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Effect of scorpion venom, *Androctonus australis* (Linnaeus,1758) against some bacterial strains

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ABSTRACT

Abstract: *This work consists to evaluate in vitro the activity of scorpion venom Androctonus australis (Linnaeus, 1758) which is the most abundant and most dangerous species in Algeria, against five bacterial strains: Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, Staphylococcus aureus MRSA and Micrococcus luteus. Biological tests by the use of different concentrations of venom aims to investigate the minimum inhibitory concentration is measured by optical density by the method of diffusion in a liquid medium. Whose, the bacterial inoculum using a UV-Visible spectrophotometer at a wavelength of 600 nm. While statistical analyses are applied to the observed results to conclude the antibacterial effect of scorpion venom. The results showed that no antibacterial activity of the A. australis venom against all bacterial strains tested. Nevertheless, the S. aureus MRSA strain exhibited greater resistance than other bacterial strains.*

I. Introduction

Scorpions are terrestrial Arthropods [1]. Currently, zoologists have discovered more than 2520 species [2]. Scorpion venom is composed of several toxins having various chemical and toxic properties, pharmacokinetics and pharmacodynamics [3] and other composites having antibacterial effect or even antifungal effect [4].

The presence of antibacterial peptides in the venoms of different species of scorpions such as androctonin, hadrurin and scorpion and other antifungal peptides such as opiscorpin and antiparasitic such as panscorpin [4,5] opens up research horizons to fight the emergence of bacterial resistance because a large part of antibiotics causes a planetary problem due to these impacts on health and the environment [6].

Although, several researchers are oriented into the use of various antimicrobial agents to fight this emergence by extracting new molecules from different types of animal venom [7]. But, Research on using of scorpion venom in this horizon remains weak.

Consequently, our work aims to research the effect of scorpion venom *Androctonus australis* (Linnaeus, 1758) which is the most abundant species in Algeria against some bacterial strains isolated, identified and purified at the laboratory of Ghardaia University, Algeria.

II. Materials and method

II.1. Animale Material

In our work, we used 140 adult specimens of *Androctonus australis* (Scorpiones: Buthidae). This species is the most abundant in the region of Ghardaïa region (Centre algérien) with an abundance exceeds 42% [8] and it is typical of urban areas [8, 9,10]. This species is ranked among the most dangerous in the world [11] and it is the first responsible for mortality [12].

II.2. Bacterial material

Five (05) isolated bacterial strains identified and purified at the laboratory of Ghardaïa University (Algeria) are used to check for a possible inhibitory effect of the venom of *Androctonus australis*. The characteristics of these bacterial strains are detailed in Table 1.

Table 1. *Bacterial strains used*

Strains	References	Resistance / sensitivity profile to antibiotics
Bacillus subtilis	ATCC 6633	Sensible to 44 µg/ml of vancomycin
Listeria monocytogenes	ATCC 13932	Sensible to 100 µg/ml of streptomycin
Micrococcus luteus	ATCC 9314	Sensible to 11 µg/ml of vancomycin
Staphylococcus aureus	ATCC 43300	Sensible to 100 µg/ml of vancomycin
Staphylococcus aureus	MRSA 2	Sensible to 100 µg/ml of vancomycin (multiresistant strain)

NB : The sensitivity of the strains was tested in the laboratory.

II.3. Methode

II.3.1. Venom collection

The extraction of the venom is carried out according to the technique proposed by Miranda et al. (1964) [13], which consists of stimulating the poison glands using a 12 Volt electric current. The venom obtained is subsequently dried in a desiccator and then kept at a temperature of 4 °C.

II.3.2. Biologic tests

The liquid solution of the venom is prepared by diluting 1 mg of the venom in 1 ml of sterile distilled water. The antibacterial activity is tested using the liquid diffusion method [14]. This method involves contacting each suspension of a bacterial strain with increasing doses of venom.

The culture medium used is Mueller-Hinton–Bouillon, composed of meat extract (2.0 g), casein acid hydrolysate (17.5 g), starch (1.5 g) and distilled water (1L).

Fourteen (14) increasing doses of venom are tested: 10 g, 20 g, 30 g, 40 g, 50 g, 60 g, 70 g, 80 g, 90 g, 100 g, 150 g, 200 g, 250 g and 300 g. Cultures are incubated using an shaker incubator (Precise Shaking Incubator WIS-10, Korea) at 37°C with constant agitation of 160 turns/min for 24 hours. The negative control was not prepared because the initial bacterial load tested is known for all bacterial suspensions. The bacterial growth rate is estimated by reading the optical density by a UV-Visible spectrophotometer with a wave length of 600 nm, an optical density (O.D.) of 0.08 to 0.1 corresponds to $1x10^8$ CFU/mL (0.5 Mc Farland) which corresponds to all initial bacterial suspensions tested. The optical density is measured for the second time after 24 hours of incubation.

III. Results

The results of the variation in bacterial load of the tested strains relative to the increasing doses of venom are summarized in Figure 1.

Overall, the optical density (O.D.) seems to vary greatly depending on the bacterial strain. Figure 1 shows that the optical density values obtained by *Bacillus subtilis* average 0.68699 ± 0.07967 (maximum 0.83626 and minimum 0.55087) (Figure 1A) and the optical density values obtained by the strain *Listeria monocytogenes* vary between 0.69133 and 0.48014 with an average of 0.60109 ± 0.06868 (Figure1B).
Staphylococcus aureus strain (Figure 1C) the O.D. values range from 0.95166 to 0.57691 with an average of 0.72801 ± 0.12110). The O.D. values for the *Micrococcus luteus* strain range from 0.75225 to 0.52652 (0.64597 ± 0.05678 on average) (Figure 1D). Finally, the optical density for *Staphylococcus aureus* MRSA ranges from 0.64128 to 0.40577 with an average of 0.53819 ± 0.06893 (Figure 1E).

In order to determine the difference between the mean bacterial loads of tested bacterial strains in the presence of the venom, an ANOVA test was applied. The results showed a significant difference in the growth of the various strains tested (p=0 much lower than the significance threshold 0.05). The variation in O.D. depending on bacterial strains is shown in Figure (2).

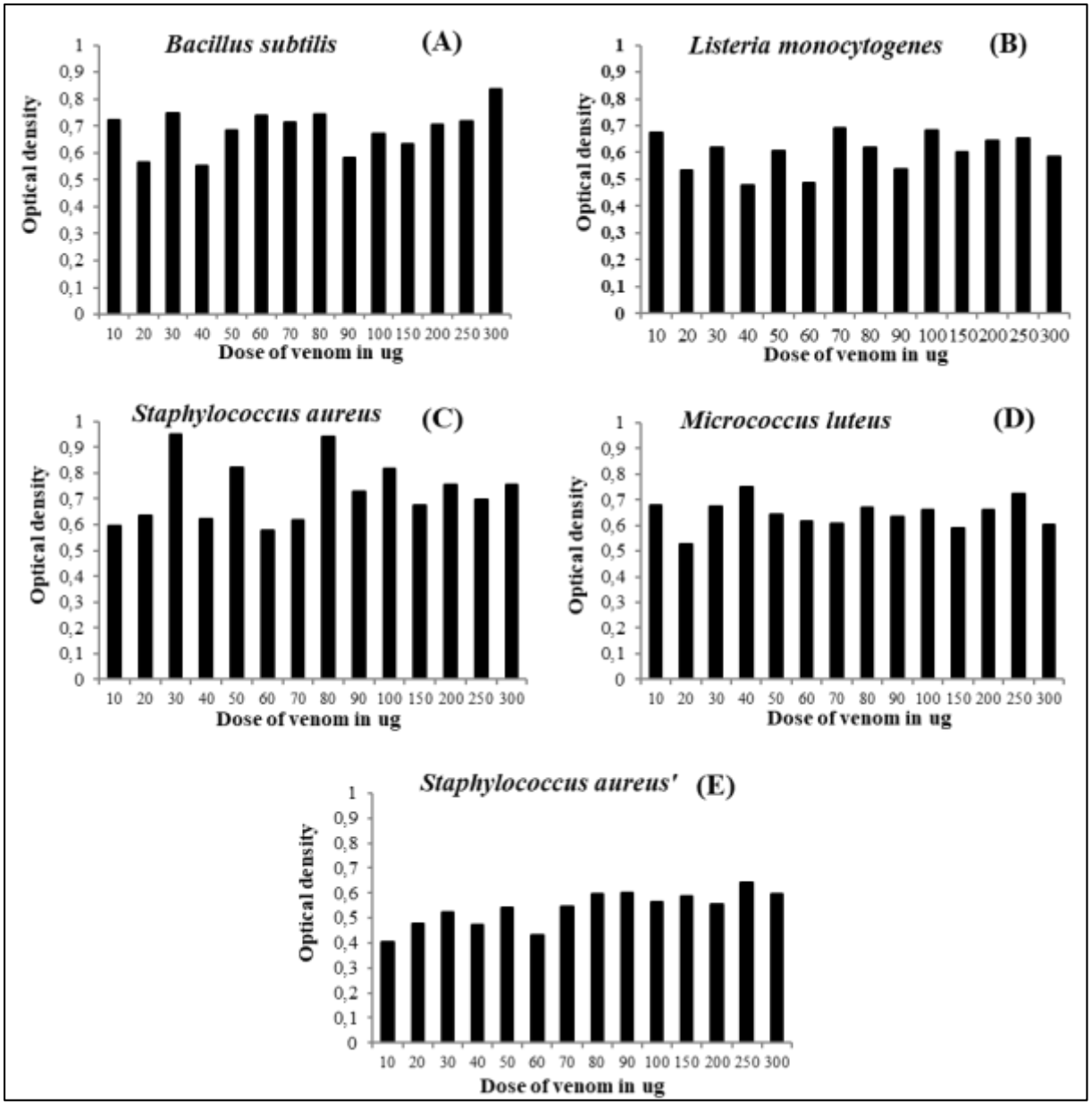

Figure 1. *Variation of bacterial load compared to increasing doses of the venom*

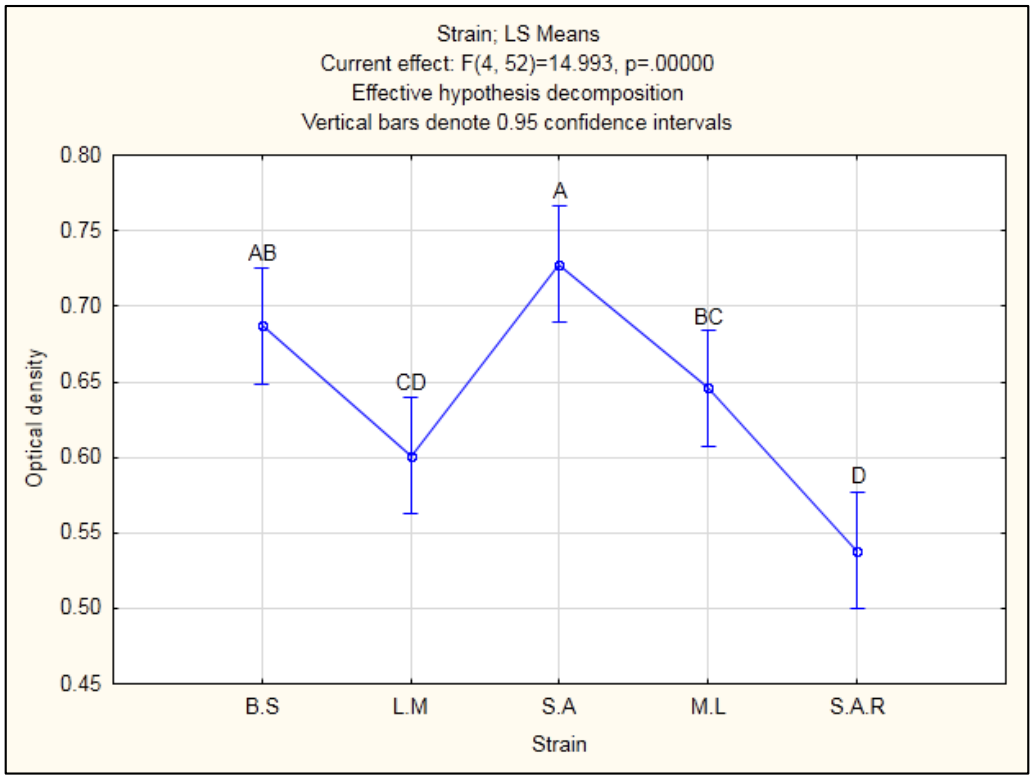

Figure 2. *Variation of optical density depending on bacterial strains.*
B.S : Bacillus subtilis. L.M : Listeria monocytogenes. M.L : Micrococcus luteus. S.A : Staphylococcus aureus. S.A.R : Staphylococcus aureus MRSA.

Figure 2 and the Tukey test (Table 2) show that *Staphylococcus aureus* (S.A) has the highest value in terms of optical density (group A). In second, *Bacillus subtilis* (B.S) and *Listeria monocytogenes* (L.M) with mean values of O.D. classified respectively in group AB and BC. While, *Micrococcus luteus* (M.L) and *Staphylococcus aureus* MRSA (S.A.R) are represented by an average value of low optical density, therefore they are classified in groups CD and D respectively.

Table 2. *Tukey test.*

Strain	Mean of optical density	*Group D*	*Group C*	*Group B*	*Group A*
S.A.R	0.538199	****			
L.M	0.601091	****	****		
M.L	0.645976		****	****	
B.S	0.686991			****	****
S.A	0.728016				****

B.S : Bacillus subtilis. L.M : Listeria monocytogenes. M.L : Micrococcus luteus.
S.A : Staphylococcus aureus. S.A.R : Staphylococcus aureus MRSA

Figure 3 shows the variation in O.D. according to venom doses. The objective of this test is to study the difference between bacterial loads mean and different doses of venom. This test revealed an O.D. value of 0.00734 ($p < 0.05$). That's indicate a bacterial growth dependent to the applied venom-dose.

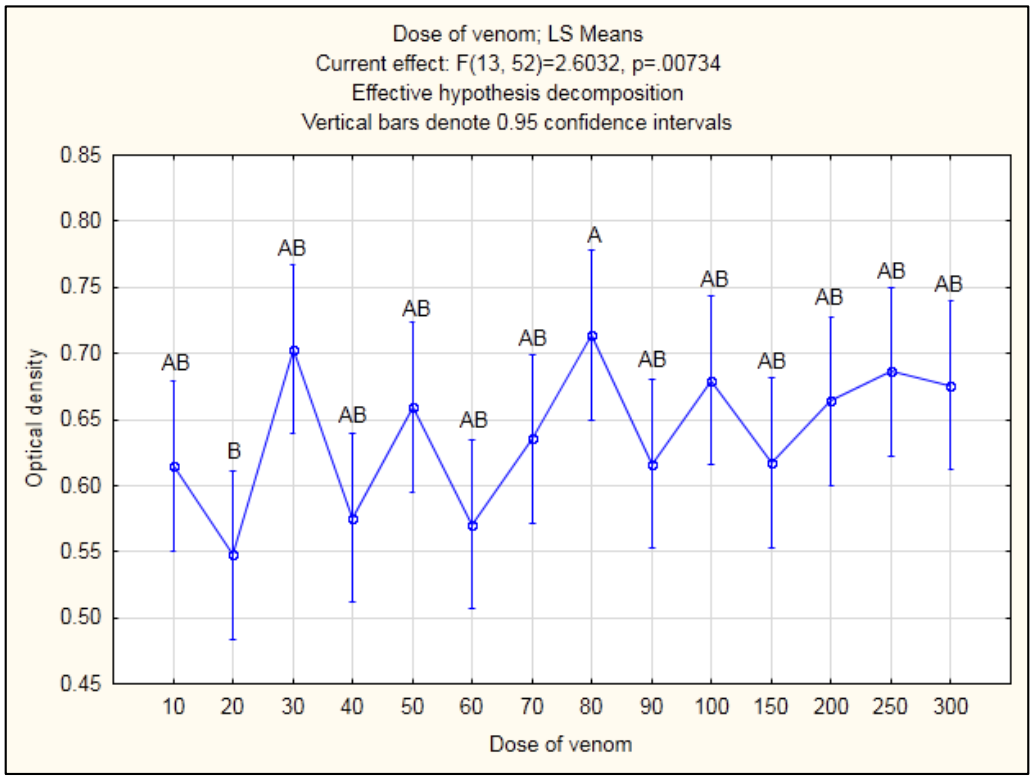

Figure 3. *Variation of optical density according to doses of venom.*
A : High mean of optical density; B : Low mean of optical density; AB : Intermediate mean of optical density

The Tukey test (Table 3) shows the presence of three groups of optical density. The dose 80 µg has the highest average and it is classified in group A of the optical density values, while the dose 20 µg has the lowest average (classified in group B). The other doses of venom are represented by the AB group of optical density values.

Tableau 3. *Tukey test.*

Dose of venom	Mean of optical density	*Group B.*	*Group A*
20	0.547558	+	-
60	0.570686	+	+
40	0.575704	+	+
10	0.615100	+	+
90	0.616626	+	+
150	0.617442	+	+
70	0.635536	+	+
50	0.659474	+	+
200	0.663910	+	+
300	0.675880	+	+
100	0.679810	+	+
250	0.686204	+	+
30	0.703226	+	+
80	0.713606	-	+

Table 4. *Significance test of the significant correlation between the bacterial load and the venom doses*

	Correlations Marked correlations are significant at p <0.05				
Variable	Optical density. : B.S.	Optical density. : L.M.	Optical density. : S.A.	Optical density. : M.L.	Optical density. : S.A.'
Dose	0.4461	0.1920	0.0796	0.0341	0.6978
P	0.110	0.511	0.787	0.908	0.006

According to Table 4, which presents the significance of the correlation test is studied between the increasing doses of venom and the optical density of the strains, the p-value is much lower than the significance level 0.05 for the bacterial strain *Staphylococcus aureus* MRSA (S.A') with a value of 0.006.

The coefficient of this significant correlation is 0.6978, indicating that the correlation is significant positive linear; the bacterial growth of this strain increases with the increase in the dose of venom used, despite the average optical density of this strain is lower compared to other strains tested with a standard deviation equal to 0.07393.

Figure 4 shows the relationship between the optical density of the bacterial strain *Staphylococcus aureus* MRSA and the increasing doses of venom used. The table 5 gives the values of the optical density of the bacterial strains which are used for the negative control (bacteria without venom).

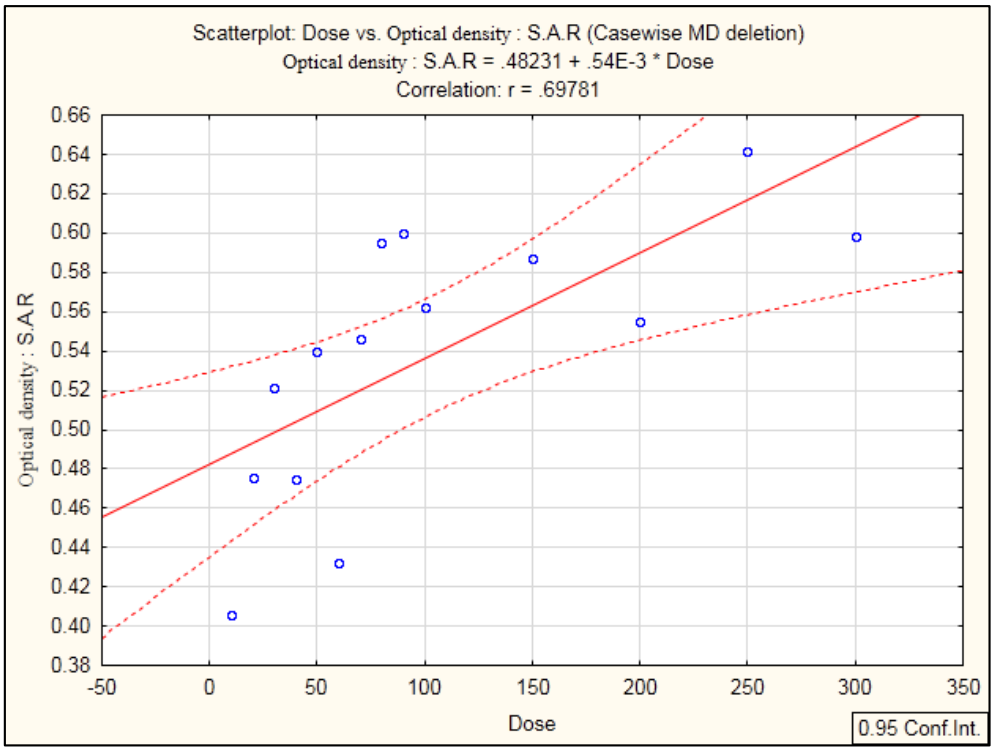

Figure 4. *Scatterplot representing the relationship between the optical density of Staphylococcus aureus MRSA and the doses of the venom used.*

Table 5. *Optical density of bacterial strains of the negative control*

Bacterial strains	Optical density of the negative control
Bacillus subtilis	0.62819
Listeria monocytogenes	0.40894
Staphylococcus aureus	0.52039
Micrococcus luteus	0.8112
Staphylococcus aureus MRSA	0.69342

IV. Discussion

The variation in the optical density of bacterial strains relative to increasing doses of venom shows that these bacterial strains mark an aleatory growth from one dose to another for each strain. This variation in bacterial load may depend on the adaptation of the bacteria to different doses of venom.

The ANOVA test indicates that bacterial behavior varies from strain to strain compared to venom, meaning that each bacterium reacts in the presence of venom in a different way from strain to strain and from dose to dose of venom.
The results show that the optical density values of the bacteria are close to the negative control values. This similarity in values reflects the absence of antimicrobial activity of the venom against the bacterial strains tested.

The same results were found in Egypt on the venoms of the scorpionic species of Androctonus amoreuxi and *Androctonus australis* where the effects of these two venoms on microbes are absent. However, they noted that the venom of the scorpion *Leiurus quinquestriatus* has a significant antibacterial effect against the two strains tested: *B. subtillis* and *C. freundi* [15].

Other research has shown that venom composites can interact with specific molecules of certain bacteria without affecting other strains. For example, the scorpion species *Heterometrus xanthopus*, its venom had an antibacterial effect on *B. subtillis*, while *E. coli* showed resistance against the same venom [16] and the same for *Buthus martensii* its venom showed an effect against Gram negative bacteria only [3].

Moreover, our results also showed that *Staphylococcus aureus* MRSA marks a growth positively correlated with increasing levels of venom, which probably means a natural resistance of this strain against the venom of *Androctonus australis*. Although, the venom of this species contains androctonin (peptide toxin) well known for these powerful antibacterial activities against bacteria (Gram-positive and Gram-negative) and even powerful antifungal activities [17].

Mandard et al (1999) have stadied the structure and composition of androctonin and show that it is a highly cationic antimicrobial peptide, that does not exhibit an amphiphilic character and the efficacy of androctonin on Gram-positive and Gram-negative bacteria (specificity for the membrane bacterial) are in fact related to its strong hydrophily [18].

Further work on the mode of action of androctonin on bacteria shows that their target is not the bacterial membrane, which proved that it can been a target for most amphipathic lytic peptides, it binds only to negatively charged lipid vesicles and induces marker leakage at high concentrations and with slow kinetics, contrary to alpha-amphipathic antimicrobial peptideshelical cells that bind and impregnate negatively charged vesicles, and to a lesser extent also zwitterionic vesicles [19].

Purification of venom composites from *Androctonus australis* scorpions may lead to antibacterial peptides that have effective antibacterial activity against resistant bacteria.

V. Conclusion

The aim of our work was to investigate the inhibitory effect of the venom of a very venomous species of scorpions from North Africa, specifically from the Algerian center (Ghardaïa): *Androctonus australis* (Linnaeus, 1758), against five bacterial strains: *Bacillus subtilis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus* and *Staphylococcus aureus* MRSA.

The results of the study show that the venom has no an antibacterial effect against the bacterial strains tested. Although the strain *Staphylococcus aureus* multi-resistant marks its growth positively correlated with increasing doses of venom.

The behavior of the bacterial strains studied differs from one strain to another. These strains are also characterized by the heterogeneity of the distribution of the values of their bacterial loads (the optical density values).

The venom of scorpions has polypeptide composites that are very important in the fight against bacterial resistance. Research in this area is needed to better develop new antimicrobial agents from the venoms of different scorpionic species.

Finally, Algeria has more than 40 species of scorpions [20, 21], more than 50% are endemic. This important species diversity certainly reflects a diversity of venoms. deserves further investigation in order to test these venoms against different bacterial strains.

VI. References

1. Pisani, D.; Poling, L.; Lyons-Weiler, M.; Blair, S. The colonization of land by animals: molecular phylogeny and divergence times among arthropods. *Bio Med Central Biology* 2 (2004) 1-10.
2. Rein, J.O. The Scorpion Files. https://www.ntnu.no/ub/scorpion-files/ (Update 30.06. 2020).
3. Polis, G.A. Ecology. In: Polis GA (Ed. 1996) The biology of scorpions. Stanford University Press, Stanford, California (1990) 247-293.
4. Gopalakrishnakone, P. ; Possani, L. D. ; Schwartz, E. F. ; Rodríguez de la Vega, R. C. Scorpion Venoms. Edition Springer Netherlands (Series Toxinology) 2542-761X (2015) 575p.
5. Gao, B.; Tian, C.; Zhu, S. Inducible antibacterial response of scorpion venom gland. *Peptides* 28 (2007) 2299 – 2305.
6. Guillot, J. F. Apparition et évolution de la résistance bactérienne aux antibiotiques. *Annales de Recherches Vétérinaires* 20 (1989) 3–16.
7. Ziad-Meziane, H-F. ; Laraba-Djebari, F. Purification d'une molécule bactéricide issue du venin de serpent *Cerastes cerastes*. *SAGREN* 2 (2018) 23-33
8. Sadine, S. E. La faune scorpionique du Sahara septentrional algérien : Diversité et Ecologie. Thèse de Doctorat ès sciences. Université Kasdi Merbah-Ouargla. Algérie (2018) 112p.
9. Sadine, S.E. ; Bissati, S. ; Ould El-Hadj, M.D. Premières données sur la diversité scorpionique dans la région du Souf (Algérie). *Arachnides* 61(2011) 2-10.
10. Sadine, S. E. ; Alioua, Y. ; Kemassi, A., Mebarki, M. T. ; Houtia, A. ; Bissati, S. Aperçu sur les scorpions de Ghardaïa (Algérie). *Journal of Advanced Research in Science and Technology* 1(2014) 12-17.
11. Goyffon, M.; Billiald, P. Envenimations. Le scorpionisme en Afrique. *Médecine Tropicale* 67 (2007) 439-446.
12. Chippaux, J.P.; Goyffon, M. Epidemiology of scorpionism: a global appraisal. *Acta Tropica* 107 (2008) 71-79.
13. Miranda, F. ; Rochat, H. ; Lissitzky, S. Sur les neurotoxines de deux espèces de scorpions nord africains. *Toxicon* 2 (1964) 51- 138.
14. Bouhaddouda, N. Activités antioxydante et antimicrobienne de deux plantes du sol local : *Origanum vulgare* et *Mentha pulegium*. Thèse de doctorat, Option de Biochimie appliquée, Université Badji Mokhtar –Annaba (2016) 167p.
15. Salama, W.; Geasa, N. Investigation of the antimicrobial and hemolytic activity of venom of some Egyptian scorpion. *Journal of Microbiology and Antimicrobials* 6 (2014) 21-28.
16. Ahmed, U.; Mujaddad-Urrehman, M.; Khalid, N.; Fawad, S. A.; Fatima, A. Antibacterial activity of the venom of *Heterometrus xanthopus*. *Indian Journal of Pharmacology* 44 (2012) 509–511.
17. Wang, K.; Li, Y.; Xia, Y.; Liu, C. Research on Peptide Toxins with Antimicrobial Activities. *Annals of Pharmacology and Pharmaceutics* 1 (2016) 1-8.
18. Mandard, N.; Sy, D.; Maufrais, C.; Bonmatin, J-M.; Bulet, P.; Hetru, C.; Vovelle, F. Androctonin, a Novel Antimicrobial Peptide from Scorpion *Androctonus australis*: Solution Structure and Molecular Dynamics Simulations in the Presence of a Lipid Monolayer. *Journal of Biomolecular Structure and Dynamics* 17 (1999) 367-380.
19. Hetru, C.; Letellier, L.; Oren, Z.; Hoffmann, J.A.; Shai, Y. Androctonin, a hydrophilic disulphide-bridged non-haemolytic anti-microbial peptide: a plausible mode of action. *Biochemical Journal* 345 (2000) 653-664.
20. Sadine, S.E. On the contribution of Wilson R. Lourenço to the knowledge of the scorpion fauna of Algeria. *Arachnida - Rivista Aracnologica Italiana* 17 (2018) 12-17.
21. Sadine, S. E.; Djilani, S.; Kerboua, K. E. Overview on Scorpions of Algeria. *Algerian Journal of Health Sciences* 2 (2020) 8-14.

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