

## DISCUSSION

Infection of *Biomphalaria* species by the larval stages of *S. mansoni* leads to an intimate, long-term association that mainly challenges the snail's physical integrity and severely compromises its biological fitness.<sup>(207)</sup> Although the infective stages (miracidia) are small relative to the host, yet once inside the host's body, they initiate major developmental changes to serve their extensive growth and transformation to more specialized larval stages.<sup>(208)</sup> Based on a postulate of the short life span of miracidium in water, that is in the same time gives rise to hundreds of cercariae, schistosome development inside its snail intermediate host can be considered the weakest link in the parasite's life cycle. Additionally, while a given parasite strain can only develop within a compatible snail, no such discrimination occurs in the human host. Hence, snail control is highly effective in elimination of schistosomiasis and is at the same time easier than changing human habits and their treatment. For this reason, searching for the snail host factors that prevent parasite development will help in designing tools that can be utilized for control of schistosomiasis.<sup>(209)</sup>

Genetic background and internal defense system are snail related factors that are known to affect compatibility of *Biomphalaria* to *S. mansoni*.<sup>(123, 163)</sup> Age dependent variability in susceptibility to *S. mansoni* has been studied in *B. glabrata*.<sup>(152, 210, 211)</sup> It was found that the number of genes controlling resistance trait differs with snail's age, which in turn affects the compatibility pattern.<sup>(155, 212)</sup> Moreover, the number of genes controlling resistance vary between different *Biomphalaria* species.<sup>(155, 212, 213)</sup> This explains the importance of studying the effect of age on the compatibility pattern of *B. alexandrina*.

Internal defense of *Biomphalaria*'s is the most important defensive mechanism against *S. mansoni* infection. Hemocytes constitute a major element in IDS. These cells produce cytosolic superoxide dismutase enzyme (Cu/Zn SOD) (SOD<sub>1</sub>), an important enzyme that catalyzes the conversion of superoxide anion (O<sub>2</sub><sup>-</sup>) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Hydrogen peroxide produced by *Biomphalaria* snails kill *S. mansoni* through its potential oxidative damage, and hence affects the susceptibility of the snails.<sup>(123, 146)</sup>

Goodall *et al.* 2006, found that in *B. glabrata*, SOD<sub>1</sub> enzyme expression differs among susceptible and resistant snails.<sup>(154)</sup> However, the effect of snail's age on the activity of this enzyme has to be evaluated.

In this context, the current work is concerned about studying the effect of *B. alexandrina* snails' age on the compatibility pattern of the snails and on the activity of SOD<sub>1</sub> (as a representative for IDS). The present work was conducted, by involving parasitological and biochemical approaches. This was achieved by including 200 snails in each of the four experimental subgroups, being; Young susceptible (Ia), Adult susceptible (Ib), Young resistant (IIa) and Adult resistant (IIb). In each subgroup, 100 snails were dedicated for parasitological examination that started four weeks post exposure to miracidia and continued for three weeks. The other 100 snails were used for biochemical study, that was performed four weeks post exposure to infection.

In the current study, we investigated the effect of snails' age on their compatibility pattern. Moreover, the effect of interaction of snail's age, genetics and internal defense on the outcome of infection was studied.

Results revealed that younger snails whether belonging to susceptible or resistant groups, showed higher susceptibility when compared to their adult peers. Young susceptible subgroup members possessed the shortest range of pre-patent period (PPP), where the majority of the shedding snails were recorded by the 28th day being 37 out of 85 shedding snails. Moreover, the rest of shedding snails in the same subgroup showed their first shedding to be before the 40th day post infection, where 27 shed cercariae for the first time at the 32nd day, 16 shed at the 36th day and 5 snails shed at the 40th day.

Regarding Adult susceptible subgroup (Ib) members, out of 74 shedding snails, only 11 snails shed for the first time at the 28th day post infection. The maximum number of shedding snails was 16 and 17 that were recorded by 36th and 40th days respectively. Additionally, in 7 snails, PPP extended to 49th day.

Njiokou *et al.* (2004) and Abou El Naga *et al.* (2011) stated that, high compatibility of a snail to trematode infection is characterized by short PPP. The significant differences observed between subgroups Ia and Ib in the shedding durations indicate that subgroup Ia (Young susceptible) carry higher susceptibility to *S. mansoni* infection, with rapid development of the parasite inside the snail tissue. <sup>(34, 214)</sup>

As for subgroup IIa (young resistant) shedding members, the maximum number of shedding snails for the first time was 11 that were recorded by 49th day, with only 2 snails that shed for the first time by the 28th day. On the other hand, members belonging to adult resistant subgroup IIb, displayed only resistant phenotype, with no recorded PPP. When compared to subgroup IIb (Adult resistant) that contained only resistant members, subgroup IIa (Young resistant) showed higher susceptibility. These significant differences between subgroups (Ia) and (Ib), and between (IIa) and (IIb) can be attributed to the effect of age.

It was not surprising that, compatibility pattern also affected the outcome of infection evidenced by shown significant differences noted between subgroups Ia and IIa, and between subgroups Ib and IIb. Moreover, the significant differences recorded between (young resistant) susceptible members and (adult susceptible) susceptible members (IIa and Ib) could be attributed to the effect of interaction between age and genetics of the snails on their compatibility pattern. This was represented by longer PPP in susceptible members in subgroup IIa, than that recorded in members of the aforementioned adult susceptible subgroup.

In the current study, some members of subgroups (IIa) and (Ib) possessed a PPP of 49 days. The delay in development of the parasite within these snails indicates lower susceptibility to infection. Comparison between young resistant (IIa) subgroup and adult susceptible (Ib) subgroup showed 11 snails to shed for the first time at 49th day in the former and only 7 in the latter. However, this difference was not statistically significant, indicating that both age and compatibility pattern are important in determination of outcome of infection.

Richards, 1984, stated that, genetic factors determining *B. glabrata* juvenile non susceptibility operate throughout snail's life masking the presence of genetic factors for adult susceptibility, thus, unfortunately complicating studies on influence of age on snail susceptibility. <sup>(210)</sup>

The highest infection rate in the present study was exhibited by the snails belonging to the young susceptible subgroup Ia members. 92% of subgroup Ia (young susceptible), 74% of subgroup Ib (adult susceptible), and 37% of subgroup IIa (young resistant) were susceptible. As for subgroup IIb (Adult resistant), all members were resistant. Noteworthy that, even the dead snails during the time course of experiment were included in the statistical analysis. After their death, perished snails were examined for any developing sporocysts.

Although our experiment was carried out on snails resulting from self-reproduction, however resistant snails were obtained in the progeny of susceptible members indicating dominance of resistance character in *B. alexandrina*. In their studies, both; Shoukry *et al.* 1997 and Abou El Naga *et al.* 2010 observed the appearance of resistant members in snails that originated from crossing of susceptible parents.<sup>(182, 183)</sup> A probable explanation for the resistant members obtained in the susceptible subgroups, is that, although resistance alleles are sometimes hidden from being shown in the snail phenotype, they group together, forming resistant phenotype to appear. Their parents carried unexpressed resistance genes, and the resistance alleles grouped among successive generations. Abdel-Hamid *et al.* (2006) and El-Nassery *et al.* (2013) found that, both susceptibility and resistance in *B. alexandrina* are hereditary characters which are genetically controlled.<sup>(215, 216)</sup> Similarly, resistance character in *B. glabrata* snails is inherited in a dominant way.<sup>(158, 217)</sup>

In contrary to Lewis *et al.* (2002), who found that susceptible *B. glabrata* parents did not give rise to any resistant progeny, susceptible subgroups in the current study gave rise to 8%, 26% resistant members at different age groups.<sup>(217)</sup> This could be explained by the assumption proposed by Abou El Naga *et al.* (2010) that, *B. alexandrina* may contain more resistance alleles in their susceptible population than those present in *B. glabrata*, thus accounting for the appearance of resistant progeny originating from completely susceptible parents.<sup>(182)</sup> In the current study, although the two susceptible subgroups contained resistance members, nevertheless the significant difference noted between them, points to that the resistant alleles obtained from (susceptible group) F1 parents were potentiated by the impact of age resulting in the appearance of more resistant members in the adult susceptible subgroup (Ib).

In the current work, no susceptible members were obtained in the adult resistant subgroup (IIb). This result was also met in other studies using different species.<sup>(182, 213, 217)</sup> On the other hand, 37 susceptible members were recorded in our young resistant subgroup (IIa). This reinforces our assumption of effect of *B. alexandrina* age on the snails' compatibility pattern. The appearance of susceptible members in the young resistant subgroup could be also explained by its weaker internal defense when compared to the adult resistant subgroup. This was proved by significant difference noted in SOD<sub>1</sub> enzyme activity between the resistant members in the young and adult resistant subgroups, being  $0.68 \pm 0.04$  and  $0.90 \pm 0.07$  Units/gram tissues respectively.

Anderson *et al.* (1982), attributed the lower infection rates in adult snails than in younger snails to different factors. First, older snails possess physical barriers to penetration imposed by its tough thickened body covering. Additionally, they produce greater quantities of mucus that is full of chemicals leading to confusion of the searching miracidia. Moreover, they own higher phagocytic capacity. They also, move faster and so, are more difficult to locate by the miracidia.<sup>(218)</sup> Furthermore, egg laying capacity could be

among the factors that cause more resistance in the adult snails, where the snail utilizes all its energy to serve its reproductive capacity as stated by Richards in 1977.<sup>(212)</sup>

The question to be addressed here is; are the resistant alleles transmitted from susceptible parent snails to their young progeny more functioning on getting adult? According to our results, the answer is; probably yes. We assume that resistance alleles in the susceptible population may be more functioning through the snail aging process, those can be accused of causing lower susceptibility met in adult *B. alexandrina* snails.

The highest susceptibility met in young susceptible subgroup was also evidenced by showing the highest TCP among the four studied subgroups, being 151002. This was followed by the adult susceptible subgroup that produced 41732 cercariae over the three weeks of shedding. Coming next, the young resistant subgroup had TCP of 9877 over the shedding weeks. According to Frandsen classification, the three subgroups laid in classes 4, 2 and 1 respectively, being well compatible, poorly compatible and not very compatible. Regarding the adult resistant subgroup, no cercariae were produced at all, putting this subgroup in Frandsen class 0 or resistant snails.<sup>(119)</sup>

Recorded mortality rates in the present study were 47%, 35%, 27% and 14% in subgroups Ia, Ib, IIa and IIb respectively. Mortality rates shown were directly proportional to infection rates in different subgroups. The higher mortality rates noted in the susceptible than in resistant groups could be explained by the devastating effects of *S. mansoni* on the snail health. *S. mansoni* is considered a disease with a selective pressure on the susceptible population. During its development, *S. mansoni* sporocysts invade *Biomphalaria*'s digestive gland and the adjacent gonads leading to destruction of these organs.<sup>(219)</sup> Thus becoming an element of natural selection, which is highly negative for the infected snails.<sup>(220)</sup> Moreover, disturbance in metallic ions concentration due to the trematodes infection is a considered cause for mortality of the parasitized snails.<sup>(221)</sup> Furthermore, exposure of susceptible snails to high dose of infection (8-10 miracidia) for each snail at the same time explains the higher mortality recorded in laboratory, as the majority of these miracidia will develop practically without restraint. In contrary to natural conditions, where it is not expected that the snail is exposed to all of these miracidia at a time.<sup>(222)</sup>

In the current study, mortality rates recorded in the two resistant subgroups were 35% and 14% in subgroups IIa (Young resistant) and IIb (Adult resistant) respectively. Mortality of resistant snails can be attributed to the high concentration of SOD<sub>1</sub> enzyme that produces H<sub>2</sub>O<sub>2</sub>, a highly reactive oxygen species. Though these reactive species are beneficial in combatting the invading parasite, nevertheless, they also seriously damage the biological membranes in host tissue. This is the so called "cost of resistance".<sup>(146, 162)</sup> Hence, reactive oxygen species are considered a double-edged sword.<sup>(223)</sup> Mangal *et al.* (2010) stated that, the exposure alone, of *B. alexandrina* snails to *S. mansoni* miracidia has a negative impact on the snails' survival rates.<sup>(224)</sup>

In the present work, we have functionally investigated the activity of SOD<sub>1</sub> enzyme in the whole snail soft tissue homogenate, 4 weeks after snail exposure to infection. Regarding the control non-infected snails, results were  $0.26 \pm 0.04$  and  $0.30 \pm 0.03$  Units/gram tissues in non-infected young and adult groups respectively. No significant difference was calculated between the two control groups. During the course of snail life, cytosolic superoxide dismutase is present to protect cells against damage by free radicals normally generated.<sup>(107)</sup>

As regards the results of the experimental subgroups in the current study; combined effects of both age and genetic background have resulted in the highest SOD<sub>1</sub> enzyme activity to be obtained in adult resistant subgroup giving mean of  $0.90 \pm 0.07$  Units/gram tissues. On the pole, the least enzyme activity in the experimental subgroups was given by susceptible members of (young susceptible subgroup), being  $0.32 \pm 0.03$ U/gram tissue. Furthermore, As shown in table (7), SOD<sub>1</sub> enzyme activities were higher in the resistant members in the studied subgroups, than in, their corresponding susceptible members. Thus indicating the importance of the SOD<sub>1</sub> enzyme in determining snail's compatibility status. Our results of finding higher SOD<sub>1</sub> activity levels in resistant than in susceptible snails, were previously obtained by Hahn *et al.* (2001), Goodall *et al.* (2004), Mahmoud and Rizk (2004), Bender *et al.* (2005) and Bayne *et al.* (2009), where they found more hemocytes generating superoxide to be present in *S. mansoni* resistant snails. The resulting free radical weapon against the parasite succeed to encapsulate or eliminate it in resistant snails, but fail in case of susceptible snails. <sup>(107,139, 153, 225, 226)</sup>

It is worthy to mention that, a study on the genes of a resistant *B. glabrata* strain has revealed that polymorphisms at the locus coding for cytosolic Cu/Zn superoxide dismutase (SOD<sub>1</sub>) associate with susceptibility/ resistance , which reinforced the role of SOD<sub>1</sub> in schistosome resistance. <sup>(146, 153, 154)</sup>

The results of the present work showed that *B. alexandrina* snails' age affects SOD<sub>1</sub> enzyme activity levels. Significant differences in SOD<sub>1</sub> enzyme activity levels between young and adult snails obtained from the same (F<sub>1</sub>) parent were observed, with higher activity to be met in adult age. Goodall *et al.* (2006), encoded *B. glabrata* SOD<sub>1</sub> enzyme by three alleles. <sup>(154)</sup> One of the alleles has been found to be significantly associated with resistance. Furthermore, some alleles controlling compatibility were found to be affected by snail's age. <sup>(117, 155, 212)</sup> In this context, we suggest that SOD<sub>1</sub> encoding genes could lie among other genes that are modified by snail's age. This suggestion can be verified after development of *B. alexandrina* gene bank.

To sum up, the significant differences in our parasitological and biochemical parameters between young and adult snails in the same group were attributed to age effect that made the resistance alleles more functioning in adults with higher superoxide dismutase enzyme activity. Moreover, the complex interaction between age, genetic background and internal defense system between susceptible and resistant subgroups has resulted in great variability in their compatibility patterns. This interaction has led to the appearance of the highest significant difference between young susceptible (Ia) and adult resistant (IIb) subgroups.

Regarding our electrophoretic study, it was done in a trial to differentiate the total protein composition in the studied subgroups. The electrophoretic separation of proteins is one of the main methods used for fractionation and characterization of all types of proteins. One of its types is Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) which is used for estimation of the protein subunit molecular weights. <sup>(180)</sup> This method was followed in our study.

Results of the current electrophoretic study indicated that young snails' tissue proteins exhibited a complex pattern of polypeptides with a total number of 9, 9, 9,10 and 11 bands in young non infected, subgroup Ia (susceptible members, resistant members) , subgroup IIa (susceptible members and resistant members) respectively. Their molecular

weights ranged from 196.739 to 2.930. On the other hand, the number of bands in adult snail subgroups were 13, 16, 16 and 17 bands in adult non infected, subgroup Ib (susceptible members, resistant members) and subgroup Iib respectively. Their molecular weights ranged from 204.353 to 2.930 KDa.

The differences between young and adult non-infected snails were recorded, where number of bands detected in non-infected young was 9, while in non-infected adult it was 13. The similarity index between the 2 non-infected groups was calculated to be 0.75. This index indicates changes in the protein content in the snail tissue with age, which may be responsible for egg laying capacity, or can be attributed to other physiological changes with aging process itself.

The importance of age is also evident on comparing adult and young snails in different subgroups, where differences in protein expression and banding pattern were shown. A new finding here is the difference between adult and young snails possessing the same genetic origin and compatibility pattern, where differences noted between susceptible members of subgroups Ia and Ib, resistant members of subgroups Ia and Ib, resistant members of subgroups Iia and Iib, being, 0.6, 0.64 and 0.62 respectively. These indices indicate that age has an impact on the protein pattern of snail tissue.

Regarding adult subgroups, many shared proteins were observed. A band of MW 55.597 KDa was shared by the 4 lanes corresponding to the adult subgroups and their control, accordingly it may be a protein that is only present in adult *B.alexandrina* whether infected or not. Bands of MW 16.738 and 14.846 KDa were shared by the 3 lanes corresponding to the exposed adult subgroups, hence they may represent proteins that appear in adult *B.alexandrina* corresponding to adult snail defense against *S.mansoni*. Additionally these shared proteins between adult subgroups whether susceptible or resistant could be responsible for the lower compatibility met in adult susceptible snails. Noteworthy that, many bands ranging from 16.738 to 12.991 were noted to appear in the three exposed adult subgroups whether susceptible or resistant, which indicates that, these proteins may be of importance in adult defense against *S.mansoni*. The highest similarity index was noticed between adult resistant subgroup and resistant members of adult susceptible subgroup, being 0.89 with 7 shared bands between them. Those possess MW of 196.739, 82.839, 28.565, 14.169, 12.991, 6.424 and 4.373. On the other hand only one band was shared by adult susceptible subgroup members of different compatibility status being 20.380. So, it is evident that internal defense in adult *B.alexandrina* is more important than its genetic origin making shared bands between snails of the same compatibility pattern and different genetic origins more than shared bands between snails of the same genetic origin and different compatibility patterns.

In contrary to adult subgroups where internal defense has the upper hand, a different scenario was present in young subgroups, where many shared proteins were observed between snails possessing the same genetic origin regardless their compatibility patterns. 5 common bands were shared between susceptible and resistant members of young susceptible subgroup of MW of 115.819, 59.448, 20.380, 16.873 and 3.158. Furthermore, 6 common bands were shared between susceptible and resistant members of young resistant subgroup of MW of 174.426, 124.635, 44.009, 20.124, 16.547 and 3.128. Hence, coefficients of 0.85 and 0.84, were calculated between (subgroup Ia resistant and susceptible members) and (subgroup Iia resistant and susceptible members) respectively.

This can be attributed to the similarities of both genetic origin and age, although there were differences in compatibility for snails in both cases. So, it can be assumed that genetic origin in young *B.alexandrina* is more important than its internal defense causing many shared bands to appear between snails of the same genetic origin and different compatibility patterns.

Noteworthy to mention that, although many shared bands possessing the same molecular weights were observed between subgroups belonging to the same age, however, each shared protein band may represent more than one protein on further molecular analysis.

Interestingly, a common shared band of MW 93.600 KDa was noticed in all lanes. This indicates that this protein is related to *B.alexandrina* obtained from Alexandria, that is neither influenced by snail's age, genetic origin nor by schistosome infection.

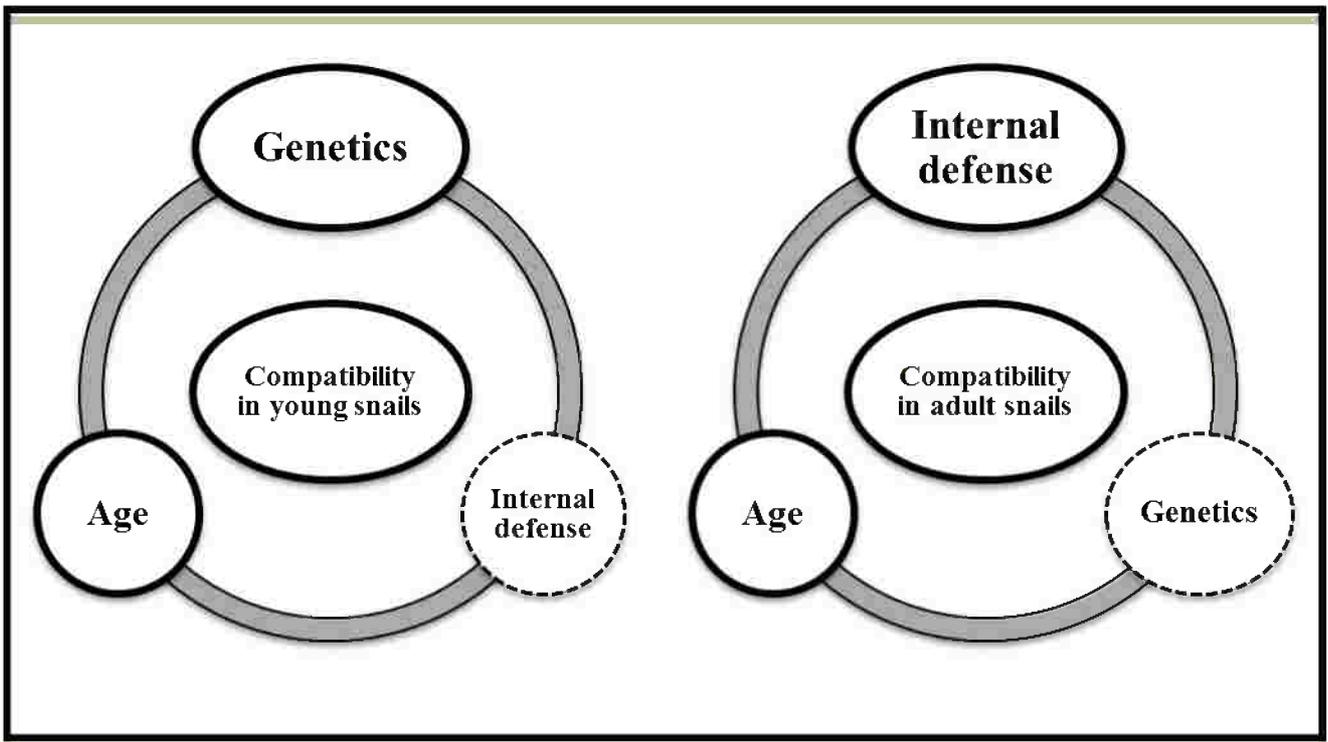
The effect of schistosome infection on the protein content within the snails can be observed between infected and non-infected peers of the same age. Indices of 0.63 and 0.7 were recorded between non-infected young with subgroup Ia susceptible members and subgroup IIa susceptible members respectively. Index of 0.7 was recorded between non-infected adult and subgroup Ib susceptible members. The effect of infection led to appearance and disappearance of several protein bands in infected compared to control, where the development of intramolluscan larval stages of the parasite within the parasitized snails has altered the electrophoretic profile of its tissue proteins. This alteration in protein fractionation can be attributed to the presence of parasite secretory-excretory products and also to the presence of some degree of immune response, whether cellular or humoral that is exhibited by the snails against the invading parasite, indicating the production of new proteins in response to infection. <sup>(147, 227)</sup> Using the same technique, many researchers reported that tissue proteins of *S.mansoni* – *B.alexandrina* complex had a different protein pattern when compared to non-infected snails. They revealed that this complex had a low similarity coefficient with the non-infected. <sup>(48, 203, 228)</sup>

The calculated similarity indices between the non-infected groups and the resistant members of the subgroups belonging to the same age were 0.67 and 0.74 between non-infected young and resistant members of subgroups Ia and IIa respectively. Additionally, indices of 0.6 and 0.6 were calculated between non-infected adult and resistant members of subgroups Ib and IIb respectively. The differences can be attributed to the activation of cellular and humoral elements of the snail's immune response against the invading parasite. <sup>(147, 227)</sup>

Accordingly, the highest similarity coefficient was found between subgroup IIb adult resistant & subgroup Ib resistant members, being 0.89, which can be attributed to combination of similarities between both age and compatibility status. Moreover, coefficients of 0.85 and 0.84, were calculated between (subgroup Ia resistant and susceptible members) and (subgroup IIa resistant and susceptible members) respectively. This can be attributed to the similarities of both genetic origin and age, although there were differences in compatibility for snails in both cases. On the other hand, the least similarity index was observed between the most resistant subgroup in our experiment (IIb adult resistant) and the most susceptible subgroup (Ia susceptible members) being 0.4, here the differences between the two subgroups are genetic, age related and compatibility status related differences.

The results of the present work including; parasitological, biochemical and electrophoretic studied revealed that the complex interaction between age, genetic background and internal defense has resulted in great variability between the studied subgroups. Additionally, the highest significant difference appeared between young susceptible (Ia) and adult resistant (IIb) subgroups, with the highest susceptibility to be met in young susceptible subgroup and the highest resistance to be met in the adult resistant subgroup. Besides, in the highlights of our results, it is concluded that, internal defense has the upper hand in adults in determination of their compatibility status, while genetic origin is more important in determination of compatibility of young snails as shown in figure (17).

Results presented herein can have potential epidemiological implications in *Biomphalaria* control. Identification of the snail's age that is most susceptible to *S. mansoni* infection will determine the best time for applying molluscicides. This in turn increases the efficacy of the applied method and hence potentiates schistosomiasis control. Additionally, by determining that adult age is in general less susceptible than young age in *B. alexandrina*, this can aid in determination of the optimum time for mass chemotherapy that is best to be applied when there is no risk of reinfection with schistosomes. Moreover, adult resistant snails could be benefited of in biological snail control, after studying the compatibility of successive generations.



**Figure 17:** The effect of complex interaction between age, genetic background and internal defense on determination of compatibility status in young and adult *Biomphalaria alexandrina* snails. Genetic background has the upper hand in young snails while internal defense has the upper hand in adult snails.

## SUMMARY AND CONCLUSION

Schistosomiasis is recognized as a major neglected tropical disease (NTD) and also as a 'silent pandemic' that affects up to 249 million people in many developing countries all over the world, with deleterious socioeconomic and public health impact.

The world-wide distribution of *S. mansoni* that causes intestinal schistosomiasis is permitted by the broad geographic range of the susceptible species of its intermediate host, *Biomphalaria* snail, where humans get infected by the larval stage of *S. mansoni* shed from this snail. Among more than 34 identified species of *Biomphalaria*, *B. alexandrina* represents the intermediate host of *S. mansoni* in Egypt.

In general, *Biomphalaria* snails are known to display a wide range of susceptibility phenotypes to *S. mansoni* infection depending on different factors. Snail's age, genetic background and internal defense system are among the snail related factors that are known to affect snail's susceptibility patterns. Considerable variety of control strategies have been proposed in eliminating schistosomiasis including chemical, physical and biological approaches, yet effective absolute permanent results have not yet been achieved. Breaking the chain of this parasite life cycle by targeting its intermediate host snail can decrease its transmission. Making intermediate host populations in the field either resistant or even less susceptible to infection could provide a better long-term solution which is ecologically safer way for breaking transmission cycles. Studying the snail's physiological phenomena that modulate its susceptibility to *S. mansoni* infection is pivotal to provide new insights in the control of the targeted mollusk.

In this context, this study aimed at elucidating impact of *B. alexandrina* snails' age on their susceptibility patterns towards *S. mansoni* infection including their genetic variability and internal defense represented in Cytosolic Cu/Zn Superoxide dismutase enzyme (SOD<sub>1</sub>). To study this impact, we have performed three different approaches; parasitological, SOD<sub>1</sub> enzyme assay and Sodium dodecyl sulphate polyacrylamide gel electrophoretic study (SDS-PAGE).

*Biomphalaria alexandrina* susceptible and resistant snails were reared singly for self-reproduction. Of their progeny, four subgroups with 200 snails for each underwent our experiment being; young susceptible (Ia), adult susceptible (Ib), young resistant (IIa) and adult resistant (IIb). Snails of all subgroups were individually exposed to *S. mansoni* miracidia. Young snails (before the start of egg laying) were exposed at two months of age and the size of 3-4 mm). Adult snails (after the start of reproductive capacity) were exposed at four months of age and the size of 8-10 mm. Four weeks later, one hundred snails in each subgroup were individually examined for cercarial shedding to determine susceptible and resistant members. This examination was repeated twice weekly for three weeks. Pre-patent period, infection rate, total cercarial production and mortality rate for the 4 subgroups were determined.

The other 100 snails in each subgroup together with 2 uninfected young and adult groups underwent homogenization step after determination of their susceptibility patterns. The supernatants of these homogenized snails were used for both; SOD<sub>1</sub> enzyme assay and SDS-PAGE study. Then, Dice similarity coefficient between total protein patterns of different subgroups was calculated.

The results of the parasitological study revealed that the most susceptible subgroup was young susceptible (Ia) subgroup; with the highest IR being 92%, the shortest PPP,

highest TCP being 151002 and highest MR being 47% . This was followed by adult susceptible (Ib) subgroup with IR of 74%, TCP of 41732 and MR of 35%. The third subgroup was young resistant (IIa) subgroup; with the IR being 37%, TCP being 9877 and MR being 27%. Regarding adult resistant (IIb) subgroup; it contained only resistant members and MR was 27%.

Recorded SOD<sub>1</sub> enzyme activities in non-infected (young and adult) groups were  $0.26 \pm 0.04$  and  $0.30 \pm 0.03$  Units/gram tissues respectively. Regarding SOD<sub>1</sub> enzyme activity in infected subgroups; the highest level of SOD<sub>1</sub> enzyme was obtained in adult resistant subgroup (IIb) giving mean of  $0.90 \pm 0.07$  Units/gram tissues, while, the least enzyme activity was given by the susceptible members of young susceptible subgroup (Ia) being  $0.32 \pm 0.03$ . The enzyme activity was higher in resistant members regardless their age, in comparison to susceptible and non-infected members. Furthermore, the SOD<sub>1</sub> activity was higher in adult subgroups than in those of young ones.

Using SDS-PAGE, differences in protein expression and banding pattern were shown between adult and young snails in different subgroups. Dice similarity coefficient (index) between different subgroups was calculated. The highest similarity coefficient was found between subgroup IIb adult resistant & subgroup Ib resistant members, being 0.89, which can be attributed to combination of similarities between both age and compatibility status. Moreover, coefficients of 0.85 and 0.84, were calculated between (subgroup Ia resistant and susceptible members) and (subgroup IIa resistant and susceptible members) respectively. This can be attributed to the similarities of both genetic origin and age, although there were differences in compatibility for snails in both cases. On the other hand, the least similarity index was observed between the most resistant subgroup in our experiment (IIb adult resistant) and the most susceptible subgroup (Ia susceptible members) being 0.4, where differences between the two subgroups are genetic, age related and compatibility status related differences.

In the view of our results, including; parasitological, enzyme assay and electrophoretic studies, we concluded that, the complex interaction between age, genetic background and internal defense has resulted in great variability between the studied subgroups. Additionally, the highest significant difference appeared between young susceptible (Ia) and adult resistant (IIb) subgroups, with the highest susceptibility to be met in young susceptible subgroup and the highest resistance to be met in the adult resistant subgroup. Besides, it is evident that internal defense has the upper hand in adults in determination of their compatibility status, while genetic origin is more important in determination of compatibility of young snails.

Results presented herein can have potential epidemiological implications in *Biomphalaria* control. Identification of the snail's age that is most susceptible to *S. mansoni* infection will determine the best timing for applying molluscicides. This in turn increases the efficacy of the applied method and hence potentiates schistosomiasis control. Additionally, by determining that adult age is in general less susceptible than young age in *B. alexandrina*, this can aid in determination of the optimum timing for mass chemotherapy that is best to be applied when there is no risk of reinfection with schistosomes. Moreover, adult resistant snails could be benefited of in biological snail control, after studying the compatibility of successive generations.