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IN VIVO

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47th ANNUAL MACUB CONFERENCE

Conference Theme

Unique Biology Opportunities in Research and Community Involvement

Saturday, November 1, 2014
Molloy College
Rockville Centre, New York

Keynote Speakers

David Micklos
Executive Director, DNA Learning Center
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

and

Donald Stearns
Professor of Biology
Wagner College, Staten Island, NY

Instructions for Authors

IN VIVO is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

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In This Issue:

Save the Date

47th ANNUAL MACUB CONFERENCE

Saturday, November 1, 2014

Molloy College

Rockville Centre, New York

Message from
Past President Gary Sarinsky

Dear Colleagues:

I am writing to let you know that I have requested that the MACUB Board of Directors allow me to step down as President of MACUB effective June 30, 2014. Over the past years, I have served MACUB in various positions which have included Member-at-Large, Vice-President and, as President since 1995. I take pride that during these past 19 years our membership increased from 31 to over 500 which includes biologists from 67 colleges and universities in the New York-New Jersey-Connecticut Metropolitan Region. Attendance at our annual meetings has ranged from 275 to 350 members each year. We have held annual conferences with exceptional programs and keynote speakers and we now average over 110 college student poster presentations. *IN VIVO* has developed into a peer reviewed on-line journal and the Benjamin Cummings/MACUB Student Research Grant was established. All of this could not have come to fruition without the active and dedicated members of our Boards of Directors over the years.

I want you to know how much I enjoyed and valued the time that we have known each other and I treasure the professionalism and personal relationships that I have developed with most of you. I will continue to support and participate in MACUB as I now take the Board of Directors' position as Past President.

I want to wish Kathleen Nolan good luck as she takes over the Presidency today. I have great confidence that she and the new Executive Board are highly capable and energetic and that MACUB will be in excellent hands.

Sincerely,

Gary Sarinsky

Molloy College is honored to host the 47th Annual MACUB Conference! Many members of the Molloy Community have a long established relationship with MACUB, and we look forward to further involving our students and faculty in your activities.

Molloy College is in a "renaissance" on a number of levels. Architecturally, MACUB guests will witness the construction of our Barbara H. Hagan Center for Nursing, a 50,000 square foot facility to house all of our nursing programs, including our two doctoral programs of study. You will be enjoying Conference keynote addresses in our new Madison Theatre, adjoining the "Public Square", housing a communal center for students, "smart classrooms", an "Information Commons" and conference rooms. A second residency hall, Maria Regina Hall, will have been completed in September, 2014, on the south side of the campus, adjacent to Fitzgerald Hall, allowing our programs to experience a more national and global attraction.

On an academic level, the Molloy faculty is industrious in creating novel advances in our undergraduate and graduate programs. With over 50 graduate and undergraduate programs to choose from, and being recognized by US News and World Report as an outstanding comprehensive university, Molloy College has achieved recognition as a place where students have a choice and can be effective competitors in their future goals.

The Department of Biology, Chemistry, and Environmental Studies is also experiencing renaissance of its own. We now have two state-of-the-art laboratories for our students and faculty to conduct cellular, molecular and biochemical research with contemporary equipment. Our student research initiative, providing research opportunities in locations such as Winthrop Medical Center, Weil Medical College of Cornell University, Downstate Medical Center and the Population Council at Rockefeller University provide excellent avenues for internships for our Biology majors. Our CERCOM (Center for Environmental Research and Coastal Ocean Monitoring) facility in West Sayville, NY, will provide enhanced opportunities for Environmental Studies and Earth Science majors, featuring new and innovative internships for any student interested in careers in these exciting and expanding fields.

We anticipate, with great excitement, the arrival of the MACUB Conference on November 1, 2014. This great organization that has done so much for the advancement of students and faculty in regional colleges and Universities now becomes an integral part of our "renaissance," more than ever, both college-wide and in our own Department of Biology, Chemistry and Environmental Studies.

Welcome, MACUB!
Anthony Tolvo, PhD.
Professor of Biology
Dean, Division of Natural Sciences, Mathematics and Computer Studies, Allied Health Sciences and Speech and Language Pathology

Keynote Address

Course Based Research Projects in Gene and Genomic Analysis

presented by

David Micklos
DNA Learning Center
Cold Spring Harbor Laboratory

David Micklos is founder and executive director of the DNA Learning Center (DNALC), the nation's first science center devoted to public genetics education. The DNALC is an operating unit of Cold Spring Harbor Laboratory, ranked #1 worldwide for citation impact in molecular biology and genetics. The DNALC has an annual operating budget of US $3.2 million and employs a multidisciplinary staff of 25 biologists, certified teachers, science communicators, designers, and computer programmers. With satellite centers in Nassau County and Harlem, the DNALC's six teaching laboratories provide hands-on science experiences to 30,000 students per year. An estimated 300,000 students per year use methods and commercial lab kits developed by the DNALC. With continuous support from the National Science Foundation since 1987, as well as other foundations, the DNALC has provided intensive lab or Internet training to more than 9,000 science teachers at workshops conducted in 47 United States, the District of Columbia, Puerto Rico, and 14 foreign countries. The DNALC was the model for teaching laboratories established at 15 museums and higher education institutions around the world, which provide labs for 130,000 students annually. The DNALC's Internet portal hosts 21 proprietary content and bioinformatics sites, along with other digital multimedia, receive 6 million visitors annually. Mr. Micklos' three textbooks, *DNA Science*, *Laboratory DNA Science*, and *Genome Science,* as well as many articles have helped popularize DNA education in high school and beginning college classrooms. He received the 1990 Dana Award for Pioneering Achievement in Education, the 2011 *Science* Prize for Online Resources in Education, and the 2012 Genetics Society of America Award for Excellence in Education. He is a Fellow of the American Association for the Advancement of Science (AAAS) and is the only CSHL staff member to receive an honorary Doctor of Science degree from its Watson School of Biological Sciences.

Keynote Address

Teaching and Learning With a Sense of Community

presented by

Donald E. Stearns
Department of Biological Science
Wagner College

Donald E. Stearns is a Professor in the Department of Biological Sciences, Wagner College, Staten Island, NY. He has spent more than 26 years in higher education, most of it as a faculty member teaching undergraduate and graduate students in the biological sciences. His degrees include the A.B. in Biology (Dartmouth College), the M.S. in Zoology (University of New Hampshire), and the Ph.D. in Zoology (Duke University). As a full-time faculty member, he taught for several years at the Universidad Autónoma de Baja California and Rutgers University before joining Wagner College in 1994. In 1998, he became the college's first representative for Project Kaleidoscope Faculty for the 21st Century, where he shared best teaching and learning practices with other STEM instructors. He has received all three Wagner College exceptional performance awards for teaching, scholarship, and college service. Donald was nominated for the Thomas Ehrlich Faculty Award for Service-Learning by the college provost in 2003 and by the president in 2004. In 2004, his First-Year Program learning community was selected as a national SENCER model by the National SENCER (Science Education for New Civic Engagements and Responsibilities) Program. In 2007, he was the principal investigator for Wagner College in a two-year, collaborative research project involving four institutions of higher education, funded by the National Science Foundation. The research was, in part, designed to determine which pedagogical protocols are most effective in enhancing the development of critical thinking and civic thinking skills in introductory science courses. In 2008, Donald was awarded the Martha Megerle Endowed Chair for his distinguished record of scholarship. In 2010, he was nominated for the 2010 U.S. Professor of the Year Award by the college provost. Recently, Donald participated in a six-week, U.S. State Department-sponsored, summer civic leadership project (*Young African Leaders Initiative*) that prepares promising Sub-Saharan Africans to take on effective civic leadership roles in their home countries.

The Effect of Neupogen® on Rodlet Cell Activity and Distribution in the Heart of the Goldfish, *Carassius auratus.* A LM and EM Study

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Abstract

Goldfish, *Carassius auratus* were treated for 4 weeks with i.p. injections of the colony stimulating factor Neupogen, known to promote granulopoiesis in higher vertebrates. By the end of the treatment period the experimental fish had a greater number of actively mature and spent rodlet cells in the bulbus arteriosus and ventricle of the heart when campared to the control fish. Our study adds to the evidence that the rodlet cell is an immune effector cell in fish, presumably of the granulocytic line. Furthermore, to our knowledge this is the first study that shows the effect of Neupogen on leucopoiesis in a lower vertebrate species.

Introduction

Since its identification over a century ago, the rodlet cell[1] (RC), in fishes has posed a major conundrum. Originally described as being a parasite[1] this cell's true origin and function have come into question over the years. Found in the tissues of both marine and freshwater species[2-6], two major hypotheses have come forth. According to the exogenous hypothesis the cell represents a parasite, most likely a member of the Apicomplexa order[4,7,8]. The tenet of the more prevalent endogenous hypothesis claims that the RC is a special immune effector cell, part of the fish's non-specific immune response[9-20]. A third hypothesis combines the two and suggests that the RC is itself a leucocyte that contains an endosymbiont in the form of the rodlets[21].

Studies on the immunological role of the RCs have focused primarily on their mobilization and recruitment in response to bacterial infection[22,23], parasitic invasion[9,10,15,24-27], stressors[17,28] and environmental toxicants[27,29-31]. Exposed tissues and organs including gills[27], intestine[10], skin[17,28], liver[11], kidney[9,24] and brain[26] invariably showed a greater number of RCs than uninfected or unexposed fish of the same species. In addition, it has been shown that RCs respond to anti-inflammatory agents such as the synthetic corticosteroid dexamethasone[18], providing further evidence as to their possible role in the immune response of the fish.

In view of the increasing evidence that RCs are active participants in the fish's immune system, it was the purpose of this study to investigate whether, as an effector cell, the RCs of the goldfish, *Carassius auratus* would respond to the colony stimulating factor, Neupogen (filgrastim) often used to promote development of granulocytes in humans suffering from neutropenia, a common side effect of chemotherapy[32]. In view of an earlier study that showed that RCs in the goldfish heart responded to the anti-inflammatory dexamethasone[18] and the fact that we were able to obtain the fish from and maintain them in a non-contaminated environment, we selected this species for our study. Our results described herein show that treatment with this chemical resulted in an increase in the number and activity of the RCs within the bulbus arteriosus and ventricle of the goldfish heart. Our findings shed further light on the putative function of the RC as a player in the immune system of fish.

Materials and Methods

Neupogen® (filgrastim)

This 175 amino acid protein is a granulocyte colony stimulating factor (G-CSF) analog (Amgen Inc.) produced by recombinant DNA technology through transfecting *E. coli*[32]. This factor is non-species specific. In addition to humans, it has proven to be effective in inducing the proliferation

and differentiation of granulocytes in monkeys, dogs, rats, mice and hamsters[33].

Experimental Animals and Design

Goldfish, *C. auratus* were obtained from a local supplier. Three groups of ten fish each were separated into 10-gallon aquaria and maintained under the controlled conditions of the laboratory as previously described[30]. They were fed twice a day with commercially prepared Tetramin goldfish food. The fish were allowed to acclimate for one week prior to the start of the experiment.

The experimental fish were each injected intraperitoneally once a week with 1.0 μl of Neupogen (obtained from Amgen Inc.) for four weeks. The drug was delivered via a 26-gauge needle mounted on a 5 μl Hamilton syringe. Two control groups were used. One group received equal volume injections of saline and the other remained uninjected to preclude any effects of stress caused by handling.

Tissue Preparation

At the time of sacrifice each fish was overanesthetized in a solution of MS-222 (tricaine methanesulphonate (Sigma)). Under the low power magnification of a dissection microscope, the heart was carefully removed from the pericardial sac and processed for LM and EM according to procedures previously described[27,30,34]. Five fish from each group were paraffin processed while five were processed for epon embedding.

For LM, paraffin sections 6 μm thick were cut, mounted on gelatin coated slides and stained with Masson's trichrome. For TEM, sectioning was carried out on a Leica Ultracut UCT ultramicrtome. Thick (1 μm) epon sections were stained with toluidine blue. Sections, 80 to 100 nm in thickness were collected on uncoated grids and stained with saturated aqueous uranyl acetate followed by lead citrate. Observatons were made at 80 Kv on an FEI Tecnai Spirit transmission electron microscope.

Biometric and Statistical Evaluation

RCs were counted at the light level from paraffin sections in the bulbus arteriosus and ventricle. The cells in each area were counted separately. Five randomly selected areas (151,976 μm^2) for each fish, stained with Masson's trichrome to facilitate RC identification, were screened. This was in accordance with the protocol of Dezfuli *et al*.[29]. Comparisons between the groups and areas were made using a two-factor ANOVA in conjunction with Tukey's post hoc test.

Results

The Neupogen treated fish had a significantly greater number ($p<0.05$) of RCs in the heart when compared to the saline injected and untreated control fish (Table I). In the experimental group, the number of RCs did not differ ($p>0.05$) between the bulbus arteriosus and ventricle while in both control groups, the number of RCs in the bulbus arteriosus exceeded those in the ventricle. In each area, bulbus arteriosus (28.9 $\pm$ 4.13) and ventricle (28.4 $\pm$ 4.95), the number of mature RCs was greatest in the experimental fish ($p<0.05$). Although the number of spent RCs was significantly lower in the same areas (2.2 $\pm$ 0.98 and 2.6 $\pm$ 1.23) in the experimental fish when compared to the mature cells, they still exceeded those in the untreated controls while they appeared to be lacking in the saline injected fish.

In the experimental group, the RCs with numerous mature rodlets were most often seen to

Table I: Number of RCs Per Area (151,976 μm^2) in Neupogen Treated and Control Fish at 4 Weeks (Mean $\pm$ S.D.)

	Mature RCs		Spent RCs	
	Bulbus Arteriosus	Ventricle	Bulbus Arteriosus	Ventricle
Neupogen	28.9 $\pm$ 4.1[a]	28.4 $\pm$ 4.9[a]	2.2 $\pm$ 1.3[a]	
Saline	8.2 $\pm$ 3.6[b]	1.5 $\pm$ 0.9[b]	----------	----------
Untreated	5.4 $\pm$ 1.7[b]	2.6 $\pm$ 2.3[b]	0.2 $\pm$ 0.4[b]	0.1 $\pm$ 0.6[b]

[a,b]Different letters in the same column indicate a significant difference (ANOVA, $p<0.05$)

be associated with the endothelium of the vascular lumen of both the bulbus arteriosus and the ventricular endocardium (Figures 1, 2, 3). They typically formed a cluster or chain-like aggregation (margination) in the former (Figure 2). Occasionally, mature RCs were seen free within the luminal space of the bulbus arteriosus and ventricular cavity (Figure 4). The mature cells were often seen discharging their rodlets into the vascular space using an apocrine mode of secretion (Figure 5). At the point of secretion the cells formed adhesion junctions with the neighboring endothelium (Figures 5, 6). The cells seemed to secrete their rodlets with ease i.e., without disrupting the surrounding endothelial cells. Free rodlets could be observed within the luminal space of the vessel (Figure 7). On rare occasions the released rodlets appeared to be taken up by macrophage-like cells (Figure 8).

In contrast to the experimental fish, the RCs of the control fish were only occasionally seen to be associated with the endothelium and margination was lacking.

There was a greater number of active RCs in the experimental fish than the control fish. The spent RCs typically were smaller than the mature cells, more rounded in appearance and became apoptotic (Figure 9). On occasion, the membrane of these cells formed a scalloped border as the fibrillar coat retracted (Figure 10). The spent cells were scattered about, sometimes co-mingling with the mature RCs (Figure 11).

Discussion

Our results indicate that Neupogen, a granulocyte stimulator, affects both the number and activity of RCs in the heart of the goldfish, *C. auratus*. The treated fish, in comparison to the controls, had a greater number of mature RCs, many of which were actively secreting, as well as spent cells that had previously released their rodlets.

RCs have been described in the heart of other species of fish including the freshwater angelfish, *Pterophyllum scalare* [22,23], swordtail, *Xiphophorus helleri* [35], platyfish, *X. maculatus*[35] and chub, *Leuciscus cephalus*[29]. Studies on the RCs of the heart focused mainly on the bulbus arteriosus and centered on the effects of anti-inflammatories such as dexamethasone[18] and toxicants such as propanil[29] (3',4'-dichloropropionanilide). Manera *et al.*[18] found that although the number of RCs in the bulbus arteriosus of dexamethasone treated goldfish, *C. auratus* did not differ significantly from the controls, these cells were unable to adhere to the endothelium of the vessel as they did in the control fish. According to Manera *et al.*, this was caused by a down regulation of the adhesion sites i.e., selectins on the endothelium and RCs induced by the anti-inflammatory action of DXM. Fish leucocytes, like those of mammals, express receptors to natural and synthetic corticosteroids[36]. Thus, Manera *et al.*[18] concluded that the RC is a piscine leucocyte most likely of the granulocytic series. Contrary to the observations of *Manera et al*., our study showed that Neupogen had a proliferative effect on the RCs and appeared to promote adhesion of these cells to the endothelium of the bulbus arteriosus and ventricle.

We observed that the RCs located close to the surface of the endothelium, particularly those discharging their rodlets, would typically form heterocellular adhering junctions in the form of desmosomes and tight junctions with the neighboring epithelial cells. This observation was consistent with those of our earlier studies on the RCs of the head kidney of the platyfish, *X. maculatus*[34] and gills of *Fundulus heteroclitus*[30] as well as those of Smith *et al*.[22,23] made on the angelfish, *P. scalare* . The latter investigators observed RCs of the angelfish to be marginated and oriented so as to discharge their rodlets between endothelial cells and into the vascular lumen of the caudal blood vessel of the eye. Although we observed margination of the RCs particularly in the endothelial layer of the bulbus arteriosus, contrary to the work of Smith et al., we found free intact rodlets as well as occasionally, whole cells within the circulation. This difference might be explained in terms of species variation and or the different locations within the animal that were the sites of focus.

Dezfuli *et al*.[29] found a two-fold effect of the herbicide propanil on the chub, *L. cephalus*. These investigators found a decrease in the number of RCs within the bulbus arteriosus accompanied by an increase in RCs within the gills coupled to several significant morphological modifications of the RCs themselves. These included varying stages of rodlet degeneration and the appearance of myelin-like bodies within the cytoplasm. Dezfuli *et al*. concluded that the bulbus arteriosus served as a storage site from which RCs could be recruited to the gills, which are anatomically and physiologically related[29], when the fish's environment became challenged. The morphological changes were most likely the effect of the toxic herbicide[29]. Contrary to Dezfuli *et al.*'s

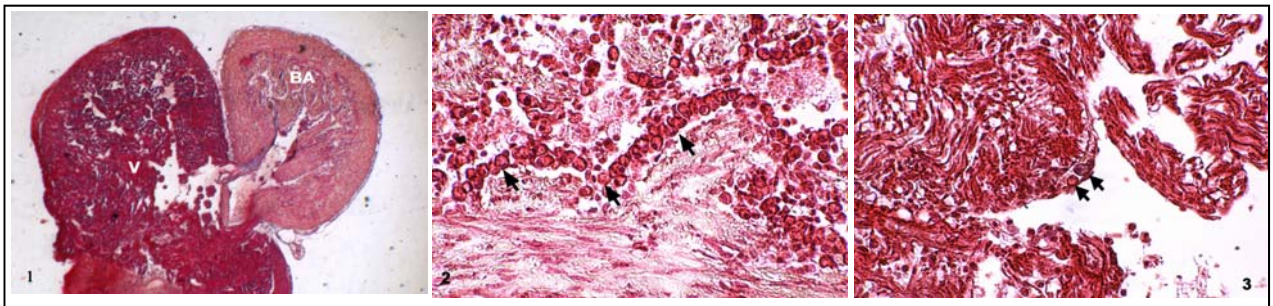

Figure 1. A longitudinal section through the bulbus arteriosus and ventricle of *Carassius auratus*. Masson's trichrome, x 220.
Figure 2. A section though the bulbus arteriosus of Neupogen treated *C. auratus* showing margination of rodlet cells (arrows). Masson's trichrome, x 750.
Figure 3. A section through the ventricle of Neupogen treated *C. auratus* showing mature RCs (arrows) underlying the endothelium. Masson's trichrome, x 750.

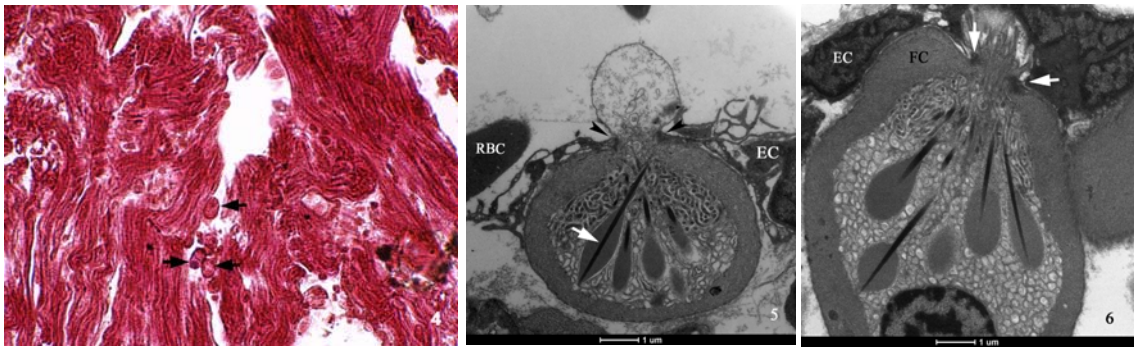

Figure 4. Free mature RCs (arrows) within the ventricular cavity of Neupogen treated *C. auratus*. Masson's trichrome, x 750.
Figure 5. A mature RC associated with the endothelium of the bulbus arteriosus of *C. auratus*, treated with Neupogen, showing early stage of apocrine secretion into the vascular lumen. Note, the heterocellular adhering junctions (arrowheads) with neighboring endothelial cells (EC). The mature rodlets contain a dense proteinaceous core (arrow) and a less dense carbohydrate cortex. A red blood cell (RBC) is also visible. TEM, x 9,700.
Figure 6. A mature RC associated with the endothelium of the bulbus arteriosus of Neupogen treated *C. auratus,* seen secreting rodlets into the vascular lumen. Note the adhering junctions (arrows) with neighboring endothelial cells (EC) and thick fibrillar coat (FC). TEM, x 9,700.

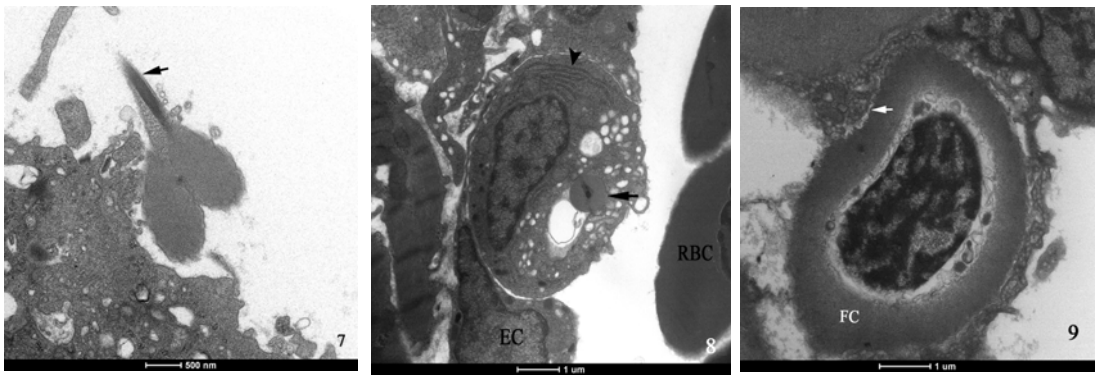

Figure 7. Free, discharged rodlets within the luminal space of the bulbus arteriosus of Neupogen treated *C. auratus.* Note the dense proteinaceous core (arrow). TEM, x 18,500.
Figure 8. A macrophage-like cell within the vascular space of the ventricle of a Neupogen treated *C. auratus.* An engulfed rodlet (arrow) is apparent as well as the ER (arrowhead). A red blood cell (RBC) and adjacent endothelial cell (EC) are also present. TEM, x 9,700.
Figure 9. A spent RC beneath the endothelium of the ventricle of a Neupogen treated *C. auratus.* Note the heterochromatic nucleus, thin cytoplasm and scalloped border (arrow) surrounding the fibrillar coat (FC). TEM, x 13,500.

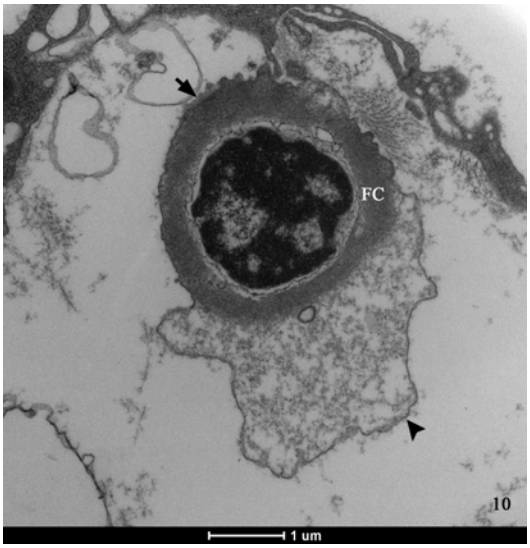

Figure 10. A spent RC with scalloped border (arrow) and extended membrane (arrowhead). Signs of apoptosis are apparent; the fibrous coat (FC) has contracted. TEM, x 13,500.

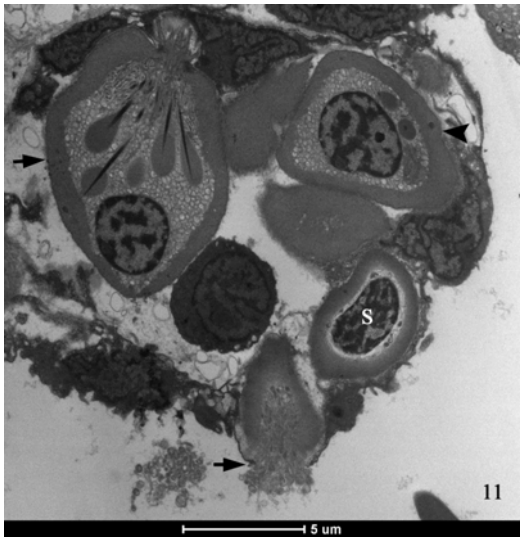

Figure 11. A group of RCs at different stages, mature (arrowhead), mature and secreting (arrows) and a spent cell (S) within the bulbus arteriosus of a Neupogen treated *C. auratus*. TEM, x 3,900.

work, we did not observe any abnormalities in the RCs and a spot check of the gills from all groups showed a paucity of RCs at this site. Our latter observation is consistent with those of Leknes[35] who found that RCs were sporadically present in small numbers within the blood vessels of the eye and gills of the swordtail, *X. helleri* and platyfish, *X. maculatus* when compared to those of the bulbus arteriosus. Thus, in our study, although Neupogen specifically affected the RCs of the heart, our observations do not preclude the possible effect of this drug on other organs of the fish which could be a factor of exposure time. Furthermore, although this study did not focus on the heart as a storage organ from which RCs may be recruited, this possibility does exist and will be the focus of future investigation.

We occasionally observed rodlets to be phagocytized by macrophage-like cells. In an earlier study, Richards *et al*.[37] observed both free rodlets as well as whole RCs to be phagocytized by macrophages and neutrophils in *Cyprinus carpio*. Richards and his co-workers interpreted their findings to mean that the RCs as well as the rodlets were parasites being neutralized by the host's immune system. The latter workers failed to recognize that the RCs and rodlets could have been defective and as such, mobilized an immune response to eliminate these defective components. The scarcity of engulfed rodlets and the lack of phagocytized RCs in our study suggest that these structures are not antigenic to the fish. On the other hand, the engulfed rodlets that we observed could have been a means of regulating the putative immune action of the rodlets i.e., a form of immunohomeostasis whereby excess rodlets would be eliminated.

In conclusion, this study supports the growing evidence that the rodlet cell is an immune effector cell in fish, most likely a component of the white blood cell line. In addition, to our knowledge, this is the first report on the efficacy of Neupogen in promoting leucopoiesis in a lower vertebrate species.

References

[1]Thelohan, P., 1892. Sur des sporozoaires indetermines parasite des poissons. J. Anat. Physiol. Paris, **28**: 161-171.

[2] Leino, R.L., 1974. Untrastructure if immature, developing, and secretory rodlet cells in fish. Cell and Tissue Research **155:** 367-384.

[3]Barber, D.L., J.E. Mills Westermann and D.D. Jensen, 1979. New observations on the rodlet cell (*Rhabdospora thelohani*) in the white sucker, *Catastomas commersoni*: LM and EM studies. Journal of Fish Biology **14:** 277-284.

[4]Mayberry, L.F., A.A. Marchiando, J.E Ubelaker and D. Kazic, 1979. *Rhabdospora thelohani* Laguesse, 1895. (Apicomplexa): new host and geographic records with taxonomic considerations. Journal of Protozoology **26:** 168-178.

[5]Bielek, E. and G. Viehberger, 1983. New aspects on the "Rodlet Cell" in teleosts. Journal of Submicroscopic Cytology **15**: 681-694.

[6]Fishelson, L., B. Russell, D. Golani and M. Goren, 2011. Rodlet cells in the alimentary tract of three genera of lizardfishes (Synodontidae, Aulopiformes): more on these enigmatic "gate-guards" of fishes. Cybium **35:** 121-129.

[7]Laguesse, E., 1895. Sur le pancreas du crenilare et particulierement sur le pancreas hepatique. Rev. Biol. Nord. **7:** 343-363.

[8]Agulleiro, B., A. Zuasti and M.T. Lozano, 1986. Rodlet cells, gamonts and differentiating microgamonts of Apicomplexa in teleosts. Acta Microscopica **9:** 23-30.

[9]Leino, R.L., 1996. Reaction of rodlet cells to myxosporean infection in kidney of the bluegill, *Lepomis machrochirus*. Canadian Journal of Zoology **74:** 217-225.

[10]Dezfuli, B.S., S. Capuano and M. Manera, 1998. A description of rodlet cells from the alimentary canal of *Anguilla anguilla* and their relationship with parasitic helminthes. Journal of Fish Biology **53:** 1084-1095.

[11]Dezfuli, B.S., E. Simoni, R. Rossi and M. Manera, 2000. Rodlet cells and other inflammatory cells of *Phoxinus phoxinus* infected with *Raphidascaris acus* (Nematoda). Diseases of Aquatic Organisms **43:** 61-69.

[12]Kramer, C.R., A. Kramer and A. Konovalov, 2005. Rodlet cell distribution in the gall bladder epithelium of *Fundulus heteroclitus*. Journal of Fish Biology **67:** 555-560.

[13]Reite, O.B., 1997. Mast cells/eosinophilic granule cells of salmonids: staining properties and responses to noxious agents. Fish and Shellfish Immunology **7:** 567-584.

[14]Reite, O.B., 2005. The rodlet cells of teleostean fish: their potential role in host defence in relation to the role of mast cells/eosinophilic granule cells. Fish and Shellfish Immunology **19:** 253-267.

[15]Dezfuli, B.S., L. Giari, R. Konecny, R. Jaeger and M. Manera, 2003. Immunocytochemistry, ultrastructure and pathology of gills of *Abramis brama* from Lake Mondsee, Austria, infected with *Ergasilus sieboldi* (Copepoda). Diseases of Aquatic Organisms **53:** 257-262.

[16]Dezfuli, B.S., L. Giari and A.P. Shinn, 2007. The role of the rodlet cells in the inflammatory response in *Phoxinus phoxinus* brains infected with *Diplostomum*. Fish and Shellfish Immunology **23:** 300-304.

[17]Iger, Y. and M. Abraham, 1997. Rodlet cells in the epidermis of fish exposed to stressors. Tissue and Cell **29:** 431-438.

[18]Manera, M., E. Simoni and B.S. Dezfuli, 2001. The effect of dexamethasone on the occurrence of rodlet cells in goldfish. Journal of Fish Biology **59:** 1239-1248.

[19]Reite, O.B. and O. Evensen, 2006. Inflammatory cells of teleostean fish: A review focusing on mast cells/eosinophilic granule cells and rodlet cells. Fish and Shellfish Immunology **20:** 192-208.

[20]Manera. M. and B.S. Dezfuli, 2004. Rodlet cells in teleosts: a new insight into their nature and functions. J. Fish Biol. **65:** 597-619.

[21]Fishelson, L. and K. Becker, 1999. Rodlet cells in the head kidney of the domestic carp (*Cyprinus carpio*): Enigmatic gland cells or coccidian parasites? Naturwissenschaften, **86:** 400-403.

[22]Smith, S.A., T. Caceci, H. E-S Marei and H.A. El -Habback, 1995. Observations on rodlet cells found in the vascular system and extravascular space of angelfish (*Pterophyllum scalare*). Journal of Fish Biology **46:** 241-254.

[23]Smith, S.A. T. Caceci and J.L. Robertson, 1995. Occurrence of rodlet cells and associated lesions in the vascular system of freshwater angelfish. Journal of Aquatic Animal Health **7:** 63-69.

[24]Palenzuela, O., P. Alvarez-Pellitero and A. Sitja-Bobadilla, 1999. Glomerular disease associated with *Polysporoplasma sparis* (myxozoa) infections in cultured seabream, *Sparus aurata* L. (Pisces: Teleostei). Parasitology **118:** 245-256.

[25]Dezfuli, B.S., L. Giari, E. Simoni, A.P. Shinn and G. Bosi, 2004. Immunohistochemistry, histopathology and ultrastructure of *Gasterosteus aculeatus* tissues infected with *Glugea anomala.* Diseases of Aquatic Organisms **58:** 193-202.

[26]Matisz, C.E., C.P. Goater and D. Bray, 2010. Density and maturation of rodlet cells in brain tissue of fathead minnows (*Pimephales promelas*) exposed to trematode cercariae. International Journal of Parasitology, **40:** 307-312.

[27]Potter, H. and C.R. Kramer, 2013. The effects of environmental toxicants on gill histopathology of two populations of killifish *Fundulus heteroclitus* (L.). In Vivo **34:** 58-66.

[28]Abraham, M., Y. Iger and L. Zhang, 2001. Fine structure of the skin cells of a stenohaline freshwater fish *Cyprinus carpio* exposed to diluted seawater. Tissue and Cell **33:** 46-54.

[29]Dezfuli, B.S., L. Giari, E. Simoni, D. Palazzi and M. Manera, 2003. Alteration of rodlet cells in chub caused by the herbicide Stam® M-4 (propanil). Journal of Fish Biology **63:** 232-239.

[30]Qama, E., J. Blaize, W.J. L'Amoreaux and C.R. Kramer, 2009. Rodlet cell distribution and activity in the gills of laboratory-maintained and wild-caught *Fundulus heteroclitus*. In Vivo **31:** 16-23.

[31]Shimada Borges, J.C., A.B.B. Salimbeni Vivai, P.C. Branco, M. Silva Oliveira and J.R. Machado, 2013. Effects of trophic levels (chlorophyll and phosphorus content) in three different water bodies (urban lake, reservoir and aquaculture facility) on gill morphology of Nile tilapia (Oreochromis niloticus). Journal of Applied Ichthyology **29:** 573-578.

[32]Zsebo, K.M., A.M. Cohen, D.C. Murdock, T.C. Boone, H. Inoue, V.R. Chazin, D. Hines and L.M. Souza, 1986. Recombinant human granulocyte colony stimulating factor: Molecular and Biological characterization. Immunology **172:** 175-184.

[33]Physician leaflet insert, Amgen Inc., 2007.

[34]Kramer, C.R. and H. Potter, 2002. Ultrastructural observations on rodlet-cell development in the head kidney of the southern platyfish, *Xiphophorus maculatus* (Teleostei: Poeciliidae). Canadian Journal of Zoology **80:** 1422-1436.

[35]Leknes, I.L., 2001. Rodlet cells and granulated leucocytes in the bulbus arteriosus of swordtail, *Xiphophorus helleri* and platy, *Xiphophorus maculatus* L. (Poeciliidae: Teleostei). Fish and Shellfish Immunology **11:** 433-436.

[36]Weyts, F.A.A., B.M.L. Verburg-van Kamenade and G. Flik, 1998. Characterization of glucocorticoid receptors in peripheral blood leucocytes of carp, *Cyprinus carp*io L. General and Comparative Endocrinology **111:** 1-8.

[37]Richards, D.T., D. Hoole, C. Arme, J.W. Lewis and E. Ewens, 1994. Phagocytosis of rodlet cells (*Rhabdospora thelohani* Laguesse, 1895) by carp (*Cyprinus carpio* L.) macrophages and neutrophils. Helminthologia **31:** 29-33.

Autism (ASD) Revisited

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This is a follow-up article to the one I wrote in the Fall, 2013 issue of *In Vivo,* on Autism Spectrum Disorder (ASD)[1]. Scientific findings have indicated that ASD has many aspects to its etiology. ASD is a complex of multiple disorders. One of the many connections to ASD is the GUT/ BRAIN connection.

Gut/Brain Connection

Recently, it has been determined that human gut bacteria have been implicated in many disorders, such as obesity, diabetes and autism. Human gut bacteria may have a positive or a negative influence upon these conditions. For example, Paul Patterson and his team at CalTech in Pasadena[2] have created mice with autism-like symptoms. They demonstrated that doses of human gut microbes helped to reverse these symptoms. The autism-like mice had lower levels of *Bacteroides fragilis* . This bacterium is a normal inhabitant of the mouse gut. When given *B. fragilis*, autism-like mice showed less abnormal behaviors. It is speculated that gut bacteria communicate via chemicals traveling through the blood stream to the brain where they may cause normal/abnormal behaviors[3]. It is too early to tell whether human ASD individuals would benefit from being administered beneficial bacteria. In addition, Gilbert, *et al*.[4] published a study showing the effectiveness of the use of probiotic treatment for autism-like behaviors in mice. Of course, mice are not humans; so much research still needs to be done.

To go along with the hypothesis that there may be harmful bacteria in the gut that may lead to ASD symptoms, a novel vaccine has been developed by University of Guelph researchers against *Clostridium bolteae,* a bacterium found in high numbers in the guts of ASD children. This is the first vaccine designed to control constipation and diarrhea caused by this bacterium. The vaccine may also control ASD-related symptoms associated with the microbe's presence[5]. This study is only the initial step in the development of a vaccine against autism-related bacteria.

Diuretic-Chloride-GABA Connection

A research study by Yehezkel Ben-Ari performed at the Mediterranean Institute of Neurology in Marseille tested the diuretic drug bumetanide on 30 ASD-children, while 30 ASD-children received a placebo. The group given the diuretic showed milder ASD symptoms. The diuretic may reduce chloride levels in cells, thereby restoring a brain neurotransmitter's (GABA) inhibitory function. Too much chloride causes brain cells to become very excitable[6] .

Biomarkers

ASD is estimated to affect one in eighty-eight children in the U.S. Biomarkers, indicators of the disorder, are extremely important to detect as early as possible so that at-risk infants may be diagnosed and receive help. Early treatment is essential so that interventions may have a positive effect upon socialization and behavior.

Examples of proposed biomarkers for ASD include:

Gene Expression Profile
Proteomic Profile
Metabolomic Profile
Head Size
Head Circumference Trajectory
MRI (Magnetic Resonance Imaging)
DTI (Diffuse Tensor Imaging)
EEG (Electroencephalogram)
ERPs (Event-related Potentials)
Eye Movement [7]

At the UC Davis M.I.N.D. Institute laboratory of Dr. Judy Van de Water[8] researchers have identified seven primary antigens of maternal autoantibody-related (MAR) autism. "Exclusive reactivity to specific antigen combinations was noted in 23% of mothers of ASD children and only 1% of controls"[8]. This is the first panel of clinically significant antigenic biomarkers.

Interventions for ASD

Some therapies have been developed over the years by physicians called DAN! (Defeat Autism Now) doctors. A list of some DAN! protocols is provided.

1. Nutritional supplements, including certain vitamins, minerals, amino acids, and essential fatty acids
2. Special diets totally free of gluten (from wheat, barley, rye, and possibly oats) and free of dairy (milk, ice cream, yogurt, etc.)
3. Testing for hidden food allergies, and avoidance of allergenic foods
4. Treatment of intestinal bacterial/yeast overgrowth (with pro-biotics, supplements and other non-pharmaceutical medications)
5. Detoxification of heavy metals through chelation (a potentially hazardous medical procedure)[9]

Note that there are other interventions not listed here.

It appears that the scientific community is getting closer to the causes and therapies for this ASD epidemic. Hopefully, effective therapies for ASD children will soon be at hand.

References

[1]Biermann, C.A., 2013. The autism controversy: Anecdotal Reports, court cases, and books vs. scientific investigations. In Vivo **35(1):** 25-29.

[2]Natalia V. Malkova, C.Z. Yu, E.Y. Hsiao, M.J. Moore and P.H. Patterson, 2012.Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain Behavior and Immunity **26:** 607-616.

[3]Reardon, S., 2013. Bacterium can reverse autism -like behavior in mice. Available online at: http://www.nature.com/news/bacterium-can-reverse-autism-like-behavior-in-mice-1.14308.

[4]Gilbert, J.A., R.Krajmainik-Brown, D.L Porazinska, S.J. Weiss and R. Knight, 2013. Toward effective probiotics for autism and other developmental disorders. Cell **155(7):** 1446– 1448.

[5]Pequegnat, B., M. Sagermann, M. Valliani, M. Toh, H. Chow, E. Allen-Vercoe and M. A. Monteiro, 2013. A vaccine and diagnostic target for *Clostridium bolteae*, an autism-associated bacterium. Vaccine **2013:** 2787-2790.

[6]Saunders, L. 2014. Common diuretic could alleviate autism symptoms. Science News **185 (5):** 8.

[7]Walsh, P., M. Elsabbagh, P. Bolton and I.Singh, 2014. In search of biomarkers for autism: Scientific, social and ethical challengers." Available online at: http://www.nature.com/nrn/journal/v12/n10/full/nrn3113.html.

[8]Braunschweig, D., P. Krakowiak, P Duncanson, R. Boyce, R.L. Hansen, P. Ashwood, I. Hertz-Picciotto, I.N. Pessah and J. Van de Water, 2013. Autism-specific maternal autoantibodies recognize critical proteins in developing brain. Translational Psychology 3 e277; doi:10.1038/lp.2013.50.

[9]Rudy, L.J., 2009. What is a defeat autism now (DAN!) autism doctor? Available online at: http://autism.about.com/od/alternativetreatments/f/dandoc.htm.

Exploring Scientific Evidence about Plant Oils Used in Different Cultures

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Abstract

Historically, plant derived oils have been used worldwide in treating a variety of human ailments. Although considerable anecdotal information about the medicinal qualities of plant derived oils exists, little scientific and clinical evidence is available to demonstrate their safety and effectiveness. This study investigates the effect of garlic, lavender, castor, and red palm oil on both prokaryotic and eukaryotic systems. The antibacterial property of these oils was compared to some antibiotics by measuring zones of inhibition using the disc diffusion method on plates inoculated with *Escherichia coli* and *Bacillus cereus.* Additionally, the growth and development of brine shrimp in the presence of the plant derived oils was measured and analyzed using the lethality test and digital microscopy.

Introduction

The medicinal use of plants is deeply rooted in human history and has been included into the traditional medicine of virtually all human cultures. The use of plant derived oils in ancient history has been documented, along with the fact that ancient cultures believed that the healing property of these oils was supernatural[1]. The use of lavender, castor, garlic, and red palm oil by different cultures around the world is worthy of scientific study in order to elucidate the mechanisms by which they promote healing.

Lavender oil, *Lavendula angustifolia*, from the Lamiaceae family, was known as the "scent sent from the heavens" by the ancient Egyptians[2]. Lavender is a small gray shrub with linear leaves and blue to violet flowers. This plant is indigenous to the mountainous regions of the countries bordering the western half of the Mediterranean. The distillation of flower spikes from the lavender plant produces lavender oil. France, England, and Italy are the main cultivators of lavender. Lavender, referred to as "spikenard" in the Bible, was believed to be a holy safeguard against evil[2]. The first account of lavender used medicinally can be traced back to Roman and Greek times[3,4]. Lavender's active ingredients are linalool, linalyl acetate, cricol, pinene, geranial, borneol, tannin, and linalyl butyrate. Lavender oil is the remedy of choice for headaches, constipation, chest pains, throat infections, insect bites, stomach aches, nervousness, anxiety, faintness, and minor burns[5]. Evidence of lavender oils' antimicrobial, anti-inflammatory and anti-analgesic properties have led to the possibility of wound healing properties[6]. It is also used as an insect repellent and a cleansing agent[5]. In France, a bottle of lavender oil is common in most households as a domestic remedy against bruises, bites and trivial aches and pains, both external and internal[7].

Garlic oil, *Allium sativum*, from the Alliaceae family, was coined "the great protector" by the ancient Greeks[8]. Garlic originally grew in Central Asia and then spread to southern Europe. It is widely cultivated in countries bordering the Mediterranean. The medicinal properties of garlic have been recorded in ancient texts. These texts include the Indian Sanskrit medical treatise, dating back from the 2nd century BC to the 2nd century AD, and the Ayurvedic Charaka Samhita and the Buddhist's Navanitaka, both dating back to the 4th century AD. Garlic has been the medicine of choice throughout different parts of the world for the treatment of asthma, diabetes, arteriosclerosis, fever, cough, chronic bronchitis, and the overall well-being of the body[8,9]. Methyl and allyl sulfide are derivatives of Allicin, which is the active ingredient in garlic produced when garlic is crushed or cut. Chemical derivatives of garlic are used medicinally as antiseptic, antifungal, antiviral, antiprotozoal, anticancer, and antimicrobial agents[9,10]. Additionally, derivatives of garlic have been shown to have potential hepatoprotective properties and wound healing activity[9]. The active ingredient Allicin is rich in important sulfur-containing compounds derived from the amino acid cysteine[11]. Allicin changes via several pathways into ajoene and thioacrolein. Ajoene is a strong antithrombotic factor meaning

that it decreases the length of time it takes for blood to clot. This is important in preventing heart diseases[12]. Ajoene also promotes low blood lipid levels in people who regularly consume garlic. Garlic oil is capable of interfering with agglutination of blood platelets[12]. Studies on the medicinal qualities of garlic are ongoing.

Red Palm Oil, *Elaesis guineensis*, from the Arecaceae family is extracted from the fruit of the oil palm plant. Red palm oil flourishes in West Africa and in other humid, tropical areas. Red palm oil is a staple in the diet of many cultures because of its high content of essential fatty acids and provitamin A carotenes[13]. People of Africa, Southeast Asia, and Latin America are the predominant users of red palm oil. They believe strongly in its nutritive value and healing qualities. Red palm oil is used to treat and prevent malnutrition, vitamin deficiency diseases, high blood pressure, high cholesterol and heart diseases. The active ingredient, tocotrienol, found in red palm oil has been shown to have anticancerous properties against an array of human cancers[14]. It is also used to boost immunity and promote healthy blood circulation[14,15].

Castor oil, *Ricinus communis*, from the *Euphorbiaceae* family, is also commonly known as Palma Christi, the palm of Christ. The castor plant is native to Africa and India. Castor oil is produced from cold pressing ripe seeds of the castor plant. The castor plant flourishes in tropical and subtropical climates. The soft wooded, small tree has had a phenomenal impact on ancient and modern medicine. Medicinal use of the castor plant from the root to the flower have shown antioxidant, antinociceptive, antiasthmatic, anti-inflammatory, antimicrobial, hepatoprotective, antihistaminic, antidiabetic, lipolytic, and wound healing activity[16].

Medicinal uses of castor oil are common in different cultures especially among people of Haitian origin. One of the authors, being from that ethnic group, uses castor oil frequently for different healing purposes which inspired authors to explore cultural myths regarding medicinal claims of castor and other oils. As a result, it was hypothesized that castor oil would have the most biological activity compared to other plant derived oils studied (lavender, garlic, red palm). The antibacterial property of these oils was compared to antibiotics by measuring zones of inhibition using the disc diffusion method on plates inoculated with *Escherichia coli* and *Bacillus cereus.* Additionally, the growth and development of brine shrimp in the presence of these plant derived oils was measured and analyzed using the lethality test and digital microscopy.

Materials and Methods for Disc Diffusion Test

Materials

Escherichia coli broth
Bacillus cereus broth
14 Tryptic Soy Agar (T-Soy) plates
castor oil
garlic oil
lavender
red palm oil
1 crushed garlic clove
antibiotic disc dispenser
24 sterile cotton swabs
5 forceps
5 petri dishes
10 filter-paper discs
metric ruler

Lavender oil, castor oil, red palm oil, and garlic clove were purchased at “Wild by Nature” Organic food store and products (2709 Long Beach Road, Oceanside, NY). All other materials were obtained from the Department of Biology, Nassau Community College, Garden City, NY.

Methodology

Using aseptic culture technique, two control plates were prepared by inoculating one plate with *E. coli* and one with *B. cereus (*Figure 1a,b*).* Seven T-soy plates were then inoculated with *E. coli* and another seven with *B. cereus*. Two plates, one of each bacterial culture, were set aside for each test agent and labeled accordingly. Using forceps, a filter paper disc was immersed in the appropriate test agent and then placed in the center of the T-soy plate. The same procedure was followed with the garlic clove except that the clove was crushed first and the filter paper disc was then rubbed in the garlic pulp.

Positive control plates were prepared using the method described above. These plates were prepared using a multi disc dispenser which included the following antibiotics: bacitracin (B2), cefoxitin (FOX30), ampicillin (AM10), tetracycline (TE30), erythromycin (E15), penicillin (P10), and streptomycin (S10).

The prepared plates were incubated at 37°C. Results were recorded 48 hours after inoculation by measuring the zones of inhibition with a metric ruler. The experiment was repeated two times.

Materials and Method for Brine Shrimp Lethality Test

Materials

Brine shrimp eggs *(San Francisco Bay Brand Easy-to -hatch Brine Shrimp Eggs)*
1 L of sterile water
sea salt *(Instant Ocean Synthetic Sea Salt from Aquarium Systems)*
24 fingerbowls
small lamp
aluminum foil
balance
castor oil
garlic oil
lavender oil
red palm oil
Motic compound microscope

All materials were obtained from Department of Biology, Nassau Community College, Garden City, NY.

Methodology

The brine shrimp egg stock was prepared by adding 3g of brine shrimp eggs to 150 ml of sterile water. The stock was kept in bright light for an hour in order to hasten hatching of eggs.

A 3.5% salt water solution was made by adding 35 g NaCl to 1L of distilled water (as per direction of brine shrimp preparation). 35 ml of saltwater stock was added to each fingerbowl preparation.

Two fingerbowls were set aside for each oil tested; 125 µl of oil (approximately 5 drops) was added to one bowl and 250 µl of oil (approximately 10 drops) was added to the other. Four fingerbowls containing only saltwater were set aside as controls. 1 ml of brine shrimp stock was added to each fingerbowl. After one week, 17.5ml from original stock was transferred to new fingerbowls with equal amounts of sea water and the appropriate initial amounts of the oils (125 µl and 250 µl). This was done in order to provide a fresh, clean environment for the growing brine shrimp.

Results

Both qualitative and quantitative observations were recorded for disc diffusion method and brine shrimp lethality test. The halo size on the petri dish was measured for each oil sample and compared to the control for antibacterial properties (Figure 1a-1h; Figure 2a-2e). The graphs (Figure 3, 4) were constructed based on the measurements of halo (s) recorded in Tables 1 and 2. Detail measurements are discussed and analyzed to compare effectiveness of different oils in the next section.

For brine shrimp experiment, number of eggs that hatched was counted and images of the developing brine shrimp were recorded with the Motic digital microscope beginning one day after the culture preparation up to fourteen days later. The data is presented in the form of images (Figures, 5a-5i; 6a-6i; 7a-7i), graphs (Figures 8, 9) and Tables (3, 4, 5). The analysis and discussion includes comparative effect of oils on brine shrimp development.

Table 1. Antimicrobial Results—Average measurements of zone of inhibition from Trial 1 & 2

OIL	ZONE of *Escherichia coli*	INHIBITION (mm) *Bacillus Cereus*
Lavender Oil	2.50	2.85
Castor Oil	1.90	2.00
Red Palm Oil	0.00	0.00
Garlic Oil	0.00	0.00
Garlic Clove	4.00	4.85
Negative Control	0.00	0.00

Table 2

ANTIBIOTICS	ZONE of *Escherichia coli*	INHIBITION (mm) *Bacillus Cereus*
Bactracin (B2)	0.00	1.40
Cefoxitin (FOX 30)	3.45	2.15
Ampicillin (AM 10)	2.45	1.35
Tetracycline (TE 30)	3.10	2.30
Erythromycin (E 15)	1.40	3.25
Penicillin (P 10)	1.20	1.00
Streptomycin (S 10)	1.80	1.80

Figure 1 (a-h)—Disc diffusion Method Demonstrating Antimicrobial properties of Oils, Antibiotics and Controls

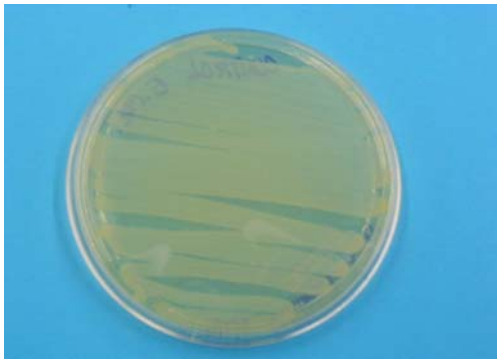

Figure 1a—Control of *E. coli*

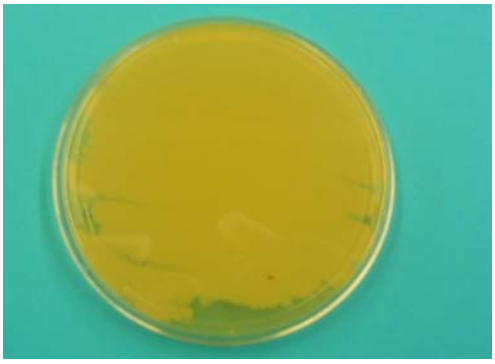

Figure 1b—Control *B. cereus*

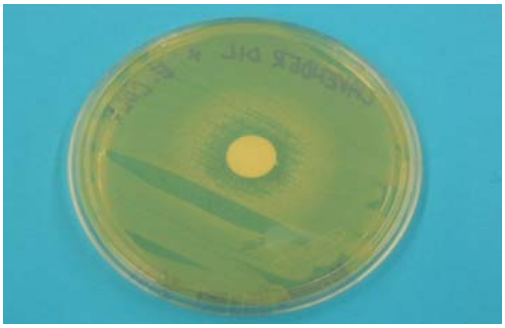

Figure 1c—Lavender Oil and *E. Coli*

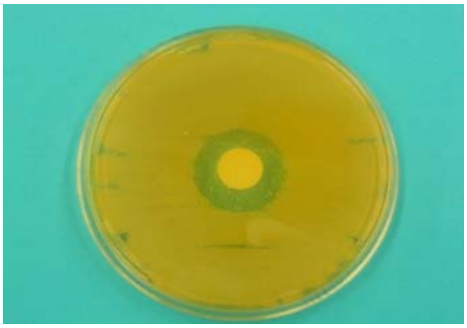

Figure 1d—Lavender Oil and *B. cereus*

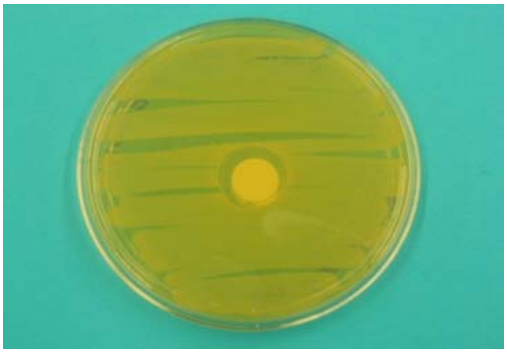

Figure 1e—Castor Oil and *E. Coli*

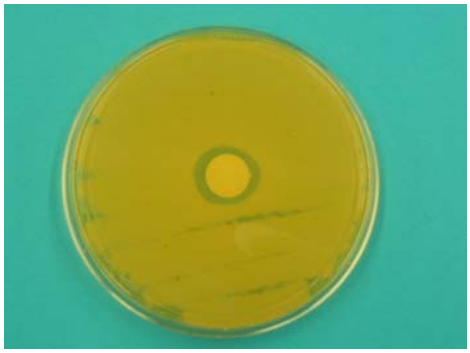

Figure 1f—Castor Oil and *B. cereus*

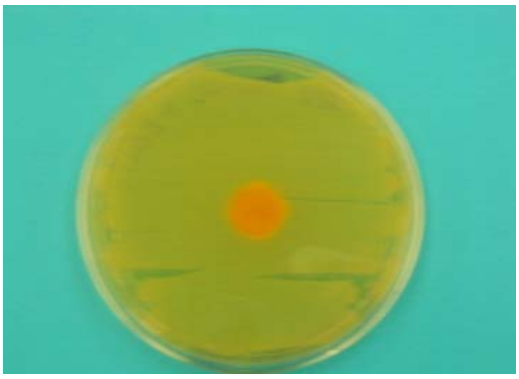

Figure 1g—Red Palm Oil and *E. coli*

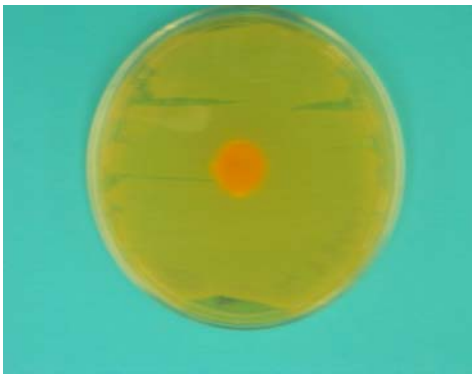

Figure 1h—Red Palm Oil and *B. cereus*

Figure 2a-e. Disc diffusion Method demonstrating Antimicrobial properties of Oils, Antibiotics and Controls

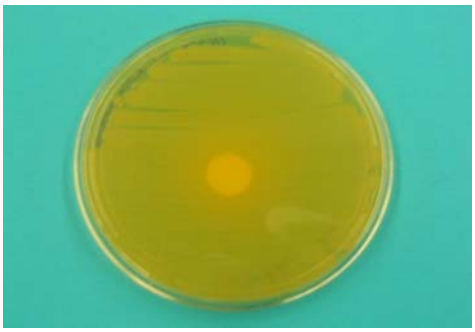

Figure 2a—Garlic Oil and *E. Coli*

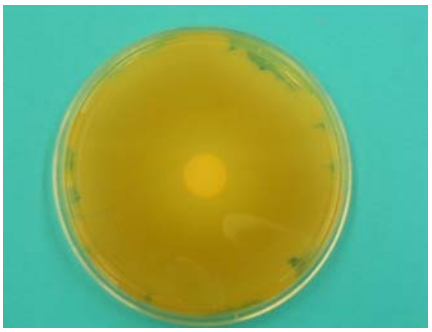

Figure 2b—Garlic Oil and *B. cereus*

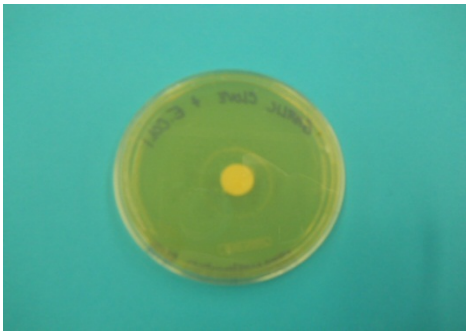

Figure 2c—Garlic Clove and *E. Coli*

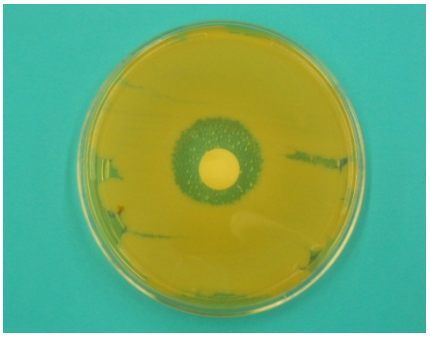

Figure 2d—Garlic Clove and *B. cereus*

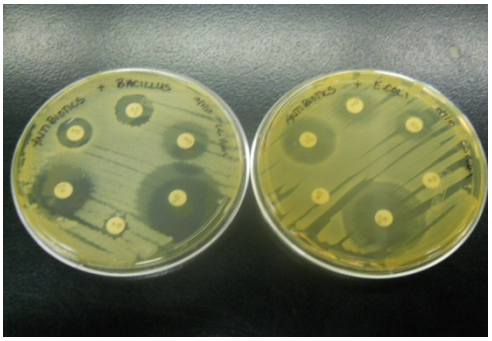

Figure 2e—Positive control showing effect of antibiotics on *B.cereus* (left) and *E. coli* (right)

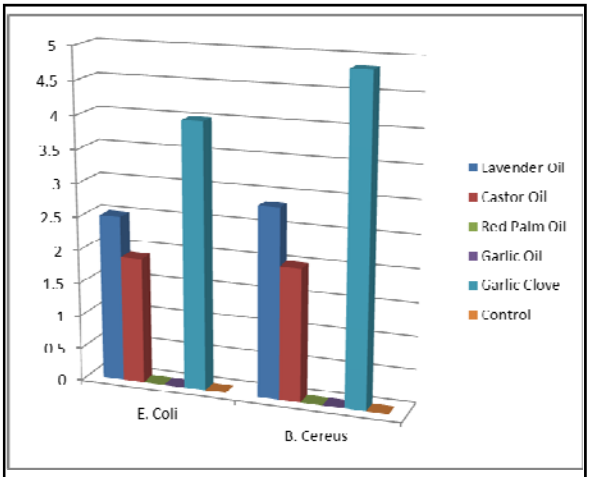

Figure 3—Measurements of Zone of Inhibition (mm) Oils against *E. coli* and *B. cereus*

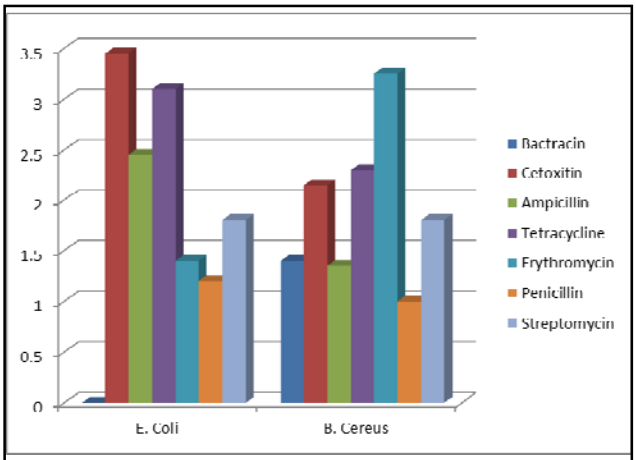

Figure 4—Positive Control Measurements of Zone of Inhibition (mm) Antibiotics against *E. coli* and *B. cereus*

Figure 5 (a-i). Results of Brine Shrimp development in Different Concentrations of Oils after One Day of Exposure

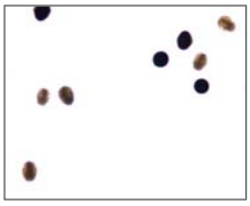

Figure 5a—Castor Oil 125 µl

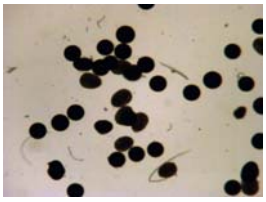

Figure 5b—Castor Oil 250 µl

Figure 5i—Control

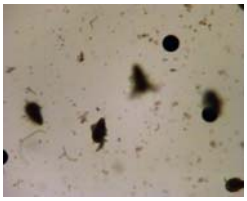

Figure 5c—Garlic Oil 125 µl

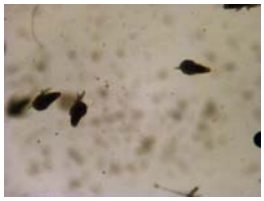

Figure 5d—Garlic Oil 250 µl

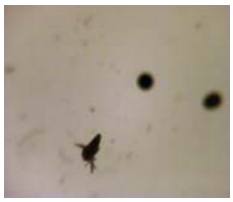

Figure 5e—Red Palm 125 µl

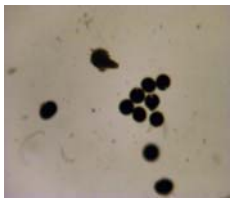

Figure 5f—Red Palm 250 µl

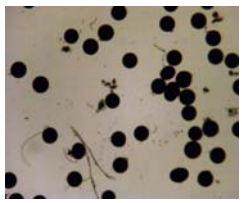

Figure 5g—Lavender Oil 125 µl

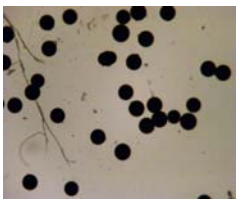

Figure 5h—Lavender Oil 250 µl

Figure 6 (a-i)—Results of Brine Shrimp development in Different Concentrations of Oils after Seven Days of Exposure

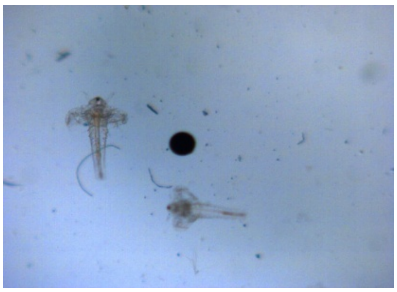

Figure 6a—Castor Oil 125 µl

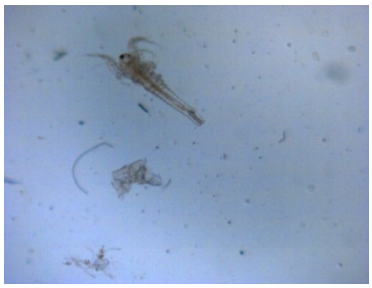

Figure 6b—Castor Oil 250 µl

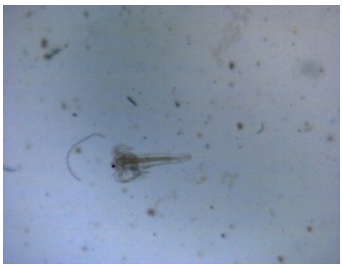

Figure 6i - Control

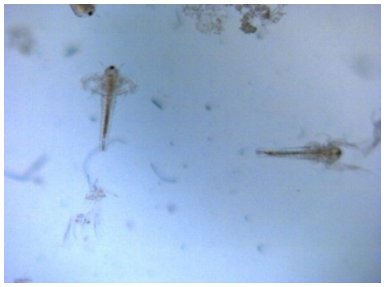

Figure 6c—Garlic Oil 125 µl

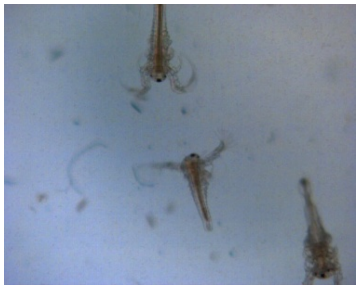

Figure 6d—Garlic Oil 250 µl

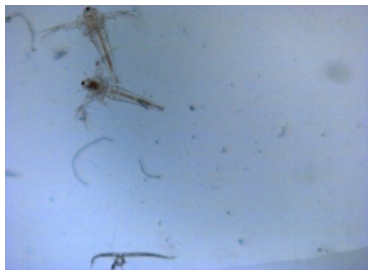

Figure 6e—Red Palm 125 µl

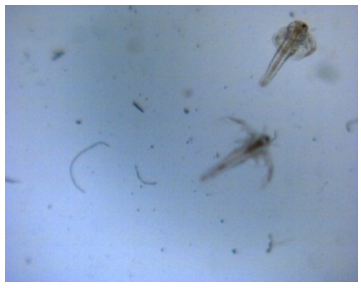

Figure 6f—Red Palm 250 µl

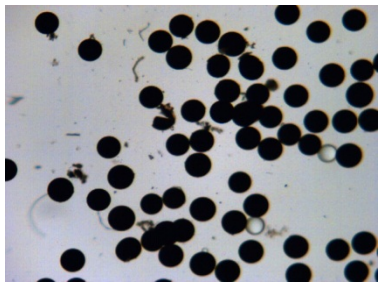

Figure 6g—Lavender Oil 125 µl

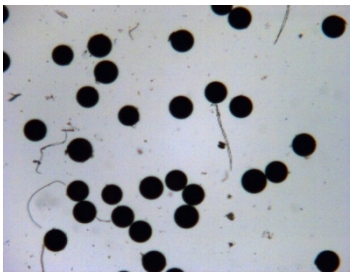

Figure 6h—Lavender Oil 250 µl

Figure 7 (a-i)—Results of Brine Shrimp Development in Different Concentrations of Oils after Fourteen Days of Exposure

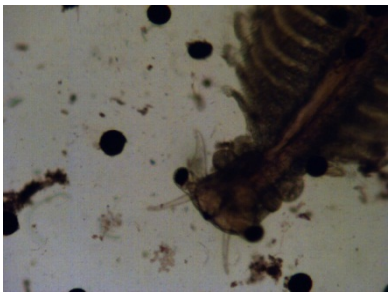

Figure 7a—Castor Oil 125 µl

Figure 7b—Castor Oil 250 µl

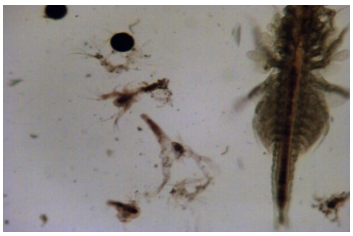

Figure 7i - Control

Figure 7c—Garlic Oil 125 µl

Figure 7d—Garlic Oil 250 µl

Figure 7e—Red Palm Oil 125 µl

Figure 7f—Red Palm Oil 250 µl

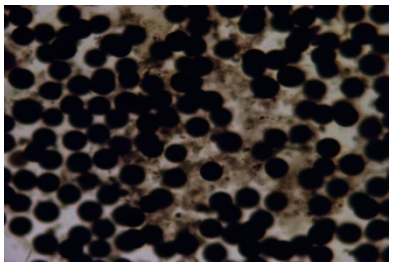

Figure 7g—Lavender Oil 125 µl

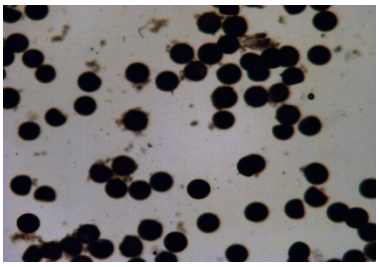

Figure 7h—Lavender Oil 250 µl

Brine Shrimp Results—Average of 1mm Drop Count of Brine Shrimp from Trial 1 & 2

Table 3
One day after preparation

OIL	125µl of Oil	250µl of Oil
Garlic Oil	15	10
Castor Oil	5	7
Red Palm Oil	5	3
Lavender Oil	0	0

CONTROL	COUNT
Control	10

Table 4
Seven days after preparation

OIL	125µl of Oil	250µl of Oil
Garlic Oil	10	17
Castor Oil	10	15
Red Palm Oil	5	7
Lavender Oil	0	0

CONTROL	COUNT
Control	8

Table 5
Fourteen days after preparation

OIL	125µl of Oil	250µl of Oil
Garlic Oil	5	8
Castor Oil	8	3
Red Palm Oil	2	1
Lavender Oil	0	0

CONTROL	COUNT
Control	2

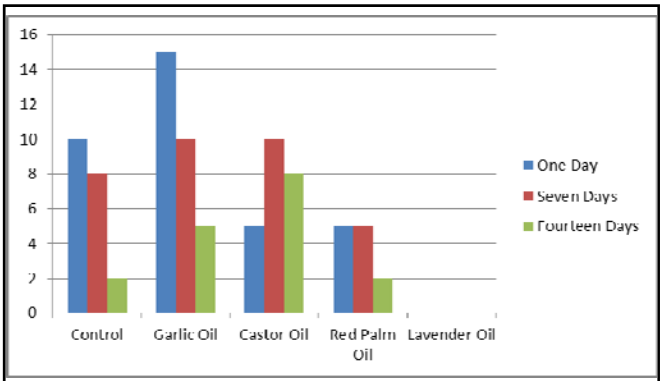

Figure 8—Brine Shrimp Lethality Test, 1ml drop count of Brine Shrimp in 125µl of Oil

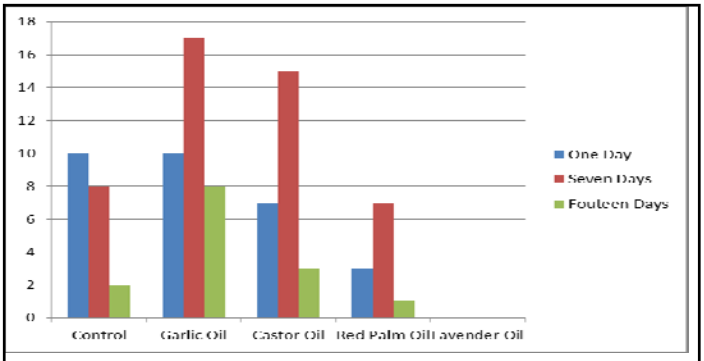

Figure 9—Brine Shrimp Lethality Test, 1ml drop count of brine shrimp in 250 µl of Oil

Discussion

Bacterial Experiment

The antibacterial test was repeated two times. The results revealed that lavender oil and castor oil possess antibacterial activity against *E. coli* and *B. cereus.* Lavender oil showed significant activity with zones of inhibition measuring an average of 2.5 mm against *E. coli* and an average of 2.85 mm against *B. cereus (*Figure 1c, 1d*)*. Castor oil also displayed antibacterial activity producing zones of inhibition measuring an average of 1.9 mm against *E. coli* and 2 mm against *B. cereus (*see Figure. 1e, 1f*)*. One of the active ingredients in castor oil is ricinoleic acid which has been reported to inhibit the growth bacteria[17]. A halo was not observed with red palm oil against *E. coli* or *B. Cereus (*Figure 1g, 1h*)*. Surprisingly, garlic oil did not exhibit any antibacterial activity against *E. coli* and *B. cereus (*see Figure 2a, 2b*)*. However, the garlic clove, a well-known natural antibacterial agent, exhibited the best activity against *E. coli* and *B. cereus* producing halos that measured 4.8 mm and 4.85 mm, respectively (see Figure 2c, 2d and Table 1). The results for garlic oil and naturally crushed garlic clove against the bacteria are dramatically different with garlic oil showing no activity and naturally crushed garlic clove showing the greatest activity. Garlic comes from the family Alliaceae, and the oil contains the active ingredient, Allicin, which has antifungal and antimicrobial properties[10]. Garlic oil is produced by steam-distillation which might have destroyed the antibacterial property of Allicin. Results of this study confirmed that crushed garlic was a stronger antibacterial agent than Penicillin. Natural garlic produced a zone of inhibition that measured 4.8 mm against *E. coli* and 4.85 mm against *B. cereus* whereas Penicillin produced smaller zones that measured 1.2mm against *E. coli* and 1.9mm against *B. cereus (*Figure 2e and Table 1, 2*)*. One significant advantage of garlic is that the body does not seem to build up a resistance to it as it does with many modern antibiotics[18]. Figure 3 is a graphical illustration that summarizes the activity of oils against *E. coli* and *B. cereus* whereas Figure 4 illustrates the activity of antibiotics against *E. coli* and *B. cereus*.

Brine Shrimp Experiment

Brine shrimp development occurred at both concentrations of garlic oil, castor oil, and red palm oil (Figure 5a-f, 6a-f, 7a-f). Brine shrimp grown in saltwater containing 250 µl of garlic oil had a significant number of mature brine shrimp compared to castor oil and red palm oil in the first trial. After fourteen days of exposure to the oils, more than half of the brine shrimp in castor oil and red palm oil died at the early stages of cellular division and differentiation (Table 3, 4, 5). Brine shrimp did not hatch in the environment concentrated with 125µl and 250 µl of lavender oil for both trials (Figure 5g, 5h, 6g, 6h, 7g, 7h*)*. Development of brine shrimp was observed in the control set *(*Figure 5i, 6i, 7i*)*. However by the fourteenth day the number of mature brine shrimp in the control was less than the number of mature brine shrimp developed in garlic and castor oil *(*Table 5*)*. Figure 8 and 9 illustrate the growth and development of brine shrimp in different concentrations of oils. Although our hypothesis was not supported by the experiment, the effect of lavender oil on prokaryotic and eukaryotic organisms is of special interest.

Conclusion

Ethnobotanists have a difficult task of researching plants, and maintaining their credibility in the field of modern medicine. Their work and value are often underrated. Plants have been used as a source of medicine for centuries and should still be studied with vigor. This study further supports the usefulness of plants in medicine. Castor oil as well as lavender, red

palm, and garlic oil are used in various cultures without scientific evidence. The active ingredients in oils are vital in discovering the oils' medicinal properties. Using the disc diffusion method this study provides evidence for antimicrobial properties of oils as well as evidence for the impact of oils on the growth and development of brine shrimp with the lethality test. Lavender oil and fresh garlic in this study were found to be as powerful if not more powerful than modern antibiotics. The measurements of the halos for lavender oil against *E. coli* and *B. cereus* were similar to the antibiotic, Cefoxitin. Lavender oil interfered with the process of cell division and differentiation in brine shrimp also. This indicates that lavender oil impacts both the prokaryotic and eukaryotic system. Even though red palm oil and garlic oil did not show antibacterial activity against *E. coli* and *B. cereus,* this does not mean that they cannot inhibit the growth of other types of bacteria. Detailed investigation of phytochemicals and developmental studies using plant oils on different prokaryotic and eukaryotic systems may help us understand effectiveness and exact mode of actions of these oil.

Acknowledgments

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References

[1]Dubick, M.A., 1986. Historical perspectives on the use of herbal preparations to promote health. Journal of Nutrition **11:** 1348-54.

[2]Spector Platt, E., 2009. *Lavender: How to Grow and Use the Fragrant Herb (2nd Edition)*. New York, NY: Stackpole Books.

[3]Charles, D.J., 2013. Lavender. Antioxidant Properties of Spices, Herbs and Other sources. Springer **2013:** 63-369.

[4]Rapper, S.,G. Kamatou, A. Viljoen, S. Vuuren. 2013. The in *vitro* antimicrobial activity of *Lavendula angustifolia* essential oil in combination with other aroma-therapeutic oils. Evidence-Based Complementary and Alternative Medicine **2013:** 1-10.

[5]Lis-Balchin, M., 2002. *Lavender: The Genus Lavandula*. New York, NY: Taylor & Francis Inc.

[6]Kutlu, A.K., D. Cecen, S.G. Gurgen, O. Sayin, F. Cetin, 2013. A comparison study of growth factor expression following treatment with transcutaneous electrical nerve stimulation, saline solution, povidone-iodine, and lavender oil in wounds healing. *Evidence-Based Complementary and Alternative Medicine* 2013:1-9.

[7]Grieve, M., 1971. *A modern herbal: Lavender*, *Vol. II*. Mineola, NY: Dover Publications,Inc.

[8]Harris, J.C., S. Cottrell, S. Pummer and D. Lloyd, 2001. Antimicrobial properties of Allium (garlic). Applied Microbiology and Biotechnology **57:** *282-286.*

[9]Asgarpanah, J. and B. Ghanizadeh, 2012. Phamacologic and medicinal properties of *Allium hirtifolium* Boiss. *African Journal of Pharmacology* **6:** 1809-1814.

[10]Ankri, S. and D. Mirelman, 1999. Antimicrobial properties of allicin from garlic. Microbes and Infection **1:** 125-129.

[11]Mehta, J., A. Sharma, N. Sharma, S. Megwal, G. Sharma, P. Gehlot and R. Naruka.,2013. An improved method for callus culture and *In vitro* propagation of garlic (*Allium sativum L*.). International Journal of Pure and Applied. Bioscience **1:** 1-6.

[12]Hanson, B.A., 2005. *Understanding medicinal plants*: *Their chemistry and therapeutic action*. Binghamton, NY: The Hansworth Herbal Press.

[13]Manorama, R., G.N.V. Brahmam and C. Rukmini, 1996. Red palm oil as a source of β-carotene for combating vitamin A deficiency. Plant Foods for Human Nutrition **49:** 75−82.

[14]Timsina, B., M. Shukla and V.K. Nadumane, 2012. A review of few essential oils and their anticancer property. Journal of Natural Pharmaceuticals **3:** 1-8.

[15]Kritchevsky, D., 2000. Impact of red palm oil on human nutrition and health. Food and Nutrition Bulletin **21:** 182-188.

[16]Jena, J. and A.K. Gupta, 2012. Ricinus communis Linn: A phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences **4:** 25-29.

[17]Narasimhan, B., V.K. Mourya and A.S. Dhake, 2007. QSAR studies of antibacterial rincinoleic acid derivatives. Pharmaceutical Chemistry Journal **41:** 133-139.

[18]Uzodike E.D. and O.U. Ogbonna, 2008. Comparative analysis of the efficacy of Allium Savitum (Garlic) and Erythromycin on *Streptococcus pyogenes*. Journal of the Nigerian Optometric Association **14:** 18-21.

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