



*IN-VIVO* ASSAY FOR THE EFFICACY OF *Pochonia chlamydosporia*  
AS POTENTIAL BIOLOGICAL CONTROL AGENT AGAINST  
*Fasciola* spp. IN WATER BUFFALOES (*Bubalus bubalis*)

NORIEL BIHASA ESTEBAN

An Undergraduate Thesis Submitted to the Faculty of the Department of  
Biological Sciences, College of Arts and Sciences, Central Luzon  
State University, Science City of Muñoz, Nueva Ecija,  
Philippines, In Partial Fulfillment of the  
Requirements for the Degree

**BACHELOR OF SCIENCE IN BIOLOGY**

**JUNE 2017**




COLLEGE OF ARTS AND SCIENCES  
Department of Biological Sciences

**APPROVAL SHEET**

The Undergraduate Thesis entitled: *IN VIVO* ASSAY FOR THE EFFICACY OF *Pochonia chlamydosporia* AS POTENTIAL BIOLOGICAL CONTROL AGENT AGAINST *Fasciola* spp. IN WATER BUFFALOES prepared and submitted by **NORIEL B. ESTEBAN** in partial fulfilment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY** is hereby approved and accepted.

  
**KHRISTINA J. CRUZ**  
Adviser  
08 JUNE 2017  
Date Signed

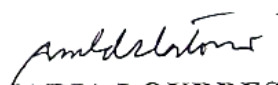
  
**CLARO N. MINGALA, DVSM, MVSt, Ph.D.**  
Co-Adviser  
Date Signed

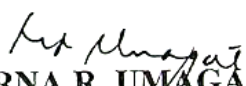
  
**MARY JHANE G. VALENTINO, MSc**  
Critic  
Date Signed

  
**RICH MILTON R. DULAY, MSc**  
Department Research Coordinator  
Date Signed

Accepted in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY**.

  
**EVARISTO A. ABELLA, PhD**  
Department Chair  
Date Signed

  
**ANNA MARIA LOURDES S. LATONIO PhD**  
College Research Coordinator  
Date Signed

  
**MYRNA R. UMAGAT PhD**  
Dean  
Date Signed



## BIOGRAPHICAL SKETCH

### PERSONAL INFORMATION

Name: Noriel Bihasa Esteban  
Date of Birth: February 7, 1996  
Place of Birth: Cabanatuan City, Nueva Ecija  
Residence: Brgy. Quezon District, Cabanatuan City,  
Nueva Ecija  
Religion: Roman Catholic  
Parents: Mr. Norito Fulgencio Esteban Sr.  
Mrs. Marilou Bihasa Esteban



### EDUCATIONAL BACKGROUND

*TERTIARY* : **Central Luzon State University**  
(A.Y. 2012-2017) Science City of Muñoz, Nueva Ecija  
Bachelor of Science in Biology

*SECONDARY* : **Nueva Ecija High School**  
(A.Y. 2008-2012) Burgos Street, Cabanatuan City, Nueva Ecija  
1<sup>st</sup> Honorable Mention

*ELEMENTARY* : **Cabanatuan East Central School**  
(A.Y. 2004-2008) Cabanatuan City, Nueva Ecija  
6<sup>th</sup> Honorable Mention

### SEMINARS ATTENDED

**“Information/Education Campaign on University Comprehensive Environmental Management Plan”** held on July 18, 2012 at the Research, Extension and Training, CLSU, Science City of Muñoz, Nueva Ecija.

**“Breathless: Smoking and Its Impact on One’s Health”** held at the University Auditorium, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines on September 14, 2012.



2013 Chemistry Symposium with the theme **“Chemistry is Fun”** held during the College of Arts and Sciences (CAS) Festival, on the 19<sup>th</sup> day of February at the University Auditorium, Central Luzon State University, Science City of Muñoz, Nueva Ecija.

Symposium on **“Rice Biotechnology”** with the theme **“Direct Seeding: A Response to Economical and Technological Challenges in Rice Farming”** held at the University Auditorium, Central Luzon State University, Science City of Muñoz, Nueva Ecija, on the 7<sup>th</sup> day of September 2013.

**“Current Trends in Food Safety and Quality Assurance”** held at the University Gymnasium, Central Luzon State University, Science City of Muñoz, Nueva Ecija, on the 30<sup>th</sup> day of August 2014.

**“HIV: AIDS Survival of the Fittest. The Human Culture Media”** held at the University Auditorium, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, on November 21, 2015.

**“1<sup>st</sup> CLSUGraduate Students Research Colloquium”** held at the College of Arts and Sciences Little Theater Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines on May 4, 2016.

**“Philippine Biodiversity and the National Museum”** held at the University Auditorium, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines on March 22, 2016.

**“ASEAN Academic Research Summit”** held at College of Arts and Sciences Little Theater, Central Luzon State University on March 25, 2015.

**“1<sup>st</sup> CLSU – University of Tsukuba Bilateral Student Research Congress”** held at Central Luzon State University, College of Arts and Sciences Little Theater on March 5, 2015.

**“22<sup>nd</sup> Psychology Student Research Congress”** held at University Auditorium, Central Luzon State University on Feb. 27, 2015.

**“16<sup>th</sup> Congress of Campus Leaders”** held at RET Amphitheatre, CLSU, Science City of Muñoz, Nueva Ecija on August 13, 2013.

**“Saliksikan 2012: Serye ng Pananaliksik sa Wika, Kultura at Panitikan”** held at University Auditorium, CLSU, Science City of Muñoz, Nueva Ecija on August 16, 2012

**“Saliksikan 2013: Serye ng Pananaliksik sa Wika, Kultura at Panitikan”** held at University Auditorium, CLSU, Science City of Muñoz, Nueva Ecija on August 19, 2013



“1<sup>st</sup> National Biotechnology Summit” held at University of the Philippines- Los Baños on August 27, 2016

**MEMBERSHIP**

**CLSU BIOLOGICAL SOCIETY**

College Based Organization

**Treasurer**

June 2014 - February 2015

**Vice President – Internal**

June 2015 – April 2016

**BIOLOGY SUPREME STUDENT COUNCIL**

Department Student Council

**President**

August 2014–September 2015

**ON THE JOB TRAINING:**

Plant Breeding and Biotechnology Division, Philippine Rice Research Institute, Science City of Muñoz, Nueva Ecija, Philippines (April-May 2015)



## ACKNOWLEDGEMENT

The author would like to express his deepest gratitude to his Thesis Adviser and Co-Adviser, Mrs. Khristina J. Cruz and Mr. Sonny C. Ramos, for their guidance, motivation, encouragement, patience, willingness and well-founded knowledge. Their deep understanding on some of my situations helped me to keep moving forward when I'm about to step back.

The author would also like to acknowledge the rest of the thesis committee for their guidance, patience, motivation, enthusiasm, and knowledge.

The author would also like to express his sincere gratitude and thoughtful appreciation to his thesis buddy, Ms. Victoria Joy C. Cuaresma, and to Ms. Vicky B. Salting for their understanding, help, support and extending their time with the author in the laboratory. Also, to the people of the Animal Health Laboratory of Philippine Carabao Center for allowing him to conduct this study at their laboratory; and to Dr. Virginia M. Venturina for her supervision during the collection of liver fluke eggs.

The author would like to thank his BSBio friends. Lmao.

The author would also like to express his deepest gratitude and special heartfelt appreciation to his parents, Mr. Norito F. Esteban, Sr., and Mrs. Marilou B. Esteban, whom after all the disappointments and not meeting their expectation, they still continue to believe and trust me, for their undying love, encouragement, words of wisdom, constructive criticisms and financial support to continue my study. Also, the author give appreciation to his siblings- Norito Jr., Michelle, Norimar, Norman, Mark Angelo and his beloved grandmother Rosalinda F. Esteban.



The author express deep gratitude to his friend, Charisse Joyce C. Leabres, for her time, encouragement, prayers, patience, understanding, sacrifice, and love to the author that will be always valued and cherished. Ms. Lynne Cabiling for extending her helping hands and guidance to the author during his financial difficulties. Their efforts and prayers will be always appreciated and valued.

And most of all, the author would like to thank God in his infinite blessings, grace, care and unconditional love that made this undergraduate thesis possible. Thank you very much.

**NORIEL B. ESTEBAN**



## TABLE OF CONTENTS

	PAGE
TITLE PAGE	i
APPROVAL SHEET	ii
BIOGRAPHICAL SKETCH	iii
ACKNOWLEDGEMENT	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF APPENDICES	xii
LIST OF APPENDIX TABLES	xiii
LIST OF APPENDIX FIGURES	xiv
ABSTRACT	xv
INTRODUCTION	
Background of the Study	1
Objectives of the Study	5
Significance of the Study	5
Scope and Limitations of the Study	6
Time and Place of the Study	6
REVIEW OF RELATED LITERATURE	
Genus <i>Fasciola</i> : The Flukes	8
Fasciolosis	11
Nematophagous Fungi as Biological Control Agent	13
<i>Pochonia chlamydosporia</i> , Egg-parasitic Nematophagous Fungi	16



**MATERIALS AND METHODS**

Pure Culture of <i>P. chlamydosporia</i>	20
<i>Fasciola</i> spp.eggs	20
Experimental Animals and Treatments	21
Mass Production of <i>P. chlamydosporia</i>	21
<i>In-Vivo</i> Assay	22
Data Gathered	24
Statistical Analysis	24

**RESULTS AND DISCUSSION**

Ability of the Fungus to Pass Through the Gastrointestinal Tract of the Water Buffalo	25
Duration of Viability of <i>P. chlamydosporia</i> in Natural Conditions after Passing through the Gastrointestinal Tract of the Water Buffalo	25
Evaluation of the Ovicidal Activity of <i>P. chlamydosporia</i> on the <i>Fasciola</i> spp. Eggs after Passing through the Gastrointestinal Tract of the Water Buffalo	26

**SUMMARY, CONCLUSION AND RECOMMENDATON**

Summary	30
Conclusion	31
Recommendation	31

**LITERATURE CITED**

32

**APPENDICES**

Appendix A- Statistical Output	42
Appendix B- Experimental Samples and Procedures	43



LIST OF TABLES

TABLE		PAGE
1	Percentages of ovicidal activity for the nematophagous fungus <i>Pochonia chlamydosporia</i> (VC21) and the control, without fungal treatment, against <i>Fasciola</i> spp. eggs at faeces collection times 12, 18, 24, 48, 72, 96 h afer 35 days of observation	27



LIST OF FIGURES

FIGURE		PAGE
1	Life Cycle of the Liver Fluke (Boray, 2007)	9
2	Adult Liver Fluke (Boray, 2007)	10
3	Interaction hyphae of the fungus <i>P. chlamydosporia</i> on the <i>Echinostoma paraense</i> eggs. (Lelis <i>et al.</i> , 2014)	23
4	<i>P. chlamydosporia</i> hypha around <i>Fasciola</i> spp. eggs under Type 1 effect	28



LIST OF APPENDICES

APPENDIX		PAGE
A	Statistical Output	42
B	Experimental Samples and Procedure	43



LIST OF APPENDIX TABLES

APPENDIX TABLE		PAGE
1	Descriptive Statistic	42
2	Ranks of Type 1 effect	42



LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	Pure Culture of <i>Pochonia chlamydosporia</i> (VC21)	43
2	Mass Production of <i>Pochonia chlamydosporia</i> (VC21)	44
3	Collection of <i>Fasciola</i> spp. Eggs	45
4	Harvesting, Air-Drying and Weighing of Fungal Mycelial Mass	46
5	Mixing of the fungal mycelial mass to commercial feeds pellet and administering it to the treated group.	47
6	<i>In-Vivo</i> Assay	48



## ABSTRACT

**ESTEBAN, NORIEL B.**, Bachelor of Science in Biology, Department of Biological Science, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, June 2017, ***IN-VIVO* ASSAY FOR THE EFFICACY OF *Pochonia chlamydosporia* AS POTENTIAL BIOLOGICAL CONTROL AGENT AGAINST *Fasciola* spp. IN WATER BUFFALOES (*Bubalus bubalis*)**

Manuscript No.: BIO-M- 2<sup>nd</sup>17-038

Adviser: Khristina J. Cruz

Co-Adviser: Claro N. Mingala, DVSM, MS St., Ph. D.

This study determined the ability of *P. chlamydosporia* to pass through the gastrointestinal tract of the water buffalo, retain its viability and assess the ovicidal activity of the nematophagous fungus against *Fasciola* spp. eggs. Twelve previously dewormed water buffaloes with a mean age of 7 months and normal weight ranging from 200-250 kg were divided into control and treated groups. Each animal of the treated group received a mycelial mass of *P. chlamydosporia* mixed in commercial feed pellets. The control group received pellet without the fungus. Fecal samples were collected from both group at intervals of 12, 18, 24, 48, 72 and 96 h after pellet administration and placed in petri dishes containing 2% water agar. Approximately five hundred *Fasciola* spp. eggs were then plated on the petri dishes of control and treated group with six replicates, and incubated in the dark for 35 days. At the end of the experiment, one hundred eggs were observed from each petri dish and classified according to the following parameters: type 1, physiological and biochemical effect without



morphological damage to eggshell, with hyphae adhered to the shell; type 2, lytic effect with morphological change in the eggshell and embryo without hyphal penetration, and type 3, lytic effect with morphological change in the eggshell and embryo, with hyphal penetration and internal egg colonization. Hyphal growth and formation was observed in petri dishes of the treated group. Based on the results, *P. chlamydosporia* remained viable after passing through the gastrointestinal tract of water buffalo but exhibited no ovicidal activity (type 3 effect) against *Fasciola* spp. eggs at all collection interval. Thus, the mycelial mat of the fungus was not efficient as a biological control agent against *Fasciola* spp. eggs.



## LITERATURES CITED

- ABROL, DHARAM P., and U. SHANKAR. (2012).** *Integrated pest management: principles and practice*. Centre For Agriculture And Biosciences International.
- APT, W., AGUILERA, X., VEGA, F., ZULANTAY, I., APT, P., GONZALEZ, V., RETAMAL, C., RODRIGUEZ, J. and J. SANDOVAL (1993).** Prevalence of fascioliasis in humans, horses, pigs, and wild rabbits in 3 Chilean provinces. *Boletín de la Oficina Sanitaria Panamericana*, 115:405–414.
- ARAUJO, J.V., FREITA, B.W., VIEIRA, T.C. and A. K. CAMPOS. (2004).** Evaluation of nematode predacious fungus *Duddingtonia flagrans* on infective *Haemonchus contortus* and *Strongyloides papillosus* larvae of goats. *The Brazilian Journal of Veterinary Parasitology*, 15(2): 76-9.
- ARAUJO, J.V., MOTA, M.A. and A.K. CAMPOS. (2004).** Controle biológico de helmintos parasitos de animais por fungos nematófagos. *The Brazilian Journal of Veterinary Parasitology*, 13:165–170.
- AYRES M., AYRES J.R.M., AYRES D.L., and A.S. SANTOS. (2003).** Aplicações estatísticas nas áreas de ciências biológicas, Belém: Sociedade civil mamiraua:Brasilia Conselho Nacional de Desenvolvimento Científico e Tecnológico, 290.
- BENGTSSON, TORBJORN. (2015).** Biological control of root-knot nematodes (*Meloidogyne* spp.) by the fungus *Pochonia chlamydosporia* – A review. US Department of Agriculture
- BORAY, J. C. (1985).** Flukes of Domestic Animals. In: Gaafar, S.M., Howard, W. S., Marsh, R. E., (Eds.), *Parasites, Pests and Predators*. Elsevier, New York, 178-218.
- BORAY, J. C. (2007).** Primefact 446, Liver fluke disease in sheep and cattle. NSW Agriculture. New South Wales Department of Primary Industries.
- BRAGA, F. R. and J. V. ARAUJO. (2010).** Viability of the nematophagous fungus *Pochonia chlamydosporia* after passage through the gastrointestinal tract of horses. *Veterinary Parasitology* 168: 264-268.



- BRAGA, F.R., ARAUJO J.V., CAMPOS, A.K., ARAUJO, J.M., CARVALHO, R.O., SILVA, A.R. and A. O. TRAVELA. (2008).***In-vitro* evaluation of the action of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Fasciola hepatica* eggs. *The World Journal of Microbiology and Biotechnology* 24: 1559-1564.
- CARVALHO, R. O., ARAÚJO, J. V., BRAGA, F. R., ARAUJO, J. M. and C. D. F. ALVES. (2010).** Ovicidal activity of *Pochonia chlamydosporia* and *Paecilomyces lilacinus* on *Toxocara canis* eggs. *Veterinary Parasitology*, 169(1): 123-127.
- CLARKE, S.L. (2004).** Improvements in Nematophagous Fungi to Control Gastro-intestinal Parasites. *MS Thesis*. Massey University.
- CHEN S, and D.W. DICKSON. (2012).** Biological control of plant-parasitic nematodes. Pp. 761–811 in R. H. Manzanilla-López and N. Marbán-Mendoza, eds. *Practical plant nematology*. Guadalajara, México: Colegio de Postgraduados and Mundi-Prensa, Biblioteca Básica de Agricultura.
- CHEN, M.G. and K.E. MOTT. (1990).** Progress in assessment of morbidity due to *Fasciola hepatica* infection. *Tropical Disease Bulletin*, 87:1–37.
- COPLAND, R. S. and L. F. SKERRATT. (2008).** Options for the control of liver fluke, Overcoming liver fluke as a constraint to ruminant production in South-East Asia, Canberra, Australian Centre for International Agricultural Research, 55-68.
- CUOMO, M.J., NOEL, L.B., and D.B. WHITE. (2006).** Diagnosing Medical Parasites: A Public Health Guide to Assisting Laboratory and Medical Officers. PH Source. Chapter 6: 92-98.
- DALTON, J.P. (1999).** Fasciolosis, 1st edition. University Press, Cambridge
- DALTON, J. P., NEILL, S. O., STACK, C., COLLINS, P., WALSH, A., SEKIYA, M., and S. M. DONNELLY. (2003).** *Fasciola hepatica* cathepsin L-like proteases: biology, function, and potential in the development of first generation liver fluke vaccines. *International Journal for Parasitology*, 33(11): 1173-1181.



- DANGPRASERT, T. W., KHAWSUK, A., MEEPOL, C., WANICHANON, V., VIVANANT, E., UPATHAM, S., WONGRATANACHEEVIN, and P. SOBHON. (2001). *Fasciola gigantica*: Surface topography of the adult tegument. *Journal of Helminthology*, 75: 43-50.
- DARGIE, J. (1987). The impact on production and mechanism of pathogenesis of trematode infections in cattle and sheep. *International Journal of Parasitology*, 17: 453-463.
- DE, S., SANYAL, P.K., SARKAR, A.K., PATEL, N.K., PAL, S. and S.C. MANDAL. (2008). Screening for Indian isolates of egg-parasitic fungi for use in biological control of fascioliasis and amphistomiasis in ruminant livestock. *Journal of Helminthology*, 82:271-277.
- DIAS, A. S., ARAUJO, J. V., BRAGA, F. R., PUPPIN, A. C. and W. R. PERBONI, (2013). *Pochonia chlamydosporia* in the biological control of *Fasciola hepatica* in cattle in Southeastern Brazil. *Parasitology Research* 112(6): 2131-2136.
- ESTEBAN, J.G., BARGUES, M.D., and S. MAS-COMA. (1998). Geographical distribution, diagnosis and treatment of human fascioliasis: a review. *Research Review in Parasitology*. 58:13-42.
- ESTEBAN, J.G., FLORES, A., ANGLES, R., and S. MAS-COMA, (1999). High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 93: 151-156.
- FAO,(1994) Diseases of domestic animals caused by liver flukes: epidemiology, diagnosis and control of *Fasciola*, paramphistome, *Dicrocoelium*, *Eurytrema* and schistosome infections of ruminants in developing countries. FAO: Rome.
- GAMS, W. and R. ZARE. (2001). A revision of *Verticillium* sect. *Prostrata*. III. Generic classification. *Nova Hedwigia* 73:329-337.
- HILLYER, G.V., and W. APT, (1997). Food-borne trematode infections in the Americas. *Parasitology Today*, 13: 87-88.
- IRVING, F., and B. R. KERRY. (1986). Variation between strains of the nematophagous fungus *Verticillium chlamydosporium* Goddard. Factors affecting parasitism of cyst nematode eggs. *Nematologica*, 32:474-485



- KARAKAS, M., BENLI, M., and S. CEBESYOY. (2012).** Effects of Some Cultural Conditions on the Growth of Nematophagous Fungus *Pochonia chlamydosporia* (Fungi:Clavicipitaceae) Isolated from *Meloidogyne incognita* Eggs. *Journal of Animal and Veterinary Advances* 11(24): 4644-4647
- KEISER J. and J. UTZINGER. (2007).** "Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes," *Trends in Parasitology*, 23(11): 555–562.
- KERRY, B.R. (1997).** Biological control of nematodes: Prospects and opportunities. FAO Corporate Document Repository. Plant nematode problems and their control in the Near East Region. FAO Plant Production and Protection Paper, 144.
- KERRY, B. R. (2000).** Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology* 38:423–441.
- KERRY, B.R., and L. HIDALGO. (2004).** Application of *Pochonia chlamydosporia* in the integrated control of root-knot nematode on organically grown vegetable crops in Cuba. *International Organization for Biological and Integrated Control/ West Palearctic Regional Section Bulletin* 27:123–126.
- KERRY, B. R., KIRKWOOD, I. A., DE LEIJ, F. A. A. M., BARBA, J., LEIDJENS, M. B., and P.C. BROOKES. (1993).** Growth and survival of *Verticillium chlamydosporium* Goddard, a parasite of nematodes in soil. *Biocontrol Science Technology*, 3: 355-365.
- KERRY, B.R. and P. R. HIRSCH. (2011).** Ecology of *Pochonia chlamydosporia* in the rhizosphere at the population, whole organisms and molecular scales. Pp. 71–182 in K. Davies and Y. Spiegel, eds. *Biological control of plant-parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms. Progress in biological control, Volume 11.* Dordrecht, The Netherlands: Springer.
- KUMAR, V. (1998).** *Trematode Infections and Diseases of Man and Animals.* AA Dordrecht, The Netherlands: Kluwer Academic Publishers.
- LARSEN, M. (1999).** Biological control of helminths. *International Journal of Parasitology* 29:139–146



- LE, T.H., DE, N.V., AGATSUMA, T., Thi NGUYEN, T.G., NGUYEN, Q.D., McMANUS, D.P., and D. BLAIR. (2008). Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. *International Journal of Parasitology*. 38: 725–730.
- LELIS, R. T., BRAGA, F.R., DE CARVALHO, L.M., DE PAULA, A.T., ARAUJO, J.M., FAUSTO, M.C., JUNIOR, A.M., RODRIGUES J.V.F., DE FREITAS SOARES, F.E., GARCIA, J.S., and J.V. DE ARAUJO. (2014). Effect of the fungus *Pochonia chlamydosporia* on *Echinostoma paraensei* (Trematoda: Echinostomatidae). *Acta tropica*, 139: 88-92.
- LOPEZ-LLORCA, L.V., GOMEZ-VIDAL, S., MONFORT, E., LARRIBA, E., CASADO-VELA, J., ELORTZA, F., JANSSON, H.B., SALINAS, J., and J. MARTIN-NIETO. (2010). Expression of serineproteases in egg-parasitic nematophagous fungi during barley root colonization. *Fungal Genetics Biology* 47: 342–351.
- LOPEZ-LLORCA, L. V., MACIA-VICENTE, J. G., and H. B. JANSSON. (2008). Mode of action and interactions of nematophagous fungi In: *Integrated management and biocontrol of vegetable and grains crops nematodes*. Ciancio, A., Mukerji, K.G. (Eds.). Dordrecht. The Netherlands: Springer, 51-76.
- LYSEK, H. (1976). Classification of ovicide fungi according to type of ovicidity. *Acta Universitatis Palackianae Olomucensis Facultatis Medicae* 76:9–13.
- LYSEK, H., FASSATIOVA, O., PINEDA, N. C. and N. L. HERNANDEZ. (1982). Ovicidal fungi in soils of Cuba. *Folia Parasitologica* 29:265-270.
- LYSEK, H. and J. STERBA. (1991). Colonization of *Ascaris lumbricoides* eggs by the fungus *Verticillium chlamydosporium* Goddard. *Folia Parasitologica* 38:255–259.
- MANZANILLA-LOPEZ, R.H., ESTEVES I, FINNETI-SIALER M, and B.R. KERRY. (2011). *Pochonia chlamydosporia*: Biological, ecological and physiological aspects in the host-parasite relationship of a biological control agent of nematodes. Pp. 267–300 in F. Boeri and J. A. Chung, eds. *Nematodes, morphology and management strategies*. New York: Nova Science



- MARTINEZ-GONZALES, B., DIEZ-BANOS, N. and F. A. ROJO-VASQUEZ. (1998).** An epidemiological study of gastro intestinal parasitism in dairy sheep flocks in Leon (NW) Spain. *Small Ruminant Research* 27:25-30
- MAS-COMA, S., ANGLES, R., ESTEBAN, J.G., BARGUES, M.D., BUCHON, P., FRANKEN, M., and W. STRAUSS. (1999).** The Northern Bolivian Altiplano: a region highly endemic for human fascioliasis. *Tropical Medicine and International Health* 4:454-467.
- MAS-COMA, S., BARQUES M.D., ESTEBAN J.G., and J.P. Dalton. (1998).** "Human fasciolosis Fasciolosis" Oxon, Wallingford, UK: The Centre for Agriculture and Bioscience International, 411-447:447
- MAS-COMA, S., BARGUES, M. D., and M. A. VALERO. (2005).** "Fascioliasis and other plant-borne trematode zoonoses," *International Journal for Parasitology*, 35(11-12):1255-1278.
- MAUCLINE, J. H., KERRY, B. R. and P. R. HIRSCH. (2002).** Quantification in soil and rhizosphere of the nematophagous fungus *Verticillium chlamydosporium* by competitive PCR and comparison with selective plating. *Applied and Environmental Microbiology*, 68: 1846-1853.
- MILIOTIS, and M., J. BIER. (2003).** *International Handbook of Foodborne Pathogens.* Imprint, New York: Marcel Dekker Incorporated.
- MOLINA, E. C., GONZAGA E. A. and L. A. LUMBAO. (2005).** Prevalence of infection with *Fasciola gigantica* and its relationship to carcass and liver weights, and fluke and egg counts in slaughter cattle and buffaloes in Southern Mindanao, Philippines. *Tropical Animal Health and Production* 37(6), 215-221.
- MORGAN-JONES, G., WHITE J.F. and R. RODRIGUEZ-KABANA.(1984).** Phytonematode pathology ultrastructural studies. 2. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. *Nematropica*, 14: 57-71.
- MORGAN-JONES, G., WHITE J.F. Jr. and R. RODRIGUEZ-KABANA.(1983).** Phytonematode pathology: ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. *Nematropica*, 13: 245-260.



- NAGESH, M., HUSSAINI, S. S., RAMANUJAM, B. and R. RANGESWARAN. (2007).** Molecular identification, characterization, variability and infectivity of Indian isolates of the nematophagous fungus *Pochonia chlamydosporia*. *Nematologia Mediterranea*, 35: 47-56.
- NORDBRING-HERTZ, B., JANSSON, H-B., and E. FRIMAN. (1995).** Nematophagous Fungi. Film No C1851 Go ttingen, Germany: Institut fur den Wissenschaftlichen Film.
- NORDBRING-HERTZ, B., JANSSON, H-B. and A. TUNLID. (2011).** Nematophagous Fungi. *Encyclopedia of Life Sciences*.
- PILE, E., DOS SANTOS, J. A. A., PASTORELLO, T., & M. VASCONCELLOS. (2001).** NOTA PRÉVIA *Fasciola hepatica* em búfalos (*Bubalus bubalis*) no município de Maricá. *Brazilian Journal of Veterinary Research and Animal Science* 38(1): 42-43.
- RAMAJO, V., OLEAGA, A., CASANUEVA, P., HILLYER, G. V. and A. MURO. (2001).** Vaccination of sheep against *Fasciola hepatica* with homologous fatty acid binding proteins. *Veterinary Parasitology*, 97 (1): 35-46.
- RAZA, M. A., AVAZ, M. M., MURTAZA, S. and M. S. AKHTAR. (2013).** Prevalence of GIT helminths in cattle at the vicinities of Tehsil Jatoi, Punjab, Pakistan. *Science International (Lahore)*, 25(2), 305-309.
- REDDINGTON, J. J., LEID, R. W., and R. B.WESCOTT. (1982).** Isolation and Purification of *Fasciola hepatica* eggs. *Veterinary parasitology*, 11(2-3): 179-183.
- RODRIGUEZ-KABANA, R. and G. MORGAN-JONES.(1988).** Potential for nematode control by mycofloras endemic in the tropics. *Journal of Nematology*, 20: 191-203.
- RULL, G., TIDY, C., and A. BONSALE. (2014).** October 20. *Fasciola hepatica*. Symptoms and treatment of *Fasciola hepatica* | Patient. Retrieved November 5, 2015, from <http://patient.info/doctor/fasciola-hepatica>
- RYAN, K.J. and C.G. RAY. (2004).** *Medical Microbiology* (4th ed.). United States of America: The McGraw-Hill Companies.



- SALEHA, A.A. (1991). Liver fluke disease (fascioliasis): epidemiology, economic impact and public health significance. *The Southeast Asian Journal of Tropical Medicine and Public Health* 22:361–4
- SINGH, R.K., SANYAL, P.K., PATEL, N.K., SARKAR, A.K., SANTRA, A.K., PAL, S. and S.C. MANDAL. (2010). Fungus–benzimidazole interactions: a prerequisite to deploying egg-parasitic fungi *Paecilomyces lilacinus* and *Verticillium chlamydosporium* as biocontrol agents against fascioliasis and amphistomiasis in ruminant livestock. *Journal of Helminthology* 84:123–131.
- SILVA, A.R., ARAUJO, J.V., BRAGA, F.R., ALVES, C.D.F. and L.N. FRASSY. (2010). *In vitro* ovicidal activity of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium* and *Pochonia chlamydosporia* on *Trichuris vulpis* eggs. *Veterinary Parasitology* 172:76-79.
- SMITH, M. C., and D. M. SHERMAN. (2009). *Goat Medicine* (2nd ed.). Ames, Iowa: Wiley-Blackwell.
- SPITHILL, T. W, SMOOKER, P. M. and D. B. COPEMAN. (1999). *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. In: Dalton, J.P. (Ed), *Fasciolosis*. The Centre for Agriculture and Bioscience International, Cambridge, 465–525.
- STEWART, S. (1999). "*Fasciola hepatica*" (On-line), Animal Diversity Web. Accessed November 09, 2015 at [http://animaldiversity.org/accounts/Fasciola\\_hepatica/](http://animaldiversity.org/accounts/Fasciola_hepatica/)
- STIRLING, G.R. (1991). Biological control of plant parasitic nematodes. Centre for Agriculture and Biosciences International, Wallingford
- STIRLING, G.R., and L. SMITH. (1998). Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. *Biological Control*. 11:231–239
- SUTHERLAND, I.A. and D.M. LEATHWICK. (2011). Anthelmintic resistance in nematode parasites of cattle: a global issue? *Trends in Parasitology* 27:176–181.
- TERRILL, T.H., M. LARSEN, O. SAMPLES, S. HUSTED, J.E. MILLER, R.M. KAPLAN, and S. GELAYE (2004). Capability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce infective larvae of gastrointestinal nematodes in goat feces in the southeastern United States:



dose titration and dose time interval studies. *Veterinary Parasitology* 120:285–296

- THEODOROPOULOS, G., THEODOROPOULOU, E., PETRAKOS G., KANTZOURA V., and J. KOSTOPOULOS. (2002).** “Abattoir condemnation due to parasitic infections and its economic implications in the region of Trikala, Greece,” *Journal of Veterinary Medicine*, 49(6):281–284.
- WANG, K., RIGGS, R. D., and D. CRIPPEN. (2005).** Isolation, Selection, and Efficacy of *Pochonia chlamydosporia* for control of *Rotylenchulus reinformis* on cotton. *Phytopathology*, 95(8): 890-893
- WILCOX J. and H.T. TRIBE.(1974).** Fungal parasitism in cysts of Heterodera. I. Preliminary investigations. *Transactions of the British Mycological Society*, 62: 585-594.
- WORLD HEALTH ORGANIZATION (2007).** Report of the WHO Informal Meeting on Use of Triclabendazole in Fascioliasis Control. Geneva: The Organization.
- ZARE R., and W. GAMS. (2001).** A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicium* gen. nov. *Nova Hedwigia* 73:1-50.