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Retama Raetam's In-Vitro Acaricidal Action on The Hyalommaaegyptium Tick, Infecting Testudo Graeca Turtles in The Southern Algeria District of Laghouat

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Abstract
This research develops the narrative on investigating the efficacy of the plant extracts of Retama raetam that is essential in treating the tick, Hyalommaaegyptium, developing in Testudo graeca. These Tortoise species lives in the Laghouat region of Southern Algeria.Various extracting solvents are used to extract the essential components of the Retama raetam plant. The Hyalommaaegyptium tick is a member of the species of tortoises, which pose a risk to other animals' existence, particularly that of turtles. Solvents, including methanol, ethanol, and acetone, extract the retama raetam plant. These extracts examine the plant extract to treatHyalommaaegyptium ticks. Ethanol-based Retama raetam plant extract has the highest efficacy. 100% of the ticks die after being exposed for 15 days. The mortality rate of acetone is 33%, while methanol has a 42% mortality rate. Medium lethal concentration tests (LC50) determined the extract concentration, which required the death of 50% of Hyalommaaegyptium ticks. Plant extracts based on ethanol tests have a concentration of 70 mg/ml, methanol; LC50 tests have an 80 mg/ml concentration, and acetone; LC50 tests have a 95 mg/mL concentration. These ticks can be effectively treated using ethanol-based plant extracts, which is crucial for animals' well-being and survival.

Keywords: Laghouat, Retama raetam, Testudo Graeca Tortoise, HyalommaAegyptium Tick, Solvent-based Plant Extracts.

Introduction

Testudo graeca, a tortoise species, is located in Laghouat, Southern Algeria. Linnaeus explored this species in 1857, thus belonging to the Testudinidae family (Fritz et al., 1996). Moorish turtles are found in humid regions, arid regions, and coastal areas of high altitudes. Their population has declined and become extinct. The IUCN (International Union for Conservation of Nature) has defined the extinction as alarming (Tiar et al., 2019).

These species of tortoise have numerous health issues that involve contracting bacterial infections and germs (Bouamer and Morrand, 2000); (Bouaamer et al., 2003); (Díaz et al., 2015). The turtles (Testudo graeca) also contract ticks and other infections. It threatens turtles' health and other diseases, such as cat scratch disease (Borrelia) (Ernst and Lovich, 2009).

In the southern part of Algeria, Hyalommaaegyptiumticks are also termed pathogens that have resulted in the extinction of tortoises. They contract the Rickettsiaaeschlianniidisease and Crimean-Congo hemorrhagic fever (Bitam et al., 2009); (Kautman et al., 2016). The susceptibility of these pathogens has affected the tortoises and their endurance. It has also raised concerns in understanding the potential of turtles to transmit diseases (McBride et al., 2009).

Plant extracts have been used for medicinal purposes to dissect microbial infections and cure diseases. These medicines are inspired by traditional medicines that help treat infections. These plant extracts are under investigation to determine their efficacy in managing parasites and viral infections. Plant extracts are known to contain various chemical substances that offerphysiomedical and therapeutic values. These compounds are useful in: inhibiting viruses, preventing their development and growth as well as in preventing them from developing in host animals (Hoste et al., 2006); (Athanasiadou et al., 2007).

Retama raetam is a native shrub from South East Asia, with a perennial life cycle and is used to cure diseases. Some diseases that are treated has to include diabetes mellitus, rheumatism, inflammations, eczemas, and, hypertension. These medicines also apply to the treatment of the after-effects of snake bites. From the various findings by most of the researchers, it is evident that various part of Retama raetam notably, the aqueous extracts, has the potential to act like a diuretic in addition to reducing blood glucose levels in diabetic rats. The attempt to believe that this plant contains carbohydrates, fatty acids, phenolics, terpenes, steroids, and alkaloids has been made due to the pharmacological effects of the plant and the study of the chemical composition. The researcher has also explained about the use of all the compounds is discussed along with the biological activities like the elements used for antibacterial, anti-inflammatory, antioxidant, anti-proliferative, anti-ulcerative, antiviral, and hepato-protective. (Hammouche-Mokrane et al., 2017); (Gonza´lez-Mauraza et al., 2014); (El-Toumy et al., 2011); (Belayachi et al., 2013); (Edziri et al., 2008); (Omara et al., 2009b); (Koriem et al., 2010).

These findings are realized when the researchers did not find any new knowledge or medical research, made and originated from Algeria, that the locally known Retama raetam plant has broader acaricidal compounds or parts present in it.This paper evaluates the possibility of using the plant and also evaluates the potential of using the extracts from the Retama raetam plant in the preparation of medicines. The plant extracts of Retama Raetam are also used to treat and/or control the ticks in Testudo graeca which is a tortoise species in the Laghouat region of Southern Algeria.

1. Results

Figure 1 shows the values of LC50 and LC90, representing the lethal concentrations of plant extract at 50% and 90%, respectively. The plant is extracted with different solvents to treat Hyalommaaegyptiumtick, whichinfects Testudo graeca. All extracts showed a mixed toxic effect on the Hyalommaaegyptium tick. However, the ethanolic extract found the highest mortality; LC50 was recorded at 70 mg ml^{-1} and LC90 at 140 mg ml^{-1}. It indicates that ethanol has greater efficacy than acetone, thus requiring lower concentrations to kill the parasites at 50% and 90%.The LC50 was recorded at 95mg ml^{-1} and the LC90 at 146mg ml^{-1} for acetone. It indicates that the concentration of Retama raetam extract in acetone, utilized to kill 50% of parasites, had LC50 at 95mg ml^{-1}.

In comparison, 90% of parasites could be killed by LC90, at the concentration level of 146mg ml^{-1}.For methanol, the LC50 was recorded at 80mg ml^{-1} and the concentration at LC90 was recorded at 146mg ml^{-1}. Although the LC50 of methanol is slightly higher than that of ethanol,the LC90 of methanol is similar to that of acetone, suggesting comparable efficacy between the two extracting solvents. The analysis of the results also indicates that ethanol has resulted in showing the highest efficacy rate, in order to fight against the Hyalommaaegyptium tick, followed by methanol having the second highest efficacy, and acetone acquiring having the most negligible efficacy to fit against ticks and parasites.

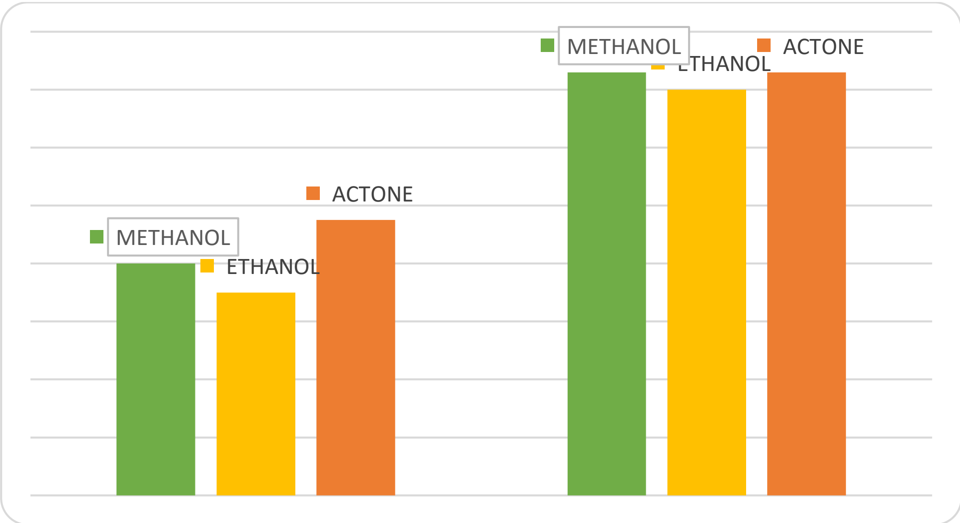

Figure1:LC50 and LC90 of Retama Raetam Extract Obtained for Different Solvents to fight against Hylommaaegyptium Tick

2.1 Association for Computing Machinery (ACM) Analysis

The purpose of the ACM analysis is to visualize the quantitative and qualitative factors in the map. Following this research, the ethanolic extract has the highest efficacy rate, in comparison with the methanolic and acetone extracts by relation to the F2 axis, which represents 30.97% of the variable, and the F1 represents 48.39% of the variables, making a cumulative of 79.35.

According to the research findings presented in Figure 1, all the extracts of Retama raetam demonstrated anti-tick properties against Hyalommaaegyptiumticks, tested at all concentrations tested. It is essential to observe that all three types of extracts (acetonic, methanolic, and ethanolic) demonstrate a rise in the death rate of the tick as the concentration increases. It also indicates that all the extraction solvents efficiently extract chemicals that harm the Hyalommaaegyptium tick.Acetone extract increases the mortality of the tick as the concentration increases. It increases from 15% to 100% mortality with the increase in concentrations, ranging from 25 mg/ml to 150 mg/ml.

Similarly, a methanolic extract shows an increase in the mortality of ticks with increasing concentrations, ranging from 20% to 100% mortality in the same concentration range as the acetonic extract.The ethanolic extract follows the same

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trend, with a gradual increase in the tick mortality rate as the concentration increases, reaching 100% mortality at 150 mg/ml.The negative control shows a mortality of 0% for all concentrations, confirming that the mortality observed in the other groups is due to the effect of the extract and notother factors.

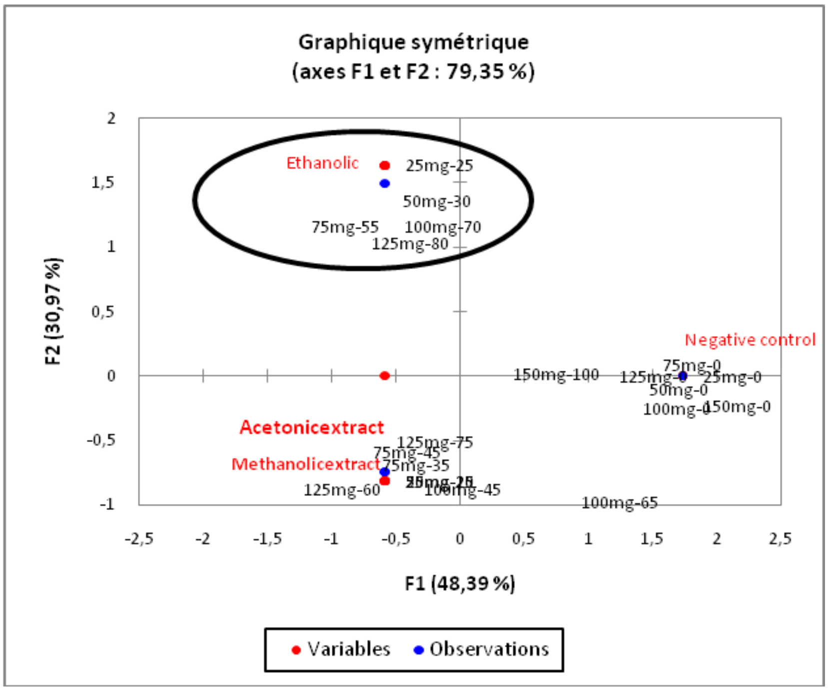

Figure 2: ACM of tick mortality rate as a function of the extraction solvent and Retama raetam extract concentration

Table 1 shows the percentage of tick mortality for each immersion period and solvent. The mortality rate generally increases with increasing immersion time. In addition, mortality is higher with ethanol, followed by methanol and then acetone.

Table 1: Mortality rate of the tick Hyalommaaegyptiumas a function of immersion time and solvent used.

		1st			**3rd**			**7th**			**9th**			**12th**			**15th**		
Immersion time (min.)		30	60	120	30	60	120	30	60	120	30	60	120	30	60	120	30	60	120
Mortality (%)	**Ethanol**	7	12	20	25	32	35	45	50	55	65	70	74	83	87	90	96	99	100
Mortality (%)	**Methanol**	2	3	6	7	9	11	12	13	14	16	18	26	30	32	36	37	40	42
Mortality (%)	**Acetone**	1	3	3	4	5	6	7	9	11	12	13	15	15	17	20	25	29	33
Control (%)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0

Table 1 shows that the mortality rate of the Hyalommaaegyptium tick reaching 20%, 35%, 55%, 74%, 90%, and 100%. The 1st, 3rd, 7th, 9th, 12th, and 15th days, in the case of 70mg/ml concentration of ethanolic extract, will be 30 minutes to 120 minutes. In the case of 70 mg/ml concentration of the methanolic extract, the tick mortality rate reached 6%, 11%, 14%, 26%, 36%, and 42% on the 1st, 3rd, 7th, 9th, 12th and 15th days at 30 minutes to 120 minutes.In the case of the acetone extract, the tick mortality rates reached 3%, 6%, 11%, 15%, 20%, and 33% on the 1st, 3rd, 7th, 9th, 12th, and 15th days at 70 mg/ml concentration for 30 minutes to 120 minutes. In the control group, the mortality rate was 6% at the end of the treatment experience. The low level of mortality for all periods of immersion indicates that the mortality observed in the other groups is due to the extraction of solvents and their effects. The results suggest that the effectiveness of solvents in tick mortality depends on the time of immersion. Thus, with the increase in immersion time, the mortality rate is also subjected to increase.

2. Discussion

In recent decades, the field of tick control has faced significant hurdles. Extensive research has been conducted to analyze the acaricide and insecticidal properties of various plant species to manage phytophagous pests, mosquitoes, mites, and ticks (Calmasur et al., 2006); (Mukandiwa et al.,2014); (Kim et al., 2004); (Nong et al., 2013a); (Lori et al., 2005). Prior

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research has primarily examined the impact of chemical acaricides on different insects and mites, both in controlled laboratory settings and in real-life conditions, along with the level of toxicity (Wilson, 1948); (Guilhon, 1950); (Arthur, 1951); (Hadani et al., 1969).

Ticks are obligatory ectoparasites that feed on blood and infest approximately 80% of livestock on a global level. Hyalommaaegyptiumtick is well studied with respect to external parasites that consume animals as their source of nutrition. These parasites transmit illnesses that result in economic losses in the livestock industry (Sajid, 2017). Chemical miticides are used as conventional approaches to control ticks. These applications are limited to environmental considerations. The invasive species and the development of insecticides and acaricides produce unintended impacts on the health of humansand the environment. Thus, this also raises doubts about the utilization of insecticides (Cafarchia et al., 2011).

This study assesses the extent to which the extract of Retama raetam plant can be implement to get rid of the ticks. As pointed out by the researcher of this study, this is the first time that attempts the research the impacts of the extracts of Retama raetam plant and come up with positive results towards acaricidal activities. But these plant extracts have in the recent past been applied in controlling horticultural pests such as the plant eating insects and Mosquitoes (Balandrinet al. , 1995). [Finding/Solution]: It is apparent; the outcome of this study indicates that extract of Retama raetam possesses toxicological impacts on Hyalommaaegyptiu tick from a range of extracting solvents. These findings also suggest that the plant leaves have certain bioactive compounds with acaricidal activity so that the plant could be used for controlling ticks. This research indicates the difference in effectiveness between the extracting solvents used to extract significant compounds from the plant material. As per the analysis results, ethanol has the highest efficacy rate, followed by methanol, which acquires mild efficacy, and acetone, which has the most negligible efficacy. This assumption is underlined by LC50 and LC90, which point to the fact that lower concentrations of ethanol extract are needed to obtain the toxic effect as compared to methanol and acetone extracts are needed for it.

This study's output has the potential to generate advanced biological control strategies for parasites, especially the ticks. The combination and application of plant extracts can be effective and efficient in protecting animals from being infected by bacteria and parasites whether through creams or food supplements while it is environmentally friendly and safer than using synthetic chemical products.

Recently, several plant products, raw extracts, and essential oils have been evaluated. It is to ascertain their efficacy in repelling and eliminating economically important tick species at their life cycle stages, including adult, pupa, larva, and egg. These assessment results have indicated a high potential (Chungsamarnyart et al., 1988); (Chungsamarnyart et al., 1990); (Chungsamarnyart et al., 1991a);(Mehlhorn et al., 2005), (Coskun et al., 2008), (Demon et al., 2009), (Magadum et al., 2009), (Monteiro et al., 2009); (Monteiro et al., 2012); (Clémente et al., 2010); (Kamaraj et al., 2010); (Zorloni et al., 2010)(Ghosh et al., 2011); (Koc et al., 2012); (Singh et al., 2014). Moreover, numerous periodic assessments are published on the aspects and characteristics of anti-tick and acaricidal effects (Kaaya, 2000); (Copping and Menn, 2000); (Flamini, 2003); (Nerio et al., 2010); (garEbadollahi, 2011); (Zoubiri andBaaliouamer, 2011); (Maïa and Moore, 2011); (Borges et al., 2011); (Andreotti et al., 2014); (Georges et al., 2014); (Ghosh and Ravindran, 2014).

Plant extracts have been used to control pests since they are cost-effective and environmentally friendly (Reverter et al., 2014). Many plant species are evaluated for their efficacy against ticks and bacterial infections(Rodriguez-Vivas et al., 2018). Most plant extracts are analyzed to cure different species of Hyalommaaegyptiumtick (Singh et al., 2017).

These remedies are in the form of chemicals obtained from plant extracts that is used to treat illnesses. These chemical substances listed above CCO enable Inhibition of the mating of viral cells, prevent the formation of the Exoskeleton, slow down the multiplication of the bacterial cells, and also the eye-laying capability. Researchers also do ongoing studies about the effectiveness and efficacy of ethanol-based extracts on different ticks (Pereira and Famadas, 2004). The substances present in the stems of Retama raetam plants that can be extracted are Polyphenols, Piscidic acid, Quinic acid andProanthocyanidin Pinocembrin (Touati et al. , 2017). The solvent with a high content of flavonoids, tannins, and alkaloids involves ethyl acetate (Edziriet al. , 2007). Few plant extractswere soluble in water and had innate antibacterial solid qualities, and polar activity (Mariem et al., 2014). Employee engagement: a study for Boussahel et al. (2017).

Plant extracts based on methanol have been used to study the fruits of the Retama raetam plant. These fruits are anti-inflammatory and antioxidant-rich. Plant extracts based on methanol exhibit mild acaricidal properties. They have larval mortality and can also inhibit the hatching of eggs on ticks. (Balan and colleagues, 2017) has researched various plant extracts with the help of extracting solvents. In comparison with methanol-based extracts and acetone-based extracts, ethanol-based extracts are highly effective in controlling ticks.

Solvents, such as hexane, acetone, and ethanol, are used as extraction agents or solvents. However, ethanol has been a solvent that has shown promising results in controlling ticks. In a study conducted by (Gonçalves et al., 2007), the impact of solvents and surfactants on adult female and cow tick larvae was assessed. The results indicated that acetone and methanol exhibited the highest level of toxicity among the solvents, while ethanol demonstrated a moderate level of toxicity. Nevertheless, (Ravindran and colleagues, 2011a); (Ravindran and colleagues, 2011b) observed that methanol can dissolve plant extracts to assess acaricidal activities. It is worth noting that although aqueous solvents are frequently employed in ethnoveterinary medicine, organic solvents may be more effective in acaricidal bioassays. The tick cuticle, made primarily of exterior waxes and inside proteins, allows for more effective penetration by non-polar chemical

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substances (Balashov, 1972); (Chagas et al., 2002). The tick also represents a significant economic issue, responsible for severe losses related to various aspects such as tick anxiety, blood loss, skin damage, injection of toxins and transmission of diseases. Worldwide, extracts from nearly 55 plants belonging to 26 different families have been evaluated for their efficacy against ticks (Borges et al., 2011).
Nevertheless, despite the comprehensive research conducted on assessing plants' anti-tick and acaricidal characteristics, certain constraints have been recognized. One challenge in comparing different investigations is the absence of defined testing procedures or protocols for making extraction agents. These challenges disrupt the ability of plant extracts to establish a relationship between the research findings and their practical implementation in animals who contract ticks and bacterial infections.

4. Experimental

4.1 Investigation of the Geographical Region

The Laghouat region, in Southern Algeria, is 400 kilometers away from Algiers. The region spans around 25,052 square kilometers and has a population of 520,188 residents. The climate in this region exhibits continental characteristics, with precipitation levels ranging from 300 to 400 mm. Conversely, in the central and southern parts of the region, the climate transitions to a Saharan and arid environment characterized by much-reduced precipitation. In the central area, rainfall amounts to approximately 150 mm, while in the south, it drops to a mere 50 mm. In 2013, Andi characterized winters as having white frosts and summers as having high temperatures and sandstorms.

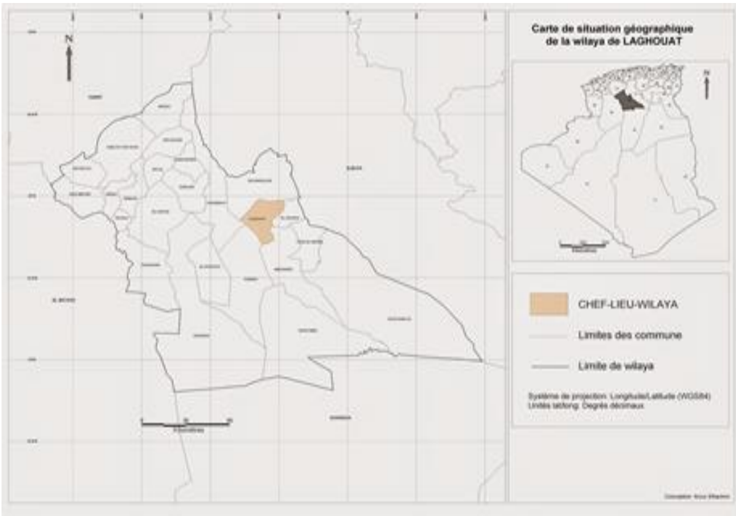

Figure 3: Map of the wilaya of Laghouat, Southern Algeria

4.2 Sampling of the Retama Raetam Plant

The sampling points for the Retama raetam plant were carefully chosen to reflect the diversity of its habitat and growing conditions. It helped the researchers obtain high-quality data to carry out the research. The researchers have also identified the potential sites of the Retama raetam plant, which helps them collect samples of the plant leaves from all locations. The soil type, sun exposure, altitude of the plant, and rainfall frequency are factors that the researchers analyze.
Detailed surveys have been conducted to analyze the presence of the Retama raetam plant. For this research, the researchers selected healthy plants and avoided deceased or isolated plants. The plants were harvested to collect sample sizes that were similar in characteristics. Samples taken from the plant were marked with relevant information that was essential to carry out the research.

4.3 Extraction of Plant Materials

This information was gathered by Dr. Salim Zarrok of the Department of Biology, University of Laghouat, Southern Algeria, after examining the Retama raetam plant. It was done to determine the taxonomical implications of plant leaves although he used specific extraction solvents to prepare the test medium. The extraction solvents were used to extract chemical compounds. Chemical compounds were extracted through the extracting solvents. The leaves were left to dry in sheltered areas for 7–10 days at a temperature range of 25–37°C. The leaves of the plant were then spat and ground until they turned into fine powder with the assistance of the mortar and pestle. With regard to the extracting solvent for each extraction, The following experimental conditions were applied according to Kumar et al., 2011: At least for a period of sixteen hours, solvents like ethanol and acetone were used to extract the chemicals existing in the plant. The extracts were separated from the residues after 16 hours of boiling process by filtration.
The extract was filtered through anhydrous sodium sulfate to eliminate any remaining alcohol (Tabassum et al., 2008).

The obtained extract was concentrated under reduced pressures and controlled temperatures of 45°C and 22-26 mmHg, respectively. This step helps remove any access solvents, leaving behind the concentrated residue, to test the compounds for medicinal purposes.
To prepare a methanolic aqueous extract, the powdered plant leaf was extracted with a methanol-based aqueous solvent in a ratio of 70:30 (70 ml extracting solvent and 30 gms leaf powder) for 72 hours. After filtration, the crude extract was obtained after the solvent evaporation in an evaporator. Later, the concentrated residue was used at different concentrations.

4.4 Ticks

Bloodstained adult female ticks were collected and identified from naturally infested turtles. The ticks were transferred to the Parasitology Laboratory on Petri dishes with perforated covers, allowing air circulation. The ticks were put through the Adult Immersion Test immediately after arrival (Cen-Aguilar et al., 1998).

4.5 Adult Immersion Test (AIT)

The Total Immersion Analysis (TIA) was used to analyze the effectiveness of killing blood-engorged female ticks (Drummond et al., 1967). The plant extract was tested on 210 ticks. These ticks were divided into seven groups, with ten individuals each. The extracts were diluted at 25, 50, 75, 100, 125, and 150 mg/ml, while a control group was treated with distilled water. Each group underwent three duplicates. The ticks in the experimental groups were submerged for 30, 60, and 120 minutes, respectively, whereas the ticks in the control group underwent the same treatment using distilled water. The ticks were affixed and placed in Petri dishes, where they were subjected to incubation at a temperature of 27 ± 1.5°C and relative humidity of 70-80%, for 14 days, followed by the protocols outlined by (Cen-Aguilar et al., 1998). The ticks were subsequently inspected using a stereoscope at three-day intervals. Ticks exhibiting darkened cuticles, absence of movement in the Malpighian tube, and skin lesions with bleeding were classified as deceased. The calculation of mortality was performed using the corrected mortality formula developed by (Abbott, 1925) following the guidelines of the Food and Agriculture Organization (FAO) in 2004.

4.6 Statistical Analysis

Excel was used to input and process data. Correlation analysis was used as a statistical method to examine the relationship between two or more category variables. MCA analyzes and interprets the qualitative data, while PCA analyzes and interprets quantitative variables. Maps show qualitative variables and observation distances. MCA extends PCA in situations with more than two variables.

5. Conclusion

This research demonstrates the acaricidal efficacy of Retama raetam extract based on the result analysis obtained from the LC50 and LC90 tests. Based on the inference of the result analysis, this study investigates the efficacy of Retama raetam plan extract to cure the Hyalommaaegyptium tick in turtles. Retama raetam plant extract has significant mortality rates when interacting with bacterial sites. However, ethanol has been one of the best extracting solvents for curing the Hyalommaaegyptium ticks. The plant extract also has the potential to be efficient in acaricidal activities when observed at low concentrations. The Retama raetam plant extract has become the best solution to cure the tick and thus prevent the population of turtles from going extinct. It also serve as a natural replacement for the synthetic chemicals and is being regarded as the traditional medicine, for curing ticks and bacterial-infected elements. Simply, the researchers require analyzing the functioning of the plant extract under the current environmental amenities in order to investigate its effectiveness on turtles in the long run. But, it further emphasizes on the need to look at the variations in the species of the ticks and the animals that may become infected with viruses and bacteria. This will enable the researchers to determine level of effectiveness of Retama raetam plant extract in reducing the secretion of ectoparasites.

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