Anthelmintic Effect of *Moringa oleifera* Lam. in Wild-caught *Achatina achatina* Linnaeus, 1758 from the Sefwi Wiawso District, Ghana

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Abstract

Parasitic infection in edible snail species such as *Achatina achatina* has the potential of reducing growth and requires investigation.This study assessed the anthelmintic effect of *Moringa oleifera* in *A. achatina*. Using dissecting and microscopic techniques, the proportion of parasitic infection in *A. achatina* group fed with *M. oleifera* was significantly lower than that of the control group ($\chi^2(1) = 14.97$; $P = 0.0001$). The mean parasite intensity recorded for the kidney of both treated (2.17) and control (3.33) groups of snails were significantly different (bootstrap $t = 2.31$; $P = 0.041$). Similar observation was made in the lung of treated (1.43) and control (3.14) snail groups (bootstrap $t = 3.54$; $P = 0.005$). However, no significant mean parasite intensity in the spermoviduct of treated (1.80) and control (1.96) snail groups was observed (bootstrap $t = 0.475$; $P = 0.627$). The results generally highlight anthelmintic value of *M. oleifera* in the control of parasites in *A. achatina*. Fresh foliage of *M. oleifera* may serve as useful addition to the feed of reared edible snails.

Introduction

Snail rearing is an important source of livelihood in West and Central Africa (Ngenwi *et al*., 2010) and has become imperative due to the considerable decline in wild snail populations resulting mainly from anthropogenic activities such as deforestation, pesticide use, slash-and-burn agriculture, spontaneous bush fires, overexploitation (Cobinnah, 1993; Ngenwi *et al*., 2010) and climate change indicated by extremely high temperatures and low rainfall (Ngenwi*et al*., 2010). It also seeks to respond to the global demand for edible snail meat (Gheoca, 2013; Murphy, 2001; Paoletti, 2005), which serves as a special delicacy in homes and restaurants (Oyenike, 2008). The demand for snail meat is based on its high nutritional value (Eruvbetine *et al*., 1997; Imevbore, Ademosun, 1988; USDA, 2006) and low cholesterol, making it a very healthy meat.

The parasitic nematode, *Angiostrongylus cantonensis*, that causes Angiostrongyliasis, the most common cause of eosinophilic meningitis in Southeast Asia and the Pacific Basin (Baheti *et al*., 2008) use snails as primary intermediate hosts and may infect humans through ingestion of infective larvae in raw or undercooked snails (Wang *et al*., 2008 & New *et al*., 1995). Parasitic infections have been detected in some organs of snails (Aboagye *et al*., 2014; Sowemimo, Asaolu, 2004), faecal samples and mucous secretions of land snails (Ohlweiler *et al*., 2010). It is, therefore, imperative to investigate parasitic

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control in edible snails and its impact on their growth performance.

Moringa oleifera is a perennial evergreen or deciduous plant that is native to Indian subcontinent (Moyo *et al*., 2011), but widely cultivated in tropical and subtropical areas around the world (Anwar *et al*., 2007). It has been noted to have nutritional value (Khalafalla *et al*., 2010; Moyo *et al*., 2011), anthelmintic properties (Monzon, 1995) and to show potent anthelmintic activity against Indian earthworm, *Pheritima posthuma* (Rastogi *et al*., 2009). This study assessed the anthelmintic effect of *Moringa olei fera* leaves in *Achatina achatina*.

Materials and methods

Study design and snail maintenance

Fifty adult *Achatina achatina* with weight and length ranging from 38.2 g to 159.3 g and 7.3 cm to 11.5 cm, respectively, were collected from the wild in the Sefwi Wiawso District (6.22° N, 2.49° W) in the Western Region of Ghana and used in the study conducted at the Department of Animal Biology and Conservation Science (DABCS), University of Ghana, Legon from November 2014 to April 2015. The snails were kept in two different hutch boxes of similar dimensions, 74 cm × 46 cm × 44 cm in length, breadth and height respectively, at average ambient temperature and humidity of 26 ^{o}C and 77%, respectively. Each hutch box was one-third filled with loamy soil, to provide suitable habitat for the snails, and covered with non-medicated mosquito net and wire mesh to keep them in the box.

The snails were randomly assigned to hutch boxes 1 and 2 containing Control and Treatment Groups of snails, respectively. Both groups of snails were fed, at two-day interval for ten weeks, with fresh foliage of *Corchorus tridens* (165 g), *Talinum triangulare* (235 g) and *Colocasia esculenta* (235 g). In addition, the Treatment Group was fed with leaves of *Moringa oleifera* (165 g). The content of each hutch box was watered every two days to create moderate temperature and humid environment to facilitate normal growth. The snails were dissected and microscopically examined for parasites after ten weeks of feeding.

Snail dissection, examination and data collection

Each *A. achatina*, from both groups, was de-shelled in a laboratory pan using bones forceps and the body placed into a Petri dish. The kidney, spermoviduct and lung of each snail were cut off, with the help of a pair of forceps, using a pair of scissors. The contents of the kidney and spermoviduct were washed with saline solution (0.25%) into labeled Petri dishes, but the lung was put in a Petri dish with 0.25% saline solution and macerated with a scalpel.

The content of each organ was mounted under a dissecting microscope and examined for endoparasites at eight times magnification. Images of endoparasites were captured on computer using an inbuilt camera of the dissecting microscope and LAS EZ software to aid image capture. Endoparasites were counted and transferred, using forceps, into a labeled container with 70% alcohol for preservation and identification. These images were subsequently saved and identified as roundworms. Proportions of parasitic infection in the Control and Treatment Groups of snails were compared using Chi-squared test in MedCalc Software 15.6. Parasite quantities and levels of infection in the respective organs of snails were assessed using bootstrap test and Mood's median test in Quantitative Parasitology 3.0 (Rozsa *et al*., 2000).

Results

All the *A. achatina* in the Control Group (n = 25) were infected with roundworms indicating 100% prevalence. However, 12 (48%) out of the 25 snails in the Treatment Group (TG) were observed to be infected. The proportion of parasitic infection in snails in the TG was significantly lower than that in the Control Group (CG) ($\chi^2(1) = 14.97$; $P = 0.0001$). Out of the 25 snails in the CG examined, 24 (96%) of them were observed to have their kidneys infected with roundworms as opposed to 6 (24%) snails in the TG. The percentage prevalence of parasitic infection in *A. achatina* kidney in the Treatment (24%) and Control (96%) Groups were significantly different ($P < 0.0001$) by unconditional exact test (Table 1).

The mean intensities of parasites in the kidney of both Treatment (2.17) and Control (3.33) Groups of snails were significantly different (bootstrap $t = 2.31$; $P = 0.041$). Similar observation was made in the lung of the Treatment (1.43) and Control (3.14) Groups (bootstrap $t = 3.54$; $P = 0.005$). Interestingly, the mean intensities of parasites in the spermoviduct of the Treatment (1.80) and Control (1.96) Groups were not significantly different (bootstrap $t = 0.475$; $P = 0.627$), Table 2. Fig. 1 to 3 show unidentified roundworms observed in spermoviduct, lung and kidney respectively.

Discussion

Edible snail species such as *Achatina achatina* and *Achatina fulica* are vulnerable to parasitic infection (Aboagye *et al*., 2014; Sowemimo & Asaolu, 2004) and require investigation to assess parasitic control and its impact on snail growth. This study assessed anthelmintic effect of fresh foliage of *Moringa oleifera* as part of feed for wild-caught *A. achatina* and recorded significant difference in proportions of parasitic infection in treated and control groups of snails. The proportion of parasitic infection in *A. achatina* fed with fresh foliage of *M.oleifera* was significantly lower than that in the control group ($\chi^2(1) = 14.97$; $P = 0.0001$).

TABLE 1

Prevalence of roundworm infection in organs of A. achatina under different treatment conditions

Snail organ and treatment status		*Percentage prevalence (95%CI)*	**P-value*
Kidney	Control group	96.0 (79.6, 99.9)	<0.0001
	Treatment group	24.0 (9.4, 45.1)	
Lung	Control group	88.0 (68.8, 97.5)	<0.0001
	Treatment group	28.0 (12.1, 49.4)	
Spermoviduct	Control group	96.0 (79.6, 99.9)	<0.0001
	Treatment group	40.0 (21.1, 61.3)	

**P* -values for observed differences in prevalence in Control and Treatment Groups of snails by unconditional exact test.

TABLE 2

Mean and median intensities of roundworm infection in selected organs of A. achatina under different treatments

Snail organ and treatment status		*Mean intensity of parasite (95%CI)*	*Bootstrap t-test; P-value*	*Median intensity*
Kidney	Control group	3.33 (2.75, 3.92)	2.314;0.041	3.0
	Treatment group	2.17 (1.50, 3.00)		2.0
Lung	Control group	3.14 (2.45, 4.18)1.	3.541;0.005	2.5
	Treatment group	3 (1.00, 1.71)		1.0
Sperm oviduct	Control group	41.96 (1.54, 2.42)	0.475;0.627	2.0
	Treatment group	1.80 (1.30, 2.20)		2.0

* *P* -values for observed differences in mean and median parasite intensities using bootstrap t-test and Mood's median test, respectively.

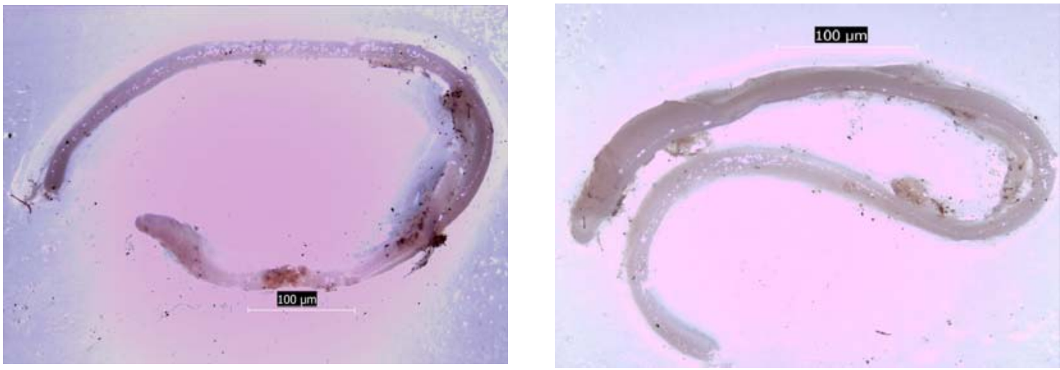

Fig. 1: Unidentified species of roundworm observed in the spermoviduct of *A. achatina*

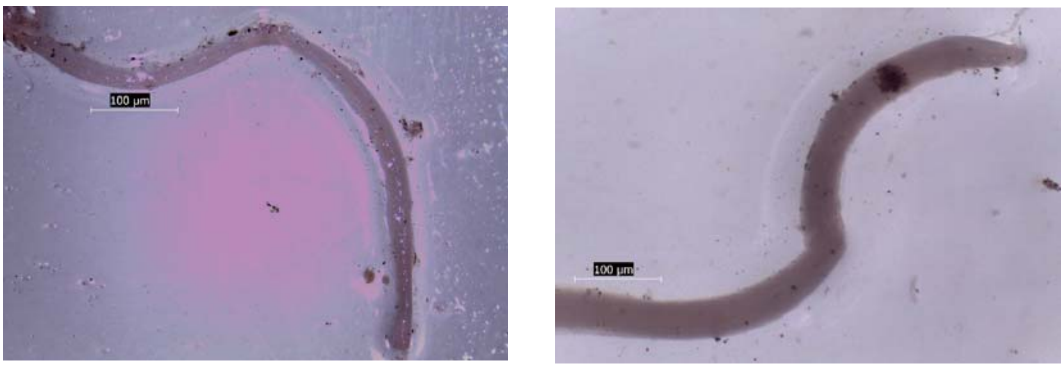

Fig. 2: Unidentified species of roundworm observed in the lung of *A. achatina*

Fig. 3: Unidentified species of roundworm observed in the kidney of *A. achatina*

Contrary to the spermoviducts, the respective kidneys and lungs of both treated and control groups of snails were observed to have significant mean parasite intensities by bootstrap analyses (Table 2).

M. oleifera, a medicinal plant, does not only have natural antifungal (Nwosu & Okafor, 1995) and antimicrobial (Bhattacharya *et al*., 1982; Nepolean *et al*., 2009) activity, but also anthelmintic activity (Monzon, 1995; Rastogi *et al*., 2009; Tayo*et al*., 2014). Tayo *et al*. (2014) observed *in vitro* that ethanolic and aqueous leaf extracts of *M. oleifera* are efficient in inhibiting egg embryonation and hatching as well as inducing about 100% mortality of L1 and L2 larvae of *Haemonchus contortus* at 5 mg/ml. Similarly, ethanolic leave extract of *M. oleifera* has also been reported to show potent anthelmintic activity against Indian earthworm, *Pheritima posthuma* (Rastogi *et al*., 2009). The results of this study highlight the anthelmintic activity of *M. oleifera* in *A. achatina*, in conformity with *in vitro* studies on the subject (Rastogi *et al*., 2009; Tayo*et al*., 2014).

The nutritional value of *M. oleifera* is well documented (Anjorin*et al*., 2010; Anwar *et al*., 2007; Khalafalla *et al*., 2010; Oduro *et al*., 2008; Reyes *et al*., 2006).There is rich composition of vitamins, minerals and amino acids in *M. oleifera* leaves (Anwar *et al*., 2007; Moyo *et al*., 2011). Not only does its leaves, flowers and fresh pods serve as vegetables for human consumption, but also livestock feed (Anjorin *et al*., 2010). The rich mineral and vitamin composition in snail meat (USDA, 2006) suggests that *M. oleifera* leaves could be an important source of nutrients to partially meet nutritional requirement of edible snails. Leaves of *M. oleifera* may, therefore, serve as useful addition to feed for reared edible snails and would be interesting to assess its impact on growth potential and feed conversion ratios in the snails.

Edible snails are consumed globally and, at global scale, it is estimated that demand exceeds offer by 90% (GNA, 2012). The flourishing international trade of snails and their short supply, with only 17% of the demand satisfied (FAO, 2015), call for commercial snail farmers to develop management systems that will enhance normal snail growth. The commercial snail farmer, in formulating feed for snails, should not only be mindful of their nutritional requirements, but also less studied factors such as parasitic infections likely to adversely affect normal snail growth. Fresh foliage of *M. oleifera* is cheap, nutritious and has anthelmintic value and may serve as a useful component of feed for optimal snail growth and better nutritional and economic value. In order to reduce cost of production and to maximize economic returns, it will be useful to study various plants, reported to have anthelmintic value, for the purpose of controlling helminth infections. In this regard, various extracts of sub-Saharan African plants such as *Acacia polyacantha* and *Cassia sieberiana* (Waterman *et al*., 2009), South African plants, *Aloe marlothii* and *Artemisia afra* (McGaw *et al*., 2000) and other non-African plants (Devi *et al*., 2014) with anthelmintic activity will be very useful.

In conclusion, the results emphasize the anthelmintic activity of *M. oleifera* leaves in *A. achatina*. Parasitic infections may hamper normal growth in edible snails particularly in heavily infected cases. Therefore, feed for edible snails should not only be nutritious, but also have anthelmintic value. The results suggest that fresh foliage of *M. oleifera* may serve as useful addition to the feed of reared edible snails on account of its anthelmintic value as well as its nutritional value previously documented. This approach to edible snail rearing has the potential of enhancing their growth, feed conversion ratios as well as their nutritional and economic value.

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References

Aboagye I. F., Nsiah-Yeboah F. and **Aboagye-Antwi F.** (2014). Assessment of Roundworm Infection in the Organs of Wild-caught *Achatina Achatina* from two Districts in the Eastern and Central Regions of Ghana. *Int. J. Nat. Appl. Sci.* **10**(1): 34–39.

Anjorin T. S., Ikokoh P. and **Okolo S.** (2010). Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. *Int. J. Agric Biol*., **12**: 431–434.

Anwar F., Sajid L., Muhammad A. and **Anwarul H. G.** (2007). *Moringa oleifera*: A Food plant with Multiple Medicinal Uses. *Phytother. Reseach* **21**: 17–25.

Baheti N. N., Sreedharan M., Krishnamoorthy T., Nair M. D. and **Radhakrishnan K.** (2008). “Eosinophilic meningitis and an ocular worm in a patient from Kerala, south India”. *Journal of Neurology, Neurosurgery and Psychiatry* **79**(3): 271.

Bhattacharya S. B., Das A. K. and **Banerji N.** (1982). Chemical investigations on the gum exudates from Sonja (*Moringa oleifera*). *Carbohydrate Res*, **102**: 253–262.

Devi A. S., Fung C. L. Y., Prakash P and **Lydia T.** (2014). Comparative study on Anthelmintic property of Medicinal Plants. *Int. J. Drug Dev. & Res*. **6**(1): 149–152.

Eruvbetine D., Oguntona E. B. and **Adoye U. A.** (1997). Preliminary studies comparing different diets for intensive rearing of snails. In *Proceedings of the 1997 Biennial Conference of the Ecological Society of Nigeria (ECOSON)* held at the University of Agriculture, Abeokuta. 23–27th November. (S. Ogunyemi and L.O. Ojo, eds.), pp. 93–97.

Food and Agricultural Organization (2015). Profitable business with edible snails. Available at: http://www.fao.org/family-farming/detail/en/c/288443/ [Accessed October 2015].

Gheoca V. (2013). Edible land snail *Helix pomatia's* exploitation in Central Romania -legislation, evolution, perspectives. *Advances in Environment, Ecosystems and Sustainable Tourism*. Available: at http://www.wseas.us/elibrary/conferences/2013/Brasov/STAED/STAED-21.pdf

Greek News Agenda (2012). Snail Farming in Greece. Available at: http://www.greeknewsagenda.gr/2011/11/snail-farming-in-greece.html [Accessed October 2015]

Imevbore E. A. and **Ademosun A. A.** (1988). The nutritive value of the African Giant Land snails (*Archachatinamaginata*). *J. Anim. Prod. Res*. **8**: 76–87.

Khalafalla M. M., Abdellatef E., Dafalla H. M., Nassrallah A. A., Aboul-Enein K. M., Lightfoot D. A., El-Deeb F. E. and **El-Shemy H. A.** (2010). Active principle from *Moringa oleifera* Lam

Leaves effective against two leukemias and a hepatocarcinoma. *Afr. J. Biotechnol*. **9**(49): 8467–8471.

McGaw L. J., Jager A. K. and **van Staden J.** (2000). Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *J. Ethnopharmacol.* **72:** 247–263

Monzon R. B. (1995). Traditional Medicine in the treatment of parasitic diseases in the Philippines. *Southeast Asian Journal of Tropical Medicine and Public Health*. **26**(3): 421–428.

Moyo B., Masika P. J., Hugo A. and **Muchenje V.** (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *Afri. J. Biotechno.* **10**(60): 12925–12933.

Murphy B. (2001). *Breeding and growing snails commercially in Australia: a report for the Rural Industries Research and Development Corporation*. Rural Industries Research and Development Corporation, Barton, A.C.T. Available at: http://trove.nla.gov.au/work/32143264. [Accessed June 2015].

Nepolean P., Anitha J. and **Renitta R. E.** (2009). Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica*, **3**: 33–39.

New D. Little M. D. and **Cross J.** (1995). *Angiostrongyluscantonensis* Infection from Eating Raw Snails. *N. Engl. J. Med*. **332**:1105–1106.

Ngenwi A. A., Mafeni J. M., Etchu K. A. and **Oben F. T.** (2010). Characteristics of snail farmers and constraints to increased production in West and Central Africa. *Afri. J. environ. Sci. Techno.*,**4**(5): 274–278.

Nwosu M. O. and **Okafor J. L.** (1995). Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. *Mycoses*, **38**:191–195.

Oduro I., Ellis W. O. and **Owusu D.** (2008). Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Sci. Res. Essays*. **3**(2): 057–060.

Ohlweiler F. P., Guimaraes M. C. D. A., Takahashi F. Y. and **Eduardo J. M.** (2010). "Current distribution of *Achatina fulica*, in the State of Sao Paulo including records of *Aelurostrongy lusabstrusus* (Nematoda) larvae infestation". *Revista do Instituto de Medicina Tropical de São Paulo* **52**(4): 211.

Oyenike A. (2008). Introduction to snail and snail Farming. Real solution computers, 23 Quarry Road, Abeokuta.

Paoletti M. G. (2005). *Ecological Implication of Mini-livestock – role of insects, rodents, frogs and snails for sustainable development*. Science Publishers, USA. pp. 608.

Rastogi T., Bhutda V., Moon K., Aswar P. B. and **Khadabadi S. S.** (2009). Comparative Studies on Anthelmintic Activity of *Moringa oleifera* and *Vitex negundo*. *Asian J. Res. Chem*. **2**(2): 181–182.

Reyes S. N., Sporndly E. and **Ledin I.** (2006). Effects of feeding different levels of foliage from *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. *Livest. Sci.* **101**(1–3): 24–31.

Rozsa L., Reiczigel J. and **Majoros G.** (2000). Quantifying parasites in samples of hosts. *J. Parasitol.* **86:** 228–232.

Sowemimo O. W. and **Asaolu S. P.** (2004). The prevalence of *Angiostrongyluscantonensis* (Chen) in *Archachatinamarginata* (Swainson)in Ile-Ife, Nigeria. *Ife J. Sci.* **6**(1): 1–5.

Tayo, M.G., Poné, J.W. Komtangi, M.C., Yondo, J. Ngangout, A.M. and Mbida, M. (2014). Anthelminthic Activity of *Moringa oleifera* Leaf Extracts Evaluated *in Vitro* on Four Developmental Stages of *Haemonchuscontortus* from Goats. *Am. J. Pl. Sci.* **21**(5): 1702–1710.

United States Department of Agriculture, **USDA** (2006). Nutritional value of the snail. From USDA Standard Reference 19. 2006. Available at: www.usda.gov/. [Accessed May 2015].

Wang Q. P., Lai D. H., Zhu X. Q. Chen X. G. and **Lun Z. R.** (2008). Human Angiostrongyliasis. *The Lancet Infectious Diseases*, **8** (10): 621–630.

Waterman C., Smith R. A., Pontiggia L. and **Der Marderosian A.** (2009). Anthelmintic screening of sub-Saharan African plants used in traditional medicine. *J. Ethnopharmacol*. **127**(2010): 755–759.