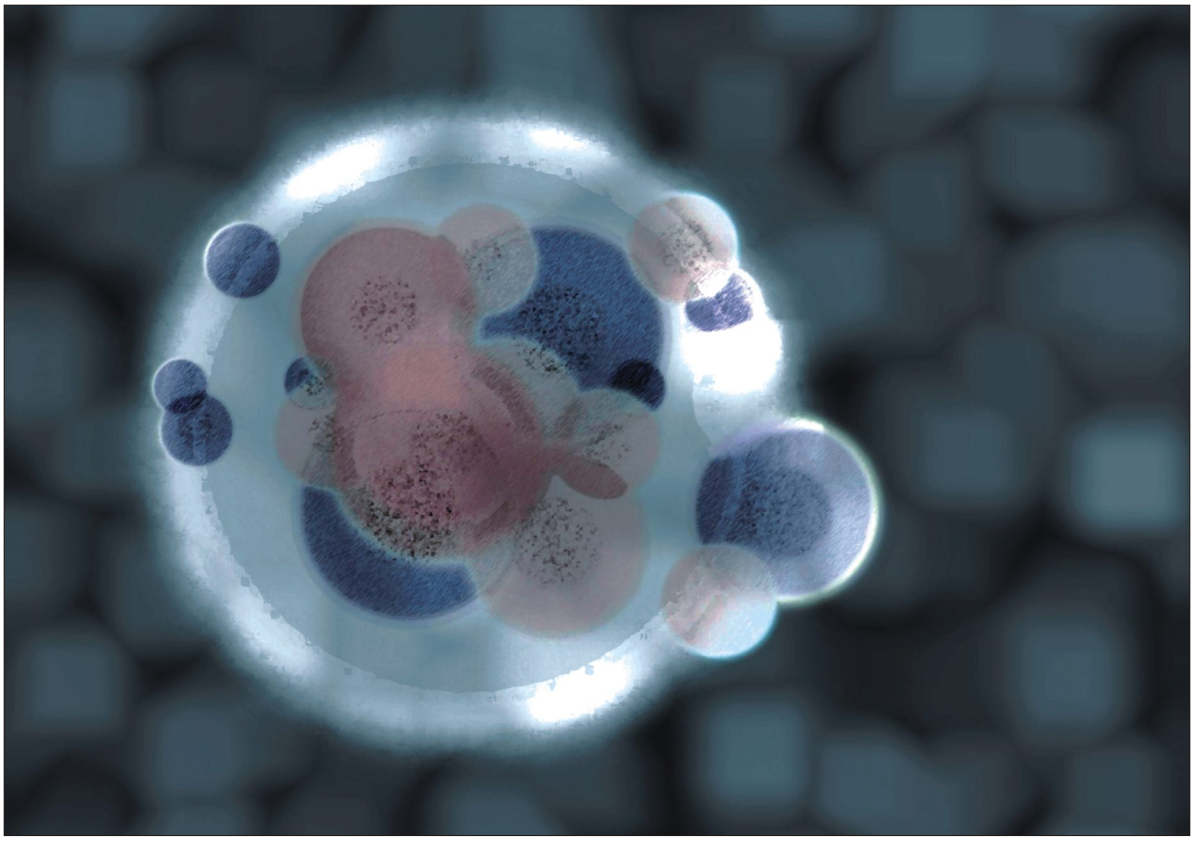

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Research Article
Observations on the Digestive Enzymes in the Giant African Land Snails (*Archachatina marginata*): Significance and Prospects

Andrew Ayodeji Ajayi and Love Abolade Babatunde

Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

Background and Objective: Determining digestive enzyme activity is a vista to obtain and understand valuable information about snail digestive physiology since digestion is an integral process of snail metabolism. This study investigated the activity of digestive enzymes in the gut regions of *Archachatina marginata* to determine the distribution of the enzymes and relate them to their functional properties. **Materials and Methods:** Thirty snail samples were analysed using the spectrophotometry method. **Results:** Lipase has the minimum activity and glucosidases are the most active enzymes in all the gut regions. The highest enzyme activity was found in the crop, followed by the small intestine. The salivary gland has the lowest enzyme activity. **Conclusion:** This study concludes that *A. marginata* is well-equipped with necessary enzymes that catalyse the digestion of its numerous diets. Cellulase secreting microbes can be isolated from the gut of *A. marginata* as a natural biocatalyst for the bioconversion of renewable energy resources to meet the demands of the future and sustainable world.

Key words: Digestive enzyme, enzyme activity, *Archachatina marginata*, microbiota, spectrophotometry

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Corresponding Author: Andrew Ayodeji Ajayi, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Snails generally belong to the phylum Mollusca and class Gastropoda. Gastropods are the largest group of molluscs and are one of the most successful and diverse animal groups in terrestrial ecosystems[1]. There are about 40,000 reported species that comprise over 80% of living molluscs[2]. Molluscs have long been of economic importance to human societies as food, medicine, crop pests, vectors of parasites, tools, personal ornaments and currency in trade[3].

A relatively small fraction of the global terrestrial gastropod diversity has proved to be highly adaptive to environmental changes brought about by human activity and often becomes a highly abundant and characteristic component of invertebrate faunas in modified habitats[4-6]. Snails, like other terrestrial gastropods, are highly susceptible to passive dispersals associated with anthropogenic activities[7-9]. They are notable crop pests in agriculture and disease vectors of helminth parasites in humans and livestock[10,11].

Snails are a perfect model for scientific studies in population genetics and neurophysiology. In ecological research, snails are great bioindicators of environmental pollution. They bioaccumulate heavy metals in their soft tissues and shells[12,13]. Moreover, they are excellent decomposers, contributing to nutrient recycling. In recent years, gastropod communities are being disturbed or lost through unabated human-induced habitat degradation and loss[1].

Snails are well-equipped with a wide array of digestive enzymes, particularly carbohydrases[14,15]. The digestive enzymes in some species of edible snails have been studied over the years in various conditions and patterns. A complex system of enzymes hydrolysing disaccharides and polysaccharides exists in the crop juice of the snails[16]. Ademolu *et al.*[17] reported that aestivation remarkably impedes the activity of digestive enzymes in *A. marginata*, resulting in reduced nutrient uptake from the consumed diet. Digestive enzymes observed in the gut of aestivated snail are negligible compared with active snail[18].

Enzymatic activity in snails fluctuates seasonally and depends on the physiological status, nature of the diet and amount of food available[15]. Studies on the enzyme activity of the foot muscle of *A. marginata* revealed that amylase, cellulase and α-glucosidase were present in the foot muscle of *A. marginata*, which correlates well with the high carbohydrate content of the diet which the snails were fed[19]. Garcia-Esquivel and Felbeck[20] and Ademolu *et al.*[21] reported the presence of cellulase, α-glucosidase, lipase, protease and trypsin in the digestive tract of snails.

The activities of the gut enzymes in snails were significantly affected by the growth phase as the adult stage recorded the highest enzyme activity followed, by the juvenile stage, and the snailet recorded the least[21]. Ademolu and Oyinloye[22] reported that glucosidases and lipase were present in the columellar muscle of *A. marginata*, *A. achatina* and *A. fulica* at different levels and amylase had the highest activities while α-glucosidase had the least activity in the columellar muscle.

Many microorganisms inhabit the gut regions of the snails, though there were more bacteria species than fungi species[23-25]. The presence of symbiotic microorganisms like *Pseudomonas* and *Bacillus* in the guts of land snails makes cellulose digestion possible[26]. Amylolytic, cellulolytic and proteolytic bacteria aid in the digestive processes of *A. marginata* and *A. achatina*. However, *Bacillus* species from the stomach region exhibited the highest enzymatic activities in the digestion of amylase, cellulase and protease[27].

Feed types and stocking density are some of the factors that influence the growth and development of snails[28]. Ademolu *et al.*[21,29] reported that *A. marginata* feeds at night-time (between 19:00 and 4:00 GMT), while no feeding occurs in the daytime.

Archachatina marginata is a potential source of high-quality nutrients[30,31]. Information about the proximate and mineral composition of *A. marginata* is abundant in the literature, while studies on the digestive enzymes in *A. marginata* have received very little attention. Its large size, rapid growth and prolific habit have made this snail an ideal subject for cultivation. However, its farming and consumption are still on a small scale here in Nigeria, whose need for such food resources is well known. Detailed knowledge of the digestive physiology of the snail would aid its successful and profitable culture. Therefore, the objectives of this study were to determine the distribution of digestive enzymes in the gut regions of *Archachatina marginata* and review the identified digestive enzymes based on their activity and functional properties.

MATERIALS AND METHODS

Study area: *Archachatina marginata* is highly dispersed and ubiquitous. Thirty snail samples, used for the study, were procured in July, 2021 from peasant farmers who handpicked the snails on their farmlands around the Ile-Ife axis of Osun State, Nigeria.

Collection and handling of snails: The procured snails were immediately transferred to the snail pen in the Department of Zoology, Obafemi Awolowo University, Ile-Ife and raised on

pawpaw leaves and the rind of watermelon in the laboratory till they were dissected. The snails were on an average of 257 g in weight.

Dissection of snails: The snails were carefully removed from their shell. The main body organs were washed thoroughly with distilled water to remove body slime. This will enable a proper dissection, where the various parts (head, mantle, trunk and foot) were identified.

Snails were dissected on a tray containing ice to arrest any enzymatic activity. The dissection of the snail started from the inner part of the mantle (collar region) that connects to the head. This reveals the beginning of the digestive tract which is infused into the head, the oesophagus and the salivary gland. The outer layer covering the visceral hump is cut open for a clear view of the organs and the digestive tract is traced from the head, around the mantle to the visceral mass which holds the bulk of the digestive system i.e., the digestive gland where the stomach and small intestine are embedded. The organs, carefully separated, are immediately stored in the deep freezer (-18°C) till the analysis of the enzymes.

Enzymes assay and analyses: The snail organs were buffeted and extracted into a solution. The specific activity of the enzymes was expressed as the enzyme activity per mg of protein. The analyses included the protein assay to measure the protein concentration, the lipase assay to measure the activity of lipase, the protease assay to measure the activity of protease and the glucose substrate enzyme assay (α-amylase, cellulose and sucrase). All experiments were conducted in triplicate measurements.

Assay of protein: The protein concentration was determined using the Bradford method[32]. Bovine serum albumin (BSA) was used as the known standard protein. The Bradford method is a typical colorimetric protein assay which is based on absorbance shift of dye Coomassie brilliant blue G-250. Six test tubes were set up with the various quantity of BSA, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL, respectively and distilled water (in various concentrations as well) after which the Bradford reagent (the colour developer) was added to each test tube, 0.2 mL each. The absorbance rate was taken at 595 nm (the standard for protein concentration).

Glucose substrate enzyme assays: The assay for the glucose substrate enzymes was carried out using the 3, 5 dinitrosalicylic acid method (DNSA) as described by Ademakinwa and Agboola[33]. The enzymatic reaction mixtures contained 500 mL of 1% (w/v) of carboxymethyl cellulose, starch and sucrose respectively in 0.5 mM acetate (buffer pH 5.5) and 200 mL of the enzyme. The reaction mixture was incubated at 25°C and the reaction terminated using DNSA reagent after 20 minutes. The obtained mixture was boiled for 10 min and the reducing sugars present were quantified appropriately. A control experiment was set up such that the enzyme was pre-incubated with the DNSA before adding the substrate.

Protease assay: Protease activity was assayed by the method described by Cupp-Enyard[34] with some modifications[35]. The substrate was prepared using 0.65% casein solution dissolved in 50 mM Tris-HCl buffer pH 7.4. The reaction mixture was made up of 5 ml of the casein substrate and 1 ml of the centrifuged enzyme supernatant. The reaction was terminated after 30 min of incubation (37°C) by adding 5 mL of 110 mM trichloroacetic acid (TCA). The 5 mL of 0.5 M sodium carbonate and 1 mL of Folin's reagent were added to each test tube. Absorbance was measured using a UV spectrophotometer at 660 nm. The concentration of protease produced was measured using a standard graph of tyrosine obtained in the range of 10-100 μg mL^{-1}. One unit of protease activity was defined as the amount of the enzyme resulting in the release of 1 μg mL^{-1} of tyrosine per minute under the assay conditions.

Lipase assay (kinetic assay): The activity of lipase was analysed using p-nitrophenyl laurate (p-NPL) according to the method of Vorderwulbecke *et al.*[36] with slight modification[37]. The 1 mL of isopropanol containing 0.001 g of p-NPL was mixed with 9 mL of 0.05 M Tris-HCl buffer (pH 7.5) containing 50 μL Triton X-100 and 0.01 g gum Arabic to form an emulsion. 300 μL of the diluted enzyme solution was mixed with 700 μL of the prepared substrate solution. Using a spectrophotometer, the liberated p-nitrophenol was observed by the change in absorbance at 410 nm. One unit of enzyme activity is defined as the amount of enzyme that released 1 μ mol of p-nitrophenol from p-NPL in one minute under the assay conditions.

Statistical analysis: Descriptive statistics were employed to compare the mean values for the enzyme activities in each organ. One-Way ANOVA with Tukey's HSD test was used to determine statistical difference at ($p<0.005$) level of significance using IBM SPSS Statistics 25 software.

RESULTS

Enzyme activities: α-amylase, cellulase, sucrase, protease and lipase activities in the digestive tract viz: The salivary gland,

Table 1: Enzyme activity in the digestive tract of *Archachatina marginata* (μmol min^{-1})

Enzyme	Salivary gland	Crop	Stomach	Digestive gland	Small intestine
α-Amylase	590.20±17.326^{dD}	286.10±9.168^{cB}	412.14±6.931^{dC}	562.19±24.988^{cD}	168.06±0.000^{bA}
Cellulase	148.05±12.494^{cA}	594.20±41.583^{dC}	240.08±12.004^{cB}	98.03±17.326^{bA}	532.18±28.364^{cC}
Sucrase	34.01±3.465^{bA}	126.04±30.010^{bB}	190.06±30.209^{bB}	136.05±17.326^{bB}	180.06±41.583^{bB}
Protease	16.80±1.482^{abB}	16.71±0.983^{aB}	28.00±0.560^{aC}	8.59±1.987^{aA}	10.73±1.060^{aA}
Lipase	1.74±0.150^{aC}	1.68±0.205^{aC}	0.46±0.023^{aA}	1.64±0.182^{aC}	1.13±0.026^{aB}

Mean±SD followed by the same lowercase letters in the columns and capital letters in the rows, are not significantly different ($p<0.05$) by the Tukey HSD test

Table 2: Specific enzyme activity in the digestive tract of *Archachatina marginata* (μ mol^{-1})

Enzyme	Salivary gland	Crop	Stomach	Digestive gland	Small intestine
α-Amylase	82.26	112.01	76.28	42.61	65.79
Cellulase	20.63	232.63	44.43	7.43	208.35
Sucrase	4.74	49.35	35.18	10.31	70.49
Protease	2.34	6.54	5.18	0.65	4.20
Lipase	0.24	0.66	0.09	0.13	0.44

crop juice, digestive gland, stomach and small intestine of *Archachatina marginata* were summarized in Table 1.

α-amylase: In the digestive tract of *A. marginata*, α-amylase activity (590.20±17.326 μmol min^{-1}) in the salivary gland was the highest, followed closely by in the digestive gland (562.19±24.988 μmol min^{-1}) as shown in Table 1. α-amylase in the crop has the highest specific enzyme activity (112.01 μ mol^{-1}). The digestive gland recorded the lowest specific enzyme reaction (42.61 μ mol^{-1}) as shown in Table 2. There was a significant difference ($p<0.05$) between α-amylase activities across the organs. However, α-amylase activity was not significantly different between the salivary gland and the digestive gland (Table 1).

Cellulase: Cellulase activity in the crop (594.20±41.583 μmol min^{-1}) was the highest, this was followed by cellulase activity in the small intestine (532.18±28.364 μmol min^{-1}) as shown in Table 1. The digestive gland recorded the lowest cellulase activity (98.03±17.326 μmol min^{-1}). The specific enzyme activity of cellulase in the other organs is relatively low compared to the crop and small intestine (232.63 and 208.35 μ mol^{-1}) respectively as shown in Table 2. There was a significant difference between cellulase activities ($p<0.05$) across the organs.

However, cellulase activity was not significantly different between the salivary gland and the digestive gland and between the small intestine and the crop (Table 1).

Sucrase: Sucrase activity (190.06±30.209 μmol min^{-1}) was highest in the stomach of *A. marginata*. The salivary gland recorded the lowest sucrase activity (34.01±3.465 μmol min^{-1}) as shown in Table 1. The specific enzyme activity of sucrase was lowest in the salivary gland (4.74 μ mol^{-1}) as shown in Table 2. There was no significant difference in sucrase activity across the organs except in the salivary gland (Table 1).

Protease: The highest level of protease activity (28.00±0.560 μmol min^{-1}) was recorded in the stomach followed by the salivary gland (16.80±1.482 μmol min^{-1}). Protease activity in the digestive gland was the lowest (Table 1). The specific enzyme activity of protease in the crop was the highest and the lowest in the digestive gland as shown in Table 2. There was a significant difference between protease activities across the organs ($p<0.05$). However, protease activity was not significantly different between the salivary gland and the crop and between the small intestine and digestive gland (Table 1).

Lipase: Lipase activity was highest in the salivary gland (1.74±0.150 μmol min^{-1}), followed by the crop. The stomach recorded the lowest enzyme activity (0.46±0.023 μmol min^{-1}) as shown in Table 1. The specific enzyme activity of lipase is the lowest among other enzyme activities. There was a significant difference between lipase activities across the organs ($p<0.05$). However, lipase activity was not significantly different between the salivary gland, the crop and the digestive gland (Table 1).

There was a significant difference between the enzyme activities across all the organs. However, protease and lipase activities in each organ were not significantly different. The activities of sucrase and protease in the salivary gland, cellulase and sucrase in the digestive gland and α-amylase and sucrase in the small intestine were not significantly different.

DISCUSSION

The presence of enzymatic activities of α-amylase, cellulase, sucrase, protease and lipase in the gut regions of *A. marginata* affirms that snails are omnivores equipped with adequate enzymes necessary for the digestion of their multivarious food items. α-amylase aids the hydrolysis of starch into simple sugars while cellulase catalyses the

breakdown of cellulose polysaccharides into glucose which is essential for digesting fruits and vegetables. Sucrase catalyses the hydrolysis of sucrose to simple sugars (glucose and fructose). These three enzymes have the glucose substrate in common. However, lipase catalyses the breakdown of lipids by hydrolysing the esters of fatty acids and protease catalyses the breakdown of proteins into smaller polypeptides or single amino acids.

This study revealed that the highest enzymatic activities were found in the glucosidases (α-amylase, cellulase and sucrase) across all the organs suggesting that *A. marginata* would prefer a plant-based diet. Lipase has the lowest enzyme activity across all the organs, however, the stomach has the lowest lipase activity.

The presence of substrates stimulates the secretion of digestive enzymes[18]. The presence but relatively low enzyme activity of protease and lipase suggests that *A. marginata* feeds more on a carbohydrate diet. The high activity of α-amylase was anticipated as it is needed to digest starch which is the major component of foliage and rind consumed by the snail. The present study agrees with the findings of Ademolu *et al.*[17] who reported higher α-glucosidase activity in the gut regions of *A. marginata*.

The highest specific enzyme activity was recorded in the crop of *A. marginata*. The crop is the portion of the alimentary canal used to store food before digestion. In a related study by Charrier and Rouland[16], the bulk of carbohydrate digesting activity was concentrated in the crop juice of *Helix aspersa*. The high enzymatic activities recorded in the crop region might be associated with the presence of symbiotic microbes that have been reported[27] to aid in the snail's digestive processes. However, Ademolu *et al.*[21] reported that the bulk of enzyme activity was found in the stomach of the snail while Garcia-Esquivel and Felbeck *et al.*[20] recorded the highest enzymatic activities in the hepatopancreas of *Haliotis rufescens*. Valarmathi and Asokan[15] reported the highest enzyme activity in the digestive gland and the lowest activity in the stomach of *Cryptozona bistrialis*. According to Vineetha *et al.*[18], the intestine of *A. fulica* showed the highest quantities of enzymes than other regions of the gut.

The activity of lipase was generally very low and almost not present in the stomach region. Vineetha *et al.*[18] also reported very low lipid digestion in *Achatina fulica.* This could suggest that snails rarely feed on a diet rich in lipids. Since *A. marginata* feeds more on vegetative and decaying matter, it is not surprising that cellulase and α-amylase activities are found in large amounts in their tracts. This was similar to Ozioko *et al.*[38] who reported high cellulase activity in the digestive tracts of *Achatina achatina*.

The cellulose digestion in the gut of snails depends on the metabolic activities of the gastro-intestinal microbiota[39]. The highest enzyme activity for cellulase and α-amylase was found in the crop and salivary gland respectively. Thus, the crop of *A. marginata* serves as the active site for cellulose digestion. Cellulase activity recorded in the crop (594.198±41.583 μmol min^{-1}) and small intestine (532.180±28.364 μmol min^{-1}) can be associated with the high microbial flora in the two regions. Cardoso *et al.*[40] reported a complex holobiont system containing diverse and abundant microbial communities in the gut of *Achatina fulica.* However, Pinheiro *et al.*[41] observed that the gut microbes only assist (but are not essential for) the digestion of cellulose since snails treated with antibiotics (to eliminate bacteria) retained the capacity to digest polysaccharides.

The enormous economic and nutritional benefits derived from giant African land snails have necessitated increased cultivation. Shells, mucin and flesh have all been investigated and found to be rich raw materials for industries[24,42,43]. Cellulase from the digestive tracts of *A. achatina* possesses high thermostability and acid/alkali stability which indicate the prospective commercial significance of cellulase in the degradation of cellulose-containing materials[38]. Successful and profitable cultivation of *A. marginata* relies on adequate knowledge of the digestive physiology of snail.

The current study recommended that due to the high activity of cellulase recorded in the gut of *A. marginata*, cellulase secreting microbes can be isolated from the gut of *A. marginata* as a natural biocatalyst for the bioconversion of renewable energy resources, biofuel etc. to meet the demands of the future and sustainable world. Snails are highly nutritious gastropods that can be used as animal protein, Heliciculture is a lucrative business. A correctly formulated feed for snails can rapidly increase their growth size and greatly reduce the cost and time of rearing. Snails can be easily cultured on budget with domesticated tools and environmental resources because of their ability to feed on various food items including some of our domestic wastes.

CONCLUSION

This study revealed the high preference for a glucose substrate-based diet (carbohydrate) over any other feed type considering the high level of α-amylase, cellulase and sucrase activity found in the gut of the snail. This study showed that the activity of enzymes in *A. marginata* is dependent on their feeding habits. Revitalization of some wild species like snails would assist in combating the insufficiency of animal protein in many developing countries. An adequate study of the

digestive enzymes in snails will aid in the proper recommendation of feeds, feeding methods and nutrition for snails. The study of digestive enzymes can also provide useful information for the feed formulation of snails to relate it to their feeding method and developmental stage.

SIGNIFICANCE STATEMENT

The study identified the digestive enzymes in *Archachatina marginata*. The activity of the enzymes and the functional properties were discussed. This study will guide the appropriate choice/formulation of feed for successful snail rearing in Nigeria.

REFERENCES

1. Barker, G.M., 2002. Molluscs as Crop Pests. CABI Publishing, Wallingford, Oxfordshire, England, ISBN: 9780851993201, Pages: 468.
2. Rosenberg, G., 2014. A new critical estimate of named species-level diversity of the recent mollusca. Am. Malacol. Bull., 32: 308-322.
3. Cowie, R.H., R.T. Dillon, D.G. Robinson and J.W. Smith, 2009. Alien non-marine snails and slugs of priority quarantine importance in the United States: A preliminary risk assessment. Am. Malacol. Bull., 27: 113-132.
4. Belhiouani, H., M.E.H. El-Okki, F.Z. Afri-Mehennaoui and L. Sahli, 2019. Terrestrial gastropod diversity, distribution and abundance in areas with and without anthropogenic disturbances, Northeast Algeria. Biodiversitas J. Bio. Divers., 20: 243-249.
5. Maheshini, P.W.D.B., K.G.D.D. Thilakarathne, G.N. Hirimuthugoda, K.B. Ranawana and S. Kumburegama, 2019. The distribution of terrestrial pest gastropods and their damage to agricultural crops in Kandy and Nuwara Eliya Districts in Sri Lanka. Ceylon J. Sci., 48: 177-184.
6. Zaidi, N., L. Douafer and A. Hamdani, 2021. Diversity and abundance of terrestrial gastropods in Skikda Region (North-East Algeria): Correlation with soil physicochemical factors. J. Basic Appl. Zool., Vol. 82. 10.1186/s41936-021-00239-6.
7. Bergey, E.A., L.L. Figueroa, C.M. Mather, R.J. Martin and E.J. Ray *et al*., 2014. Trading in snails: Plant nurseries as transport hubs for non-native species. Biol. Invasions, 16: 1441-1451.
8. Barbato, D., A. Benocci, T. Caruso and G. Manganelli, 2017. The role of dispersal and local environment in urban land snail assemblages: An example of three cities in Central Italy. Urban Ecosyst., 20: 919-931.
9. McKinney, M.L., N.S. Gladstone, J.G. Lentz and F.A. Jackson, 2019. Land snail dispersal, abundance and diversity on green roofs. PLoS ONE, Vol. 14. 10.1371/journal.pone.0221135.
10. Sneha, J.A., N.H. Khamis, R. Chakravarthi, M. Harun and Y.K. Yeow, 2018. Acoustic identification of snail for pest control in agriculture. J. Adv. Res. Dyn. Control Syst., 10: 2349-2356.
11. Sneha J.A., R. Chakravarthi, N.H. Khamis, M. Harun and Y.K. Yeow, 2018. Sound based identification of snail pest in agriculture. Int. J. Eng. Technol., 7: 267-270.
12. Baroudi, F., J. Al Alam, Z. Fajloun and M. Millet, 2020. Snail as sentinel organism for monitoring the environmental pollution; A review. Ecol. Indic., 10.1016/j.ecolind. 2020.106240.
13. Louzon, M., Q. Devalloir, F. Gimbert, B. Pauget, D. Rieffel and A. de Vaufleury, 2021. From bioavailability to risk assessment of polluted soil using snails: Link between excess transfer and inhibition of sexual maturation. Environ. Sci. Pollut. Res., 28: 17343-17354.
14. Ndlovu, T.M. and J.P.H. van Wyk, 2021. Temperature dependent bioconversion of waste paper by garden snail (*Cornu aspersum*) cellulase into glucose a feedstock for bio-product development. Int. J. Pharm. Sci. Res., 12: 3985-3993.
15. Valarmathi, V. and S. Asokan, 2017. Study of enzyme activity in the land snail *Cryptozona bistrialis* in Nagapattinam, Tamil Nadu India. Int. J. Zool. Appl. Biosci., 2: 77-82.
16. Charrier, M. and C. Rouland, 2001. Mannan-degrading enzymes purified from the crop of the brown garden snail *Helix aspersa* Müller (Gastropoda Pulmonata). J. Exp. Zool., 290: 125-135.
17. Ademolu, K.O., O.D. Fakeye, G.A. Dedeke, O.A. Ajayi and A.B. Idowu, 2013. Digestive enzymes in African giant land snail (*Archachatina marginata*) during aestivation. Arch. Zootec., 62: 73-77.
18. Vineetha V.S., S. Sreedharan and K.A. Krishnan, 2018. Gut content analysis of Achatinafulica with digestive enzymes-A comparative study. Int. J. Res. Anal. Rev., 5: 440z-445z.
19. Ademolu, K.O., O.D. Fakeye, G.A. Dedeke and A.B. Idowu, 2009. Activities of glycosidases in the foot muscles of African giant land snail, *Archachatina marginata* during aestivation. Ethiopian J. Biol. Sci., 8: 165-170.
20. Garcia-Esquivel, Z. and H. Felbeck, 2006. Activity of digestive enzymes along the gut of juvenile red abalone, *Haliotis rufescens*, fed natural and balanced diets. Aquaculture, 261: 615-625.
21. Ademolu, K.O., V.O. Ojo, J.A. Bamidele, A.B. Adelabu, I. Ebenso and A.B. Idowu, 2017. Feeding pattern and digestive enzyme activity of the giant African snail (*Archachatina marginata*) during growth phases. Arch. Zootec., 66: 29-34.
22. Ademolu, K.O. and N.A. Oyinloye, 2016. Composition, enzymes analysis and retraction time of columellar muscles ofgiant African land snail (*Archachatina marginata*) in response to externalstimuli. Niger. J. Anim. Sci., 18: 329-335.
23. Kim, J.Y., S.M. Yoon and Y.S. Kim, 2015. Cellulase activity of symbiotic bacteria from snails, *Achatina fulica*. J. Korean Wood Sci. Technol., 43: 628-640.

24. Belouhova, M., E. Daskalova, I. Yotinov, Y. Topalova, L. Velkova, A. Dolashki and P. Dolashka, 2022. Microbial diversity of garden snail mucus. MicrobiologyOpen, Vol. 11. 10.1002/mbo3.1263.
25. Clec'h, W.L., S. Nordmeyer, T.J.C. Anderson and F.D. Chevalier, 2022. Snails, microbiomes, and schistosomes: A three-way interaction? Trends Parasitol., 38: 353-355.
26. Idowu, A.B., O.M. Somide and K.O. Ademolu, 2008. Comparative analysis of the chemical composition of the haemolymph, flesh and the microflora content of the guts of some African land snails in Abeokuta, Nigeria. Trop. Vet., 26: 20-29.
27. Okeniyi, F.A., O.A. Osinowo, O.A. Ladokun, A.K. Akinloye, O.S. Bamidele and D.M. Sanni, 2015. Bacteria and digestive enzymes in the alimentary tract of the giant African land snails, *Archarchatina marginata* and *Achatina achatina*. Niger. Soc. Anim. Prod., 42: 28-36.
28. Omole A.J., O.O. Obi, K.O. Soetan and T.O. Olaseinde, 2010. Influence of stocking rate on growth and reproductive performance of breeding snails (*Archachatina marginata*). J. Cell Anim. Biol., 4: 064-067.
29. Ademolu, K.O., O.A. Jayeola, A.B. Idowu and I.O. Elemide, 2011. Circadian variation in locomotor and feeding periods of two land snail species. Arch. Zootec., 60: 1323-1326.
30. Çağıltay, F., N. Erkan, D. Tosun and A. Selçuk, 2011. Amino acid, fatty acid, vitamin and mineral contents of the edible garden snail (*Helix aspersa*). J. FisheriesSciences.com, 5: 354-363.
31. Uboh, F.E., I.O. Williams and N.C. Essien, 2014. Effect of processing on the proximate and mineral composition of *Archachatina marginata* and *Achatina achatina*. Food Public Health, 4: 10-14.
32. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
33. Ademakinwa, A.N. and F.K. Agboola, 2019. Kinetic and thermodynamic investigations of cell-wall degrading enzymes produced by *Aureobasidium pullulans* via induction with orange peels: Application in lycopene extraction. Prep. Biochem. Biotechnol., 49: 949-960.
34. Cupp-Enyard, C., 2008. Sigma's non-specific protease activity assay-casein as a substrate. J. Visualized Exp., Vol. 19. 10.3791/899.
35. Park, M.H., B.C. Walpola and M.H. Yoon, 2013. Purification and characterization of protease enzyme from *Burkholderia stabilis*. Afr. J. Biotechnol., 12: 1408-1418.
36. Vorderwuelbecke, T., K. Kieslich and H. Erdmann, 1992. Comparison of lipases by different assays. Enzyme Microbial Technol., 14: 631-639.
37. Ayinla, Z.A., A.N. Ademakinwa and F.K. Agboola, 2017. Studies on the optimization of lipase production by *Rhizopus* sp. ZAC3 isolated from the contaminated soil of a palm oil processing shed. J. Appl. Biol. Biotechnol., 5: 030-037.
38. Ozioko, P.C., S.O.O. Eze and F.C. Chilaka, 2013. Partial purification and characterization of cellulases from digestive tracts of the African giant snail (*Achatina achatina*). Turk. J. Biol., 37: 199-205.
39. Dar, M.A., K.D. Pawar and R.S. Pandit, 2017. Gut Microbiome Analysis of Snails: A Biotechnological Approach. In: Organismal and Molecular Malacology, Ray, S. (Ed.), InTechOpen, London, UK, ISBN: 978-953-51-4711-4, pp: 189-218.
40. Cardoso, A.M., J.J.V. Cavalcante, R.P. Vieira, J.L. Lima and M.A.B. Grieco *et al*., 2012. Gut bacterial communities in the giant land snail *Achatina fulica* and their modification by sugarcane-based diet. PLoS ONE, Vol. 7. 10.1371/journal.pone.0033440.
41. Pinheiro, G.L., R.F. Correa, R.S. Cunha, A.M. Cardoso and C. Chaia *et al*., 2015. Isolation of aerobic cultivable cellulolytic bacteria from different regions of the gastrointestinal tract of giant land snail *Achatina fulica*. Front. Microbiol., Vol. 6. 10.3389/fmicb.2015.00860.
42. Adeyeye, S.A.O., O.T. Bolaji, T.A. Abegunde and T.O. Adesina, 2020. Processing and utilization of snail meat in alleviating protein malnutrition in Africa: A review. Nutr. Food Sci., 50: 1085-1097.
43. Ademolu, K.O., M.Y. Akintola, A.O. Olalonye and B.A. Adelabu, 2015. Traditional utilization and biochemical composition of six mollusc shells in Nigeria. Int. J. Trop. Biol., 63: 459-464.