Original Article

Effects of *Trypanosoma cruzi* infection in Balb/c and NIH mouse strains

DOI: 10.5377/alerta.v7i2.16425

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Efectos de la infección del *Trypanosoma cruzi* en ratones de las cepas Balb/c y NIH

Suggested citation:
Ramírez Merches CB, Campos Portillo WM, González Pérez AM, Mejía Valencia JG. Effects of *Trypanosoma cruzi* infection in Balb/c and NIH mouse strains. Alerta. 2024;7(2):152-160. DOI: 10.5377/alerta.v7i2.16425

Editor:
Edgar Quinteros.

Received:
July 18, 2023.

Accepted:
June 27, 2024.

Published:
July 24, 2024.

Author contribution:
CBRM[1], WMCP[2], AMGP[3], JGMV[4]: literature search, data analysis, writing, revising and editing. CBRM[1], WMCP[2]: data collection. AMGP[3], JGMV[4]: manuscript design. WMCP[2]: study design and data or software management.

Conflicts of interest:
The authors declared there are not conflicts of interest.

Abstract
Introduction. Chagas disease is an infection caused by the parasite *Trypanosoma cruzi* and transmitted by the vector *Triatoma dimidiata*, known in El Salvador as "chinche picuda". This disease has always been of scientific interest in animal models. **Objective.** Identify the effect of *Trypanozoma cruzi* infection in mice of different strains (BALB/c and NIH) and sex. **Methodology.** Eight groups were established: four infected with *Trypanosoma cruzi* and four uninfected groups, distributed by strain and sex, with five mice per group. The body weight of the mice was recorded for six weeks. In addition, blood samples from the infected groups were prepared on slides for parasitemia counts. At the end of the study, the spleen and heart were extracted from both groups for statistical analyses. **Results.** The infected groups showed an increase in weight compared to their control groups. In the NIH strain, females had higher parasitemia, whereas in the BALB/c strain, males had higher parasitemia. The organs of the infected groups were significantly larger compared to those of the control groups, except in the heart of the BALB/c strain. Regarding organ weight, significant differences were observed only in the heart of the male BALB/c strain, while the opposite was true for the spleen. **Conclusion.** Males of the BALB/c strain are more susceptible to *Trypanosoma cruzi*, presenting higher levels of parasitemia among the groups studied.

Keywords
Trypanosoma cruzi, Parasitaemia, Blood, Animal Experimentation, Chagas Disease.

Resumen
Introducción. La enfermedad de Chagas es una infección causada por el parásito *Trypanosoma cruzi* y transmitida por el vector *Triatoma dimidiata*, conocido en El Salvador como «chinche picuda». Esta enfermedad siempre ha sido de interés científico en modelos animales. **Objetivo.** Identificar el efecto de la infección por *Trypanosoma cruzi* en ratones de diferentes cepas (BALB/c y NIH) y sexo. **Metodología.** Se establecieron ocho grupos: cuatro infectados con *Trypanosoma cruzi* y cuatro grupos no infectados, distribuidos por cepa y sexo, con cinco ratones por grupo. Durante seis semanas se registró el peso corporal de los ratones. Además, se prepararon muestras de sangre de los grupos infectados en láminas para realizar los conteos de parasitemia. Al final del estudio, se extrajeron el bazo y el corazón de ambos grupos para los análisis estadísticos. **Resultados.** Los grupos infectados mostraron un incremento de peso en comparación a sus grupos controles. En la cepa NIH, las hembras presentaron una mayor parasitemia, mientras que en la cepa BALB/c fueron los machos los de mayor parasitemia. Los órganos de los grupos infectados fueron significativamente más grandes comparados a los de los grupos de control, excepto en el corazón de la cepa BALB/c. Respecto al peso de los órganos, se observaron diferencias significativas únicamente en el corazón de los machos de la cepa BALB/c, mientras que en el bazo ocurrió lo contrario. **Conclusión.** Los machos de la cepa BALB/c son más susceptibles al *Trypanosoma cruzi*, presentando niveles de parasitemia más altos entre los grupos estudiados.

Palabras clave
Trypanosoma cruzi, parasitemia, sangre, experimentación animal, enfermedad de Chagas.

Introduction

The flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*) is the causative agent of American trypanosomiasis or Chagas disease, a disease of public health importance that persists as endemic in large areas of Latin America. Transmission occurs mainly using triatomine vectors, nocturnal hematophagous insects belonging to the subfamily Triatominae of the order *Hemiptera*, family *Reduviidae*, which feed on mammal blood.[i]

The natural reservoirs are armadillos, marsupials, rodents, bats, wild primates, and domestic animals such as dogs and cats. The parasite has been isolated in more than 150 species of domestic and wild mammals.[ii]

The life cycle of *T. cruzi* is complex, involving transmission by insect vectors and infection of vertebrate hosts, most of which are mammals, including humans, which are susceptible hosts.[iii]

Understanding the pathophysiology of *T. cruzi* infection in laboratory animals is a significant achievement in studying Chagas disease. Controlled experimental models allow the analysis of different parameters related to the host and the parasite, which cannot be addressed in humans for practical and ethical reasons.[iv,v]

Most of the studies of the acute phase of the disease are performed in animal models since this phase of the disease, generally in humans, goes unnoticed because it is self-limited and has non-specific clinical manifestations, which leads to most cases going undiagnosed. The most widely used model to study this disease is the laboratory mouse since it is one of the best-studied species from the immunological point of view. The experimental acute infection progresses differently depending on the parasite strain, mouse strain, virulence, inoculation dose, number of parasites, age, sex, and genetic profile, among other factors.[v,vi,vii] Pathogenic strains of *Trypanosoma cruzi* generally cause acute disease and high mortality in susceptible strains of mice, although these models are considered accurate representations of the infection process in humans. However, they are crucial for investigating immune responses, endocrine and metabolic states, and their interactions since this acute stage goes unnoticed in humans, except in cases of oral infection.[vii]

Different strains of mice differ in susceptibility or resistance to infection, evidencing a complex genetic control of parasitemia levels and survival of infected animals.[viii,ix,x] Therefore, in this study we evaluated the effect of *Trypanozoma cruzi* infection in mice of different strains (BALB/c and NIH) and sex.

Methodology

Experimental design

A total of 40 healthy mice were used. They underwent clinical checks to evaluate their fur, eyes, skin, and mucous membranes. The mice were divided into eight groups, each consisting of five mice. Four groups were infected with parasites (cases), and four remained healthy (controls). The groups were categorized based on strain (NIH and BALB/c) and sex (refer to Table 1).

The control group was assessed for body weight, heart and spleen size, and weight. All mice were randomly chosen and were around five to six weeks old. The study lasted six weeks, with the first week dedicated to parasite inoculation and the following five weeks for counting parasite levels twice a week. Each infected mouse was evaluated ten times using a Leica microscope.

Currently, there is no ethics committee for laboratory animals in El Salvador. However, the study was conducted by international ARRIVE standards.[xi]

Parasite collection

Trypanosoma cruzi parasites in their metacyclic trypomastigote stage were obtained from a sample of feces of the vector *Triatoma dimidiata* collected in the Huisiltepeque canton, city of Tenancingo, department of Cuscatlán, El Salvador. Vector feces were diluted in 0.5 mL of 0.9 % normal saline, and the presence of *Trypanosoma cruzi* was verified by direct observation using a bright-field microscope. This preparation was provided

Table 1. Initial body weight, final body weight and percentage increase in grams of the experimental groups

Group	Initial weight	Final weight	Increase %	p-value
NIH (females) Control	24.63 ± 2.62	26.93 ± 2.50	9.56 ± 5.06	-
NIH (females) Infected	22.94 ± 2.21	28.44 ± 2.29	24.22 ± 5.70	0.005*
NIH (males) Control	26.60 ± 5.23	31.08 ± 5.63	17.17 ± 4.53	-
NIH (males) Infected	22.15 ± 7.64	33.43 ± 4.16	60.49 ± 18.64	0.102
BALB/c (females) Control	24.40 ± 0.87	27.63 ± 0.85	13.33 ± 5.65	-
BALB/c (females) Infected	27.08 ± 0.93	33.24 ± 1.37	22.80 ± 4.84	0.030*
BALB/c (males) Control	21.78 ± 0.77	28.98 ± 0.81	33.20 ± 6.04	-
BALB/c (males) Infected	17.32 ± 0.83	30.10 ± 2.96	73.69 ± 13.08	0.001*

Values are expressed as the mean ± standard deviation. Significant differences between groups are expressed when *p-value < 0.05.

Weight expressed in grams.

by the Laboratory in Vector Entomology of the Health Research and Development Center of the University of El Salvador.

Experimental animals

Mice of the NIH and BALB/c strains from the Animal Laboratory of the Center for Health Research and Development of the University of El Salvador were used. The mice were fed a diet of Tecnutral pelleted concentrate for rodents and free access to water. All animals were kept at a temperature of 22 ± 2 °C and a controlled relative humidity between 50-60 %, with a light-dark cycle of 12/12 hours. For individual identification, they were individually marked with picric acid.

Parasite inoculation

The mouse with the highest parasitemia (blood trypomastigotes) was identified and blood was drawn from the submandibular vein to obtain 1×10^5 parasites/mL in saline. This amount was necessary to reach to infect the mice. Subsequently, mice were restrained and immobilized and each mouse was inoculated intraperitoneally with 1×10^4 parasites/0.1 mL of the previously prepared solution using a 1 mL tuberculin syringe. The following formula was used to calculate the number of parasites required to achieve infection,[xii] where:

C_1: 1×10^5 parasites

V_1: 1 mL of 0,9 % normal saline

$$V_2 = \frac{C_1 V_1}{C_2}$$

C_2: Amount of parasites in the infected mouse

V_2: Amount of blood needed

Body weight

The initial body weight in grams of all groups was recorded using a COBOS scale before parasite inoculation. Subsequently, weighing was performed once a week until the end of the experiment.

Parasite count

Perform the count, a blood sample of approximately 10 µL was drawn from the tail of each infected mouse. Then, 5 µL of blood were taken with an Accumax® micropipette, which were deposited on a slide and immediately covered with a coverslip to obtain a thin and homogeneous layer. Subsequently, it was taken to the microscope for observation at 40x and a parasite count was performed in 50 fields, counting from left to right and vice versa.

Organ size and weight

Once the parasitemia counts were completed, all animal groups underwent cervical dislocation for euthanasia. The organs (heart and spleen) were then removed to evaluate their appearance, size (measured in centimeters), and weight (measured in grams).

Statistical analysis

The data was analyzed using IBM® SPSS 21 and Microsoft Excel 365 for statistical analysis. The Student's T-test was used to compare body and organ weights, while a one-way analysis of variance (ANOVA) was used for parasite counts. A difference between experimental groups was considered significant when $p < 0.05$. The results are mostly expressed as Mean ± Standard Deviation.

Results

Body weight

Table 1 indicates that the *Trypanosoma cruzi* groups obtained a greater percentage increase in body weight compared to their control groups (uninfected mice), with most having a p-value < 0.05 except for the male NIH strain group, which obtained a p-value = 0.102.

Parasite counts

Mice infected with *T. cruzi* of NIH and BALB/c strains presented different results in terms of sex, reflected in Table 2 and Figure 1. All groups of mice evaluated survived until the end of the study. The peak of parasitemia occurred at counts seven and eight.

Figure 1-A graphically compares the number of parasites observed during the investigation. In the NIH strain, it can be seen that the group of females presented a higher parasitemia through time, having its maximum point at parasite count seven. Figure 1-B shows the BALB/c strain, where the males presented the highest parasitemia, reaching its maximum at the seventh parasite count.

Organ size and weight

Once the parasite count was completed, the heart and spleen were extracted from the

eight groups of mice. Table 3 shows that the size of both organs in the groups infected with *T. cruzi* showed an increase when compared with their control groups, except in the heart of the BALB/c strain where no significant differences in size were obtained, while in the spleen almost all showed significant differences, with the exception of the male group of the BALB/c strain that obtained a p value = 0.053 with respect to its control group.

The weight of the organs analyzed between the control and infected groups can be seen in Table 4. They showed significant differences for the heart only in the group of the BALB/c strain males with a p-value = 0.008. On the other hand, in the spleen, the only group that did not show differences compared to their control was the BALB/c strain males with a p-value = 0.061, despite having a very high mean.

Discussion

The fundamental interest in this study was to demonstrate the acute phase of *Trypanosoma cruzi* infection in an animal model using BALB/c and NIH strain mice after a six-week observation period.

However, the results obtained in this study contradict the findings of other authors[xiii,xiv,xv,xvi,xvii] who have shown that infection with this parasite causes a statistically significant decrease in body weight in BALB/c and Cba/j mice approximately thirty days after infection.[xiii,xiv,xv] This weight loss is attributed to a marked depletion of body fat and an increase in water retention, suggesting that the disease induces true cachexia, which is considered one of the manifestations of the inflammatory reaction to this infection.[xvi,xvii]

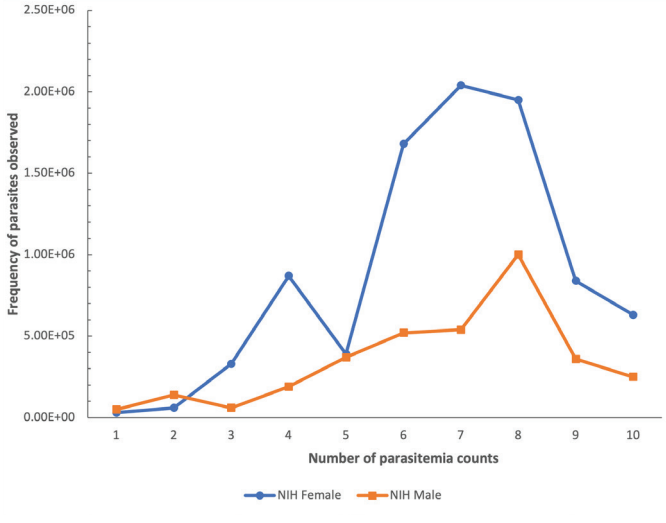

Figure 1. (A) Behavior of parasite counts (*T. cruzi*) of the NIH strain for both sexes over time (six weeks)

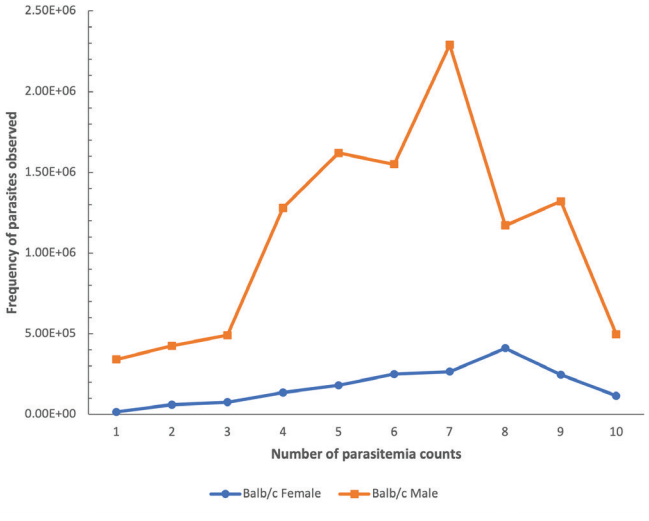

Figure 1. (B) Graphical behavior of parasite counts (*T. cruzi*) of the BALB/c strain for both sexes over time (six weeks)

Table 2. Median values of the number of parasites (*T. cruzi*) per milliliter (mL) in the experimental groups

Groups/Count	NIH (females)	NIH (males)	BALB/c (females)	BALB/c (males)
1	0	5.00E+04	0	3.00E+05
2	0	5.00E+04	7.50E+04	4.00E+05
3	1.50E+05	5.00E+04	5.00E+04	3.00E+05
4	7.50E+05	1.00E+05	1.25E+05	8.75E+05
5	7.50E+05	3.00E+05	1.00E+05	1.60E+06
6	7.50E+05	3.50E+05	2.00E+05	1.10E+06
7	7.50E+05	5.00E+05	1.50E+05	2.20E+06
8	7.50E+05	8.00E+05	4.75E+05	1.10E+06
9	7.50E+05	3.50E+05	2.50E+05	1.60E+06
10	7.50E+05	1.00E+05	1.50E+05	6.00E+05

Values expressed as median, significant differences (*) between groups are expressed when p-value < 0.05.

Besides, these studies show weight loss is associated with pronounced hypoglycemia and is a consequence of a multifactorial process that includes the increase in proinflammatory cytokines, the reduction in food intake at the end of the infection, the hepatic involvement caused by the parasite, leading to deficiencies in the gluconeogenic pathway, and the high energy demand caused by the activation of the immune system.[xv]

Variations in body weight can be inversely influenced by metabolic disorders and hormonal imbalances. Major hormones influencing body weight include glucocorticoids, prolactin, dehydroepiandrosterone, growth hormone, testosterone, and leptin. Alteration in the structure or resistance to the hormone leptin can inhibit satiety and lead to energy imbalance, resulting in obesity and increasing susceptibility to infections and

Table 3. Size of organs (heart and spleen) in centimeters mainly affected in experimental mice

Organ	Group	Mean ± S.D.	p-value
Heart	NIH (females) Control	0.68 ± 0.17	
	NIH (females) Infected	0.86 ± 0.09	0073
	NIH (males) Control	0.87 ± 0.24	
	NIH (males) Infected	0.88 ± 0.08	0.966
	BALB/c (females) Control	0.95 ± 0.10	
	BALB/c (females) Infected	0.82 ± 0.08	0.071
	BALB/c (males) Control	1.00 ± 0.08	
	BALB/c (males) Infected	0.94 ± 0.09	0.334
Spleen	NIH (females) Control	1.88 ± 0.15	
	NIH (females) Infected	2.66 ± 0.15	0.000*
	NIH (males) Control	1.75 ± 0.33	
	NIH (males) Infected	2.48 ± 0.11	0.002*
	BALB/c (females) Control	2.00 ± 0.08	
	BALB/c (females) Infected	2.52 ± 0.13	0.000*
	BALB/c (males) Control	1.73 ± 0.38	
	BALB/c (males) Infected	2.56 ± 0.63	0.053

Values are expressed as the mean ± standard deviation (S.D.). Significant differences between groups are expressed when *p < 0.05.

Table 4. Weight in grams of affected organs (heart and spleen) in experimental mice

Organ	Group	Mean ± S.D.	p-value
Heart	NIH (females) Control	0.17 ± 0.01	
	NIH (females) Infected	0.14 ± 0.03	0.060
	NIH (males) Control	0.16 ± 0.03	
	NIH (males) Infected	0.14 ± 0.06	0.685
	BALB/c (females) Control	0.16 ± 0.04	
	BALB/c (females) Infected	0.21 ± 0.03	0.076
	BALB/c (males) Control	0.20 ± 0.04	
	BALB/c (males) Infected	0.13 ± 0.02	0.008*
Spleen	NIH (females) Control	0.13 ± 0.03	
	NIH (females) Infected	0.37 ± 0.15	0.020*
	NIH (males) Control	0.14 ± 0.02	
	NIH (males) Infected	0.32 ± 0.08	0.007*
	BALB/c (females) Control	0.13 ± 0.01	
	BALB/c (females) Infected	0.38 ± 0.07	0.001*
	BALB/c (males) Control	0.16 ± 0.09	
	BALB/c (males) Infected	0.35 ± 0.16	0.061

Values are expressed as mean ± standard deviation (SD). Significant differences between groups are expressed when *p-value <0.05.

inflammation,[xviii,xix] which could explain the findings observed in this study.

Concerning parasitemia, the results show that in the BALB/c strain, the females showed greater resistance than the males, presenting a lower number of parasites in the blood during the acute phase of Chagas disease, as previously reported.[xx] In contrast, male mice of the NIH strain were the most resistant to parasitemia.

It is important to note that the blood trypomastigotes of *T. cruzi*, as in this research, are visualized more rapidly compared to when they are inoculated in their metacyclic form, obtained directly from the insect or from transformations carried out *in vitro*, as has been documented in different studies with other strains of mice.[xxi,xxii] Some similar investigations mention a variety of patterns in the relationship between the parasite and the host. In this case, experimental mice acquire the disease using an established or predetermined inoculation that can be modulated by multiple variables that depend on the host, among which we have sex and genetic composition.[xix] Furthermore, infection in laboratory strains of mice can vary from highly resistant to highly susceptible strains, suggesting a genetically established basis.[vii,xxiii,xxiv] Despite the different strains of mice and parasites, it is clear that multiple factors can influence infection, such as inoculum size (amount of parasites administered) and host age, although experimentally, young adults are used for this kind of *in vivo* models.[vii,xxi]

It has also been demonstrated that there are genes involved in resistance or susceptibility to the disease, and among these, the most important in determining the number of parasites are those related to the response of the immune system.[xxv] It has been documented that the virulence capacity of *T. cruzi* to establish the infection of a host is associated with the state-specific expression of genes and their polypeptide products; many of these molecules are regulated during the differentiation and development of the different stages of the parasite that are related to the evasion of the host's defenses.[xxvi,xxvii,xxviii] Among the genetic factors associated with resistance or susceptibility to a variety of infectious agents are those usually linked to the Major Histocompatibility Complex (MHC), whose genes code for proteins responsible for the induction of the immune response against a variety of antigens.[xxix]

Infection by protozoa such as *T. cruzi* is also usually influenced by the control that sex hormones can exert.[xxx,xxxi] In the case of females, this may be due to the effect produced by estrogens that stimulate the phagocytic activity of the macrophage and increase the local inflammatory response.[xxxii,xxxiii,xxxiv] The development of infection with this parasite seems to have different responses between males and females in murine models; in some cases, sex does not seem to be a determinant in the number of parasites in infected animals.[vii] On the other hand, host defense against aggression seems to depend on the state of development of the immune system for both sexes and on the stimulation of the response of this system, possibly acting in association with some hormonal and genetic factors but independently.[xviii,xx]

In Chagas disease, some of the main organs affected by the parasite in mammals are the heart and spleen, and sometimes the intestine.[xxxv] It is reported in this research that the group of infected BALB/c females showed an increase in heart weight, which is plausible since the parasite may be lodged in the tissue, causing the organ to increase in size. There was only a significant change in the decrease in heart weight for the male BALB/c strain group, contrary to what happened with the spleen. As for organ size, the infected groups showed an increase, except for the heart of the BALB/c strain.

No studies have been found that compare the size and weight of affected organs at the macroscopic level. Instead, they are directly evaluated at the microscopic level using histological sections. One study mentions that an increase in heart size may be caused by the ablation of fats, leading to endoplasmic reticulum stress and mitochondrial oxidative stress, causing biventricular dilatation and increased parasite load in infected mice during the early chronic stages of infection.[xxxvi] The increase in spleen size may be related to an important parasite antigen called cruzipain, which increases the number of cells of this organ, granularity, and size.[xxxvii] On the other hand, the parasite uses the antigen to propagate within the host.[xxxviii] Furthermore, evidence shows that adipose tissue is the major reservoir for *T. cruzi*, which can be reactivated during periods of immunosuppression and create a state of inflammation that affects a variety of metabolic pathways.[xxxix]

One of the limitations of this research was not knowing to which type of strain or discrete taxonomic units (DTU) the parasite belonged; these are methods for genetic classification that have been developed previously to determine the different ways of interacting with its host and its geographic distribution; some studies reveal that in El Salvador the so-called *Trypanosoma cruzi* I (TcI) prevails according to this classification, but it must be taken into account that it

can vary from TcI to TcVI in Latin America.[xl,xli] Another important limitation was not having histological sections of the possible infected organs, mainly the spleen, which would have allowed us to observe the absence or presence of amastigote nests in the tissues and to confirm which of the strains and sexes in this study were more resistant or vulnerable to the parasite according to the damage caused.[xli]

Some of the most outstanding recommendations are to perform molecular biology studies such as gene expression analysis through the quantitative polymerase chain reaction (qPCR) technique and immunohistochemistry techniques, which allow measuring protein expression and thus obtaining more detailed information for the interpretation of the results based on the genes involved to combat this disease on the part of the host.[xxviii,xlii] It is important to consider that other tests, such as *in vitro* tests, can provide more reliable and conclusive results.

Conclusion

The group of male mice of the BALB/c strain was the most susceptible, presenting the greatest increase of parasites in the blood compared to the other infected groups. On the other hand, The female group of this strain showed the highest observed resistance to parasite load. However, contrary patterns were observed in the NIH strain, so it is difficult to affirm that sex is a determining factor in the behavior of this disease. Regarding body weight, the size, and weight of the organs, mainly in the heart, did not show significant differences, which could be because some of them were probably not so infected at that time, as was the case with the spleen.

Regarding body weight, the size and weight of the organs (heart and spleen) show results that are difficult to explain.

Acknowledgements

To the students of the School of Biology who participated in this research to obtain the results.

Funding

No external funds were received for this work.

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