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Pomacea canaliculata BIOLOGICAL RESPONSES TO SILVER NANOPARTICLES

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Abstract

The potential Bio-indicator species such as *Pomacea canaliculata* (Family: Ampullariidae) resources could be utilized as a biological model for monitoring the anthropogenic pollution in freshwater environments. In the present study, the *P. canaliculata* biological responses to silver nanoparticles (synthesized via the green method) were evaluated. The similarity values among the estimated snail samples were calculated based on the analysis of morphometric (shell morphology) and molecular variations (via Inter simple sequence repeats). The normality of morphometric results was checked before scouting quantitative shell measurements. The results did not differ significantly from that which is normally distributed. The snail individuals were reared for certain time intervals under silver nanoparticles' stresses. Saline soluble protein subunits' separations and some isozyme systems (Glucose6 phosphate dehydrogenase, Superoxide dismutase, Esterase, and Malate dehydrogenase) were applied to detect biochemical tags due to the treatment effects. No differences were detected among the evaluated samples concerning the Glucose 6 phosphate dehydrogenase separations. The separations of the other systems exhibited some tags between the control and the treated individuals. The numbers and/or the intensities (as sensitive biochemical probes of differential gene expression in the snails 'foot muscle samples) of isozyme bands were affected by the silver Nanoparticles treatment under the experiment condition. More informative markers could be detected and evaluated for monitoring aquatic ecological health at water pollution level in the future Eco-Toxicological studies.

Keywords: *P. canaliculata*; Biological responses; morphometric variations; electrophoresis; Silver Nanoparticles; Tags

1. Introduction

Assessing the superiority of certain ecosystems in terms of biological patrimony and functioning is a vital significance in the intensified anthropogenic activities. From this point of view, the potential bio-indicator species such as *P. canaliculata* resources could be utilized as a biological model for monitoring the anthropogenic pollution in freshwater environments **[1, 2].**

Pomacea canaliculata survives against numerous ecological stressors due to their wonderful adaptation mechanisms. So, these organisms could be used as good bioindicators in the Eco-toxicological investigations **[3, 4].**

Many of the *Pomacea* species have been introduced from their original habitats via the pet trade. On the other hand, they also introduced promoting biological control of other aquatic organisms such as weeds. The ecological influences and the resistances of *P. canaliculata* to many ecological stresses including salinity were discussed by some investigators **[3, 5].**

Many cosmetics products (including moisturizers, makeup, sunscreen, and hair care products), dietary supplements, and paints were manufactured using silver nanoparticles as active ingredients. These nanomaterials are now being used in cosmetics products to give protection (antimicrobial factor). The explanation of its protective and toxicity properties is still unclear. It is proposed that bacterial cell walls could be disrupted under certain AgNPs concentrations **[6].**

The byproducts of such materials are mainly moved to many water bodies including the sewer system. These materials have unexpected consequences for the environment and human health.

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So, the conception of various Silver Nanoparticles' effects on aquatic organisms is mainly significant **[7, 8]**.

The accurate effects of the green synthesized nanoparticles (a harmless alternative to chemically synthesized) on aquatic organisms such as aquatic snails are not yet saturated relatively with the other known pollutants **[9].**

A lot of techniques were applied to detect the heavy metal accumulation in various organism systems and/or tissues such as Atomic absorption, High-performance liquid chromatography **[10]**, and Gas chromatography **[11]**.

More biochemical tags should be developed to explore aquatic environmental health in future Eco-toxicological studies**.** Developing various biochemical tags (as bioindicators) for ecological stresses is an urgent tool for exploring the status of our environment. Isozyme electrophoresis techniques are easy and sensitive tools for developing such tags at Lab. level **[8]**.

The separation of protein subunits, Dehydrogenases, and hydrolysis isozymes' systems offered many informative markers in this field in different animal taxa including aquatic organisms **[12- 14].**

Al-amri et al [8] confirmed that the numbers and intensities of Superoxide dismutase, Esterase and Malate dehydrogenase in certain fish body organs were changed after exposure to AgNPs. So, allozyme polymorphisms were proven to be good bioindicators for environmental stresses.

The various effects of the Silver Nanoparticles (AgNPs) on various aquatic organisms **[8]** including aquatic snails **[15, 16]** such as *P. canaliculata* should be evaluated.

There are various molecular methods that could be applied for molecular identification such as COI barcoding system and/or homogeneity testing such as Inter simple sequence repeats (ISSR).

The ISSR is a sensitive DNA method wildly applied for exploring the molecular variations among and within certain aquatic organisms **[8, 17, and 18].**

The current investigation was planned for developing some biomarker tags for screening the probable *P. canaliculata* genome responses for AgNPs (silver nanoparticles) under definite treatment conditions.

2. Experimental:

Source of samples

P.canaliculata (family Ampullariidae) individuals were achieved from an ornamental fish market.

Shell morphometric investigation

The morphometric parameters among the investigated ***P. canaliculata*** were measured. A total of four parameters were recorded for investigating the morphological differences among the evaluated snails' samples. The morphological parameters were the shell width, aperture width, aperture height, and shell height.

The similarity among the investigated samples at the morphological characterization level was calculated by the PAleontological Statistics, (PAST) Version 3.22 **[19].**

Evaluation of the genetic similarity among the evaluated samples

DNA purification and amplification

The DNA samples were extracted from snail foot tissues (2mg) as mentioned by **Hillis et al [20].**

Analysis of Inter Simple Sequence Repeats (ISSR)

The ISSR primers were examined for detect the homogeneity and/or similarity within the evaluated snail samples **(Table1)**.

PCR reactions were prepared according to **Saad and Elsebaie [18]** with minor modifications. The PCR program was designed as the following: one cycle for 2 min. at 96^{o}C, 35 cycles for (45 sec. at 96^{o}C, 30 sec. at 41 ^{o}C & 60 sec. at 72^{o}C) and one cycle for 15 min. at 72^{o}C.

The PCR products were separated using agarose gels (1.5%) electrophoresis.

Synthesis of the silver nanoparticles (AgNPs)

The silver nanoparticles were prepared using the *Commiphora gileadensis* bark extracts as described by **Al-amri et al [8]**.

The nanoparticles were prepared by mixing the AgNPs (0.108 g/L) solution with the aqueous plant bark extracts in the ratio solution of 6:1 (v/v).

The conversion of Ag+ to AgNPs (70-100 nm) was detected via the inductively coupled plasma atomic emission spectroscopy **[8]**.

Experiments

Calculation of LC50

Snail samples (n=75) with an average shell height of 30.6±0.4mm and shell width of 26.5 ±0.5mm were acclimated for 15 days. The averages of pH and water temperature values were 8.2±0.07 and 22.05±0.03 respectively.

All samples were fed green lettuce leaves.

The samples were separated into five groups (five snails of each group, in three replicates) each as follows: First group (water, control group), Second group (water with the *C. gileadensis* bark extract), and the Third group 3 (18.33 µg/L AgNP), Group 4 (36.66µg/L AgNP) and Group 5 (73.33 µg/L AgNP).

The values of the LC50 were determined via the AAT Bioquest, Inc. as described by **Al-amri et al [8].**

Snails' genome responses under Silver Nanoparticles' effects

An experiment was accomplished (The light: dark regime was set to 16:8 h) for estimating the effects of Silver Nanoparticles on the snail genome responses during three-time intervals (t1= 24h, t2= 48h, and t3= 72h) compared with the control (a and b).

A total of 15 containers (20L) were divided into 5 categories (water, Water with plant extract and Silver Nanoparticles (15µg/L) solution for t1, t2, and t3). The snails were exposed to Silver Nanoparticles based on a semi-static exposure system.

The snail individuals were separated into the tanks (n=3). According to a certain schedule time, the samples were removed, killed and washed with deionized water.

Saline soluble protein extraction

The Saline soluble protein subunits were extracted from each snail foot tissue. A total of 0.08g from each sample was extracted in 500µl of 0.85% NaCl solution (pH= 8.1). The protein samples were purified **[8]** and prepared for electrophoresis separation.

Separation of protein subunits via SDS-PAGE

A total of 40µl from each protein sample was separated using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (15%). The gels' preparation, Staining was optimized as mentioned by **Mansour et al [21].**

Separation of isozyme bands

Malate dehydrogenase (Mdh), Superoxide dismutase (Sod), Esterase (Est) with substrates (a-naphthyl acetate), and Glucose 6 phosphate dehydrogenase (G6PD) systems were applied for detecting the biomarker variations among the control and treated snail's samples. Electrophoretic conditions and gel preparation were applied as mentioned by **Mansour et al [21].**The gels were stained as described by **Rashed et al [22]** and **Saad et al [23].**

Statistical analysis:

Some snail morphometric (Shell width, height, aperture width and height) measurements and analyses were conducted as described by **Gouveia et al., [24].**

Calculation of the correlation coefficient values and the reconstruction of Dendrogram among the evaluated samples (n=27) were carried out based on the four morphological characters' differences using PAleontological Statistics, (PAST) Version 3.22 **[19]**.

The regression Coefficient values were calculated using Linear Regression Calculator (https://www.graphpad.com/quickcalcs/linear2/).

The lethal concentrations were evaluated as mentioned by **Gulec et al [25].**

Isozyme banding patterns were identified according to **Mansour et al [21].**

The Inter simple sequence repeats' banding patterns were detected and analyzed as described by **Saad and Elsebaie [18].**

3. Results:

Shell morphometric characterization

A total of four morphological characters' differences (Aperture height, Shell height, Aperture width, and Shell width) were quantitatively measured.

The shell morphological characters for quantitative measurements achieved from an aperture view of the shell were presented in **Figure (1a).** The averages of these values (mm) were (22.42±0.44), (30.65±0.49), (16.33±0.39) and (26.59±0.54) for Aperture height, Shell height, Aperture width, and Shell width respectively.

The normality of morphometric results was checked before scouting quantitative shell measurements. The results did not differ significantly from that which is normally distributed (p-value > α).

The correlations (r) and the coefficient of determinations (R2) among the shell height and other characters were calculated (**Table 2)**. The results exhibited positive correlations between the shell height values and
each of aperture height (r=0.828), aperture width (r=0.5687), and shell width (r=0.5687). A weak positive correlation value (r= 0.3413) was calculated between Aperture width and Shell width.

Regressions of variable discrete shell characteristics of *P. canaliculata* were presented in **(Figure 1b)**. The statistical probabilities are presented for the effect of shell height on the dependent variables (A, B, and C). The regression slopes for A, B, and C measurements were positive and significant.

The similarity among the evaluated snails

Concerning the shell characters (Aperture height, Shell height, Aperture width, and Shell width), the similarity (homogeneity) records among the estimated samples were reflected using the Dendrogram analysis (**Figure 2)**.

The ISSR banding pattern variations among the evaluated *P. canaliculata* samples are presented in **Figure (3).** The ISSR primers (n=8) were tested to generate the ISSR fragments. The results were explored for calculating the similarity values among the evaluated snail samples.

The numbers of ISSR bands were calculated **(Table 1)**. It ranged from 5 bands (Primers HB1 and HB15) to 9 bands (primer IT3). The analyses showed low polymorphic loci. The DNA band frequencies (as average values) were presented in **Figure (4).**

The homogeneity (similarity) values among the estimated snail samples were high based on the analysis of both the shell morphology (0.97) and the molecular variations (0.99).

Calculation of the LC50

Each the snail group was exposed to a definite silver nanoparticles' concentration.

Diverse mortalities were detected in the snail groups (3, 4 and 5). No mortality was detected in the first and second groups. The LC50 value of silver nanoparticles for the estimated snails was 35.21µg/L.

***P. canaliculata* responses to silver nanoparticles**

Separation of protein bands

Analysis of the protein banding pattern showed high similarity levels among the separated protein samples.

A total of 26 snail foot muscle protein bands were detected according to their relative frons (Figures 5 and 6).

The distributions of the bands were similar among the estimated soluble protein samples except for bands numbers 21(RF= 0.675) and 22 (RF=0.71) due to treatment effects.

The total number of foot muscle protein bands (26) was accessible in **Table (3)**. The Numbers of foot muscle protein bands in each time interval were presented in **Table (4)**.

Isozyme banding pattern analysis

Our results exhibited some variable tissue responses among the time intervals under a definite AgNPs concentration.

The banding patterns and the numbers of isozymes were showed in **Figure (**7**)** and **Table (3)** respectively. The band frequencies (BF) and the relative fronts (RF) of all evaluated isozyme bands were presented in **Figure (8).**

Mdh polymorphism

The banding pattern of the Mdh allozyme (Figures 7A) in the snail muscle tissues exhibited informative Mdh bands.

A total of three common Mdh bands (RF=0.349, 0.444, and 0.539) were identified. On the other hand, a total of seven Mdh bands were polymorphic. A total of seven Mdh bands were specific for treatment 2 (t2) at the relative fronts (RF=0.111, 0.190, 0.222, 0.555, 0.619, 0.873 and 0.920).

The band frequencies of the detected Mdh isozyme pattern were presented in **Figure (8A).**

Sod polymorphism

The banding pattern of the Superoxide dismutase (Sod) isozymes was accessible in the Figure (7B).

Out of the six identified Sod bands (Figure 7B), three bands were polymorphic. Out of them, two bands (at the relative fronts 0.333 and 0.444) were absent from the control (a and b) and (t1) samples. the third band (at RF=0.444) was specific for (t3).

The band frequencies and relative fronts for the evaluated Sod isozyme pattern were accessible in **Figure (8B).**

G6PD polymorphism

Only two Glucose 6 phosphate dehydrogenase isozyme bands **(Figure 7C)** were identified (as common bands) in the snail foot muscle tissues (RF=0.28 and 0.33).

The relative fronts and the distribution of band frequencies for the evaluated G6pdh isozyme pattern were accessible in Figure (8C).

Est polymorphism

The pattern of the Esterase isozymes **(Figures 7D)** showed distinguished Esterase bands. A total of 13 bands were recorded **(Table 3).**

The numbers and intensities of recorded bands were variable among the evaluated time intervals.

There are 11 Est. bands (common bands) that were recorded at definite relative fronts.

A total of two Esterase bands (at RF= 0.57 and 0.60) were polymorphic.

The band number 8 (at RF= 0.60) is an informative marker for treatment 3 (t3).

The relative fronts for the evaluated Esterase isozyme pattern were accessible in **Figure (8D).** The Numbers of foot muscle isozymes in each time interval were presented in **Table (4)**.

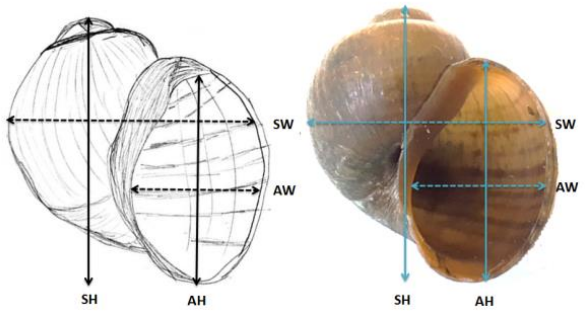

Fig.1a. Shell morphological characters for quantitative measurements are achieved from aperture view of the shell: aperture width (AW), shell width (SW), shell height (SH) and aperture height (AH).

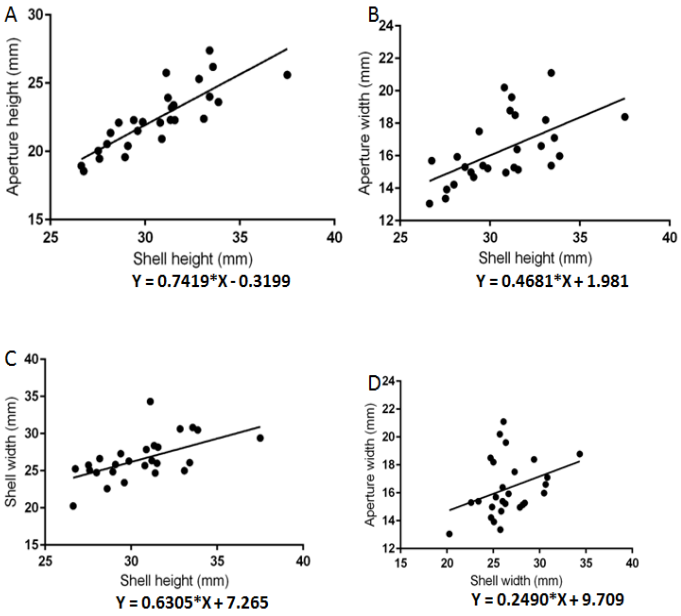

Fig.1b. Regressions of variable discrete shell characteristics of *P.canaliculata* (N = 27). Statistical probabilities are presented for the effect of shell height on the dependent variables (A, B and C). The regression slopes for A, B and C measurements were positive and significant.

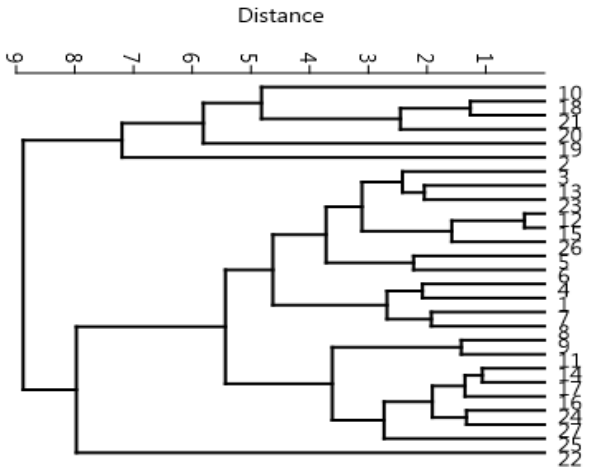

Fig. 2. Dendrogram analysis based on the shell (Aperture height, Shell height, Aperture width and Shell width) morphological characterization among the evaluated *P.canaliculata* samples.

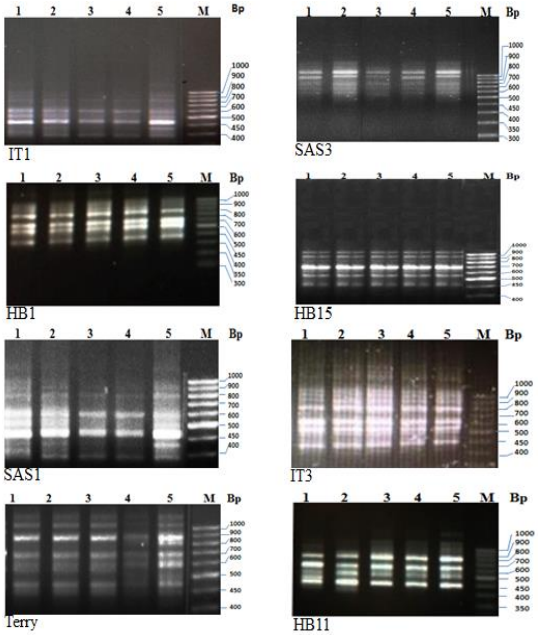

Fig.3: ISSR banding pattern variations among the evaluated *P. canaliculata* samples. M= DNA marker and Bp= base pair.

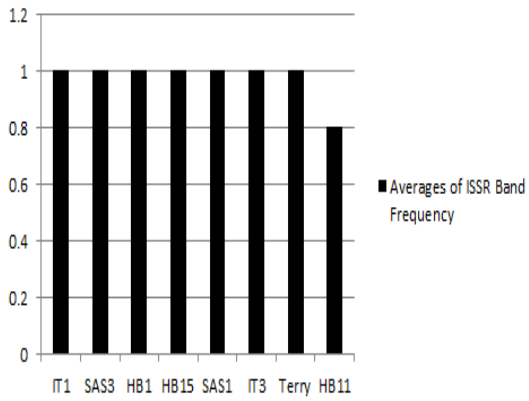

Fig.4. Average of Inter simple sequence repeats' band frequencies for each tested primer.

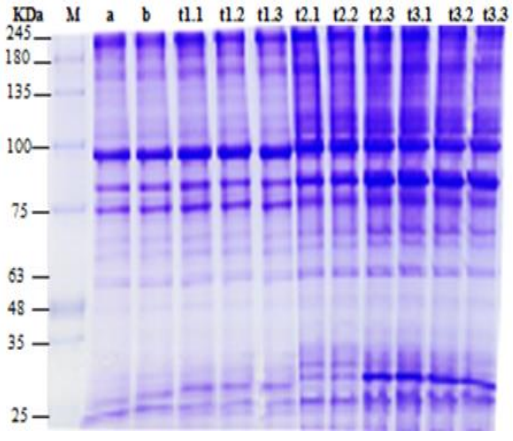

Fig.5. Protein banding pattern for the snail foot tissue extracts. t1=24h, t2=48h and t3=72h, a= control 1(water) and b= control 2 (water with plant extract).

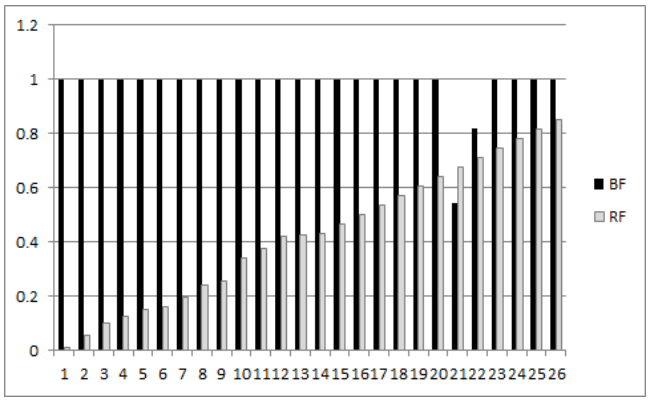

Fig.6. Average of separated protein band frequencies for each the evaluated snail foot muscle samples under treatment conditions. BF=Band frequency and RF= Relative front.

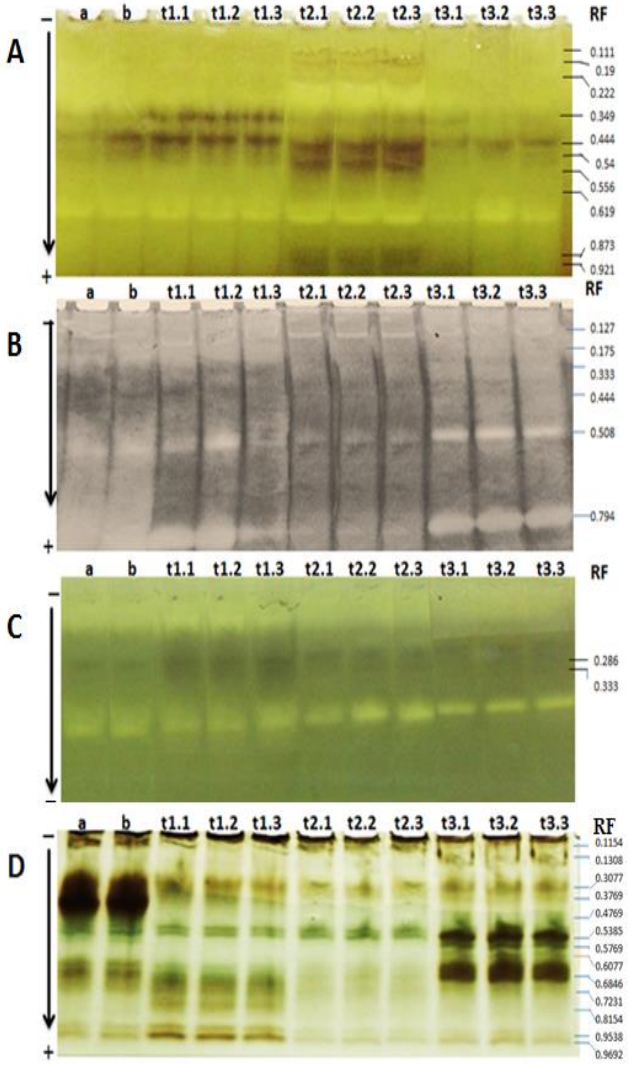

Fig. 7. The electrophoretic patterns of isozymes from snail foot tissue extracts. t1=24h, t2=48h and t3=72h, a= control 1(water) and b= control 2 (water with plant extract). RF= Relative front. A= Malate dehydrogenase isozymes (Mdh), B= Separation of Superoxide dismutase isozymes (Sod), C= Glucose6 phosphate dehydrogenase isozymes (G6PD) and D= Esterase isozymes (Est).

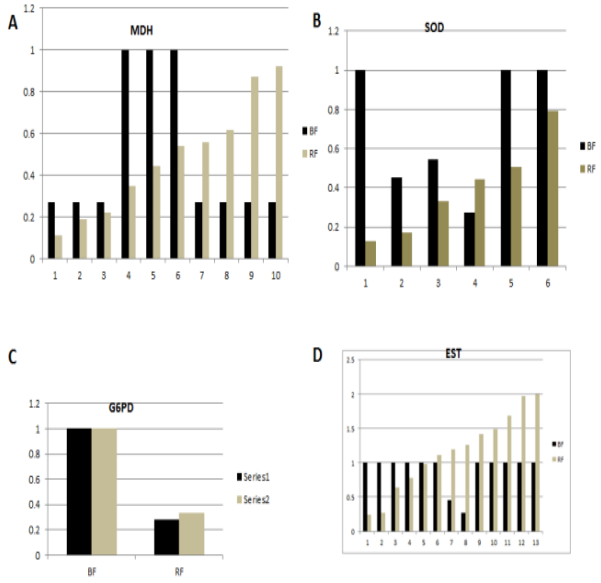

Fig. 8. Average of the separated isozyme band frequencies for each the evaluated snail foot muscle samples under treatment conditions. BF=Band frequency and RF= Relative front. A= Malate dehydrogenase isozymes, B= Separation of Superoxide dismutase isozymes, C= Glucose6 phosphate dehydrogenase and D= Esterase isozymes.

Table 1: The primer codes, sequences and the number of recorded ISSR bands.

Code	Sequence	TNB	PB	SB
IT1	5'CACACACACACACACAGT3'	6	0	6
IT3	5'GAGGAGGAGGAGAG3'	9	0	9
SAS1	5'GTGGTGGTGGTGGC3'	6	0	6
SAS3	5'CAGGAGGAGGAGG3'	6	0	6
HB1	5'CAA CAA CAA CAA CAA3'	5	0	5
HB11	5' GTGTGTGTGTGTCC3'	8	2	6
HB15	5'GTGGTGGTGGC3'	5	0	5
Terry	5'GTGGTGGTGGTGC3'	6	0	6

TNB= Total number of bands, SB= Shared bands and PB= Polymorphic bands.

Table 2: The Correlation (Under diagonal) and the coefficient of determination (above diagonal) values among different shell morphometric variation analysis.

	AH	SH	AW	SW
AH		0.685	0.509	0.392
SH	0.828		0.334	0.323
AW	0.714	0.578		0.116
SW	0.627	0.5687	0.3413	

SH=Shell height, SW= Shell width, AH= Aperture height and AW= Aperture width.

Table 3: The isozymes and protein electrophoresis resolutions of the *P.canaliculata* foot tissues.

System	EC	Clas.	Res.	NDB	PB
Glucose-6-phosphate dehydrogenase	1.1.1.49	Oxi.	+	2	0
Malate dehydrogenase	1.1.1.37	Oxi.	+++	10	7
Superoxide dismutase	1.15.1.1	Oxi.	+++	6	2
Esterase	3.1.1.1	Hy.	+++	13	2
SDS-PAGE			+++	26	2

SDS=Sodium Dodecyl Sulfate, +++= strong, ++= Moderate, += Weak, PB = Number of Polymorphic bands, NDB= Number of detected bands, Clas.= Classes, Res. = Resolution, Oxi.=. Oxidoreductases and Hy.= Hydrolases.

Table 4: Numbers of foot muscle isozymes and protein bands in each time interval.

	Est	Sod	Mdh	G6ph	SDS
a	12	4	3	2	24
b	12	4	3	2	24
t1	11	4	3	2	25
t2	11	4	10	2	26
t3	13	5	3	2	26
ABF	0.902	0.712	0.490	1	0.966
SD	0.24	0.32	0.351	0	0.11

t1=24h, t2=48h and t3=72h, a= control 1(water) and b= control 2 (water with plant extract). G6ph =Glucose6 phosphate dehydrogenase, Sod =Superoxide dismutase, Est =Esterase, and Mdh =Malate dehydrogenase) and SDS-sodium dodecyl sulfate. ABF= Average of band frequency and SD= standard deviation.

4. Discussion

Silver nanoparticles are enormously valuable in a various range of industrial products and biomedical facilities. Nevertheless, their impact on the environment is still under investigation **[16]**.

In the present study, *P. canaliculata* (invasive freshwater snail species spread throughout the humid tropics and sub-tropics) individuals were reared for certain time intervals under silver nanoparticles stresses. This nanomaterial was synthesized by the green method **[8, 9]** due to its low cost and eco-friendly.

The snails such as *P. canaliculata* morphometric variation analysis could be applied to identify a trait of various environmental effects via characterizing the probable phenotypic plasticity responses**.**

The morphometric data coupled with suitable statistical analysis were suitable **[26]** in discrimination among *P. canaliculata* populations (collected from different Philippian geographical locations).

Rama et al [27] discussed some diagnostic snail shell characters that were used to evaluate the morphological variations within the genus *Pomacea*. However, they reported some problems in the use of some shell characters such as shell thickness, color, and pallial lip pigmentation for species identification.

Many studies recommended the development of molecular markers for inferencing the accurate variations within and among biological taxa. So, molecular investigations have recently led for identifying of numerous cryptic species complexes within morphologically ambiguous species **[18, 28].**

The efficiency of combining the morphometric and genetic analysis in *Pomacea* species discrimination and characterization was discussed in many biological studies **[29 - 31].**

Some of the snails' characteristics such as shell thickness and surface characteristics in *Pomacea* and other ampullariids were supposedly divergence within certain species because such characteristics are associated with water pH and calcium contents **[27]**. So, many scientists recommended the development of molecular characterization for detecting the true *Pomacea* species evolutionary variations **[27, 31, 32].**

In the present study, the shell height could be used as a representative for the other shell size parameters. Also, the shell height was applied as a covariate in the regression evaluation of all remaining linear metrics of shell size. All shell measurement parameters exhibited significant relations for shell height. The results exhibited positive correlations between the shell height values and each aperture height (r=0.828), aperture width (r=0.5687), and shell width (r=0.5687).

The normality of morphometric results was checked before the exploration of quantitative shell measurements. The results did not differ significantly from that which is normally distributed (p-value > α).

The analyses of the characteristics are applied to generate the numerous hypotheses needed for understanding the relationships of the evaluated snails. These results should be carefully analyzed, their homogeneity tested, and their character variation determined. Some molecular marker data such as Inter simple sequence repeats (ISSR) from the extant taxa of snails might be helpful.

The sensitivity of the Inter simple sequence repeats in measuring the homogeneity among the snails' samples was confirmed by many authors in different aquatic organisms **[8, 18, 33].**

Our results showed that morphometric (Aperture height, Shell height, Aperture width, and Shell width) and molecular (ISSR) analyses were

useful in inferencing the biological variations within the evaluated snail individuals. The similarity (homogeneity) values among the estimated snail samples were calculated based on the analysis of shell morphology (0.97) and molecular variations (0.99) via Inter simple sequence repeats. These high values within the evaluated snails were also confirmed by the high ISSR fragment frequencies.

Concerning the snail's biological responses to silver nanoparticles studies **[16]**, many biological probes such as various biochemical markers including isozyme polymorphisms could be developed and tested for monitoring ecological health, particularly in the term of Eco-toxicological studies **[34, 35]**.

Some isozyme systems (Glucose 6 phosphate dehydrogenase, Esterase, Superoxide dismutase and Malate dehydrogenase) and protein subunit separations were applied to detect biochemical tags due to treatment effects.

Comparatively, with other aquatic taxa such as fish, the expression levels of some isozymes are affected after exposure to AgNPs. For example, the Glutathione S-transferase A in the liver of *Oryzias latipes* exposed to AgNPs was increased **[36, 34]**.

The number and intensity of some isozyme bands (as actual probes in certain organism body organs) could be changed due to exposure to nanoparticle materials such as silver nanoparticles. For example, **Al-amri et al [8]** detected some informative isozymes (eye Esterase and Superoxide dismutase) variations in *Pterophyllum scalare*. They proposed that the intensity and numbers of some isozymes in certain fish body organs could be changed under silver nanoparticle stress. Also, they confirmed that the Superoxide dismutase and Esterase isozymes' separation exhibited good markers for differential gene expression in certain tissue samples **[37].**

Some other isozymes were not affected under certain experimental conditions. For example, in the present study, no differences were detected among control and treated samples concerning the Glucose 6 phosphate dehydrogenase separations.

The separation of the other systems such as the Sod, Est, and Mdh and protein subunit separations exhibited some informative tags between the control and the treated individuals. Regarding the Mdh isozyme pattern, major differences have been found between the control and treated snails' samples. The highest polymorphic bands (7 bands) were revealed by the Mdh isozymes separation. All of these biomarkers were specific for (t2) treatment.

The detected electrophoretic pattern of the Superoxide dismutase isozymes (Sod), in the snail foot muscle tissues, exhibited informative Sod bands. The identified band numbers were variables among the evaluated Sod lanes. The antioxidant groups (which protects cells against oxidative stress) such as glutathione peroxidase and superoxide dismutase were act like the cell prelude defense mechanisation to hold up numerous oxidative pressures **[38, 39].**

Esterase isozyme (with various naphthyl ester substrates) as a good bioindicator for environmental stresses was identified under many experimental conditions in many Eco-toxicological investigations **[8, 21, 23]**. These isozymes might be a housekeeping protein that aids many metabolic activities in various cell categories **[40].**

These detected band variations might be due to changes in transcription and/or translation alterations in one or more series of cell levels under the experiment condition.

Vogler [41] believed that the geometric tools should be considered valuable analytical methods for exploring the snails' shape variability. In addition, these methods should be combined with other methods including anatomy, morphology, ecology, and genetics to the revision of this wonderful of organisms. Therefore, in the present study, the homogeneity values among the evaluated snail samples were calculated based on the analysis of morphometric (shell morphology) and molecular variations (via Inter simple sequence repeats) to avoid the effects of individual variations.

Our results highlight the utility of the *P. canaliculata* as a bioindicators aquatic animal for silver nanoparticle pollution bio-monitoring, especially, the use of biochemical and/or molecular tags of pollutants and/or stresses effects as applicants to be included in a multi-biomarker strategy.

5. Conclusion:

Our results suggested that *P. canaliculata* occupies an interesting systematic position, its biomarker characteristics distinguishing the control and the treated snail's samples. Also, detection of the isozyme variations was considered a useful biochemical marker of certain environmental pressures in certain aquatic environments. More informative markers could be detected and evaluated for monitoring aquatic ecological health in water pollution levels in future Eco-Toxicological studies.

6. Conflicts of interest

The authors have no financial or personal relationship with other people or organizations that could influence or bias this paper inappropriately.

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