.5	0	0	25	0	0	25	0	0	25
50	0	1	24	0	0	25	0	0	25
62.5	0	0	25	0	0	25	1	1	24
75	0	0	25	0	0	25	0	0	25
87.5	0	0	25	0	0	25	2	0	25
100	0	0	25	0	0	25	0	0	25

Table 2. Table of the percent mortality induced by *Padina minor, Sargassum cristaefolium, and Turbinaria ornata* solutions on *Anopheles flavirostris* larvae taken after 24 hours of exposure to treatment

Solution Concentrations (%)	Percent Mortality (%)		
	Padina minor Extract	*Sargassum cristaefolium* Extract	*Turbinaria ornata* Extract
0	0	0	0
12.5	4	0	0
25	20	91.67	4.17
37.5	100	100	100
50	96	100	100
62.5	100	100	100
75	100	100	100
87.5	100	100	100
100	100	100	100

Table 3. Table of the percent mortality induced by the positive control set-up (i.e. water based Baygon insect repellant) on *Anopheles flavirostris* larvae taken after 24 hours of exposure to treatment.

Solution Concentration (%)	Percent Mortality (%)
	Water-based Baygon Insect Repellant
0	0
12.5	100
25	100
37.5	100
50	100
62.5	100
75	100
87.5	100
100	100

Table 4. Table of the percent mortality induced by *Padina minor* extract, *Sargassum cristaefolium* extract, *Turbinaria ornata* extract, and water-based Baygon insect repellant on *Anopheles flavirostris* larvae after 24 hours of exposure to treatment.

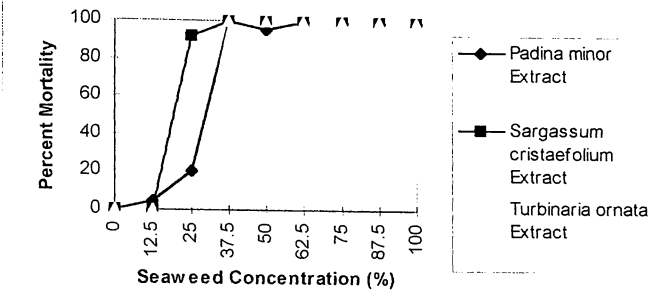
Solution Conc. (%)	Percent Mortality (%)			
	Padina minor Extract	*Sargassum cristaefolium.* Extract	*Turbinaria ornata.* Extract	water-based Baygon
0	0	0	0	0
12.5	4	0	0	100
25	20	91.67	4.17	100
37.5	100	100	100	100
50	96	100	100	100
62.5	100	100	100	100
75	100	100	100	100
87.5	100	100	100	100
100	100	100	100	100

Table 5. Table of the results of Pearson Correlation Coefficient (r) and Sample Coefficient of Determination (r^2) analysis for *Padina minor*, *Sargassum cristaefolium*, and *Turbinaria ornata*.

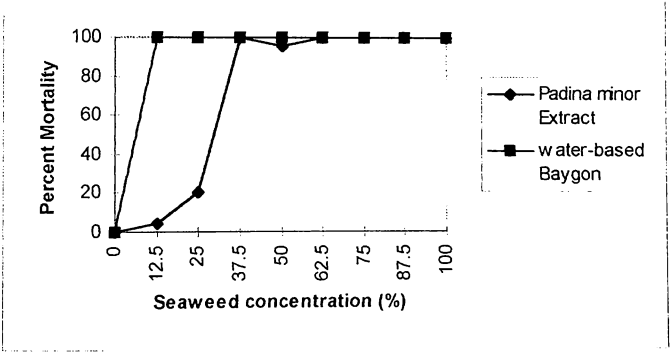
Seaweed	Pearson Correlation Coefficient (r)	Sample Coefficient of Determination (r^2)
Padina minor	0.84	0.71
Sargassum cristaefolium	0.75	0.56
Turbinaria ornata	0.83	0.69

Appendix D

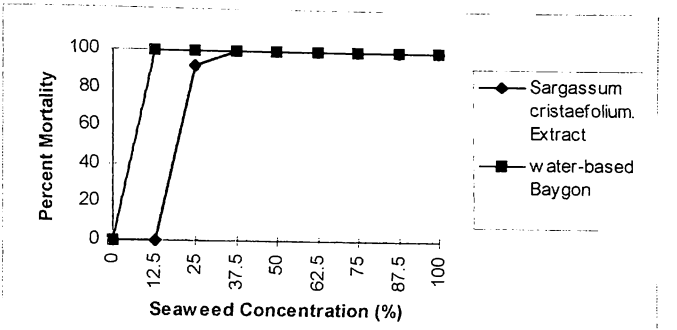
Graph 1. Line graph of the mean percentage mortality induced by the *Padina minor* extract, *Sargassum cristaefolium* extract, and *Turbinaria ornata* extract on the *Anopheles flavirostris* larvae after 24 hours of exposure to treatment



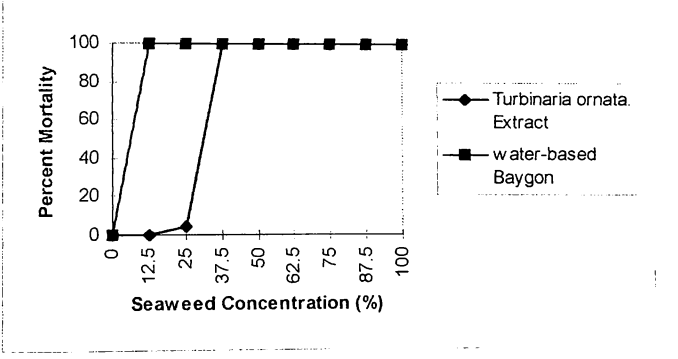
Graph 2. Line graph of the percent mortality induced by *Padina minor* extract *and* water-based Baygon insect repellant, on *Anopheles flavirostris* larvae taken after 24 hours of exposure to treatment



Graph 3. Line graph of the percent mortality induced by *Sargassum cristaefolium* extract and water-based Baygon insect repellant, on *Anopheles flavirostris* larvae taken after 24 hours of exposure to treatment



Graph 4. Line graph of the percent mortality induced by *Turbinaria ornata* extract *and* water-based Baygon insect repellant, on *Anopheles flavirostris* larvae taken after 24 hours of exposure to treatment



Appendix E

Plate 1. Draining excess water from the ice tray compartments containing 25-4th instar *Anopheles* flavirostris larvae in the laboratory of the Institute of Plant Breeding

Plate 2. Test organisms in some of the concentrations of test solutions

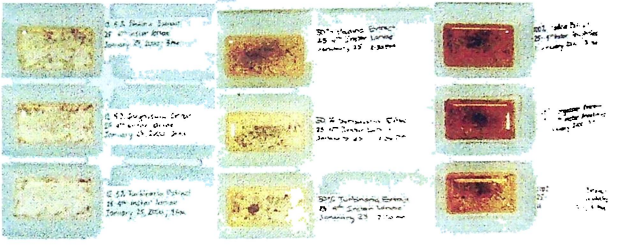


Plate 3. Test organisms in some of the positive and negative control set-ups

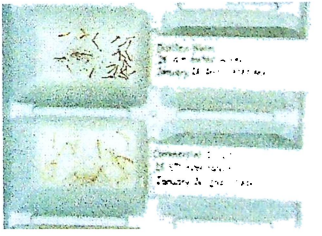


Plate 4. Collection site in Central Park, Ulugan Bay, Puerto Princesa, Palawan

Plate 5. Dried specimen of *Sargassum cristaefolium;* Habit of *Sargassum cristaefolium*

Plate 6. Dried specimen of *Padina minor;* Habit of *Padina minor*

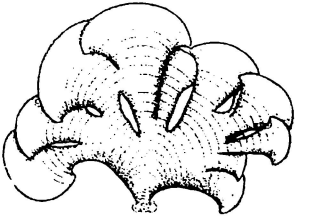
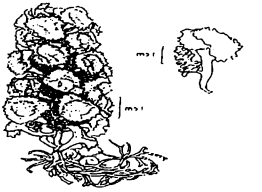


Plate 7. Dried specimen of *Turbinaria ornata;* Habit of *Turbinaria ornata*



Appendix F

1. Computation for r and r^2 of *Padina minor*.

Let X = the concentration of *Padina minor*

Y = the percent mortality of *Anopheles flavirostris* larvae

r = the value of the Pearson Correlation Coefficient

r^2 = the value of the Sample Coefficient of Determination

Extract Concentration or X (%)	Percent Mortality or Y (%)	XY
0	0	0
12.5	4	50
25	20	500
37.5	100	3750
50	96	4800
62.5	100	6250
75	100	7500
87.5	100	8750
100	100	10000

a. $\Sigma X = 0 + 12.5 + 25 + 37.5 + 50 + 62.5 + 75 + 87.5 + 100 = 450$

b. $\Sigma X^2 = 0 + 12.5^2 + 25^2 + 37.5^2 + 50^2 + 62.5^2 + 75^2 + 87.5^2 + 100^2 = 31875$

c. $(\Sigma X)^2 = (450)^2 = 202500$

d. $\Sigma Y = 0+ 4 + 20 + 100 + 96 + 100 + 100 + 100 + 100 = 620$

e. $\Sigma Y^2 = 0+ 4^2 + 20^2 + 100^2 + 96^2 + 100^2 + 100^2 + 100^2 + 100^2 = 59632$

f. $(\Sigma Y)^2 = (620)^2 = 384400$

g. $\Sigma XY = 41600$

h. $\Sigma X \Sigma Y = 279000$

i. $r = \dfrac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\sqrt{(9375)(16920.89)}} = \dfrac{41600 - 31000}{} = 0.84$

$$\sqrt{[\Sigma X^2 - \frac{(\Sigma X)^2}{n}][\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}]}$$

j. $r^2 = 0.71$

2. Computation for r and r^2 of *Sargassum cristaefolium.*

Let X = the concentration of *Sargassum cristaefolium*

Y = the percent mortality of *Anopheles flavirostris* larvae

r = the value of the Pearson Correlation Coefficient

r^2 = the value of the Sample Coefficient of Determination

Extract Concentration or X (%)	Percent Mortality or Y (%)	XY
0	0	0
12.5	91.67	1145.88
25	100	2500
37.5	100	3750
50	100	5000
62.5	100	6250
75	100	7500
87.5	100	8750
100	100	10000

a. $\Sigma X = 0 + 12.5 + 25 + 37.5 + 50 + 62.5 + 75 + 87.5 + 100 = 450$

b. $\Sigma X^2 = 0 + 12.5^2 + 25^2 + 37.5^2 + 50^2 + 62.5^2 + 75^2 + 87.5^2 + 100^2 = 31875$

c. $(\Sigma X)^2 = (450)^2 = 202500$

d. $\Sigma Y = 0+ 91.67 + 100 + 100 + 100 + 100 + 100 + 100 + 100 = 691.67$

e. $\Sigma Y^2 = 0+ 91.67^2 + 100^2 + 100^2 + 100^2 + 100^2 + 100^2 + 100^2 + 100^2 = 68403.39$

f. $(\Sigma Y)^2 = (691.67)^2 = 478407.39$

g. $\Sigma XY = 43541.75$

h. $\Sigma X \Sigma Y = 311251.5$

i. $r = \dfrac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\sqrt{[\Sigma X^2 - \frac{(\Sigma X)^2}{n}][\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}]}} = \dfrac{43541.75 - 34583.5}{\sqrt{(9375)(15247.01)}} = 0.75$

j. $r^2 = 0.56$

3. Computation for r and r^2 of *Turbinaria ornata.*

Let X = the concentration of *Turbinaria ornata*

Y = the percent mortality of *Anopheles flavirostris* larvae

r = the value of the Pearson Correlation Coefficient

r^2 = the value of the Sample Coefficient of Determination

Extract Concentration or X (%)	Percent Mortality or Y (%)	XY
0	0	0
12.5	0	0
25	4.17	104
37.5	100	3750
50	100	5000
62.5	100	6250
75	100	7500
87.5	100	8750
100	100	10000

a. $\Sigma X = 0 + 12.5 + 25 + 37.5 + 50 + 62.5 + 75 + 87.5 + 100 = 450$

b. $\Sigma X^2 = 0 + 12.5^2 + 25^2 + 37.5^2 + 50^2 + 62.5^2 + 75^2 + 87.5^2 + 100^2 = 31875$

c. $(\Sigma X)^2 = (450)^2 = 202500$

d. $\Sigma Y = 0 + 0 + 4.17 + 100 + 100 + 100 + 100 + 100 + 100 = 604.17$

e. $\Sigma Y^2 = 0 + 0 + 4.17^2 + 100^2 + 100^2 + 100^2 + 100^2 + 100^2 + 100^2 = 60017.31$

f. $(\Sigma Y)^2 = (604.17)^2 = 365009.31$

g. $\Sigma XY = 41354$

h. $\Sigma X \Sigma Y = 271872$

i. $$r = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{n}\right]\left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}\right]}} = \frac{41354 - 30208}{\sqrt{(9375)(19460.72)}} = 0.83$$

j. $r^2 = 0.68$