Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.160

RESEARCH ARTICLE

# Estimation of *In vitro* Acaricidal Activities of Ethanolic and Ethyl Acetate Extracts of *Nicotiana tabacum* against *Hyalomma* species of Livestock

Eman A. Al-Nabati[1], Ruoa Almahallawi[2], Amal M. Alzahrani[3], Nawal Al-Hoshani[4]*, Mariam S. Al-Ghamdi[5], Sally Negm[6], Attala F. El-lkott[7,8], Majed A. Bajaber[9], Soliman M. Soliman[10], Mohamed T. El-Saadony[11]

[1]Department of Biology, College of Science, Taibah University, Madinah, P.O. 344, Saudi Arabia
[2]Department of Biology, University College of Duba, University of Tabuk, Tabuk 71491, Saudi Arabia
[3]Department of Biology, Faculty of Sciences, Al Baha University, Al Baha 65779, Saudi Arabia
[4]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
[5]Department of Biology, College of Sciences, Umm Al-Qura University, Makkah 24381, Saudi Arabia
[6]Department of Life Sciences, College of Science and Art Mahyel Aseer, King Khalid University, Abha 62529, Saudi Arabia
[7]Department of Biology, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia
[8]Department of Zoology, Faculty of Science, Damanhour University, Damanhour 22511, Egypt
[9]Department of Chemistry, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia;
[10]Department of Medicine & Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt;
[11]Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
*Corresponding author: Nialhoshani@pnu.edu.sa

ARTICLE HISTORY (24-081)

| | |
|---|---|
| Received: | February 12, 2024 |
| Revised: | March 14, 2024 |
| Accepted: | March 17, 2024 |
| Published online: | April 04, 2024 |

**Key words:**
*Nicotiana tabacum*
Tobacco
Ticks
Livestock
*Hyalomma*
Botanical control
Acaricidal

**ABSTRACT**

Ticks are ectoparasites belonging to the phylum Arachnida infesting humans and animals. Besides their parasitic role, ticks also spread multiple bacterial, viral, and parasitic diseases because of their vector role. *Hyalomma* is a major genus of ticks containing species of zoonotic and pathogenic significance. Control of the ticks is the primary importance, but chemical acaricides are being avoided because of resistance and one health-related issue. In this study acaricidal and repellent activities of the ethanolic and ethyl acetate extracts of Tobacco (*Nicotiana tabacum*) against the ticks of *Hyalomma* genus. Each extract was serially diluted to have 1.25, 2.5, 5, and 10% concentrations. The parameters taken were adult mortality, larval mortality, egg hatchability and product effectiveness. The results suggested that the ethyl acetate and ethanolic extracts had statistically comparable (P>0.05) effects on adult mortality (tick immersion), larval mortality (larval immersion test), egg hatchability and tick repellency parameters. This research also revealed that extracts of *N. tabacum* were also comparable to (P>0.05) standard medicated control at the highest concentrations. Ethanolic and ethyl acetate extracts had significantly (P<0.05) different acaricidal effects from the blank control at 10% concentration. This research suggests that the *N. tabacum* can be used as an acaricidal and tick-repellent agent.

**To Cite This Article:** Al-Nabati E, Almahallawi RS, Alzahrani AM, Al-Hoshani N, Al-Ghamdi MS, Negm S, El-lkott AF, Bajaber MA, Soliman SM, El-Saadony MT, 2024. Estimation of *in vitro* acaricidal activities of ethanolic and ethyl acetate extracts of *Nicotiana tabacum* against *Hyalomma* species of livestock. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2024.160



## INTRODUCTION

Ticks are parasitic organisms that belong to the phylum Arachnida and infest animals and humans (Fogaca *et al.*, 2021). They are blood-sucking organisms and vectors of multiple diseases of viral, bacterial and parasitic origin. Additionally, ticks are among the most threatening ectoparasites because of their parasitic and zoonotic importance (Fogaca *et al.*, 2021). *Hyalomma* spp. is the major genus of ticks infecting cattle, buffalo, goats, and multiple other domesticated and non-domesticated animals (Jones and Garcia, 2021). They do not only complete a part of their lifecycle on the animals but feed on their blood as well as transmit multiple other pathogens. In unmanaged conditions, they may lead to hypovolemic shock and toxicities related to their bites (Jones and Garcia, 2021).

Control of ticks is the major concern of scientists to boost the performance of animals and avoid the transmission of zoonotic diseases. Chemical acaricides are commonly used as dips, drenches, sprays, and multiple other formulations to help control ticks in domesticated animals including cattle and buffalo (Ramzan *et al.*, 2021). These chemicals acaricide are widely used and this irrational use is creating several problems. The major problem because of these acaricides is the development of resistance. Ticks have developed resistance to multiple drugs through several mechanisms of action, which results in the wastage of capital while the problem persists (De Rouck *et al.*, 2023). Along with the resistance, the major issue with acaricidal drugs is that they are ecologically toxic. The chemical agents used in acaricidal drugs are toxic for all life raising public health problems (Bava *et al.*, 2022). The extinction of multiple symbiotic organisms and the development of pathologies that may be so severe that they may cause cancer and adverse effects on the environment have drawn the attention of scientists to develop alternatives that may be effective, ecologically safe, and economical for the control of ticks (Akpan *et al.*, 2023).

Vaccination is the most widely proposed alternative to acaricides for the management of resistance and ecological problems (Ribeiro *et al.*, 2021). The acaricidal vaccine failure leads to unmanaged tick infestation and a waste of capital (Schulze *et al.*, 2023). Other alternatives being suggested to chemical acaricides include preparations of botanical origin, organic acids, and semisynthetic acaricides to manage the resistance issues. All the alternatives have vital importance, but the botanical preparations have their promising importance (El-Mansi *et al.*, 2023). Plant-based products, especially extracts of plants contain phenolic acid and its derivatives, which have proven antiparasitic effects including acaricidal potential (Salman *et al*., 2020; Abbas *et al*., 2023; Saeed and Alkheraije, 2023). The acaricidal activity of the herbal extracts depends upon the quantity and diversity of the phenolics present in them (Bravo-Ramos *et al.*, 2021). Multiple factors are there that affect the phenolics in herbals, including the type of plant and the extraction solvent used, which are primarily important.

Tobacco (*Nicotiana tabacum*) is a flowering plant belonging to the family "Solanaceae" of flowering plants. It is widely cultivated within the subcontinent, Africa, and Latin America. Tobacco is mainly farmed for smoking and chewing purposes, but it has well-known pharmaceutical attributes. Tobacco is being used in various pharmaceutical preparations and has well-known medical importance (Al-Lahham *et al*., 2020). Antibacterial, antifungal, antiviral, and antiparasitic activities of tobacco have been reported by several scientists (Al-Snafi, 2022). Several extracts of tobacco are being searched for treatment of various diseases of a metabolic and infectious nature. The scientists have mentioned that the extraction solvent affects the medicinal activity of tobacco greatly (Al-Lahham *et al.*, 2020).

In this research, various concentrations of ethanolic and ethyl acetate tobacco extracts (*N. tabacum*) were experimented on the *Hyalomma* spp. ticks for their acaricidal. This study estimated the acaricidal activity of both extracts using the adult mortality, larval mortality, and egg hatchability assays.

## MATERIALS AND METHODS

**Extract preparation:** Leaves of tobacco were taken from a verified source and processed for ethanolic and ethyl acetate extracts. El-Mansi *et al.* (2023) methods were used for ethanolic and ethyl acetate solvent-based extraction. The tobacco leaves were inserted in the rotary evaporator in the presence of ethanol or ethyl acetate to achieve the respective extract of the *N. tabacum.*

**Collection of ticks:** Ticks were collected from the cattle and buffalo farms and identified microscopically using the keys of Estrada-Peña *et al*. (2017) *Hyalomma (H.) kumari*, *H. brevipunctata*, *H. anatolicum*, and *H. marginatum* were identified from the genus *Hyalomma* using the method similar and collected for the experiment.

**Collection of eggs of ticks:** The female ticks engorged with the eggs were identified microscopically and separated into the falcon tubes having sufficient oxygen supply. The collected eggs were counted using methods of (Figueiredo *et al*., 2018) and stored in the refrigerator (4°C) for further processing.

**Adult mortality assay:** 300 adult ticks of *Hyalomma* spp. were divided into 10 groups each divided into 3 replicates. The first 8 groups were treated with 1.25, 2.5, 5, and 10% (v/v) concentrations of ethanolic and ethyl acetate treated groups. Ticks were observed at 2-hour intervals for 12 hours and mortality was noted. Percent mortality was calculated by taking the percentage of dead ticks over the total ticks following the methods of Salman *et al*. (2020).

**Larval mortality assay:** Similar experimental patterns were followed as mentioned for the adult mortality assays. Larvae of *Hyalomma* ticks were isolated at the age of a week to two weeks and subjected to the larval immersion assay as mentioned by Salman *et al*. (2020). Larval mortality was checked at the 2-hour interval and calculated by taking the percentage of dead larvae by total larvae.

**Egg hatchability assay:** An egg hatchability assay was performed by collecting the freshly laid eggs from the female ticks of *Hyalomm* spp. the egg counts with a similar average were divided into groups according to concentrations of ethanolic and ethyl acetate extracts and positive and negative control. Eggs were divided into respective concentrations of ethanolic, and ethyl acetate extracts of the extracts of tobacco and the controls and left for 24 hours. After 24 hours the percentage of the eggs hatched was calculated (Salman *et al*., 2023). Moreover, using the egg hatchability index following parameters were also calculated.

**Reproductive index:** The reproductive index was calculated using the given formula:

$$\text{Percent reproductive index} = \frac{\text{Mass of laid eggs}}{\text{Mass of adult females befor treatment}}$$

**Product effectiveness (PE):** Product effectiveness was calculated using the methods of Salman *et al.*, (2023). In this method the reproductive efficiencies (RE) were calculated and then PE was estimated:

$$RE\% = \frac{\text{egg mass} \times \text{percent eggs hatched}}{\text{engorged female tick weight}} \times 20000$$

$$PE = \frac{\text{RE(control} - \text{treatment)}}{\text{Control RE}} \times 100$$

**Statistical analysis:** The data was analyzed using the Minitab® 26.0 statistical software. A generalized linear model was applied to data for analysis of variance and means were compared using Tuckey's pairwise tests for the statistical differences. The statistical significance ($p<0.05$) was maintained at a confidence interval of 95%.

## RESULTS

**Adult tick mortality:** The adult tick mortality was checked in the experiment and no statistically significant difference ($p>0.05$) was found between both ethanolic and ethyl acetate extracts of tobacco at the same concentration. However, both the extracts had a significant difference ($p<0.05$) from the neutral control. All the concentrations had a response in a dose-dependent manner. Table 1 shows the acaricidal effects of the ethanolic and ethyl acetate extracts of the tobacco on the adult ticks of *Hyalomma* spp.

**Larval mortality:** The larval mortality was checked in the experiment and no statistically significant difference ($p>0.05$) was found between both ethanolic and ethyl acetate extracts of tobacco at the same concentration. Both the extracts had a significant difference ($p<0.05$) from the neutral control (Table 2). All the concentrations had a dose dependent manner.

**Egg hatchability:** Percent Egg hatchability was checked in the experiment and no statistically significant difference ($p>0.05$) was found between both ethanolic and ethyl acetate extracts of tobacco at the same concentration. Both the extracts had a significant difference ($p<0.05$) from the neutral control. All the concentrations had a dose dependent manner. Table 3 shows the acaricidal effects of the ethanolic and ethyl acetate extracts of the tobacco on the percent egg hatchability of *Hyalomma* spp.

**Reproductive index:** Reproductive index was checked in the experiment and no statistically significant difference ($p>0.05$) was found between both ethanolic and ethyl acetate extracts of tobacco at the same concentration for reproductive index of *Hyalomma* ticks. Both the extracts had a significant difference ($p<0.05$) from the neutral control. All the concentrations had a dose-dependent manner (Fig. 1).

**Productive effectiveness:** Percent Egg hatchability was checked the product effectiveness was calculated by the formulas in the experiment and no statistically significant difference ($p>0.05$) was found between both ethanolic and ethyl acetate extracts of tobacco at the same concentration. Both the extracts had a significant difference ($p<0.05$) from the neutral control. All the concentrations had a dose-dependent manner. Fig. 3 shows the acaricidal effects of the ethanolic and ethyl acetate extracts of the tobacco on the percent egg hatchability of Hyalomma spp.

## DISCUSSION

Plant-based products have been used for medicinal purposes since human existence. Modern research agrees that plants and plant-based products have great pharmacological and medicinal properties (Najmi *et al.*, 2022). Herbal extracts are mixtures of compounds that are prepared by extraction using the solvent (Abubakar and Haque, 2020). The chemical type, variety amount, and molecular sizes of the molecules in extracts depend upon the species of plant and type of extraction solvent used (Salam *et al.*, 2019). Therefore, the species of the plant and type of extraction solvent are supposed to pose a diverse medicinal activity. *N. tabacum* (Tobacco) is a common plant mainly used for smoking (Al-Snafi, 2022). It is widely cultivated worldwide and is well known for its medicinal properties. Its extracts have been proven to have a lot of activity against infectious agents (Al-Snafi, 2022). Ticks are ectoparasites and infest almost all terrestrial animals. *Hyalomma* spp. is a genus comprising a diverse group of parasitic arachnids that can transmit multiple diseases (Fogaca *et al.*, 2021). They are widely present in domesticated livestock and compromise their performance because of their parasitic and vector role. Controlling ticks using botanicals is the primary research area, attracting multiple scientists (Bravo-Ramos *et al.*, 2021; Ramzan *et al.*, 2021). Scientists have proven that the compounds present in various herbal extracts can act as potent acaricidal agents (Rosado-Aguilar *et al.*, 2010; Zaman *et al.*, 2012; Alimi *et al.*, 2021). In this research, it has been shown that the herbal extracts of tobacco have the potency of controlling the *Hyalomma* ticks by showing their acaricidal and repellent activities.

**Table 1:** Percent mortality of *Hyalomma* spp. adults by the various concentrations of ethanolic (Et) and ethyl acetate (EA) extracts of tobacco leaves at 2 hour' time interval

| Groups | | Adult mortality (%) with time (hours) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| % concentration | Solvent | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| 1.25 | EA | $0\pm0^{m}$ | $3.33\pm5.77^{lm}$ | $6.66\pm5.77^{klm}$ | $23.33\pm5.77^{i-l}$ | $40\pm10^{def}$ | $60\pm10^{def}$ | $76.66\pm15.27^{bcd}$ |
| | Et | $0\pm0^{m}$ | $10\pm15.49^{klm}$ | $10\pm17.88^{klm}$ | $26.66\pm14.71^{h-k}$ | 60±8.16b$^{cd}$ | 76.66±0b$^{cd}$ | $90\pm0^{ab}$ |
| 2.5 | EA | $0\pm0^{m}$ | 13.33±5.77klm | 16.66±5.77j-m | 26.66±5.77h-k | 56.66±5.77bcd | 76.66±5.77bcd | $96.66\pm5.77^{bcd}$ |
| | Et | $0\pm0^{m}$ | 13.33±15.27klm | 20±10i-m | 36.66±15.27g-j | 90±10ab | 96.66±5.77ab | $100\pm0^{a}$ |
| 5 | EA | $0\pm0^{m}$ | 36.66±5.77g-j | 46.66±5.77e-h | 56.66±5.77d-j | 90±10a | 100±0a | $100\pm0^{a}$ |
| | Et | $0\pm0^{m}$ | 40±10m | 60±10f-i | 60±10def | 96.66±5.77def | 100±0a | $100\pm0^{a}$ |
| 10 | EA | $0\pm0^{m}$ | 90±10ab | 83.33±15.27ab | 93.33±5.77ab | 100±0a | 100±0a | $100\pm0^{a}$ |
| | Et | $0\pm0^{m}$ | 93.33±5.77ab | 83.33±5.77ab | 96.66±5.77ab | 100±0a | 100±0a | $100\pm0^{a}$ |
| Positive control | | $0\pm0^{m}$ | $93.33\pm5.77^{ab}$ | $100\pm0^{a}$ | $100\pm0^{a}$ | $100\pm0^{a}$ | $100\pm0^{a}$ | $100\pm0^{a}$ |
| Negative control | | $0\pm0^{m}$ | $0\pm0^{m}$ | $0\pm0^{m}$ | $0\pm0^{m}$ | $0\pm0^{m}$ | $0\pm0^{m}$ | $0\pm0^{m}$ |

Different superscripts across the rows and columns represent the statistically significant differences ($p<0.05$).

**Table 3:** Percent mortality of larvae of *Hyalomma* spp. ticks by the various concentrations of ethanolic (Et) and ethyl acetate (EA) extracts of tobacco leaves at 2 hours' time interval

| Groups | | Laval mortality (%) with time (Hours) | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration (%) | Solvent | 2 | 4 | 6 | 8 | 10 | 12 |
| 1.25 | EA | 7.33±5.77llm | 7.66±5.77lm | 20.33±5.77i-l | 40.67±10.33def | 60.67± 10.67def | 76.66±15.7cd |
| | Et | 15±15.41klm | 18±17.89klm | 26.66±14.91h-k | 63± 8.16bcd | 76.66±0bcd | 90±0ab |
| 2.5 | EA | 13.33±5.77klm | 16.66±5.77jklm | 26.66±5.77h-k | 56.66±5.77bcd | 76.66±5.77bcd | 96.66±5.77bcd |
| | Et | 13.33±15.27klm | 20±10i-m | 36.66±15.27g-j | 90±10ab | 96.66±5.77ab | 100±0a |
| 5 | EA | 36.66±5.77g-j | 46.66±5.77e-h | 56.66±5.77d-j | 90±10a | 100±0a | 100±0a |
| | Et | 40±10m | 60±10f-i | 60±10def | 96.66±5.77def | 100±0a | 100±0a |
| 10 | EA | 90±10ab | 63.33±15.27ab | 93.33±5.77ab | 100±0a | 100±0a | 100±0a |
| | Et | 93.33±5.77ab | 83.33±5.77ab | 96.66±5.77ab | 100±0a | 100±0a | 100±0a |
| Positive control | | 93.33±5.77ab | 100±0a | 100±0a | 100±0a | 100±0a | 100±0a |
| Negative control | | - | - | - | - | - | - |

Different superscripts across the rows and columns represent the statistically significant differences (p<0.05).

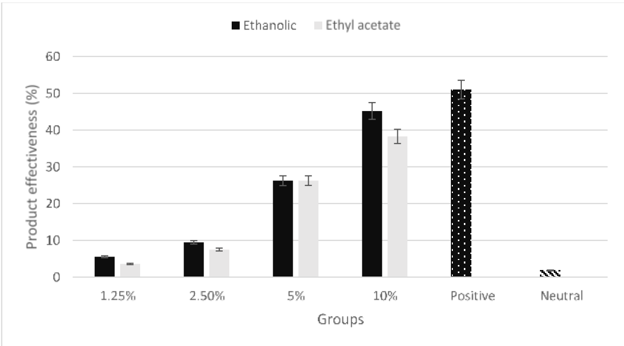


**Fig. 1:** Product Effectiveness of *Hyalomma* spp. ticks by the various concentrations of ethanolic and ethyl acetate extracts of tobacco leaves.

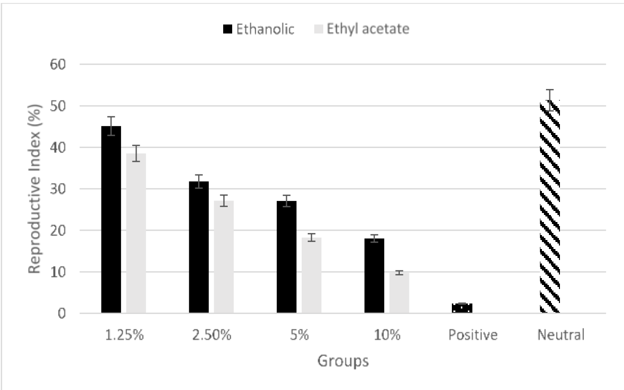


**Fig. 2:** Percent reproductive index of *Hyalomma* spp. ticks by the various concentrations of ethanolic and ethyl acetate extracts of tobacco leaves.

**Table 3:** Percent Egg hatchability of Hyalomma spp. ticks by the various concentrations of ethanolic (Et) and ethyl acetate extracts of tobacco leaves

| Concentration (%) | Solvents | Eggs hatched (%) |
|---|---|---|
| 1.25 | Ethyl acetate | 67.37±9.96$^{b}$ |
| | Ethanol | 63.57±5.93$^{bc}$ |
| 2.5 | Ethyl acetate | 57.11±5.72$^{cd}$ |
| | Ethanol | 45.73±7.6$^{de}$ |
| 5 | Ethyl acetate | 37.91±3.22$^{e}$ |
| | Ethanol | 33.87±5.05$^{e}$ |
| 10 | Ethyl acetate | 25.31±3.45$^{f}$ |
| | Ethanol | 23.91±1.28$^{f}$ |
| Positive control | | 15.99±0.49$^{f}$ |
| Neutral control | | 95.37±0.59$^{a}$ |

Different superscripts represent the statistically significant differences (p<0.05).

The research revealed that the 10% concentration of both ethanolic and ethyl acetate extracts of tobacco had significantly higher (p<0.05) mortality rates. Both the extracts were statistically comparable (p>0.05) to each other. The other concentrations had a dose-dependent response. This acaricidal activity finds its justification because of the presence of phenolic acids and derivatives (Bravo-Ramos *et al.*, 2021). The phenolics have been reported to kill the ticks by their cytotoxic and membrane rupturing effects (Lima *et al.*, 2008). These findings are like Alimi *et al.* (2021), who reported similar results using essential oil and crude extracts of *Laurus nobilis*. Similar results have been reported by Shyma *et al.* (2014) but the Rosado-Aguilar *et al.* (2010) have reported that the change in extraction solvent results in a variation of results. (Nyahangare *et al.*, 2016) and Martinez-Velazquez *et al.* (2011) also agree that the change in extraction solvent affects the acaricidal efficacy of the plants. The difference in arguments can be justified that the phenolics extracted vary because of a change in extraction solvent and result in varying acaricidal activities. Though there is a variation in the ethanolic and ethyl acetate extracts of tobacco, they both can be potent because the extracted compounds may have similar mechanisms or the same mode of action.

In our study, similar results were obtained for other acaricidal parameters like egg hatchability, larval motility, reproductive index, reproductive efficiencies, and product effectiveness. The ethanolic and ethyl acetate extracts showed significantly higher efficiencies and 80% product effectiveness. These studies were in line with the studies conducted by Shyma *et al.* (2014) and Shyma *et al.* (2014).

The results of this experiment are coherent logically with the results of other studies. Multiple research papers also show that herbal extracts or compounds of phytochemical origin have mechanisms to kill the ticks. The phenolics in plants have cytotoxic effects which, upon ingestion kill the gut cells of ticks (Bravo-Ramos *et al.*, 2021). These phenolics get into the cells of the gut and stop ATP synthesis and can imbalance the osmotic potential of the cell, leading to necrosis and destruction. The pant active compounds have also been reported to reduce feed intake of ticks because of their anti-nutritional nature and reduce the feed intake of ticks. Several other mechanisms have also been including DNA damage of the ticks, cell cycle arrest, imperfect attachment to the host reduction in ovipositioning (Salman *et al.*, 2020). Tobacco plants have also been reported to be rich in phytochemicals, including phenolics (Al-Lahham *et al.*, 2020). So, these phenolics in tobacco can be the reason for the acaricidal activities presented in this research.

This research proves that the ethyl and ethanolic extracts of tobacco can control the ticks of genus *Hyalomma* because of their active ingredients in *in vitro* trials. Both the extracts showed significant acaricidal activities in respect to adult mortality, larval mortality, egg hatchability, product effectiveness, and reproductive index parameters.

**Conclusions:** This research concludes that ethanolic and ethyl acetate extracts of tobacco have similar acaricidal activity regarding adult mortality, larval mortality, product effectiveness and reproductive efficiencies. Both the ethanolic and ethyl acetate extracts have similar effects on all the parameters and their use for further research and commercial use is recommended at 10% concentrations. The 10% concentrations of the ethanolic and ethyl acetate extracts showed the maximum effects. This research concludes that the ethanolic and ethyl acetate extracts of the tobacco can be used as acaricidal agents at 10% concentration however *in vivo* studies and long-term clinical trials may give a clear picture.

**Acknowledgments:** Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R437), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the deanship of scientific research at King Khalid University for supporting this work Under the large group grant number (R.G.P2/227/44).

**Authors contribution:** EAl-N, RA, MSA designed the research idea and protocol. SN, AFEl-l, conducted the research and collected data., MAB and NAl-H, were actively involved in the supervision of research. All authors were involved in the data analysis and final write up of the manuscript.

## REFERENCES

Abbas RZ, Saeed Z, Bosco A, *et al.*, 2023. Botanical control of coccidiosis in ruminants. Pak J Agri Sci 60(4): 473-485.

Abubakar AR and Haque M, 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharma Biallie Sci 12(1):1.

Akpan GE, Ndukwu MC, Etim PJ, *et al.*, 2023. Food Safety and Agrochemicals: Risk Assessment and Food Security Implications, One Health Implications of Agrochemicals and their Sustainable Alternatives. Springer. pp:301-333

Al-Lahham S, Sbieh R, Jaradat N, *et al.*, 2020. Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the roots of Nicotiana tabacum L. Euro J Interog Med 33:101039.

Al-Snafi AE, 2022. Pharmacological and toxicological effects of Nicotiana tabacum. World J Adva Pharmaceut Med Res 3(01):006-018.

Alimi D, Hajri A, Jallouli S, *et al.*, 2021. In vitro acaricidal activity of essential oil and crude extracts of Laurus nobilis,(Lauraceae) grown in Tunisia, against arthropod ectoparasites of livestock and poultry: Hyalomma scupense and Dermanyssus gallinae. Vet Parasitol 298:109507.

Bava R, Castagna F, Palma E, *et al.*, 2022. Phytochemical Profile of Foeniculum vulgare Subsp. piperitum Essential Oils and Evaluation of Acaricidal Efficacy against Varroa destructor in Apis mellifera by In Vitro and Semi-Field Fumigation Tests. Vet Sci 9(12):684.

Bravo-Ramos JL, Flores-Primo A, Paniagua-Vega D, *et al.*, 2021. Acaricidal activity of the hexanic and hydroethanolic extracts of three medicinal plants against southern cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). Experim Appl Acarol 85(1):113-129.

De Rouck S, İnak E, Dermauw W, *et al.*, 2023. A review of the molecular mechanisms of acaricide resistance in mites and ticks. Insect Biochem Mol Biol 153:103981.

El-Mansi AA, Alhag SK, Al-Shuraym LA, *et al.*, 2023. Antiparasitic Activity of Methanolic and Ethyl acetate Extracts of Azadirachta indica against Haemonchus contortus. Pak Vet J 43(1): 199-203

Estrada-Peña A, Pfäffle MP and Petney TN, 2017. Genus Hyalomma Koch, 1844. Ticks of Europe and North Africa: a guide to species identification: pp. 343-348.

Figueiredo A, Agnolon IC, Lopes LG, *et al.*, 2018. Comparative study of hatching estimation methods of Rhipicephalus (Boophilus) microplus eggs. Vet Parasitol 264:35-38.

Fogaca AC, Sousa G, Pavanelo DB, *et al.*, 2021. Tick immune system: what is known, the interconnections, the gaps, and the challenges. Front Immunol 12:628054.

Jones KR and Garcia GW, 2021. Teaching of Veterinary Parasitology to University students in Trinidad, West Indies; are our local wildlife species neglected? Brazilian J Biol 83; e248493.

Lima RMF, Alvarez LDG, Costa MFD, *et al.*, 2008. Cytotoxic effects of catechol to neuroblastoma N2a cells. Gen Physio Biophys 27(4):306.

Martinez-Velazquez M, Castillo-Herrera GA, Rosario-Cruz R, *et al.*, 2011. Acaricidal effect and chemical composition of essential oils extracted from Cuminum cyminum, Pimenta dioica and Ocimum basilicum against the cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). Parasitol Res 108:481-487.

Najmi A, Javed SA, Al Bratty M, *et al.*, 2022. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules 27(2):349.

Nyahangare ET, Mvumi BM and Maramba T, 2016. Acute oral mammalian toxicity and effect of solvents on efficacy of Maerua edulis (Gilg. & Ben.) De wolf against Rhipicephalus (Boophilus) decoloratus Koch, 1844 (Acarina: Ixodidae), tick larvae. BioMed Res Int 2016: 1-8.

Ramzan M, Murtaza G, Sattar A, *et al.*, 2021. Techniques for managing ticks and tick-borne diseases under changing climate; A review. Egypt Acad J Biol Sci B Zool 13(1):117-128.

Ribeiro HS, Pereira DFS, Melo-Junior O, *et al.*, 2021. Vaccine approaches applied to controlling dog ticks. Tick Tick-born Dis 12(3):101631.

Rosado-Aguilar JA, Aguilar-Caballero A, Rodriguez-Vivas RI, *et al.*, 2010. Acaricidal activity of extracts from Petiveria alliacea (Phytolaccaceae) against the cattle tick, Rhipicephalus (Boophilus) microplus (Acari: ixodidae). Vet Parasitol 168(3-4):299-303.

Saeed Z and Alkheraije KA, 2023. Botanicals: A promising approach for controlling cecal coccidiosis in poultry. Front Vet Sci 10:1157633.

Salam AM, Lyles JT and Quave CL, 2019. Methods in the Extraction and Chemical Analysis of Medicinal Plants. Method Tech Ethnobiol Ethnoecol pp. 257-283.

Salman M, Abbas RZ, Israr M, *et al.*, 2020. Repellent and acaricidal activity of essential oils and their components against Rhipicephalus ticks in cattle. Vet Parasitol 283:109178.

Salman M, Abbas RZ, Khan MK, *et al.*, 2023. Acaricidal and Repellent Efficacy of Cinnamomum verum Essential Oil Against Rhipicephalus microplus Ticks. Pak J Zool 2023: 1-9.

Schulze TL, Eisen L, Russell K, *et al.*, 2023. Community-based integrated tick management programs: cost and feasibility scenarios. J Med Entomol 60(5):1048-1060.

Shyma KP, Gupta JP, Ghosh S, *et al.*, 2014. Acaricidal effect of herbal extracts against cattle tick Rhipicephalus (Boophilus) microplus using in vitro studies. Parasitol Res 113:1919-1926.

Zaman MA, Iqbal Z, Abbas RZ, *et al.*, 2012. In vitro and in vivo acaricidal activity of a herbal extract. Vet Paarsitol 186(3-4):431-436.