AVOCADO (*Persea americana*) AQUEOUS LEAF EXTRACT AS ADDITIVE IN THE TRANSPORT MEDIUM OF NILE TILAPIA (*Oreochromis niloticus* L.) FINGERLINGS

BY

APHRODITE S. ENTOMA

An undergraduate thesis submitted to the faculty of College of Fisheries in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE IN FISHERIES

COLLEGE OF FISHERIES
CENTRAL LUZON STATE UNIVERSITY
SCIENCE CITY OF MUÑOZ, NUEVA ECIJA
PHILIPPINES

2017

CF Thesis Form No. 8

COLLEGE OF FISHERIES
CENTRAL LUZON STATE UNIVERSITY
Science City of Muñoz, Nueva Ecija

AVOCADO (*Persia Americana*) AQUEOUS LEAF EXTRACT AS ADDITIVE IN THE TRANSPORT MEDIUM OF NILE TILAPIA (*Oreochromis niloticus* L.) FINGERLINGS

By

APHRODITE S. ENTOMA

is an Undergraduate Thesis presented to the Faculty of the College of Fisheries in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE IN FISHERIES

APPROVED:

EMMANUEL M. VERA CRUZ
Adviser
06/20/17
Date

REMEDIOS B. BOLIVAR
Critic
6/29/17
Date

NOTED:

KARL MARX A. QUIAZON
Department Chair
06/29/17
Date

RECORDED:

REMEDIOS B. BOLIVAR
College Research Coordinator
6/29/17
Date

ACCEPTED:

RAVELINA R. VELASCO
Dean
06 2017
Date

BIOGRAPHICAL DATA

Personal Data

Name	Aphrodite S. Entoma
Birthday	December 13, 1996
Birthplace	Dasmariñas, Cavite
Address	Pundakit, San Antonio, Zambales
Parent	Ma. Teresa A. Sales

Educational Attainment

Elementary	Cadapli Elementary School Cadapli, Bangar, La Union
Secondary	T. R. Yangco Catholic Educational Institute San Juan, San Antonio, Zambales
Tertiary	Central Luzon State University Science City of Muñoz, Nueva Ecija

ACKNOWLEDGMENTS

The author would like to acknowledge and express her sincerest gratitude to the following people whom without their help this thesis would not be possible.

Above all, to God for His given grace, wisdom, strength, guidance, unending blessings, and everlasting love that the author feels in everyday of her life

To her adviser and also the director of the Freshwater Aquaculture Center, Dr. Emmanuel M. Vera Cruz, for his patience in helping the author write her thesis proposal and for his support during the conduct, for believing in the author and for on all his advises. Also, for his fatherly concern and positive remarks which encouraged the author to do better. Without histechnical knowledge and unending support, this paper would not have been possible.

To her critic and also the College Research Coordinator, Dr. Remedios B. Bolivar, for all her constructive criticisms and suggestions, for her knowledge and support, in making this paper more presentable. Also, for her kindness and patience which motivated the author.

The author could have not have asked for a better adviser and critic. The author will be forever grateful for stimulating the young scientist in her and for all the knowledge, lessons and pointers in the field of research she have acquired from both of you.

To Ate Angelina Arellano of the Processing Laboratory for letting the author used the electric sealer. To Mr. Eddie Boy T. Jimenez, Mr. Reynaldo N. Lanuza, Mr. Jayson

V. Isais and Mr. Dennis G. Bungar, for their support during the conduct of this study and for helping the author in packing the experimental fish used.

To Dr. Jose S. Abucay and the staffs of the Fish-Gen., for making the cart with wheels used in the simulated transportation.

To all the staffs and faculty members of the College of Fisheries-Freshwater Aquaculture Center, for their help and support. The author will always remember all the knowledges and experiences she acquired during her college life.

To BFAR III, for the financial support.

To Kuya Jon Christian Suyom and Xyrra Jerremiah Mazo, in teaching the author on how to analyze her data.

To all the friends she made during her college life: Eden Abejero, Kairone Maniquiz, Trazarah Hanoof Argarin, Ma. ReinalynTercias, Jeffrey Manansala, Jan Noelle Dennise Rimando, Lindsay Star Dacanay, Geneva Mayuyo, Jacqueline Osalbo, Arleen Madriaga, Laura Marie Benigno, Joshua Calimlim, Monique Ebreo ,Abigail Cavita, Albert Arcos, Mark Anthony Mapanao, Daniel Jeric Torno Carlo Doctolero and Patrick Resolme for all the college blues and happiness they shared with the author.

To Carissa Alarcon and Abigail Pempeña, for the short but wonderful and unforgettable memories they have shared with the author during their freshman until their sophomore years. For all the laughter and arguments they had. The author misses them so much.

To all of her friends, BSF IV Family, FAC Dorm Family and all of the people who have shared part in the author's experiences not just in doing the thesis but also in her college life. Thank you very much!

To her family, for their unending and unconditional love. To her mother, Ma. Teresa Sales, for her smart advices and pointers on how to live independently. For always believing in the author's capability and for teaching the author how to be strong and resilient. To her brothers, Apollo and Achilles, for their financial and moral support. To her sisters, Malaya Shien and Michaela Vernn, for being sweet and adorable. To her Inang Salve and Tatang Carlos, for their motivational remarks which encouraged the author to keep going during the crucial moments of being a college student. They are the author's strength and inspiration.

APHRODITE S. ENTOMA

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDIX TABLES	xi
LIST OF APPENDIX FIGURES	xiii
ABSTRACT	xiv
INTRODUCTION	
Background of the Study	1
Statement of the Problem	3
Objectives of the Study	4
Significance of the Study	4
Scope and Limitation of the Study	5
Time and Place of the Study	5
REVIEW OF RELATED LITERATURE	
Persea americana	6
Antioxidative Capacity	6
Taxonomic Classification of *P. americana*	7
Transport of Fish	7
Stress	8
MATERIALS AND METHODS	
Phase 1: Acute Toxicity Test of Different Concentrations of *P. americana* Aqueous Leaf Extract in Nile Tilapia Fingerlings	11
Preparation of *P. americana as water additive*	11
Experimental Fish	11
Experimental Lay-out and Design	12
Acute Toxicity Test	13
Data Gathered in Phase 1	13
Analysis of Data	13
Phase 2: Optimum Concentration of *P. americana* Aqueous Leaf Extract	14

During Transport of Nile Tilapia Fingerlings

Treatments 14
Preparation of *P. americana* Extract as Water Additive 14
Experimental Lay-out and Design 15
Experimental Fish 16
Experimental Procedure 16
Water Quality Analysis 16
Analysis of Data 16
Data Gathered in Phase 2 17

RESULTS AND DISCUSSION

Phase 1:Acute Toxicity Test of Different Concentrations of *P. americana* Aqueous Leaf Extract in Nile Tilapia Fingerlings 18

Mortality Rate 19

Phase 2: Optimum Concentration of *P. americana* Aqueous Leaf Extract During Transport of Nile Tilapia Fingerlings 21

Survival Rates 21
Water Quality Parameters
Temperature 23
Dissolved oxygen 27
pH 29
Total-ammonia nitrogen 31

Correlation of Survival Rates in the Water Quality Parameters 34

SUMMARY, CONCLUSION, AND RECOMMENDATION 336

LITERATURE CITED 40

APPENDICES 38

LIST OF TABLES

Table No.	Title	Page
1	The different concentrations of the aqueous leaf extract of *P. americana* used as treatment in Phase 1.	12
2	The different amount of aqueous leaf extract of *P. americana* used as treatments in Phase 2 in relation to different time intervals.	15
3	Mortality rates of Nile tilapia fingerlings during the evaluation of *P. americana* aqueous extract in the 48 h of toxicity tests	18
4	Survival rates of Nile tilapia fingerlings during the evaluation of *P. americana* aqueous extract in the 48 h of toxicity tests	21
5	The temperature levels during evaluation of *P. americana* aqueous extract at different time intervals of simulated transportation of Nile tilapia fingerlings	24
6	The dissolved oxygen levels during evaluation of *P. americana* aqueous extract at different time intervals of simulated transportation of Nile tilapia fingerlings	26
7	The pH levels during evaluation of *P. americana* aqueous extract at different time intervals of simulated transportation of Nile tilapia fingerlings	29
8	The ammonia levels during evaluation of *P. americana* aqueous extract at different time intervals of simulated transportation of Nile tilapia fingerlings	31
9	Correlation of survival rate to water quality parameters	32

LIST OF FIGURES

Figure No.	Title	Page
1	Leaves of *Persea americana*	7
2	Physical, chemical and other perceived stressors in fish	10
3	Experimental lay-out for the toxicity test of *P. americana* to Nile tilapia fingerlings	13
4	Experimental lay-out on the evaluation of *P. americana* aqueous extract on the simulated transportation of Nile tilapia fingerlings.	15
5	Mean mortality rates in different *P. americana* concentrations in the 48 h toxicity test	20
6	Mean survival rates in different *P. americana* concentrations in the 48 h simulated transportation	22
7	Mean temperature at different *P. americana* concentrations and different durations of transportation	24
8	Mean dissolved oxygen concentrations at different *P. americana* concentrations and different durations of transportation	27
9	Mean pH at different *P. americana* concentrations and different durations of transportation	29
10	Mean TAN at different *P. americana* concentrations and different durations of transportation	31

LIST OF APPENDIX TABLES

Appendix Table No.	Title	Page
1	Probit values based on the concentration	42
2	Probit calculation table for *P. americana* 48 hours exposure	42
3	Tests of Between-Subjects Effects of mortality rate on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 1	42
4	Analysis of variance on mortality rate of five treatment concentrations at different hours in Experiment 1	43
5	Tests of Between-Subjects Effects of survival rate on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	43
6	Analysis of variance on survival rate of five treatment concentrations at different hours in Experiment 2	44
7	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five treatment concentrations at 6 hours in Experiment 2	45
8	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five treatment concentrations at 12 hours in Experiment 2	45
9	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five treatment concentrations at 24 hours in Experiment 2	46
10	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five treatment concentrations at 48 hours in Experiment 2	46
11	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five durations of transportation at 0ml/l water concentration in Experiment 2	47
12	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five durations of transportation at 1.5 ml/l water concentration in Experiment 2	48
13	Analysis of variance on the temperature, pH and total-ammonia nitrogen	48

	of five durations of transportation at 3 ml/l water concentration in Experiment 2	
14	Analysis of variance on the temperature, pH and total-ammonia nitrogen of five durations of transportation at 4.5ml/l water concentration in Experiment 2	49
15	Tests of Between-Subjects Effects of temperature on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	50
16	Tests of Between-Subjects Effects of dissolved oxygen on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	50
17	Tests of Between-Subjects Effects of pH on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	51
18	Tests of Between-Subjects Effects of ammonia on Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	51
19	Pearson correlation test of survival rate, temperature, DO, pH and TAN of Nile tilapia fingerlings subjected to different concentrations of *P. americana* extract in relation to time in Experiment 2	52

LIST OF APPENDIX FIGURES

Appendix Figure No.	Title	Page
1	Avocado *P. americana* leaves	53
2	Diluting of extract with water	53
3	Counting of Nile tilapia fingerlings through bulk weight	53
4	Packaging of Nile tilapia fingerlings	54
5	Oxygenating the transport bags with Nile tilapia fingerlings	54
6	Transport bag with Nile tilapia fingerlings and oxygen.	54
7	Wheeled-cart used for simulated transportation of Nile tilapia fingerlings.	55
8	Measuring of water quality parameters	55
9	Total ammonia reading before the transportation of tilapia fingerlings	55

AVOCADO (*Persea americana*) AQUEOUS LEAF EXTRACT AS ADDITIVE IN THE TRANSPORT MEDIUM OF NILE TILAPIA (*Oreochromis niloticus* L.) FINGERLINGS[1/]

ABSTRACT

Since the introduction of the Nile tilapia in the Philippines in the 1970's the culture and production of tilapia has progressed in leaps and bounds. As production techniques improved, tilapia moved into the mainstream market. Producers practiced the method of live fish transportation and it is now a common necessity in aquaculture. Numerous chemical additives can be added to the transport water to alleviate several problems associated in the bag shipment. The objective of this study was to evaluate the effect of *P. americana* aqueous leaf extract as an additive in the transport medium of Nile tilapia fingerlings.

On the 1st phase of the experiment, toxicity test of different concentrations (0, 1, 25, 50, 75, and 100 ml) of *P. americana* aqueous leaf extract in size #22 Nile tilapia fingerlings revealed 10 percent mortality at 3.33 ml/L and 50 percent mortality at 106.42 ml/L. Mortality was attributed to the deterioration of water quality as duration of transportation increased.

On the 2nd phase, different concentrations (0, 1.5, 3 and 4.5 ml per L of water) of *P. americana* aqueous leaf extract were used as water additives in the transport medium at 6, 12, 24, and 48 h of transport. Results showed that mean survival rate was not affected by the interaction of extract concentration and duration of transport (P=0.889).Likewise, survival rates between treatment concentrations (P=0.290) were not

significantly different from each other although transport bags with extract showed numerically higher survival rates the that of the control. Meanwhile, survival rate was significantly influenced by duration of transportation ($P<0.01$). The mean survival rate on the 6th h was comparable to that on the 12th h but was significantly higher than those on the 24th, 36th and 48th h while mean survival rate on the 12th h was comparable to that on the 24th h but significantly higher than those on the 36th and 48th h. Meanwhile, mean survival rate on the 24th h was significantly higher than those on the 36th h and 48th h and that of the 36th was significantly higher than that on the 48th h.

Temperature readings and pH levels during the 48 h simulated transportation were within the ideal range for growth and survival. However, DO levels of the different concentrations decreased continuously as transport duration progressed. This scenario, however, is common in live fish transportation. DO being the most important single factor in transporting fish contributed to the mortality of the fingerlings. Total-ammonia nitrogen, on the other hand, have no significant effect on the mortality of the fish because at transportation time where the TAN levels were significantly higher (6th, 12th and 24th h) survival rates were still high.

Results showed that survival rate was significantly correlated to DO (r=0.920, P=0.000, n=48), pH (r=-0.615, P=0.000, n=48) and ammonia (r=0.878, P=0.000, n=48) but was not significantly correlated to temperature (r=0.310, P=0.320, n=48).

[1/] Undergraduate thesis presented to the faculty of the College of Fisheries, Central Luzon State University as a partial fulfillment of the requirements for the degree of Bachelor of Science in Fisheries. Prepared at the Department of Aquaculture under the supervision of Dr. Emmanuel M. Vera Cruz.

LITERATURE CITED

Agamadodaigwe, R.C. 2005. The effect of transportation stress on haematocrit level of *Oreochromis niloticus* Linnaeus. Animal Research International, 2(1): 224 – 226.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42: 517–525.

Berka, R. 1986. The transportation of live fish: A review. European inland fisheries advisory commission technical paper no. 48. Food and Agricultural Organization the United Nations. Rome, Italy. 79 p. Bolorundoro, P.I. 2001. Transporting fish for culture. National Agricultural Extension and Research Liaison Services. Ahmado Bello University. Zaria, Nigeria. p. 21.

Bower, J.P., B.N. Wolstenholme and J.M. de Jager. 1977. Incoming solar radiation and internal water status as stress factors in avocado, *Persea americana*(mill.) Cvedranol. South african avocado growers' association proceedings of the technical committee, 1: 35-40.

Carmichael, G.J., R.M. Jones and J.C. Morrow. 1992. Comparative efficacy of oxygen diffusers in a fish-hauling tank. The Progressive Fish Culturist, 54: 35-40.

Cech, J.J, S.D. Bartholow, P.S. Young and T.E. Hopkins. 1996. Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. Transactions of the American Society, 125: 308-320.

Coskun, O., M. Kanter, F. Armutcu, K. Cetin, B. Kaybolmaz and O. Yazgan. 2004. Protective effects of quercetin, a flavonoid antioxidant, in absolute ethanol-induced acute gastric ulcer. European Journal of General Medicine, 1(3): 37-42.

Delince, G.A., D. Campbell, J.A.L. Janssen and M.N. Kutty. 1987. Lectures presented in ARAC for senior aquaculturists course. Retrieved on May 1, 2016 from http://www.fao.org/docrep/field/003/ac182e/AC182E00.htm#TOC.

FAO. 2005. *Oreochromis niloticus.* Retrieved on May 1, 2016 from http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en#tcNA0112.

Ibibia, E.T. 2013. Spectroscopic determination of total phenolic and flavonoid contents, and antioxidant activity, of the leaves of *Persea americana.* International Journal of Pharmacy and Pharmaceutical Sciences, 5(3): 598-603.

Jensen, G.B. 1990. Transportation of warm water fish procedures and loading rates. Southern Regional Aquaculture Center Publication No. 392. Mississippi, USA. p 2.

Maule, A.G., C.B. Schreck, B.C. Samual and B.A. Barton. 1988. Physiological effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia River. Transactions of the American Fisheries Society, 117: 245-261.

Noga, E.J. 2000. Fish Disease Diagnosis and Treatment. Retrieved on May 15, 2017 from https://books.google.com.ph/books

Ojewole, J.A. and G.J. Amabeoku. 2006. Anticonvulsant effect of *Persea americana* Mill (Lauraceae) (avocado) leaf aqueous extract in mice. Phytotherapy Research, 20: 696-700.

Owalabi, M.A., H.A.B. Coker and S.I. Jaja. 2010. Bioactivity of the phytoconstituents of the leaves of *Persea americana*. Journal of Medicinal Plants Research, 4(12): 1130-1135.

Oyalinka, E.T., A. Ore, O.A. Adeyemo, O.S. Ola, O.O. Olotu and R.C. Echebiri. 2015. Quercetin, a flavonoid antioxidant, ameliorated procarbazine-induced oxidative damage to murine tissues. Antioxidants, 4: 304-321.

Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler and J.R. Leonard. 1982. Fish Hatchery Management. United States Department of the Interior, Fish and W ildlife Service, Washington, D.C. Retrieved on May 15, 2017 from https://books.google.com.ph/booksPopma, T. and M. Masser. 1999. Tilapia: life history and biology. Southern Regional Aquaculture Center Pub. No. 283. Mississippi, USA. p. 4.

PSA. 2015. Fisheries situationer. Retrieved on November 19, 2016 from https://psa.gov.ph/sites/default/files/fishsit_jan_mar2015.pdf.

Ranade, S.S. and P. Thiagarajan. 2015. A review on *Persea americana* Mill. (Avocado) - its fruit and oil. International Journal of Pharm. Tech. Research, 8(6): 72-77.

Rice-Evans, C. 1995. Plant polyphenols: free radical scavengers or chain-breaking antioxidants. Biochemical Society Symposia, 61: 103-116.

Rice-Evans, C.A. and N.J. Miller. 1996. Antioxidant activities of flavonoids as bioactive components of food. Biochemical Society Transactions, 24: 790-795.

Rice-Evans, C.A., N.J. Miller and G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20: 933-956.

Swann, L. 1993. Transportation of fish in bags. North Central Regional Aquaculture Center Fact Sheet Series #104. Retrieved from http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1012&context=ncrac_factsheets on May 1, 2016.

Wendalaar Bonga, S.E. 1997. The stress response in fish. Physiological Reviews, 77(3): 591-625.

Wedemeyer, G.A. and C.P. Goodyear. 1984. Disease caused by environmental stressors. p. 424-434. Retrieved from http://www.sciencedirect.com on April 10, 2016.

Wedemeyer, G.A., B.A. Barton and D.J. Mcleay. 1990. Stress and acclimation. Retrieved from https://books.google.com.ph on April 10, 2016.

Weirich, C.R. and J.R. Tomasso. 1991. Confinement- and transport induces stress on red drum juveniles: effects of salinity. The Progressive Fish Culturist, 53: 146-149.

https://en.wikipedia.org/wiki/Antioxidant

https://en.wikipedia.org/wiki/Procarbazine

http://philaquaculture.blogspot.com