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

Eman A. Al-Nabati1, Ruoa Almahallawi2, Amal M. Alzahrani3, Nawal Al-Hoshani4*, Mariam S. Al-Ghamdi5, Sally Negm6, Attala F. El-Ikott7,8, Majed A. Bajaber9, Soliman M. Soliman10, Mohamed T. El-Saadony11

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


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 Alkheraifie, 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 Hyalomma 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:

\[
\text{Percent reproductive index} = \frac{\text{Mass of laid eggs}}{\text{Mass of adult females before treatment}}
\]
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:

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

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

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 Tukey’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 acaridical 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 acaridical 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>% concentration</th>
<th>Solvent</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
</table>
| 1.25   | EA 0±0         | 3.3±3.77 | 6.6±3.77 | 23.3±3.77 | 40±10 | 60±10 | 76.6±15.27 | 53±10 | 90±5
|       | Et 0±0         | 10±15.49 | 10±17.88 | 20±10 | 40±10 | 60±10 | 76.6±15.27 | 53±10 | 90±5
| 2.5    | EA 0±0         | 13.3±15.77 | 16.6±5.77 | 26.6±4.71 | 5.6±5.77 | 60±10 | 76.6±15.27 | 53±10 | 90±5
|       | Et 0±0         | 13.3±15.27 | 20±10 | 40±10 | 60±10 | 90±10 | 96.6±5.77 | 90±5
| 5      | EA 0±0         | 36.6±5.77 | 46.6±5.77 | 56.6±5.77 | 90±10 | 100±0 | 60±10 | 90±5
|       | Et 0±0         | 40±10 | 60±10 | 90±10 | 100±0 | 60±10 | 76.6±6.77 | 90±5
| 10     | EA 0±0         | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77
|       | Et 0±0         | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77
| Positive control | 0±0       | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77
| Negative control | 0±0       | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77 | 93.3±5.77

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

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Solvent</th>
<th>Laval mortality (%) with time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>Et</td>
<td>100±0a</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>100±0a</td>
</tr>
<tr>
<td>2.5</td>
<td>Et</td>
<td>96.66±5.77bcd</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>96.66±5.77bcd</td>
</tr>
<tr>
<td>5</td>
<td>Et</td>
<td>96.66±5.77ab</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>96.66±5.77ab</td>
</tr>
<tr>
<td>10</td>
<td>Et</td>
<td>96.66±5.77ab</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>96.66±5.77ab</td>
</tr>
</tbody>
</table>

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 (EA) extracts of tobacco leaves.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Solvents</th>
<th>Eggs hatched (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>Ethanol</td>
<td>67.3±9.96</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>63.5±5.93</td>
</tr>
<tr>
<td>2.5</td>
<td>Ethanol</td>
<td>57.1±5.72</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>45.7±5.6</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>37.9±3.22</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>33.8±5.05</td>
</tr>
<tr>
<td>10</td>
<td>Ethanol</td>
<td>25.3±3.45</td>
</tr>
<tr>
<td></td>
<td>EA</td>
<td>23.9±1.28</td>
</tr>
</tbody>
</table>

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 feeding (Lima 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 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: EAI-N, RA, MSA designed the research idea and protocol. SN, AFEI-I, conducted the research and collected data., MAB and NAI-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

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.