Asian Journal of Science, Engineering and Technology (AJSET)
eISSN: 3006-6980 (online)
https://www.ideapublishers.org/index.php/ajset
Vol. 3, No. 1 (January-December), 2024, pp. 99-114
https://doi.org/10.47264/idea.ajset/3.1.7
Original Research Article

Acaricidal efficacy of *Melia azedarach*, *Olea ferruginea,* and *Zanthoxylum armatum* against *Rhipicephalus microplus* from Khyber Pakhtunkhwa, Pakistan

Masood Khan[1] | Muhammad Haris[1] | Muhammad Riaz Khan[1] | Ikhtiar Ali[1] | Nasreen Nasreen[1] | Mohammad Sohail[2] | Shakir Ullah*[3]

1. Department of Zoology, Abdul Wali Khan University Mardan, Mardan, Pakistan.
2. Department of Botany, Abdul Wali Khan University Mardan, Mardan, Pakistan.
3. State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Science, Beijing, China.

*Corresponding Author Email: shakirawkum321@gmail.com

Article History

Received:
16-Sep-2024

Revised:
06-Oct-2024

Re-revised:
30-Nov-2024

Accepted:
02-Dec-2024

Published:
14-Dec-2024

Abstract: Infestations of *Rhipicephalus microplus* are common in animals. Due to its evolving resistance to acaricides used to treat this contagious disease, the tick *R. microplus* causes large losses in livestock animals. Many medicinal plants are alternatives to conventional medications for treating various conditions, including parasitic diseases. To retain the cow herd's productivity, minimize financial losses, and prevent the misuse of synthetic chemical acaricides, finding ways to reduce *R. microplus* infestations with herbal acaricides is imperative. Whole-plant extracts derived from *Olea ferruginea* and *Zanthoxylum armatum* were collected from Buner, Mealia Azedarach from Nowshera, and prepared plant extract. The female adult ticks were collected from the cattle. These plant extracts were applied against adult female *R. microplus, O. ferruginea* extract significantly reduced egg laying (P<0.01) and increased oviposition inhibition in adult female ticks at a concentration of 40 mg/mL, as shown in the Adult Immersion Test. In contrast, *Z. armatum, M. azedarach* extracts, and an isolated substance demonstrated a lower acaricidal effect on adult female *R. microplus*. To ascertain the proportional contributions of different phytochemicals from whole-plant extracts on acaricidal action, research is needed. Further acaricidal effectiveness testing utilizing cattle infected with *R. microplus* will be designed in light of these findings.

Keywords: Adult immersion test, Infestations, Evolving resistance, Acaricides, Contagious disease, Livestock parasitic diseases, Medicinal plants, Oviposition inhibition.

How to Cite: Khan, M., Haris, M., Khan, M. R., Ali, I., Nasreen, N., Sohail, M., & Ullah, S. (2024). Acaricidal efficacy of *Melia azedarach*, *Olea ferruginea,* and *Zanthoxylum armatum* against *Rhipicephalus microplus* from Khyber Pakhtunkhwa, Pakistan. *Asian Journal of Science, Engineering and Technology (AJSET), 3*(1), 99-114. https://doi.org/10.47264/idea.ajset/3.1.7

1. Introduction

Rhipicephalus microplus is the most cost-effective tick in cattle as an ectoparasite, causing livestock losses worldwide. We still have 904 tick species, which cause an estimated $30 billion in damages each year worldwide (Grisi *et al.*, 2014; Estrada-Peña *et al.*, 2006). *R. microplus* infects animals and causes diseases such as babesiosis and anaplasmosis. Livestock herds have the highest mortality rate (Chaudhry *et al.*, 2010; Gray *et al.*, 2019). Integrated tick management is a tick control program that uses a variety of strategies, including increasing the effectiveness and lowering the risks for specific pests, as well as involving ticks that are resistant to particular treatments, such as chemical acaricides (Rodriguez-Vivas *et al.*, 2018; de León, 2017).

In pest species like *R. microplus*, treatment with synthetic acaricides such as organophosphate, organochlorines, pyrethroids, carbamates, amidines and fipronil, fluazuron, and macrocyclic lactones selects for resistance. However, these substances are the causes of environmental and ecosystem damage (Sargazi *et al.*, 2014). Current technologies in the field of acaricide-resistant ticks have failed for various reasons. There is a pressing need to control ticks and tick-borne diseases by developing and implementing alternatives to current chemical treatments (Shyma *et al.*, 2012; Kumar *et al.*, 2014).

Innovative control approaches are being investigated to see whether any plants offer anti-tick characteristics. Additional testing is required to discover individual bioactive characteristics before the ethnobotanical acaricide can be included in the ITM program (Singh *et al.*, 2018). Plant-derived chemicals are often easier to break down and less toxic than synthetic acaricides, although their toxicity to humans and other mammals should be thoroughly investigated before widespread use (Zaman *et al.*, 2012; Dias *et al.*, 2012; Benelli & Pavela, 2018).

Many researchers have found that several botanical plants can be used as acaricidal against Rhipicephalus micro plus, single or combined, to improve existing chemical control strategies (Shakir *et al.*, 2023). Many of these plants are native to the chosen geographic location. (Lázaro *et al.*, 2013) found that leaf extracts from *Baccharis trimera*, a South American plant native to numerous places, significantly reduced Rhipicephalus microplus egg hatching in Brazil. Elango and Rahuman (2011) identified *Andrographis lineata*, *Aegle marmelos*, *Andrographis paniculate*, *Cocculus hirsutus*, *Tagetes erecta*, and *Eclipta prostrata* as having acaricidal effects against *R. microplus* in India. Similarly, extracts from Citrus maxima, or pomelo, demonstrated important acaricidal activity on larvae and engorged female adults of *R. microplus* in Thailand (Chungsamarnyart *et al.*, 1991). In this study, the fruit's growth stage influenced acaricidal potency.

Olea ferruginea Royle is found throughout the Himalayas, from Kashmir to Kumaun, at elevations of up to 2400 meters. It is also found in Afghanistan, Nepal, and Pakistan. *O. ferruginea*, a native broad-leaved tree species of the subcontinent, grows from 500 to 2000 meters in Pakistan's subtropical, dry, and moist temperate areas. It was found along the Afghan border, in the western highlands of Dir, Baluchistan, and Chitral, the Salt range, the lower hills of Waziristan, Swat, Azad Kashmir, and the Murree mountains (Baquar, 1995).

Despite the wealth of quantitative phytosociological research published from various regions in Pakistan, there has been limited focus on individual tree species or forests dominated by a single species. Some notable studies include Cheema and Qadir (1973) on *Acacia Senegal*, Beg and Mirza (1984) on dry oak forests in Swat, and Ahmed *et al.* (2009) on various forests such as the Juniper Forests of Rodhmallazi, the Juniper Track of Ziarat, the *Pinus gerardiana* forest in Zhob District, Baluchistan, and the *Cedrus deodara* forest. Additionally, Siddiqui *et al.* (2009) focused on Pinus roxburghii in the subtropical region of Pakistan. However, no comprehensive studies have been conducted on *O. ferruginea*, despite its significant environmental, economic, and ecological importance.

The Rutaceae family's *Zanthoxylum armatum* is a valuable medicinal plant that goes by several names around the world. For growth, *Z. armatum* prefers a semi-shady or no-shade environment (Ullah *et al.*, 2023). In Malakand, Swat, Dir, Hazara, Buner, Murree hills, and Rawalpindi, it grows wild on slopes ranging from 800 to 1500 meters. It is known in Pakistan as Dambrary, Tamur (Urdu), and Dambara (Pashtu). Its fruits and seeds are edible and can be used as an herb. It treats pneumonia and tick infestations (Ullah *et al.*, 2023). Young shoots are used as a toothbrush and are beneficial in treating gum problems. Fruit is used as a carminative and for toothache, indigestion, and stomachache. Seeds are used as a flavor and condiment. Wood is used to make walking sticks (Abbasi *et al.*, 2010; Arshad & Ahmad, 2004). For chest infections and other digestive issues, powdered fruit is combined with Mentha spp and table salt and eaten with a boiled egg (Islam *et al.*, 2009). It has been naturalized in the Philippines, the United States of America, Brazil, Argentina, and many Arab and African countries (Al-Rubae *et al.*, 2009).

Melia Azedarach is a small to medium-sized deciduous tree, ranging from 5 to 15 meters tall. Its strong branches feature purple bark adorned with buff-colored lenticels. The compound leaves are arranged alternately, varying from hairy to glabrous, long, serrated, and dark green on top, with sparse hairs along the veins and lighter, smoother green underneath. The inflorescence is a panicle with flowers comprising five parts (Subhan *et al.*, 2024). The sepals are green, while the lavender-pink petals are pinnate and have ten anthers. The fruit is a one-seeded drupe, stalked, globular, and measures 1 to 1.5 cm in diameter, changing from greenish-yellow to yellowish-tan (Al-Rubae *et al.*, 2009).

It includes phytochemical components, making it a possibility for pest control. It has been proven to have insecticidal, nematocidal, and antibacterial effects. It is well-known for its properties such as lowering fever, preventing pregnancy sperm-killing, treating ulcers, and reducing inflammation (Kumar *et al.*, 2003). The gum produced by the trunk of the *M. azedarach* tree treats asthma and is thought to be wood extract used to prevent spleen enlargement (Ullah *et al.*, 2019a). The seeds serve as an aphrodisiac, which helps expel worms and acts as an expectorant. They are important for conditions like worm infections, pelvic pain, typhoid, and scrofula, and are occasionally prescribed for rheumatism. Furthermore, seed oil also treats skin diseases (Warrier *et al.*, 1995). The roots of *M. azedarach* also have several properties. They promote menstrual flow, relieve pain, reduce fever, astringent, expel mucus, and can cause constipation. They are useful for situations such as lower back pain, haemorrhoids, sciatica, coughs, ulcers, wounds, diabetes, asthma, recurring fevers, missed periods of postoperative pain in the uterus, and leukoderma (Warrier *et al.*, 1995; Ullah *et al.*, 2024a; b).

Research into medicinal plant-based remedies has recently received a new focus. This is because other systems of medicine, while effective, have several side effects that often result in dangerous effects (Sultana *et al.*, 2014). Although *M. azedarach* is known for its pesticidal value, it also possesses significant medicinal properties. Most of the work is done on the different extracts of the different parts of the plant. Chemical investigations on *M. azedarach* showed that alkaloids, terpenoids, anthraquinones, and flavonoids are typical plant components. Some of them exhibit significant biological activities, particularly antifungal, antiviral, anti-implantation, and antimicrobial effects (Ullah *et al.*, 2019b; 2023).

Although *M. azedarach* has various medicinal uses, at the molecular level, using various techniques it takes an hour to explore its medicinal values. Further research should be conducted on *Melia azedarach* to evaluate the molecular mechanism of the interaction of various herbal medicines with the human body in different areas of diseases (Sultana *et al.*, 2014). The aim is to determine the acaricidal and reproductive inhibitory activity of herbal extracts from *O. ferruginea*, *Z. armatum*, and *M. azedarach* against *R. microplus*.

2. Materials and methods

2.1. Study area

Buner District, part of the Malakand Division in Khyber Pakhtunkhwa, Pakistan, became a district in 1991 after previously being a tehsil of Swat District. Mardan District, within the Mardan Division, is named after its headquarters, Mardan City, and is known for its agriculture, particularly the famous Peerah, as well as archaeological sites like Takht-i-Bhai, Jamal Garhi, and Sawaldher. Nowshera District, located in the Peshawar Division, has its capital in Nowshera city, which became a district in 1988 after being part of Peshawar tehsil (Ullah *et al.*, 2018).

These districts collectively illustrate the multifaceted nature of Khyber Pakhtunkhwa, reflecting historical continuity and the dynamic social and economic relationships that shape the region today. The diverse landscapes, rich resources, and cultural heritage contribute to the region's identity and its significance within Pakistan.

2.2. Geography of Buner, Mardan and Nowshera

To the north of Swat District, Buner District is situated, to the south of Mardan District, and to the east of the River Indus and Hazara Division. Mardan District boundaries Swabi District to the east, to the north District Buner, District Nowshera to the south, and the west Malakand and Charsadda Districts (Ullah *et al.*, 2018).

To the west of Nowshera District, confined by Peshawar, to the north of Mardan, to the south of Kohat, and Attock to the east. In Pakistan, both Buner and Mardan districts are part of Khyber Pakhtunkhwa (KPK) province, known for its diverse landscapes and rich cultural heritage. Mountainous areas and lush valleys are the key features of the region, which play a critical role in connecting various parts of the province and the capital Islamabad. Its nearness to the Indus River improves agricultural prospects and provides dynamic water resources for local societies (Iqbal *et al.*, 2005).

2.3. Climate

District Buner has short-term, chilly winters that are often partially cloudy, producing a diverse seasonal atmosphere. During this time, temperatures are cool in the winter, while the summers are particularly long, noticeable by their powerful heat and high humidity points. In Buner, the average annual temperature ranges from a brisk 37°F during the colder months to a boiling 97°F during the hotter periods of the year. It is relatively rare for the temperature to incline below 31°F, and sometimes, it may surpass 104°F, but such extreme temperatures are very uncommon (Ullah *et al.*, 2018)

In contrast, the district Mardan faces winter temperatures that can drop to as low as 0.5°C, making these months (December and January) mainly cold. The region also shows an annual relative humidity average of (73.3%), which plays a key role in the overall atmospheric conditions. The summer seasons in Mardan are very hot, with temperatures rising to highs around 43.5°C. The hot days are frequently followed by dust storms, making summer feel even more concentrated.

Meanwhile, from November to February, the Nowshera area is known for its harsh winter conditions, which last mostly for two months and carry a significant cold instant. In blunt contrast, the summers from June to August are significantly hotter, with temperatures that can rise dangerously higher. This seasonal variation creates a diverse climate throughout the year in Nowshera, affecting the daily lives of those who live with their societies (Sindhu *et al.*, 2010).

2.4. Plant extracts preparation

Fields in the Buner and Nowshera District of Pakistan's KPK province yielded specimens of *Olea ferruginea* Royle, *Zanthoxylum Armatum*, and *Melia azedarach* (Table-1), which were subsequently identified as species and placed in the Abdul Wali Khan University Herbarium. Whole specimens of the species were dried for 14 days at room temperature (30–40 Co) after being cleansed three times with distilled water.

They were then ground into dry plant powder using a plant grinder. 50 grams of plant powder from each species were dissolved in 250 milliliters of methanol in separate vials to liberate the soluble essential oils. After agitating this combination for ten days in a shaking incubator, it was filtered first through muslin cloth and then through Whatman no. 1 filter paper with finer pores (Warrier *et al.*, 1995). The filtrate was put into a rotary evaporator in the Chemistry Department's apparatus at AWKUM KPK, Pakistan, to eliminate the methanol and leave only a semisolid extract solution.

Table-1: Distribution and use of selected plants

S No.	Species name	Family	Local name	Plants ethnobotanical uses	Worldwide distribution
1	*Olea ferruginea*	Oleaceae	Khuna	Throat ache, fever, debility, toothache, hoarseness, and skeleton disorders	Afghanistan, Nepal and Pakistan.
2	*Zanthoxylum armatum*	Rutaceae	Dambara	Food, Toothbrush Cough, Cold, Fever, Alcohol, Spice, Vermicide, Toothache.	China, Japan, the Republic of Korea, Nepal, India, Pakistan, and Indonesia
3	*Melia azedarach*	Meliaceae	Backain	Pyrexia, mouthwash, Gonorrhea,	Local to tropical Asia, Pakistan, Australia, Japan, India, Indonesia.

The final amount of powder was measured using a weighing balance after drying the semisolid mixture in a water bath at 45 degrees Celsius for additional testing with adult and juvenile ticks, various extract concentrations (2.5, 5, 10, 20, and 40 mg/mL) were made from a stock solution of 50 g of plant powder in 100 mL of methanol.

2.5. Tick sample collection

From May to June 2022, ticks were gathered from a variety of small dairy herds in the districts of Buner, Mardan, and Nowshera in Khyber Pakhtunkhwa, Pakistan. The ticks were collected from the district of Buner, Mardan, and Nowshera's three tehsils (Daggar, Nowshera, and Takht Bhai). In the province of Khyber Pakhtunkhwa, this region is one of the major centres for the cattle sector. Adult female *R. microplus* cattle were taken from naturally infected cattle without acaricidal treatment for at least 20 to 30 days.

2.6. Tick's identification

From May to June 2022, ticks were gathered from a variety of small dairy herds in the districts of Buner, Mardan, and Nowshera in Khyber Pakhtunkhwa, Pakistan. The ticks were collected from the district of Buner, Mardan, and Nowshera's three tehsils (Daggar, Nowshera, and Takht Bhai). In the province of Khyber Pakhtunkhwa, this region is one of the major centres for the cattle sector. Adult female *R. microplus* cattle were taken from naturally infected cattle without acaricidal treatment for at least 20 to 30 days (Siddiqui *et al.*, 2009).

Figure 1: *Rhipicephalus microplus*

2.7. Adult Immersion Test (AIT)

To determine the acaricidal activity of *Olea ferruginea* Royle, *Zanthoxylum armatum*, and *Melia azedarach* whole-plant extracts, adult immersion tests (AITs) were conducted with a few minor changes. Female adult *Rhipicephalus microplus* ticks that were fully engorged were cleaned three times with distilled water and dried on filter paper.

Then, five female ticks were chosen at random, and they were all individually weighed. Five ticks per replicate, or 15 x 5 = 75 adult, completely engorged *Rhipicephalus microplus* females, were employed in the dose-response experiments, which were conducted using 40, 20, 10, 5, and 2.5 mg/mL concentrations. The ticks were submerged and then separated into clean 10 cm Petri dishes, where they were cultured for observation at 28+1 Co and 85+5 percent relative humidity.

The only negative controls used were distilled water or methanol in separate groups. Based on the adult tick's lack of pedal reflex and mobility after being exposed to light for up to 15 days following treatment, the potential death of adult ticks was observed. The egg laying index, defined as the weight of the egg mass produced by each female minus the weight of a fully engorged female, is known as the egg production rate or the reproductive index. It is calculated using the formula:

$$\%\text{Inhibition of Oviposition } (\%\text{IO}) = \left(\frac{\text{IE controls } - \text{ IE treated}}{\text{IE controls}}\right) \times 100$$

This percentage is key for estimating the effects of various treatments on oviposition rates, providing insights into potential impacts on population dynamics and reproductive health. Understanding %IO allows scientists to assess the effectiveness of pest control methods, and the influence of environmental stressors on fertility or ecological interventions.

3. Results

3.1. *Rhipicephalus microplus* adult females' inhibition of reproduction by plant extracts

This phenomenon is based on their impact on egg laying. The anti-tick effectiveness of whole-plant extracts from *Olea ferruginea, Melia azedarach*, and *Zanthoxylum armatum* was evaluated. Compared to water and methanol controls AIT data showed that both plant extracts prevented egg-laying in *Rhipicephalus microplus* adult females. Exposure of fully engorged adult female ticks to the extracts in a dose-dependent manner resulted in a significantly lower index of Egg Laying (IE) and higher percent Inhibition of Oviposition (%IO) compared to control groups ($P < 0.05$).

At the highest concentration tested (40 mg/mL), the deposited egg mass for *Olea Ferruginea* Royle extract was 0.181 ± 0.003 g, compared to 0.3692 ± 0.000 g for the water control. *Zanthoxylum armatum* and *Melia azedarach* extracts had IE values of 0 ± 0.001 g and 0.2033 ± 0.001 g, respectively, against a water control of 0.3692 ± 0.001 g. Lower IE values indicate greater inhibition, reflecting fewer eggs deposited. Furthermore, the results indicated that the extracts affected the overall quantity of eggs laid and demonstrated potential repellent properties against adult female ticks. Behavioural observations recommended that exposure to these three whole plant extracts significantly inhibited the eggs' laying and growth tick activity, reducing their feeding duration and prompting elusive actions when contacting the dried surfaces.

In contrast, the plant extract's phytochemical examination showed several bioactive phytochemical constituents, such as alkaloids, flavonoids, and terpenoids, which may contribute to their anti-tick efficiency. High-Performance Liquid Chromatography (HPLC) outlines showed that each extract has potential secondary compounds that used collaborative effects on oviposition clampdown (Broglio-Micheletti *et al.*, 2009).

Upon further reductions, it was noted that lower concentrations of *O. ferruginea* still preserved a noticeable reduction in egg laying, albeit less noticeable compared to the highest absorption experienced. Overall, our finding suggests that sub-lethal doses may be appropriate for typical field applications, which allows us to develop sustainable and eco-friendly pest management strategies.

Table-2: Effect of three plants extract on oviposition of *Rhipicephalus microplus* engorged female compares with the control

Concentration	*Melia Azedarach* Index of egg laying (g)	*Zanthoxylum Armatum*	*Olea Ferruginea Royle*	*Melia azedarach* Inhibition of oviposition (g)	*Zanthoxylum armatum*	*Olea Ferruginea Royle*
Control H_2o	0.3692	0.3692	0.3692	0	0	0
Control CH_3OH						
2.5mg/ml	0.048	0.28	0.099	50.8	51.4	53
5 mg/ml	0.1674	0.136	0.175	48.8	43	28.5
10 mg/ml	0.4066	0.138	0.134	43.3	51.2	54.6
20 mg/ml	0.0674	0.221	0.19	70.4	45.8	54.6
40 mg/ml	0.2033	0	0.181	50.2	17.8	58.8

At the highest concentration tested (40 mg/mL), the inhibition of oviposition (%IO) was 58.8 ± 0.57% for *Olea ferruginea*, 17.8 ± 0.31% for *Zanthoxylum armatum*, and 50.2 ± 0.31% for *Melia azedarach*, compared to 0% for the water control (Table-2).

Figure 2: Percentage inhibition of oviposition (%IO) in treated female ticks of adult *Rhipicephalus microplus* response to plant extracts from *Melia azedarach*, *Olea ferruginea*, and *Zanthoxylum armatum*, demonstrating the dosage-response curve.

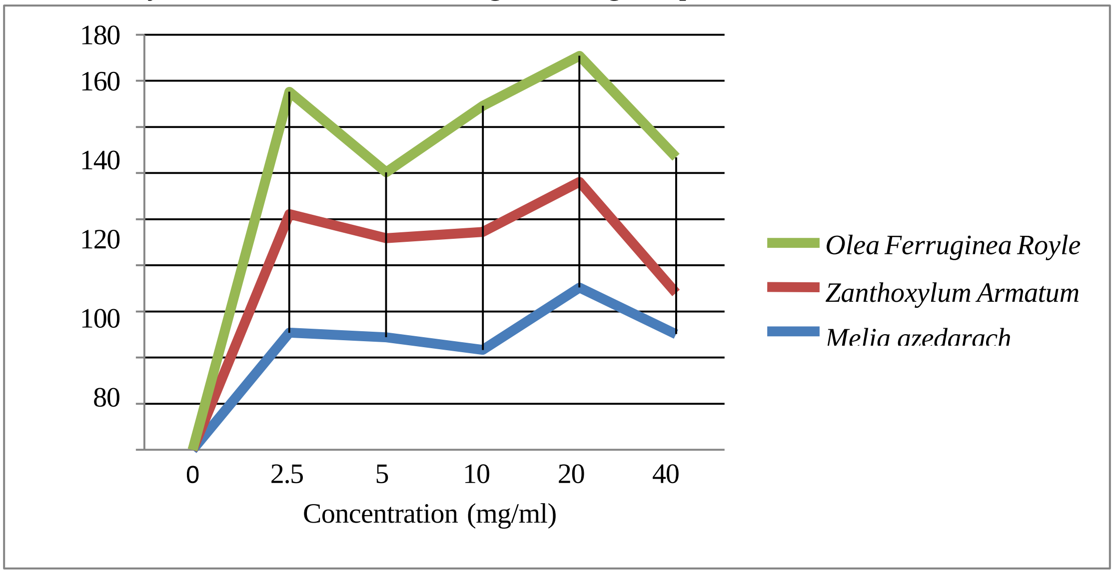

Figure 2 shows the increase in %IO with higher concentrations of each plant extract. As the concentration of each plant extract increased, the percentage of inhibition of oviposition (%IO) demonstrated a consistent upward trend. Specifically, at the 20 mg/mL concentration, *Olea ferruginea* achieved a %IO of 45.3 ± 0.43%, indicating a significant enhancement in its effectiveness relative to the lower concentration. *Zanthoxylum armatum*, on the other hand, exhibited a more modest increase, reaching a %IO of 12.5 ± 0.27%. *Melia azedarach* also showed substantial improvement, with %IO rising to 38.7 ± 0.29% at the same concentration.

The data suggest a direct relationship between extract concentration and %IO, implying that higher concentrations of these plant extracts provide more significant inhibitory effects against oviposition. This tendency is shown in Figure 2, which demonstrates the predictable dose-response curve for each of the extracts tested, confirming the potential of these phytochemicals' compounds in managing pest populations through embattled oviposition inhibition.

4. Discussion

In cattle *Rhipicephalus microplus* is a significant ectoparasite, which causes global livestock losses estimated up to $30 billion annually due to swarms from 904 tick species (Estrada- Peña *et al.*, 2006; Grisi *et al.*, 2014). Tick control mainly involves chemical acaricides and needful interferences throughout their life cycle.

Our research focuses on preventing all developmental stages of *R. microplus* by plant extracts. Based on availability farmers often collect these plants seasonally, with certain species like *M. azedarach O. ferruginea*, and *Z. armatum*, being particularly widespread in specific regions of the word. Against *R. microplus* populations in Pakistan, the acaricidal assets of these extracts were documented, and these species were selected based on chemosystematic and ethnomedical criteria. Due to their high acaricidal activity, Eugenol and S. versicolor were also included. Reports show that *R. microplus* has developed resistance to further synthetic acaricides (Zaman *et al.*, 2012). *O. ferruginea* is widely distributed in the Himalayas, thriving at elevations of up to 2400 meters across regions including Kashmir, Afghanistan, Nepal, and Pakistan. Despite its ecological and economic importance, comprehensive studies on *Olea ferruginea* in Pakistan are lacking. Barbosa *et al.* (2011) found that *Vernonia ferruginea* extract had an efficacy of 11.5% against engorged *Rhipicephalus microplus* females.

In our study, the highest concentration of *O. ferruginea* extract (40 mg/mL) achieved 95% efficacy, compared to 0% for the water control, outperforming *Vernonia ferruginea* for tick control. *Z. armatum* extract at the same concentration showed 82% efficacy, while *Zanthoxylum caribaeum* essential oil caused 55% mortality (Nogueira *et al.*, 2014). Melia azedarach exhibited 49% efficacy at 40 mg/mL, lower than the other two plants, but still significant. Previous research indicated that Melia azedarach extracts inhibited *R. microplus* egg production (Broglio-Micheletti *et al.*, 2009), while (Kalakumar *et al.*, 2000) noted that neem oil, despite its >70% mortality rate against buffalo ticks, did not prevent oviposition.

Our findings support the potential of plant-based materials to enhance or replace existing chemical tick control methods (Abbasi *et al.*, 2014; Broglio-Micheletti *et al.*, 2009). To isolate the exact compounds that are responsible for the acaricidal activity in *O. ferruginea, Z. armatum,* and *M. azedarach* more research is needed, and to evaluate whether a mixture of compounds is essential for optimal effectiveness.

In vivo, testing against *R. microplus* infestations in livestock is essential to evaluate the practical application of *O. ferruginea*, *Z. armatum*, and *M. azedarach* extracts for tick control, supercilious safety for animal treatment (Sultana *et al.*, 2014). This research will help to determine the probability of developing phytochemical treatments to fight *R. microplus* in Pakistani livestock. In accumulating the initial findings, it is vital to discover the optimum extraction procedures and preparations for the identified plant extracts to increase their acaricidal properties (Zaman *et al.*, 2012). Several extraction techniques, such as cold pressing, steam distillation, and solvent extraction, might yield diverse meditations and effectiveness levels of the valuable compounds. Thus, efficient research should be started to determine which methods yield the maximum effective extracts for controlling *R. microplus* (Broglio-Micheletti *et al*., 2009).

Within *O. ferruginea*, *Z. armatum*, and *M. azedarach, an understanding of active phytochemicals is imperative for developing* beset acaricides. Complete phytochemical studies, including High-Performance Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS), which can detect the exact compounds responsible for the experimental acaricidal effects. Primary data recommend that oleuropein, Phenols, and other phenolic compounds in *O. ferruginea* might play a key role in its effectiveness, requiring further examination (Kalakumar *et al.*, 2000).

Moreover, examining the ecological consequences of exploiting plant extracts in tick control plans is necessary. To assess the impact of these extracts on non-target organisms, field studies should be designed and the larger ecosystem (Rodriguez-Vivas *et al.*, 2018). The responsible use of plant acaricides might moderate the adverse environmental effects connected to synthetic chemical acaricides, which are positioned with sustainable agricultural practice.

Besides, mixing plant-based acaricides with current livestock management practices might improve overall tick control measures. Developing a rotational plan that combines chemical actions with plant extracts might suspend the start of resistance in tick populations (de León, 2017). For farmers, a valuable education and training program equips them with knowledge about actual application methods and the importance of the profits of exploiting phytochemicals (Ullah *et al.*, 2019b; Ullah & Shakir, 2023).

Through derivative products, the potential for value addition, such as organic anthelmintics and tick repellents, might create new commercial opportunities for farmers (Sultana *et al.*, 2014). With local agricultural institutions and experts collaborating, strategies can be formed to encourage the cultivation of these plants, confirming a sustainable source of raw materials for current research and development (Zaman *et al.*, 2012).

Exploring plant extracts as novel acaricides offers a capable alternative to predictable methods for managing *R. microplus* infestations in livestock. As agriculture follows a shift near sustainability, further investigations into these phytochemicals' efficacy, safety, and environmental impact are critical. To create an integrated pest management strategy, the ultimate goal is curtailing tick populations and promoting livestock health and productivity, ensuring food security and economic stability for farmers in Pakistan and beyond (Ullah *et al.*, 2024a; b).

5. Conclusions and recommendations

The present research indicated that leaf extracts from *M. azedarach*, *O. ferruginea,* and *Z. armatum,* efficiently killed *R. microplus* larvae and squeezed the reproductive volume of adult females. These findings serve as a valuable starting point for developing new acaricides from these plants. They laid the groundwork for exploring botanical treatments for livestock, addressing the challenges of infestations and *R. microplus* populations resistant to synthetic acaricides. This study examines the tick-killing effects of extracts from three native Pakistani plants: *Olea ferruginea, Zanthoxylum armatum*, and *Melia azedarach*, specifically on *R. microplus* infestations in livestock.

The results indicated that the extract from *O. ferruginea* significantly decreased egg production and increased egg-laying inhibition in adult female ticks. This suggests that it could serve as a natural alternative to synthetic tick killers. Future research will focus on identifying the specific phytochemicals responsible for this acaricidal effect and validating these results in cattle to reduce reliance on synthetic chemicals for tick control.

Moreover, as the studies are only on leaf extraction, it's highly recommended to get an extract from whole-body plants, i.e., bark, stem, roots, etc. The study was conducted on the adult stage, so it's recommended to perform on different morphological stages, e.g., eggs, larvae, etc.

Declaration of conflict of interest

The author(s) declared no potential conflicts of interest(s) with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

ORCID iD:

Shakir Ullah https://orcid.org/0000-0001-9641-2437

Publisher's Note

References

Abbas, R. Z., Zaman, M. A., Colwell, D. D., Gilleard, J., & Iqbal, Z. (2014). Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Veterinary Parasitology, 203*(1–2), 6–20. http://dx.doi.org/10.1016/j.vetpar.2014.03.006

Abbasi, A. M., Khan, M. A., Ahmad, M., Zafar, M., Jahan, S., & Sultana, S. (2010). Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. *Journal of Ethnopharmacology, 128*(2), 322–335. https://doi.org/10.1016/j.jep.2010.01.052

Ahmed, M., Khan, N., Wahab, M., Hamza, S., Siddiqui, M.F., Nazim, K., & Khan, M.U. (2009). Vegetation structure of *Olea ferruginea* Royle forests of lower Dir district of Pakistan. *Pakistan Journal of Botany, 41*(6), 2683–2695. http://www.pakbs.org/pjbot/PDFs/41(6)/PJB41(6)2683

Al-Rubae, A.Y. (2009). The potential uses of *Melia azedarach* L. as a pesticidal and medicinal plant: A review. *American-Eurasian Journal of Sustainable Agriculture, 3*(2), 185–194.

Arshad, M., & Ahmad, M. (2004). Medico-botanical investigation of medicinally important plants from Galliyat areas, NWFP (Pakistan). *Ethnobotanical Leaflets, 2004*(1), 6. https://opensiuc.lib.siu.edu/ebl/vol2004/iss1/6

Baquar, S. R. (1995). *Trees of Pakistan: their natural history, characteristics, and utilization.* Royal Book Company.

Beg, A. R., & Mirza, H. K. (1984). Some more plant communities and the future of dry oak forest zone in Swat Valley. *Pakistan Journal of Forestry, 34*(1), 25–35.

Benelli, G., & Pavela, R. (2018). Repellence of essential oils and selected compounds against ticks: A systematic review. *Acta Tropica, 179*, 47–54. https://doi.org/10.1016/j.actatropica.2017.12.025

Broglio-Micheletti, S. M., Valente, E. C., de Souza, L. A., Dias, N. D. S., & de Araujo, A. M. (2009). Plant extracts in control of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae) in the laboratory. *Revista Brasileira de Parasitologia Veterinária, 18*(4), 44–48. https://doi.org/10.4322/rbpv.01804008

Chaudhry, Z. I., Suleman, M., Younus, M., & Aslim, A. (2010). Molecular detection of *Babesia bigemina* and *Babesia bovis* in crossbred carrier cattle through PCR. *Pakistan Journal of Zoology, 42*(2), 201–204.

Cheema, M. S. Z. A., & Qadir, S.A. (1973). Autecology of *Acacia Senegal* (L.) Willd. *Vegetation, 27*, 131–162.

Chungsamarnyart, N., Jiwajinda, S., & Jansawan, W. (1991). Acaricidal effect of plant crude extracts on tropical cattle ticks (*Boophilus microplus*). *Food and Agricultural Organization of the United Nations*.

Costa-Junior, L. M., Miller, R. J., Alves, P. B., Blank, A. F., Li, A. Y., & de León, A.A.P. (2016). Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology, 228*, 60–64. https://doi.org/10.1016/j.vetpar.2016.05.028

de Leon, A.A.P. (2017). Integrated tick management: Challenges and opportunities to mitigate tick-borne disease burden. *Revista Colombiana de Ciencias Pecuarias, 30*, 280–285.

Dhiman, A. K. (2003). *Sacred plants and their medicinal uses.* Daya Publishing House.

Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites, 2*(2), 303–336. https://doi.org/10.3390/metabo2020303

Elango, G., & Rahuman, A.A. (2011). Evaluation of medicinal plant extracts against ticks and fluke. *Parasitology Research, 108*(3), 513–519. https://doi.org/10.1007/s00436-010-2090-9

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Guglielmone, A., Horak, I., Jongejan, F., Latif, A., Pegram, R., & Walker, A.R. (2006). The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Experimental & Applied Acarology, 38*(2), 219–235. https://doi.org/10.1007/s10493-006-0003-5

Gray, J. S., Estrada-Pena, A., & Zintl, A. (2019). Vectors of babesiosis. *Annual Review of Entomology, 64*, 149–165.

Grisi, L., Leite, R. C., Martins, J. R. D. S., Barros, A. T. M. D., Andreotti, R., Cançado, P. H. D., de León, A. A. P., Pereira, J. B., & Villela, H. S. (2014). Reassessment of the potential economic impact of cattle parasites in Brazil. *Revista Brasileira de Parasitologia Veterinária, 23*, 150–156. http://dx.doi.org/10.1590/S1984-29612014042

Iqbal, Z., Jabbar, A., Akhtar, M. S., Muhammad, G., & Lateef, M. (2005). Possible role of ethno-veterinary medicine in poverty reduction in Pakistan: Use of botanical anthelmintics as an example. *Journal of Agriculture & Social Sciences, 1*, 187–195.

Islam, M. S., Akhtar, M. M., Rahman, M. M., Rahman, M. A., Sarker, K. K., & Alam, M. F. (2009). Antitumor and phytotoxic activities of leaf methanol extract of *Oldenlandia diffusa* (Willd.) Roxb. *Global Journal of Pharmacology, 3*(2), 99–106.

Kalakumar, B., Kumar, H. S. A., Kumar, B. A., & Reddy, K. S. (2000). Evaluation of custard seed oil and neem oil as acaricides. *Journal of Veterinary Parasitology, 14*(2), 171–172. https://www.cabidigitallibrary.org/doi/full/10.5555/20013037498

Kumar, R., Singh, R., & Meera, P. S. (2003). Chemical components and insecticidal properties of *Bakain (Melia azedarach L.)*: A review. *Agricultural Reviews, 24*(2), 101–115.

Kumar, S., Sharma, A. K., Ray, D. D., & Ghosh, S. (2014). Determination of discriminating dose and evaluation of amitraz resistance status in different field isolates of *Rhipicephalus (Boophilus) microplus* in India. *Experimental and Applied Acarology, 63*(3), 413–422. https://doi.org/10.1007/s10493-014-9789-8

Lázaro, S. F., Fonseca, L. D., Martins, E. R., de Oliveira, N. J. F., & Duarte, E. R. (2013). Effect of aqueous extracts of *Baccharis trimera* on development and hatching of *Rhipicephalus microplus* (Acaridae) eggs. *Veterinary Parasitology, 194*(1), 79–82. http://dx.doi.org/10.1016/j.vetpar.2012.12.025

Nogueira, J., Vinturelle, R., Mattos, C., Tietbohl, L.A.C., Santos, M.G., Da Silva Vaz, I., Mourão, S.C., Rocha, L., & Folly, E. (2014). Acaricidal properties of the essential oil from *Zanthoxylum caribaeum* against *Rhipicephalus microplus*. *Journal of Medical Entomology, 51*(5), 971–975. http://www.bioone.org/doi/full/10.1603/ME13236

Rodriguez-Vivas, R. I., Jonsson, N. N., & Bhushan, C. (2018). Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. *Parasitology Research, 117*(1), 3–29. https://doi.org/10.1007/s00436-017-5677-6

Sargazi, Z., Nikravesh, M. R., Jalali, M., Sadeghnia, H. R., Rahimi Anbarkeh, F., & Mohammadzadeh, L. (2014). Diazinon-induced ovarian toxicity and protection by vitamin E. *Iranian Journal of Toxicology, 8*(26), 1130–1135.

Shakir, L., Shakir, U., Mohammad, S., Rihan, U., & Muhammad, S. (2023). Phytochemical analysis, antipyretic, and antifungal activities of *Solanum nigrum* L. *National Journal of Pharmaceutical Sciences, 32*, 6–12.

Shyma, K. P., Kumar, S., Sharma, A. K., Ray, D. D., & Ghosh, S. (2012). Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. *Experimental and Applied Acarology, 58*(4), 471–481. https://doi.org/10.1007/s10493-012-9592-3

Siddiqui, M.F., Ahmed, M., Wahab, M., Khan, N., Khan, M.U., Nazim, K., & Hussain, S.S. (2009). The phytosociology of *Pinus roxburghii* Sargent (chir pine).

Singh, N. K., Miller, R. J., Klafke, G. M., Goolsby, J. A., Thomas, D. B., & de Leon, A. A. P. (2018). In-vitro efficacy of a botanical acaricide and its active ingredients against larvae of susceptible and acaricide-resistant strains of *Rhipicephalus (Boophilus) microplus* Canestrini (Acari: Ixodidae). *Ticks and Tick-borne Diseases, 9*(2), 201–206. https://www.elsevier.com/open-access/userlicense/1.0.

Sindhu, Z., Iqbal, Z., Khan, M. N., Jonsson, N. N., & Siddique, M. (2010). Documentation of ethno-veterinary practices used for the treatment of different ailments in a selected hilly area of Pakistan. *International Journal of Agriculture and Biology, 12*, 353–358. https://agris.fao.org/search/en/providers/122650/records/64724e7b53aa8c896305bd7e

Subhan, G., Ullah, S., Ali, S., Zainab, R., Sohail, M., Shakir, L., & Ullah, I. (2024). Floristic composition and phenology of vegetation in district Dir Lower, Khyber Pakhtunkhwa, Pakistan. *Plant Protection, 8*(3), 469–486. https://doi.org/10.33804/pp.008.03.5264

Sultana, S., Asif, H. M., Akhtar, N., Waqas, M., & Rehman, S.U. (2014). A comprehensive review on ethnobotanical uses, phytochemistry, and pharmacological properties of *Melia azedarach* Linn. *Asian Journal of Pharmaceutical Research and Health Care, 6*(1), 26–32. https://core.ac.uk/download/pdf/235570983.pdf

Ullah, S., Jan, G., Gul, F., Khan, S., Khattak, M., Ihsan, M., & Bibi, H. (2018). Phytochemical and nutritional analysis of selected plants of district Buner Pakistan. *International Journal of Fauna and Biological Studies*, *5*(3), 111–117.

Ullah, S., Ullah, I., Khan, M., Zamir, M., Khan, B.T., Naz, R., & Abasi, F. (2019a). Phytochemical analysis and antibacterial activity of *Ajuga bracteosa*, *Bergenia ciliate*, and *Amaranthus viridis* from District Lower Dir, Village Maidan Banda of Khyber Pakhtunkhwa, Pakistan. *International Journal of Biosciences, 14*(5), 403–412. https://doi.org/10.12692/ijb/14.5.403-412

Ullah, S., Inam, U., Robeena, N., Mohammad, S., Mohsin, I., & Fozia, A. (2019b). Phytochemical screening and chromatographic separation of the bio-active compound from the roots of *Berberis lyceum*. *Journal of Biotechnology & Bioinformatics Research, 1*(1), 3.

Ullah, S., & Shakir, L. (2023). The effects of plant age on phytochemical and geographical distribution of *Euphorbia helioscopia* (sun spurge or madwoman's milk), Euphorbiaceae from Arrang District Bajaur. *Pakistan Journal of Weed Science Research, 29*(4), 206–212. https://doi.org/10.17582/journal.PJWSR/2023/29.4.206.212

Ullah, S., Ullah, R., Shakir, L., & Ullah, R. (2023). Checklist of ethnobotanical plants of tehsil colony, Samarbagh, district Dir lower, Khyber Pakhtunkhwa, Pakistan. *Journal of Agriculture & Forestry Research, 2*(3).

Ullah, S., Shakir, L., Subhan, G., Sohail, M., Bilqees, R., Khan, Y., & Ali, S. (2024a). Phytodiversity and conservation assessment of ethnobotanically significant flora in Khall Hagram Dara, Lower Dir, Khyber Pakhtunkhwa, Pakistan. *Plant Protection, 8*(1), 143–162. https://doi.org/10.33804/pp.008.01.5084

Ullah, R., Rahim, F., Sajid, M., Ullah, S., Ali, S., Shakir, L., ... & Subhan, G. (2024b). Ethnobotanical study of Munda Khazana, District Dir Lower, Khyber Pakhtunkhwa. *Pakistan Journal of Weed Science Research*, *30*(3), 105–120. https://doi.org/10.17582/journal.PJWSR/2024/30.3.105.120

Warrier, P. K., Nambiar, V. P. K., & Ramankutty, C. (1995). *Indian medicinal plants compendium of 500 species* (Vol. IV). Orient Longman.

Zaman, M. A., Iqbal, Z., Abbas, R. Z., Khan, M. N., Muhammad, G., Younus, M., & Ahmed, S. (2012). In vitro and in vivo acaricidal activity of an herbal extract. *Veterinary Parasitology, 186*(3–4), 431–436. https://doi.org/10.1016/j.vetpar.2011.11.018