Journal of Biological Sciences and Public Health ISSN 3007-9535
December, 2024, 2: 19-26

Hibernative Behavior of Scorpions in District Sheikhupura Punjab, Pakistan

Muhammad Mohsin Ahsan, Amna Iqbal, Ayesha Hafeez, Muhammad Samiullah, Muhammad Ismail

Department of Zoology, Division of Science and Technology, University of Education Lahore, Pakistan

ABSTRACT

Background: In the current study, the scorpion's hibernative behavior was observed both in field and laboratory. The study was conducted in Sheikhupura District, Punjab, Pakistan. **Methods:** For scorpion collection, different active and passive methods were adopted. The study period was two years (2021-2022). Live observations were made with ultraviolet light at night, while daytime observations were made by rolling bricks and digging muddy houses in rural areas. The Scorpions were also observed at the Department of Zoology, University of Education, Lahore Faisalabad Campus. Scorpions were maintained in a controlled environment at a temperature range of 25-30°C. The relative humidity was maintained at 60-70%. The scorpions were reared in a plastic container (15 x 20 inches) in length x width. The substrate of sandy gravel was added (1 inch) at the bottom of the boxes. **Results:** It was observed that there was only one dominant scorpion species, *Hottentota tamulus*, in Sheikhupura district. During field and laboratory observations, it was noticed that the scorpions were active during the months from March to September but became inactive and hide in the burrows from November to February. **Conclusion:** It is concluded that as the temperature starts to decrease, scorpions reduce their activity, hide in burrows, and undergo hibernation.

Key Words: Scorpion hibernation, Sheikhupura, *Hottentota tamulus*, Ecological behavior, Winter dormancy

Corresponding author:
Muhammad Mohsin Ahsan
Department of Zoology, Division of Science and Technology, University of Education Lahore, Pakistan
mohsin.ahsan@ue.edu.pk

Article Information:
Received: 02-08-2024
Revised: 12-12-2024
Accepted: 13-12-2024
Published: 22-12-2024

Author Contribution:
MMH: Conceptualized the study, collected and analyzed data, wrote and edited the manuscript. ***AI:*** Contributed to data collection and analysis, manuscript drafting, editing, and final approval. ***AH:*** Participated in data collection and analysis, manuscript drafting, editing, and final approval. ***MS:*** Involved in data collection and analysis, manuscript drafting, editing, and final approval. ***MI:*** Conducted data collection and analysis, contributed to manuscript drafting, editing, and final approval.

INTRODUCTION

The fossil record showed that scorpions are an oldest group of arachnids that first appeared during the Silurian Period (Das et al., 2021). Scorpions are venomous arthropods within the class Arachnida of the phylum Arthropoda. There are 2200 different species of these predatory arachnids. They are found in both tropical and subtropical locations worldwide, with the exception of Antarctica (Firooziyan et al., 2020). These intriguing creatures live in diverse habitats including tropical forests, temperate forests, grasslands, savannas, and caves (Mullen & Sissom, 2019).

Scorpions are predatory arachnids with a segmented, curving tail capped with a venomous stinger and a pair of grabbing pincers at the front of the body (Loria et al., 2024). They survive a wide range of ecosystems, including caves, temperate forests, grasslands, savannas, tropical forests, rain forests, snow-covered mountains, and tropical rain forests (Tobassum et al., 2020). Scorpions are nocturnal predators; at the day time, they may hide in different burrows or beneath the rocks and vegetation. They also show hiding behavior under blankets, shoes, and clothing, which often leads to accidental stings in human (Haghani et al., 2018).

Scorpions are simple to keep in captivity and require little maintenance. They are suitable for storage in a plastic bag, terrarium, jar, or plastic box. Scorpions are thigmotactic and seek shelter in soil or other rocky surface for their protection (El Hidan et al., 2017). Scorpions are among the largest terrestrial arthropods, with an average length of roughly 6 centimeters (2.5 inches). Although male scorpions have longer tailed and typically slender than females (Lowry, 2020). Many species of scorpions are sit-and-wait predators, waiting for food at and near their burrow entrance. Scorpions use mechanoreceptive and chemoreceptive hairs to detect their prey and their claws to seize them (Stockmann et al., 2016).

In terms of ecology, behavior, physiology, and life cycle, scorpions are very adaptable. Some species may endure super-cooling below the freezing point for weeks but return to normal activity within hours. The preferred temperature range for scorpions is between 11 and 40 degrees Celsius, however, they can tolerate temperatures ranging from considerably below zero to desert scorching. Desert scorpions can withstand temperatures as high as 47 °C (117 °F), many degrees higher than the temperature at which other desert arthropods expire (Pekár & Raspotnig, 2022). Scorpions can withstand a broad variety of climates, including extremely hot to extremely cold temperatures, hours submerged in water, and prolonged periods of dehydration and famine (Mullen & Sissom, 2019)).

Scorpion adaptations are the result of slow metabolic rates, water conservation efficacy, and thermoregulatory behavior. In order to regulate the temperature of their bodies, scorpions are primarily nocturnal and seek cover throughout the day in burrows and other sheltered locations. They excrete highly insoluble nitrogenous wastes, including guanine, xanthine, and uric acid, through their cuticle, spiracles, and book-lungs. Similarly, their faeces are quite dry (Mullen & Sissom, 2019). Scorpion shows different type of behaviors in their life to survive on the land: as sexual behavior, attacking and prey-capturing behavior and defensive behavior. It also shows hibernation behavior to survive.

Scorpions have highly developed sensory mechanisms. They carry a telson equipped with venom glands and show stinging behavior. This process drastically reduces its metabolic activity (Sentenská et al., 2017). Female scorpions show hibernative behavior before the onset of winter though male scorpions die before the start of winter (Bryson et al., 2016). Reportedly, during the colder months, scorpions sleep. As the temperature fall down scorpion's loss their activity and start to hibernate in muddy places (Garcia et al., 2022). Scorpions enter into hibernation early in the fall and in winter and reduce their activity (Boucherit et al., 2017).

The primary objective of this study is to investigate the hibernative behavior of scorpion fauna in both field and laboratory settings, specifically at the Arachnology Lab (A-30), Faisalabad, Pakistan. The study aims to record the duration, timing, and frequency of hibernation in scorpions found in District Sheikhupura. Furthermore, this research explores the role of environmental factors, including temperature, humidity, and daylight exposure, in inducing hibernation in scorpions.

MATERIALS AND METHODS

Study area

District Sheikhupura is situated between 31° 42′ 0″ North and 73° 59′ 0″ East, with an elevation of 236 meters (777 feet) above sea level. The district is bordered by Lahore to the east, Nankana Sahib and Faisalabad to the west, Hafizabad to the northwest, and Narowal to the northeast. Sheikhupura covers an area of approximately 3,030 square kilometers (1,170 square miles), measuring around 80 kilometers (50 miles) in length and 40 kilometers (25 miles) in width. The terrain gently slopes from northwest to southeast. Sheikhupura is known for its agricultural productivity, with major crops including wheat, rice, sugarcane, and vegetables. The district is also home to several industrial units, including textile mills and food processing plants. Sheikhupura has a humid subtropical climate, with hot summers and mild winters. The population of Sheikhupura district is approximately 3.5 million.

Figure 1: Map showing the distribution of scorpions in the District, Sheikhupura, Punjab, Pakistan.

Collections and observations

Scorpions were collected through different methods i.e., live collection, brick rolling, black light, destroying old and mud walls, pouring into burrows, and rubber bands as detailed below:

Brick-rolling

Scorpions prefer to live in old, muddy houses. They easily establish a home in muddy dwellings. They penetrate bricks according to their size. So, scorpions were collected by rolling bricks. When a scorpion was found, it was picked up by forceps measuring 12cm length. These scorpions were placed in a plastic box measuring (15x10) cm (L x W). For further studies, scorpions were carried in plastic containers measuring (75x75) cm in the Division of Science and Technology, University of Education, Lahore Faisalabad campus Arachnology lab. This is the most common method of scorpion capture (Dehghani *et al*., 2016). This technique was appropriately used method during the day time.

Ultraviolet light (UV)

Scorpions were collected and observed at night-time with ultraviolet lights (SOGO-JPN-139). The exoskeleton of scorpions shows fluorescence properties under UV light (Rowe et al., 2013). The property of fluorescence was discovered more than 60 years ago. The scorpion becomes visible by spreading UV light at a wavelength of 400 to 700nm. The flashlights were used to survey scorpions in various habitats, including burrows, and vegetation. When a scorpion was found, it was picked up with forceps 12 inches long. The captured scorpions were then placed in a plastic jar measuring 75x75 cm (LxW).

Pouring water into burrows

This approach was used for capturing scorpions that lives in tunnels of old muddy houses. Water was poured into holes where scorpions were spotted. By using this technique, about 0.3 L water was poured into the tunnels of scorpions. By utilizing a thin tube this procedure continued until the scorpion emerged from their burrows. When scorpions escape out of their holes they were captured with a forceps. The collected scorpions were placed in little plastic boxes (15×20) cm (L×W) and transferred to big plastic container (75 ×75) cm (L×W).

Destroying old and mud walls

Muddy walls provide shelter to the scorpions. Scorpions inhabit crevices in the bricks, and old mud walls. Stone walls constructed around farms for terracing or separating them from one another or watercourses provide a safe home for scorpions. In rural places, stone walls encircling farms are positioned near residences. The use of stone as a building's foundation also provides scorpions with a suitable home. Scorpions were collected by destroying the stone walls (Dehghani et al., 2016) (Figure 2).

Figure 2: A typical habitat for scorpion (*Hottentotta tamulus*) in different places of district Sheikhupura

Rubber band

For the collection of scorpions, the rubber band method was also used. This procedure involved inserting a rubber band inside the scorpion's tunnel and gently twisting it. When the rubber band reaches the burrow's end, a scorpion comes into contact with it. The scorpion grasps the rubber band with its claws. The scorpion was then carefully removed by gently pulling the band, taking care not to harm the scorpion, and placing it into a plastic jar for further lab assessments. The jar was then sealed and labeled for experiment purposes. The collected scorpions were then transported to the laboratory for further study.

Scorpion acclimatization

After collecting scorpions, they were brought to the laboratory and provided with an artificial environment that mimicked their natural habitat. To create a balanced artificial habitat, a box was prepared with small holes for providing specific aerations (Figure 3). The box was also equipped with a heat source, a humidifier, and a photoperiod simulator to maintain optimal environmental conditions. The temperature within the box was maintained between 20°C and 40°C, with a relative humidity of 60-80%. At this temperature range, the scorpions were active and visible within the box, exhibiting normal behaviors such as foraging, burrowing, and social interaction. However, when the temperature dropped below 20°C, they began to hide themselves in sandy soil which is present in boxes. Scorpions were also provided with a diet of crickets, grasshoppers, and small other insects. The scorpions were found to be most active at night, and their feeding activity peaked during this time.

Laboratory observations

For laboratory observation, live scorpions were collected from their natural habitat and transported to the laboratory in specialized plastic containers (75 x 75) cm in (L x W) to prevent escape (Figure 3). The scorpions were carefully collected from various microhabitats, including old houses, animal dung (Farm), and underground burrows. The microhabitats were thoroughly searched, and scorpions were collected using a gentle and non-invasive method to minimize stress and injury. The collected scorpions were then placed in a controlled laboratory environment, where their hibernative behavior and other characteristics were observed and recorded. The laboratory setup was designed to mimic the scorpions' natural habitat, with controlled temperature, humidity, and lighting conditions. The scorpions were provided with food, water, and shelter, and their activity patterns, feeding behavior, and social interactions were monitored.

Data recording

To ensure accurate data collection and analysis, the geo-coordinates of each sample location were recorded by using a portable Germin GPS 64s device. GIS 10.2 software was used to map the sample locations by using geo-coordinates of each sample location. The type of locality, date and time of sampling, type of habitat, temperature, and level of humidity were recorded by using this technique.

Figure 3: Habitat for scorpion (*Hottentotta tamulus*) in laboratory setup.

RESULTS

Hottentotta tamulus (Fabricius, 1798)

A total of 155 scorpions of *Hottontota tamulus* was collected from Sheikhupura and studied in field and in the laboratory (Figure 5). Scorpions were observed throughout the winter months (October to February) under varying temperature conditions (11-25°C). Seasonal temperature requirements were taken into account, and our observations showed that scorpions exhibited distinct behavioral changes in response to temperature fluctuations. Our observations indicated that scorpion activity was highest during monsoon seasons. During this time, scorpions were more active, foraging for food, and interacting with each other.

In contrast, scorpion activity was almost nonexistent in winter months (December, January, and February) at temperatures range of 8-14°C. During this period, scorpions retreated to underground burrows, reducing their activity and relying on stored energy reserves to survive. This behavioral adaptation allowed them to conserve energy and protect themselves from harsh environmental conditions. Our results also showed that scorpions exhibited a range of behavioral responses to temperature changes, including changes in activity patterns, feeding behavior, and social interactions.

Live observations

H. tamulus activity was observed directly in the field (Figure 4). These live observations enabled us to gather valuable insights into scorpion behavior and ecology. Our observations revealed that as temperatures began to decrease, scorpions reduced their activity, accompanied by a decrease in their respiration rate, and reproductive rate. In response to the cooler temperatures, scorpions started to construct burrows and hide themselves, ultimately entering a state of hibernation to cope with the temperature fluctuations. We also observed that the immature individuals of the species show hibernating activity in colder months by reducing activity by hiding in the burrows, crevices of old, muddy houses and under the bricks.

The population of *H. tamulus* varied throughout the year. Notably, they were found to have a higher number during the months of June to September, whereas the population density was significantly lower in January and February (Figure 5a,b). This fluctuation in *H. tamulus* populations suggests that they may be adapting to the changing environmental conditions, such as temperature and humidity, throughout the year. Furthermore, our observations indicated that scorpions exhibited a range of behavioral adaptations to cope with the changing environmental conditions. For example, during periods of high temperature, scorpions became more active and aggressive, while during periods of low temperature, they became more sluggish.

Figure 4: Habitat of scorpion (*Hottentotta tamulus*) in open fields.

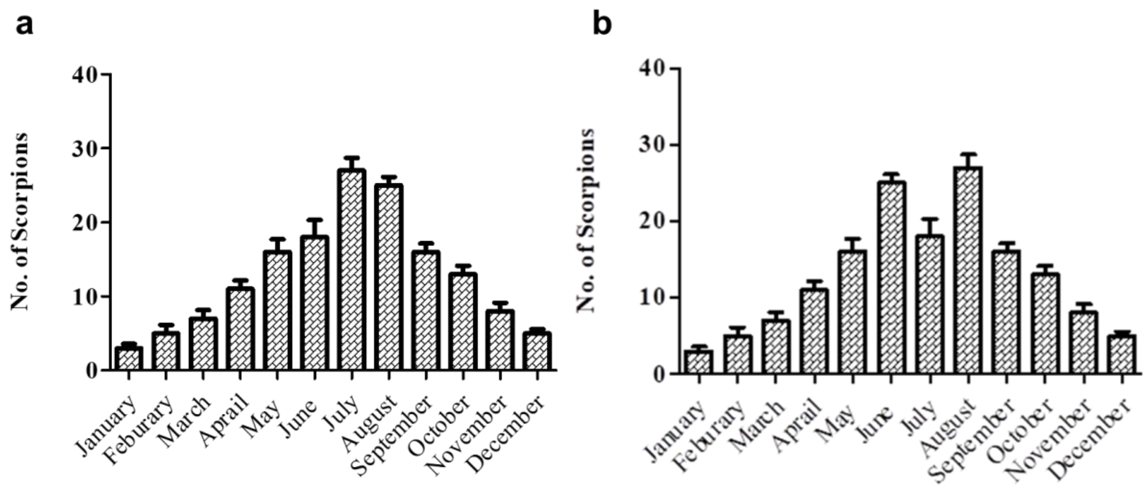

Figure 5: Scorpion's (*Hottentotta tamulus*) seasonal activity in the year 2021 (a) and 2022 (b).

Laboratory observations

Our observations revealed that as the temperature dropped, scorpions began to hide and start to dig tunnels, reducing their activity and eventually becoming inactive. This behavior was most pronounced during the winter months (January to February), when scorpions would dig burrows and hide themselves to escape the low temperatures. In contrast, during the summer months (August to September), scorpions exhibited slightly increased activity, although they were not fully active. These observations suggest that scorpions adapt to harsh winter conditions by burrowing and becoming inactive, a state of dormancy known as hibernation. Our laboratory experiments confirmed that *H. tamulus* hibernates in response to low temperatures, highlighting their ability to survive and thrive in a variety of environmental conditions.

DISCUSSION

Research presented in this study focused on hibernation behavior of scorpions *Hottentotta tamulus*. Random samples of scorpions were collected from old muddy houses, under the bricks, and from animal dung (farms) in District, Sheikhupura, Punjab Pakistan. Scorpions in different seasons were collected and observed. The majority of scorpions were not present in the winter season as compared to the summer season. Scorpions reduce their activity and hibernate in the winter season. Previously, many scientists have worked on scorpion hibernating behavior and reported the hibernation activity of scorpions (Van Aardt et al., 2016).

The hibernation behavior of scorpions has been observed by Qari et al. (2021) in various species including *Compsobuthus acutecarinatus* and *Compsobuthus schmiedeknechtii*. These species have been found to hibernate under stones in specific habitats, such as slopes, hilltops, and riverbeds. Our study on *Hottentotta tamulus* revealed similar hibernation behavior, where scorpions lose their activity by hiding under burrows, closets, garages, and indoor spaces when the temperature becomes low. This behavior suggests that *H. tamulus*, like other scorpion species, adapts to harsh winter conditions by seeking sheltered locations to conserve energy and survive. The similarity in hibernation behavior among different scorpion species highlights the importance of this adaptation in enabling scorpions to thrive in diverse environments.

Our findings are also consistent with those reported by Thapa et al. (2013), who observed that female scorpions in Nepal exhibited hibernation behavior before the onset of winter and Stockman et al., (2016), who noted that scorpions demonstrate hibernation behavior, characterized by a drastic reduction in metabolic activity. Our study expands on Thapa's and Stockman's findings by demonstrating that both male and female *H. tamulus* scorpions exhibit hibernation behavior, characterized by reduced activity and burrowing behavior, in response to decreasing temperatures (below 20 °C) and humidity. This behavior was observed in both field and laboratory conditions, suggesting that it is a robust adaptation to harsh winter conditions. Furthermore, our study underscores the need to consider the specific ecological and behavioral adaptations of scorpions when developing strategies for their conservation and management, it also highlights the importance of considering environmental factors, such as temperature, in shaping their behavior and physiology.

Current study revealed that scorpion collection rates varied significantly between dry and rainy seasons. Notably, more scorpions were collected during the dry season compared to the cold rainy season. This finding is consistent with reports from Brazil, where scorpions have been observed to exhibit hibernative behavior during the rainy season (Santos et al., 2018). Our observations indicated that the scorpion species *H. tamulus* exhibited distinct behavioral responses to rainy and humid conditions. During the summer season (May to August), on rainy nights, these animals remained in their burrows, reducing their activity due to decreased humidity. However, after rainfall, when nights were humid *H. tamulus* emerged from their burrows and became highly active, seeking new shelters as their existing burrows were destroyed by rain.

Previous studies have reported hibernation behavior in spiders in temperate regions, such as North Germany (Rahman et al., 2015). Similarly, some scorpion species have been found to exhibit different adaptations at various developmental stages to avoid cold temperatures. For instance, *Stenochronous* species hibernate in the egg stage. Our findings revealed that immature scorpions of this species exhibited long-term hibernation activity, characterized by reduced foraging and feeding activities, to cope with low temperatures. This behavior was more pronounced in immature scorpions compared to adult scorpions of the same species.

Lira et al. (2018) investigated the microhabitat choice and functional richness of scorpions in wet (Atlantic woodland) and semiarid (Caatinga) environments. While their study provided valuable insights into how environmental structure affects species establishment, it did not explore the hibernative activity of scorpions. In contrast, our study demonstrated that scorpions exhibit hibernation behavior in response to cold temperatures, which was not observed by Lira et al. (2018).

CONCLUSION

In conclusion, our study demonstrates that the scorpion species *H. tamulus* exhibits a significant change in behavior and physiology in response to decreasing temperatures. During the summer months (June to September), *H. tamulus* displays normal behavior and metabolic activity. However, as temperatures drop, they become sluggish and inactive, ultimately undergoing hibernation to survive extreme cold conditions. This adaptation enables *H. tamulus* to conserve energy and withstand low temperatures during the winter months. This highlights the diverse range of adaptations employed by scorpions to cope with environmental challenges. Our study contributes significantly to the understanding of scorpion ecology and behavior.

Acknowledgements

We are immensely grateful to the Higher Education Commission (HEC) for their support in funding this research project via the National Research Program for Universities (NRPU). Special thanks go to our research team members for their unwavering commitment and significant input.

Statement of conflict of interest

The authors declare that they have no competing or personal interest that could have appeared to influence the work reported in this manuscript.

Funding Statement

This research was funded by the Higher Education Commission (HEC) of Pakistan under the National Research Program for Universities (NRPU) Project No. 17324.

REFERENCES

Abdel-Rahman, M. A., Harrison, P. L., & Strong, P. N. (2015). Snapshots of scorpion venomics. *Journal of Arid Environments*, *112*, 170-176.

Boucherit, H., Benabdeli, K., & Benaradj, A. (2017). Contribution to the phytotherapy against scorpion sting envenomation in the Naama region (Algeria). *Mediterranean Botany*, *38*(1), 75.

Bryson Jr, R. W., Savary, W. E., Zellmer, A. J., Bury, R. B., & McCormack, J. E. (2016). Genomic data reveal ancient microendemism in forest scorpions across the California Floristic Province. *Molecular Ecology*, *25*(15), 3731-3751.

Das, B., Saviola, A.J. and Mukherjee, A.K., 2021. Biochemical and proteomic characterization, and pharmacological insights of indian red scorpion venom toxins. *Frontiers in pharmacology*, *12*, p.710680.

Dehghani, R., & Arani, M. G. (2015). Scorpion sting prevention and treatment in ancient Iran. *Journal of traditional and complementary medicine*, *5*(2), 75-80.

Dehghani, R., Valizade, R., & Mahmoodi, S. (2016). A review of the scorpion predators and the introduction of Scarites subterraneus, as a new predatory of them in Iran. *Journal of Entomological Research*, *40*(3), 291-296.

El Hidan, M. A., Touloun, O., & Boumezzough, A. (2017). Spatial relationship between environmental factors and scorpion distribution in Morocco. *Journal of Entomology and Zoology Studies*, *5*(3), 674-678.

Firooziyan, S., Sadaghianifar, A., Rafinejad, J., Vatandoost, H., & Bavani, M. M. (2020). Epidemiological characteristics of scorpionism in West Azerbaijan province, northwest of Iran. *Journal of Arthropod-Borne Diseases*, *14*(2), 193.

García, L. F., Valenzuela-Rojas, J. C., González-Gómez, J. C., Lacava, M., & Meijden, A. V. D. (2022). Pinching or stinging? Comparing prey capture among scorpions with contrasting morphologies. *Journal of Venomous Animals and Toxins including Tropical Diseases*, *28*, e20210037.

Haghani, A., Khoobdel, M., Dehghani, R., Adibzadeh, A., Sobati, H., & Aliabadian, M. (2020). Ecological modeling and distribution analysis of digger scorpions: Odontobuthus doriae, Odonthubutus bidentatus (Scorpiones: Buthidae) and Scorpio maurus (Scorpiones: Scorpionidae) in Iran using the maximum entropy method. *Applied entomology and zoology*, *55*, 17-24.

Lira, A. F. A., DeSouza, A. M., & Albuquerque, C. M. R. (2018). Environmental variation and seasonal changes as determinants of the spatial distribution of scorpions (Arachnida: Scorpiones) in Neotropical forests. *Canadian Journal of Zoology*, *96*(9), 963-972.

Loria, S. F., Ehrenthal, V. L., & Esposito, L. A. (2024). Revisiting the scorpion central nervous system using microCT. *Scientific Reports*, *14*(1), 27961.

Lowry, J. A. (2020). Venoms. In *Toxicology Cases for the Clinical and Forensic Laboratory* (pp. 437-447). Academic Press.

Mullen, G. R., & Sissom, W. D. (2019). Scorpions (scorpiones). In *Medical and veterinary entomology* (pp. 489-504). Academic Press.

Pekár, S., & Raspotnig, G. (2022). Defences of Arachnids: diversified arsenal used against range of enemies. *Entomologia Generalis*, *42*(5).

Qari, S. H., Alrefaei, A. F., Filfilan, W., & Qumsani, A. (2021). Exploration of the medicinal flora of the aljumum region in saudi arabia. *Applied Sciences*, *11*(16), 7620.

Rowe, A. H., Xiao, Y., Rowe, M. P., Cummins, T. R., & Zakon, H. H. (2013). Voltage-gated sodium channel in grasshopper mice defends against bark scorpion toxin. *Science*, *342*(6157), 441-446.

Santos, G. C. S. D. G., Dionisio-Da-Silva, W., Souza-Alves, J. P., De Albuquerque, C. M. R., & Lira, A. F. D. A. (2018). Random or clumped: How litter dwelling scorpions are distributed in a fragment of Brazilian Atlantic forest. *European Journal of Entomology*, *115*, 445-449.

Sentenská, L., Graber, F., Richard, M., & Kropf, C. (2017). Sexual dimorphism in venom gland morphology in a sexually stinging scorpion. *Biological Journal of the Linnean Society*, *122*(2), 429-443.

Stockmann, M., Turiel, C., Althoff, F., Lowe, G., & Kovařík, F. (2016). First report of Lissothus occidentalis Vachon, 1950 (Scorpiones: Buthidae) from Morocco and Western Sahara, with notes on ecology and captive breeding. *Euscorpius*, *2016*(235), 1-12.

Tobassum, S., Tahir, H.M., Arshad, M., Zahid, M.T., Ali, S. and Ahsan, M.M., 2020. Nature and applications of scorpion venom: an overview. *Toxin Reviews*, *39*(3), pp.214-225.

Thapa, R., Wongsiri, S., Lee, M. L., & Choi, Y. S. (2013). Predatory behaviour of pseudoscorpions (Ellingsenius indicus) associated with Himalayan Apis cerana. *Journal of Apicultural Research*, *52*(5), 219-226.

Van Aardt, W. J., Le Roux, J. M., Lindeque, J. Z., Mason, S., & Louw, R. (2016). The effect of temperature on the respiration and metabolism of the African burrowing scorpion (Opistophthalmus latimanus). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *20*, 50-56.