Bindura University of Science Education

COMMITMENT DISCIPLINE B U S E EXCELLENCE

FACULTY OF SCIENCE AND ENGINEERING

DEPARTMENT OF BIOLOGICAL SCIENCES

Evaluating the individual and combined acaricidal activity of *Azadirachta indica* (Neem), *Cymbopogon citratus*(Lemongrass) and *Tagetes erecta* (Marigold) extracts against several tick species.

BY

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biotechnology.

June 2026

APPROVAL FORM

The undersigned certify that they have read the dissertation titled **" Evaluating the individual and combined acaricidal activity of *Azadirachta indica* (Neem), *Cymbopogon citratus*(Lemongrass) and *Tagetes erecta* (Marigold) extracts against several tick species"** and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science, for assessment.

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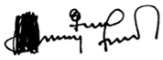
Signature of Supervisor  Date: 15 June 2026

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I, Venus M Mafusire (B223081B), declare that this research herein is my own work and has not been plagiarised from any other source(s) without acknowledgement of the concerned author(s). Where the work of others has been consulted, it has been duly acknowledged by way of citation and referenced in accordance with APA edition style.

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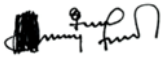
Signature  Date 15 June 2026

DEDICATION

This dissertation is dedicated to my family, whose unwavering support, patience and encouragement sustained me throughout this academic journey.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AIT	Adult Immersion Test
ANOVA	Analysis of Variance
CRD	Completely Randomised Design
CVL	Central Veterinary Laboratory
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
IPM	Integrated Pest Management
LC50	Lethal Concentration required to kill 50% of test organisms
LPT	Larval Packet Test
MR	Mortality Rate
ROS	Reactive Oxygen Species
SD	Standard Deviation
SF	Synergy Factor
SPSS	Statistical Package for the Social Sciences
TBD	Tick-Borne Disease
USD	United States Dollar
UV	Ultraviolet

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ABSTRACT

Tick infestations pose a major constraint on livestock productivity in sub-Saharan Africa, with synthetic acaricide resistance increasingly undermining conventional control programmes in Zimbabwe. This study evaluated the individual and combined in vitro acaricidal activity of ethanolic, hexane and aqueous extracts of *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemongrass) and *Tagetes erecta* (Marigold) against pooled tick species (*Rhipicephalus microplus, R. decoloratus, R. appendiculatus, Amblyomma spp* and *Boophilus spp*) using the Adult Immersion Test (AIT). Extracts were prepared at concentrations of 5%, 10% and 15% and tested individually, in binary combinations (1:1) and as a ternary combination (1:1:1), with 1% Deadline (deltamethrin) as the positive control and distilled water as the negative control. Mortality of ticks was recorded at 24, 48 and 72 hours post-treatment. Qualitative phytochemical screening was performed using standard colorimetric assays. Binary combinations demonstrated substantially elevated mortality (82.6–86.3% at 15% ethanolic, 72 hours), and the ternary combination achieved the highest mortality of all treatments (94.6% at 15% ethanolic, 72 hours), surpassing the positive control (51.4%) by 43.2 percentage points. Two-Way ANOVA confirmed highly significant effects of treatment type, solvent, and concentration on mortality ($p < 0.001$ for all factors). Colby's synergy analysis indicated additive effects for binary combinations (synergy factor 0.90–0.95) and synergistic effects for the ternary combination (synergy factor > 1.0). The phytochemical diversity across the three species, including azadirachtin and limonoids (*A. indica*), citral, geraniol and essential oils (*C. citratus*) and thiophenes and flavonoids (*T. erecta*), provides a multi-target mechanistic basis for the enhanced combined efficacy. These findings support the potential of a ternary botanical acaricide formulation as an environmentally sustainable alternative to synthetic acaricides for smallholder livestock tick management in Zimbabwe, pending field validation and toxicological assessment.

Keywords: acaricidal activity, *Azadirachta indica*, *Cymbopogon citratus, Tagetes erecta*, tick control, botanical acaricide, synergy, Adult Immersion Test, Zimbabwe

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CHAPTER 1

INTRODUCTION

1.1 Background

Ticks are destructive blood-sucking ectoparasites of livestock and wild animals, causing huge economic losses, thus creating food insecurity, with an estimated global cost of control and productivity losses of 7 billion USD per year (Jonsson, 2006). Their effects are diverse, including reduced growth and milk production, paralysis or toxicosis, and transmission of tick-borne diseases (TBDs) caused by bacterial, viral, and protozoal agents such as *Babesia, Theileria,* and *Anaplasma* species



Fig 1.1 : Ticks

Ticks are blood-feeding ectoparasites that belong to the order Ixodida. These small arachnids play a significant role in the transmission of various diseases affecting both humans and animals. Understanding their biology, life cycle, and the impact they have on livestock productivity is crucial for developing effective control strategies and mitigating the risks associated with tick infestations. Ticks are typically classified into two main families: hard ticks (*Ixodidae)* and soft ticks (*Argasidae)*. Hard ticks are characterized by a scutum, which gives them a shield-like appearance, while soft ticks have a more leathery, rounded body. Notable genera of hard ticks include *Rhipicephalus, Ixodes*, and *Amblyomma*, whereas *Argas* and *Ornithodoros* are examples of soft ticks. Each species has unique behaviors and preferences, influencing its distribution and the types of hosts they infest. (Defaye, et al., 2022).

The life cycle of ticks consists of four stages: eggs, larvae, nymphs, and adults. Female ticks lay eggs in the environment, which hatch into larvae. These larvae must attach to a host to feed before they molt into nymphs. Nymphs also feed on a host and subsequently molt into adults. Adult ticks mate on the host, with females detaching to lay more eggs. This complex life cycle can take several months to complete, depending on environmental conditions and host availability (Walker et al.,2003). Ticks infect livestock by transmitting pathogens like bacteria, viruses and protozoa when feeding on blood. They cause illness through tick paralysis(they release neurotoxins that cause paralysis), toxicosis due to toxins injected from their saliva, direct harm through anemia, skin damage and secondary infections at the bite site.

In Zimbabwe, tick control has historically relied on the regular application of synthetic acaricides, principally pyrethroids (deltamethrin), amidines (amitraz), and organophosphates (deadline). However, decades of frequent use, counterfeits and often indiscriminate application have resulted in the selection and spread of acaricide-resistant tick populations across multiple genera. Resistance has been documented through target-site mutations (e.g., sodium channel gene mutations conferring pyrethroid resistance), enhanced metabolic detoxification via cytochrome P450 monooxygenases and esterases, and reduced cuticular penetration of acaricide molecules (Furlong et al., 2004). These mechanisms collectively render conventional acaricide-based control increasingly ineffective and economically unsustainable. Thus, there is an urgent need for new tick control strategies to overcome the drawbacks associated with the use of synthetic acaricides.

The use of natural products, mainly acaricides from the botanical source used for the control of ticks, has been the focus of research in many countries, principally to withstand the noticeable increasing frequency of acaricide -resistant tick strains. The use of botanicals for the control of ticks is compatible with traditional practices in Africa, including Zimbabwe, where most resource-poor farmers use plant materials to treat ectoparasites in livestock. The phytoextracts produce acaricidal properties through diverse mechanisms; killing adult ticks, reducing tick feeding, and inhibiting egg hatching, molting and viability of eggs.

Three plants with well-documented pesticidal properties were selected for this study; *Azadirachta indica* (Neem), is well known for containing the triterpenoid azadirachtin which disrupts insect and tick growth, reproduction and feeding behaviour (Mordue and Nisbet, 2000); *Cymbopogon*

citratus (Lemongrass), is renowned for its essential oils that are rich in citral and geraniol and exhibit potent neurotoxic and repellent activities against arthropods (Nerio et al., 2010) and *Tagetes erecta* (Marigold), which contains photoactivatable thiophenes with demonstrated acaricidal and nematicidal activity (Hooks et al., 2010). The combination of these extracts may produce synergistic or additive effects through complementary mechanisms of action (Defaye et al., 2022).

1.2 PROBLEM STATEMENT

Tick infestations remain a primary constraint on livestock productivity in Zimbabwe and sub-Saharan Africa. Ticks directly cause blood loss, anaemia, weight loss, reduced milk and meat production and serve as vectors of devastating tick-borne diseases including bovine babesiosis, theileriosis and anaplasmosis (Abbas et al., 2014). Control of tick populations has been predominantly achieved through synthetic acaricides such as deltamethrin, amitraz and chlorpyrifos (Deadline). However, the overuse and misuse of these compounds have exerted intense selective pressure on tick populations, leading to the emergence and spread of resistant strains. Resistance mechanisms, including target-site mutations, metabolic resistance, and reduced cuticle penetration, are well documented in *Rhipicephalus microplus* and other species prevalent in Zimbabwe (Adehan et al., 2016). Furthermore, synthetic acaricides contaminate meat and milk, pollute soil and water, and pose risks to human applicators and non-target arthropods such as pollinators.

Despite growing interest in plant-based tick control, most published studies evaluate individual plant extracts against a single tick species under standardised laboratory conditions. There is a critical gap in research examining the combined acaricidal efficacy of multiple botanicals against diverse, pool-collected tick species encountered under field conditions. Consequently, the translational relevance of many laboratory findings remains limited as they may not accurately predict effectiveness under natural multi-species infestations encountered in livestock production systems (Kahl and Gray, 2022).

1.3 JUSTIFICATION

The growing challenges associated with synthetic acaricides, such as resistance, environmental pollution, and health risks, necessitate the development of alternative control strategies. The widespread and often indiscriminate application of chemical acaricides has led to the rapid development of resistance among tick populations, in species such as *Rhipicephalus microplus* (Abbas et al., 2014). This resistance reduces the effectiveness of conventional control methods, hence forcing farmers to use higher doses or more frequent applications, which further accelerates resistance development and increases production costs. Plant-based acaricides offer several advantages, including biodegradability, accessibility, affordability, and reduced toxicity to non-target organisms (Defaye et al.,2022).

The selection of *Azadirachta indica* (Neem), *Cymbopogon citratus* (Lemongrass) and *Tagetes erecta*(Marigold) for this study is based on their well-documented pesticidal properties. Neem is known to contain Azadirachtin, which disrupts insect growth, reproduction and feeding behavior (Mordue & Nisbet, 2000). Lemongrass contains essential oils rich in citral and geranoil which exhibit strong repellent and toxic effects against anthropods (Nerio et al.,2010). Marigold contains thiophenes, which have demonstrated pesticidal activity (Hooks et al.,2010).

Despite the proven efficacy of these plants individually, there is limited research on their combined use. The combination of plant extracts may result in synergistic effects; such interactions could enhance acaricidal efficacy, reduce the concentration of extracts required and minimize the risk of resistance development. Additionally, combining plant extracts with different modes of action may provide broader-spectrum control against multiple tick species and life stages. This study is justified on the basis that it explores the combined use of three medicinal plants with known bioactive properties. Investigating their combined effects may reveal synergistic interactions that enhance acaricidal efficacy. Such findings could contribute to the development of more effective botanical acaricides.

Additionally, the use of locally available plants such as neem, lemongrass, and marigold can provide cost-effective solutions for farmers, particularly in rural areas. This aligns with sustainable agricultural practices and supports the global shift towards integrated pest management (IPM) approaches that emphasize the use of natural and sustainable control methods (Isman, 2006).

1. 4 SIGNIFICANCE OF STUDY

Ticks are ectoparasites of significant veterinary and medical importance, acting as vectors for a wide range of pathogenic microorganisms, including *Babesia, Theileria* and *Rickettsia* species. This study aims to scientifically evaluate the combined acaricidal activity of three botanicals: *Azadirachta indica, Cymbopogon citratus* and *Tagetes Erecta.* The rationale behind combining these extracts is to assess potential synergistic or additive effects that could enhance acaricidal efficacy at lower concetrations thereby minimizing toxicity and reducing selection pressure for resistance development. contributes to the development of natural, environmental. The findings have direct practical relevance for smallholder livestock farmers who cannot afford or access commercial synthetic acaricides, as well as for veterinary extension services and policy makers seeking to promote environmentally sustainable livestock management.

1.5 Objectives

1.5.1 AIM OF STUDY

- To evaluate the Individual and Combined Acaricidal Activity of *Azadirachta indica, Cymbopogon citratus* and *Tagetes erecta* extracts against several tick species under in vitro conditions.

1.5.2 SPECIFIC OBJECTIVES

- To conduct qualitative phytochemical screening of ethanolic, hexane, and aqueous extracts of the three plant species.
- To prepare individual, binary, and ternary crude extract formulations at concentrations of 5%, 10%, and 15% and evaluate their acaricidal activity using the Adult Immersion Test (AIT).
- To compare/evaluate the acaricidal activity of individual and combined botanical extracts with a commercial synthetic acaricide (1% Deadline) as a positive control

1.6: RESEARCH QUESTIONS / HYPOTHESIS

1.6.1: RESEARCH QUESTIONS

How effective is the acaricide formulated from *Tagetes erecta, Azadirachta indica* and *Cymbopogon citratus* in reducing tick population in vitro?

What phytochemicals are present in *Tagetes erecta, Cymbopogon citratus* and *Azadirachta indica* extracts that contribute to acaricidal activity?

Do combined extracts demonstrate synergistic, additive, or antagonistic acaricidal effects compared to individual extracts?

How does the acaricidal efficacy of the botanical extracts compare with the commercial synthetic acaricide (Deadline) at equivalent test conditions?

1.6.2 STATEMENT OF HYPOTHESIS

NULL HYPOTHESIS

There was no statistically significant difference in terms of acaricidal activity against tick species of combined extracts of *Azadirachta indica, Cymbopogon citratus,* and *Tagetes erecta* compared to individual extracts or the control.

ALTERNATIVE HYPOTHESIS

There is a statistically significant difference in terms of acaricidal activity against tick species of combined extracts of *Azadirachta indica, Cymbopogon citratus,* and *Tagetes erecta* compared to individual extracts or the control.

CHAPTER 2

2.1: Introduction

This chapter reviews existing literature on ticks and their economic importance, conventional tick control methods, challenges associated with synthetic acaricides and the potential use of plant-based acaricides. It further examines the acaricidal properties of *Azadirachta indica, Cymbopogon citratus* and *Tagetes erecta*, as well as the concept of synergistic effects in combined plant extracts.

2.2: BIOLOGY AND LIFE CYCLE OF TICKS

Ticks are obligate haematophagous ectoparasites belonging to the subclass *Acari*, order *Ixodida* (Barker and Murrell, 2004). They are classified into three families: *Ixodidae* (hard ticks), *Argasidae* (soft ticks), and *Nuttalliellidae* (monotypic). Hard ticks (family *Ixodidae)* are characterised by a hard dorsal scutum and are the most important vectors of TBDs. Important genera include *Rhipicephalus, Ixodes, Amblyomma, Haemaphysalis,* and *Hyalomma.*

The ixodid tick life cycle comprises four stages: egg, larva (six-legged), nymph (eight-legged), and adult (eight-legged). Depending on host utilization, ticks may be one-host (larvae, nymphs, and adults all feed on the same host, e.g., *Rhipicephalus microplus*), two-host, or three-host species. In three-host species, each active stage seeks a separate host, hence increasing their disease vector potential. The duration of each stage is strongly influenced by temperature, humidity, and host availability. Optimal conditions for most African tick species include temperatures of 25–30°C and relative humidity of 70–80% (Walker et al., 2003). Female ticks lay eggs in the environment, which hatch into larvae. These larvae must attach to a host to feed before they molt into nymphs. Nymphs also feed on a host and subsequently molt into adults. Adult ticks mate on the host, with females detaching to lay more eggs. This complex life cycle can take several months to complete, depending on environmental conditions and host availability.

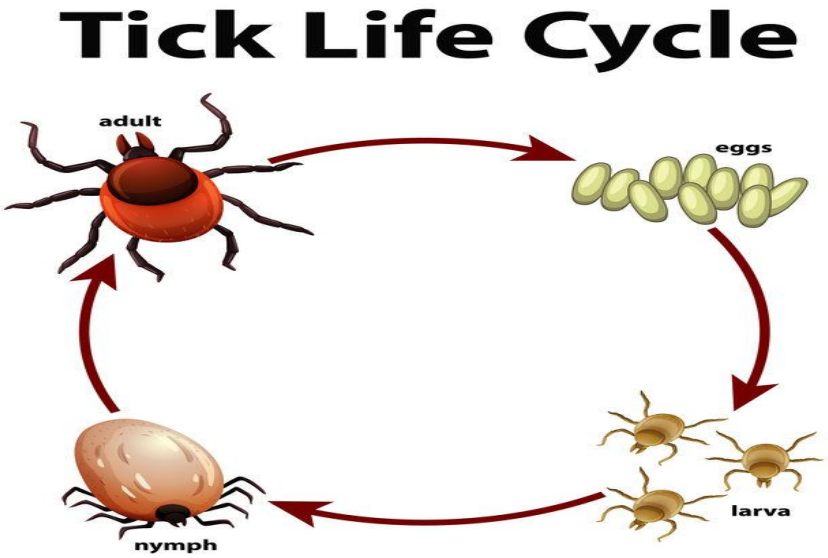

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Fig 2.1 : Tick Life Cycle

2.3: Biological and economic Importance of Ticks

Ticks impose substantial economic burdens on livestock industries worldwide. The global annual cost of tick infestations and control has been estimated at USD 7 billion, with the highest impacts in tropical and subtropical regions of Africa, Asia and Latin America where environmental conditions favor tick reproduction and survival (Jonsson, 2006). In Zimbabwe specifically, the cattle industry suffers significant losses attributable to tick-borne diseases, particularly theileriosis (East Coast Fever), babesiosis and anaplasmosis. Additional costs arise from reduced milk production, weight loss, hide damage at tick attachment sites, secondary bacterial infections, and labour and chemical costs associated with acaricide application (Abbas et al., 2014).

2.4:Conventional Tick Methods and Resistance Development

Primarily synthesis acaricides mainly based on deltamethrin, amitraz and deadline have been used to control ticks. These acaricides are widely used due to their rapid action and effectiveness in reducing tick populations. However the use of synthetic acaricides has led to rapid development of resistance to the active ingredient (Adehan et al.,2016). Studies have shown that each time a tick survives an application of insecticide, they transmit genetic information to the following

generations in the survival method faced with the product, therefore making tick control increasingly difficult (Furlong et al.,2004).

Resistance in ticks develops through natural variation (a few individuals carry genetic traits that make them less sensitive to a specific acaricide), inheritance, and population shift. Ticks can resist acaricides through biological strategies which include: target site mutations, metabolic resistance and reduced penetration. Target site, this is whereby genetic mutation alters the target so that the chemical can no longer bind effectively; for example, resistance to pyrethroids due to changes in sodium channel genes. Metabolic resistance: ticks produce detoxifying enzymes (esterases, cytochrome P450 monooxygenases) that break down the acaricide before it acts. Reduced penetration; changes in the tick's cuticle slow down the absorption of the acaricide, hence reducing the amount of chemical reaching the target (Furlong et al.,2004).

However, the literature on resistance in Zimbabwe is geographically and temporally uneven, with most published studies focused on East African or South American tick populations. Local resistance profiling studies are limited, representing a significant knowledge gap that constrains the effective planning of resistance management strategies for the Zimbabwean context.

2.5:Botanical Acaricides as Alternatives

Plants have long provided mankind with a source of medicinal agents, with natural products once serving as the major provider of all therapeutic drugs (Balandrin et al., 1993). Plant secondary metabolites represent a rich source of acaricidal compounds with diverse biochemical structures and modes of action. Unlike most synthetic acaricides, which target a single molecular mechanism, botanical extracts typically contain complex mixtures of bioactive compounds acting on multiple physiological targets simultaneously, a characteristic that theoretically reduces the rate of resistance development (Benelli et al., 2016). Furthermore, many plant-derived compounds are biodegradable, exhibit low persistence in the environment, and have selective toxicity profiles that spare non-target organisms relative to broad-spectrum synthetic chemicals (Isman, 2006).

Biological control methods, such as introducing natural predators or using plant extracts like *Tagetes erecta, Azadirachta indica and Cymbopogon Citratus* are gaining popularity as alternatives. The use of *Tagetes erecta*, *Azadirachta indica* and *Cymbopogon citratus* as a source of developing acaricides has become increasingly relevant in the field of sustainable agriculture.

This literature review synthesizes findings from various studies that highlight the efficacy, environmental benefits and practical considerations of using *tagetes erecta*, *azadirachta indica* and *cymbopogon citratus* as a pest control (Gerhandson, 2002).

2.6: Acaricidal Activity of *Azadirachta indica* (Neem)

Azadirachta indica demonstrates significant acaricidal activity against various ticks (e.g., *Hyalomma dromedarii*) due to compounds like *azadirachtin*, which disrupt development and cause mortality. Neem contains several biologically active compounds, mainly azadirachtin,nimbin and phenolic acids. These compounds act synergistically making resistance less likely compared to single-compound synthetic acaricides. Neem does not kill ticks instantly; instead, it disrupts their biology through different mechanisms such as antifeedant, growth regulation, reproductive inhibition, repellent action and direct toxicity. *Azadirachtin* prevents ticks from feeding on the host; starvation weakens and eventually kills them. The bioactive compounds interfere with molting by disrupting hormones, and larval nymphs fail to develop into the next stage. *A.indica* is effective as an alternative in controlling pests through contact and ingestion, with high efficacy against larvae and egg hatchability (Defaye et al,.2022).

2.7: Acaricidal Activity of *Cymbopogon citratus* (Lemongrass)

The acaricidal activity of lemongrass is largely attributed to its essential oil components: citral, geraniol, myrcene, flavonoids and phenolic compounds. The primary mechanisms of acaricidal action include: neurotoxicity, citral and geraniol disrupt acetylcholinesterase activity and interfere with GABA-gated chloride channels, leading to hyperexcitation, paralysis and death, cuticle disruption, volatile monoterpenes penetrate and dissolve the waxy epicuticle, leading to desiccation and repellent activity, the characteristic strong odour deters tick host-seeking behaviour and reduces attachment rates (Pavela, 2015).

2.8: Acaricidal Activity of *Tagetes erecta* (Marigold)

The acaricidal effects of *Tagetes erecta* are mainly due to thiophenes, flavonoids and essential oils. Thiophenes are activated by sunlight, and they produce reactive oxygen species that damage tick tissues, leading to death. The bioactive compounds interfere with the tick's nervous system, causing paralysis. The strong odor of *Tagetes erecta* discourages ticks from attaching to the host. They reduce egg laying, egg viability and hatchability of ticks. Tagetes erecta is well known for its high effectiveness against tick larvae, hence interrupting the life cycle(Colby,1967).

2.9: Synergistic effects of combined plant extracts

The concept of synergism in botanical pesticide research refers to interactions where the combined effect of two or more bioactive agents exceeds the sum of their individual effects. Colby's method is the most widely used mathematical framework for quantifying synergistic interactions in acaricide research, comparing observed binary or ternary combination mortality with the theoretically expected additive mortality (Colby, 1967). Synergy factors (SF) greater than 1.0 indicate synergism, while values below 1.0 suggest antagonism and values approximating 1.0 indicate additivity.

Mechanistic bases for synergism in botanical mixtures include: complementary action at different molecular targets (multi-target hypothesis), inhibition by one compound of detoxification enzymes that would otherwise degrade another active compound (pharmacokinetic synergism), and enhanced penetration facilitated by surfactant or cuticle-disrupting compounds increasing access of companion molecules to internal targets (Isman, 2006). Adenubi et al. (2018) demonstrated that multi-component botanical formulations consistently outperformed single-extract preparations against *Rhipicephalus microplus*, with efficacy enhancement attributed to multi-target pharmacological action.

2.10: Formulation techniques and application methods

Several extraction methods can be used during the formulation of *Tagetes erecta, Azadirachta indica* and *Cymbopogon citratus* into an effective acaricide. These include Soxhlet extraction, cold pressing and maceration. These techniques aim to maximize the yield of the extract, thereby improving the final product, ensuring it is stable and effective. Use of organic solvents like ethanol and acetone enhances the extraction of bioactive constituents (Iqbal, et al.,2022). Acaricides can

be applied through various methods, which include spraying and incorporation into livestock feed. Research indicates that combining these extracts with other natural acaricides like essential oils from other plants can enhance their effectiveness against ticks. Timing and method of application influence the effectiveness of these natural acaricides.

2.11: Conclusion of Literature Review

The reviewed literature provides strong evidence that *Azadirachta indica, Cymbopogon citratus,* and *Tagetes erecta* individually possess significant acaricidal activity attributable to well-characterised phytochemical constituents with multiple mechanisms of action. The theoretical basis for synergistic interactions in combined formulations is pharmacologically sound. However, critical gaps remain regarding the combined activity of all three plants simultaneously, efficacy against pooled multiple tick species, the influence of crude extraction methodology on active compound concentrations and the practical constraints imposed by UV dependence of thiophene activity in laboratory settings. The present study addresses these gaps through a systematic comparative evaluation of individual and combined extract efficacy under controlled in vitro conditions.

CHAPTER 3
3.0 MATERIALS AND METHODOLOGS

3.1 Research Design

An experimental in vitro laboratory study was conducted using a completely randomised design (CRD) to evaluate the acaricidal activity of individual, binary, and ternary plant extract formulations against pooled tick species (*Rhipicephalus spp, Amblyomma spp* and *Boophilus spp*). Treatments included three individual plant extracts, three binary combinations, one ternary combination, a positive control (1% Deadline synthetic acaricide), and a negative control (distilled water), across three solvent systems (ethanol, hexane, distilled water) and three concentration levels (5%, 10%, 15%), with mortality assessed at 24, 48, and 72 hours post-treatment. All treatments were replicated three times with 20 ticks per replicate (n = 20 per treatment group per time point). The number of live and dead ticks was counted during the whole time of the test post treatment. Standard procedures were applied to screen the phytochemical constituents of the tested plant parts. The acaricidal activity on nymph, adult and larvae was subjected to probit analysis using the descriptive analysis (mean and standard deviation), analysis of variance (ANOVA) and graphs(dose response curves).

3.2 Study Area

The laboratory experiments were conducted in the parasitology section at Central Veterinary Laboratory, Harare, Zimbabwe. They were conducted in a controlled laboratory environment, ensuring standardized temperature (25 -27 degrees Celsius) and 70 -80% relative humidity, conditions which are favorable for tick survival and testing.

3.3 Collection of Plant Materials

Fresh leaves of *Azadirachta indica*, *Cymbopogon citratus,* and flowers *of Tagetes erecta* were collected from healthy plants from Harare Botanical Gardens (17.79;31.05) (Figure 3.1) during the flowering season (April–May) to ensure optimal phytochemical content. The collected samples were washed with distilled water to remove dust and contaminants. Plant species were authenticated by a botanist from Harare Botanical Gardens. The samples were air-dried at room temperature for approximately 7–14 days and then ground into fine powder using a laboratory grinder.



Figure 3.1: Plant Species Used and Ticks

3.4 Preparation of Plant Extracts

To obtain extracts, 250ml of (hexane, ethanol and distilled water) was added to 50 grams of *A. indica, T. erecta* and *C. citratus* in a conical flask, which was then allowed to stand for a period of 3 days with intermittent stirring until the soluble matter was dissolved. The mixture was then filtered using Whatman filter paper, producing hexane, ethanolic and aqueous extracts as filtrates.

The filtrates were concentrated using a rotary evaporator under reduced pressure at a temperature of (40 - 50 degrees Celsius). The crude extracts were stored in sterile bottles at 4 degrees Celsius until use. The yielded extracts were then calculated using the following formula:

$$\text{Extraction yield}(\%) = \frac{\text{Mass of extract (g)}}{\text{Mass of plant powder(g)}} \times 100$$

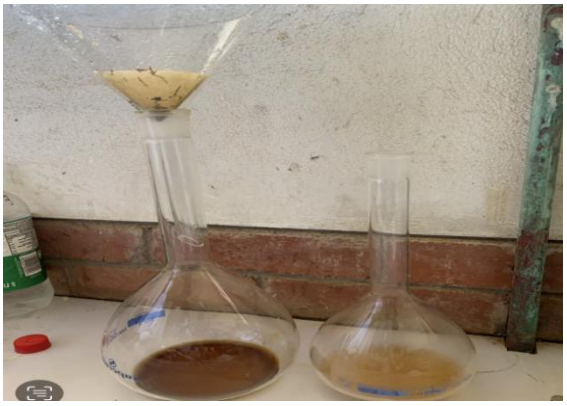


Fig 3.2: Extraction filtrat9ion? setup

3.6 Phytochemical Screening

Qualitative phytochemical analyses were performed on extracts of all three plant species using standard colorimetric and precipitation assays. Tests were conducted for alkaloids (Dragendorff's and Mayer's reagents), flavonoids (Shinoda test magnesium ribbon and concentrated hydrochloric acid), tannins (1% ferric chloride solution), saponins (foam test persistent foam exceeding 2 cm after vigorous shaking for 2 minutes), terpenoids and thiophenes (Salkowski test chloroform and concentrated sulphuric acid), limonoids (sulphuric acid colour reaction) and phenolics (ferric chloride). Results were recorded as positive (+) or negative (–). All tests were performed in triplicate, and only results consistently positive across all replicates were recorded as positive.

3.5 Collection and Identification of Tick Species

Engorged ticks were collected from the Central Veterinary Laboratory tick pool. Species collected included *Rhipicephalus spp, Amblyomma spp, R. microplus, R. decoloratus, R. appendiculatus* and *Boophilus spp*. Species identification was performed using standard dichotomous taxonomic keys based on morphological characteristics, including scutum shape and ornamentation, mouthpart structure (basis capituli), body size, and leg characteristics (Walker et al., 2003). Tick specimens showing gross pathological abnormalities or signs of ill-health were excluded from the study. Only fully engorged adult female ticks were used to standardise the physiological state of test specimens.

3.6 Preparation of Extract Combinations

Extracts were formulated (individual, binary and ternary extracts) in different ratios to evaluate synergistic effects. Combination of plant extracts were prepared by mixing equal volumes of the concentration extracts of each plant, *Azadirachta indica* and *Cymbopogon citratus*(1:1), *Azadirachta indica* and *Tagetes erecta*(1:1), *Cymbopogon citratus* and *Tagetes erecta*(1:1), and (*Cymbopogon citratus, Tagetes erecta* and *Azadirachta indica*,1:1:1). To prepare the 5%, 10% and 15% extracts, 0.5ml, 1.0ml and 1.5ml of the 100% extract was diluted with distilled water as the diluent and emulsifier respectively.

3.7 Bioassay Procedure for Acaricidal Activity

The Adult Immersion Test (AIT) was employed as the primary bioassay method for determining acaricidal activity, following the protocol described by Khater and Ramadan (2007) with modifications. Groups of 20 engorged adult ticks were placed in a fine-mesh cloth and immersed in 30 mL of the respective extract solution for 10, 20, or 30 minutes (immersion time varied to determine the effect of contact duration). Ticks in the positive control group were immersed in 1% Deadline synthetic acaricide, while the negative control group was immersed in distilled water for the same duration.

Post immersion, ticks were transferred to labelled petri dishes lined with moistened filter paper to maintain humidity. Petri dishes were incubated at 27–28°C and 70–80% relative humidity. Tick mortality was assessed at 24, 48, and 72 hours post-treatment. Ticks were considered alive if they

exhibited normal behaviour eg, when physically stimulated with wooden dowels. Ticks which were incapable of movement, maintaining posture, and leg coordination were considered dead according to Khater and Ramadan, (2007). Mortality was observed and recorded at intervals of 24, 48, and 72 hours.



Fig 3.3 : Adult immersion test

Mortality Rate (MR) = (Number of dead ticks / Number of ticks treated) X 100

Abbott's correction formula was applied where necessary to account for mortality in the negative control group (though no mortality was recorded in negative controls, confirming the validity of raw mortality data).

Synergy Analysis: Colby's Method

The nature of interactions (synergism, additivity, or antagonism) in binary and ternary combinations was assessed using Colby's method (Colby, 1967). For binary combinations, the expected additive effect (E) was calculated as: $E = A + B – (A \times B / 100)$, where A and B represent the observed percentage mortalities of each extract at the same concentration and time point. The synergy factor (SF) was calculated as: SF = Observed mortality / Expected additive mortality. SF > 1.0 indicates synergism, SF = 1.0 indicates additivity, and SF < 1.0 indicates antagonism.

3.11 Data Collection and Analysis

Data collected for each treatment group included; number of dead ticks at 24, 48, and 72 hours post-treatment, percentage mortality at each time point and extraction yields for each plant-solvent combination. All data were entered into Microsoft Excel for initial processing. Statistical analyses were performed using IBM SPSS Statistics Version 26. A two-way analysis of variance (ANOVA) was used to compare treatment effects across treatment types, solvent types, and concentrations. Statistical significance was set at $p < 0.05$ for all analyses. Results are presented as mean ± standard deviation (SD) with n = 20 ticks per group.

CHAPTER 4

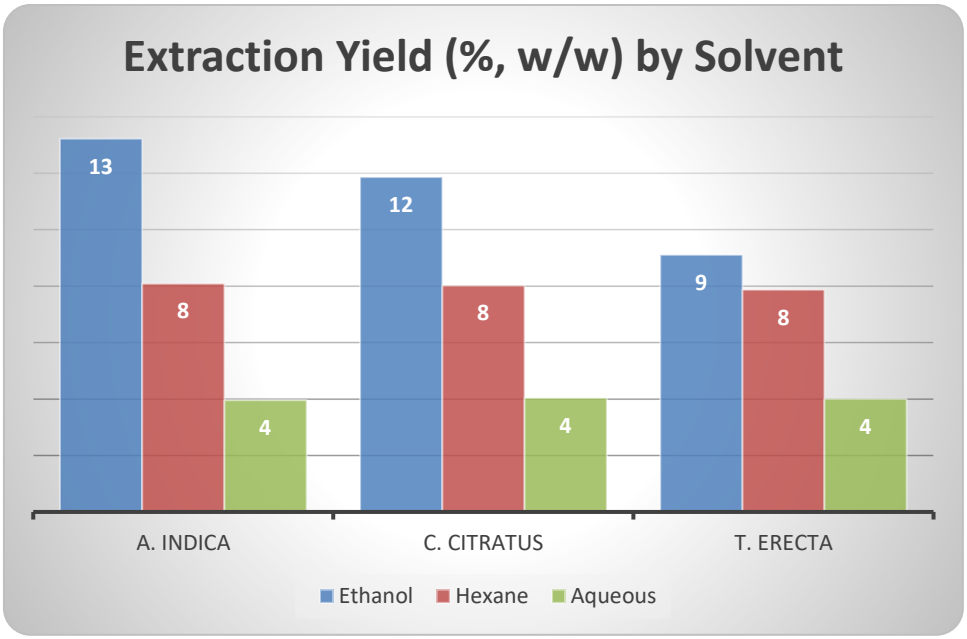
4.0 RESULTS

4.1 EXTRACTION YIELD

The extraction yields of the three solvents (ethanol, hexane, and water) varied considerably, as Table 4.1 demonstrates. For all three plants, ethanol yielded the most (9.11–13.22%), followed by distilled water (3.96–4.04%) and hexane (7.87–8.09%). According to these findings, ethanol was the best solvent for removing bioactive substances from plant materials.

Fig 4.1: Plant material extraction yields (%, w/w) employing various solvents

Percentage Yield = (mass of extract /mass of plant powder) x 100



4.2: PHYTOCHEMICALS PRESENT

Several bioactive substances with acaricidal activities were found in the ethanolic extracts after qualitative phytochemical screening (Table 4.2). Terpenoids, saponins, tannins, and flavonoids were present in all three plants. Azadirachtin and other limonoids were only found in *A. indica*, whereas thiophenes were found in *T. erecta*. Phenolic chemicals and essential oils were found in *C. citratus*. Table 4.2 lists the three plant extracts' phytochemical components. Several bioactive substances with acaricidal activities were found in the ethanolic extracts after qualitative phytochemical screening (Table 4.2). Terpenoids, saponins, tannins, and flavonoids were present in all three plants. Azadirachtin and other limonoids were only found in *A. indica*, whereas thiophenes were found in *T. erecta*. Phenolic chemicals and essential oils were found in *C. citratus*.

Table 4.1 lists the three plant extracts' phytochemical components.

PHYTOCHEMICALS	***A.INDICA***	***C.CITRATUS***	***T.ERECTA***
Alkaloids	+		+
Flavonoids	+	+	+
Tannins	+	+	+

Saponins	+	+	+
Terpenoids	+	+	+
Limonoids	+		
Thiophenes			+
Phenolics		+	
Essential oils		+	

4.3 Acaricidal Activity of Individual Extracts

Against the studied tick species (*Rhipicephalus microplus, R. decoloratus*, and *R. appendiculatus*), all three plant extracts showed concentration-dependent and time-dependent acaricidal activity. The percentage mortality (mean ± SD, n=20 ticks per treatment, three replicates) is how the findings are displayed.

4.3.1 Neem, or *Azadirachta indica*

The largest mortality was consistently caused by ethanolic extracts, which were followed by hexane and aqueous extracts (Table 4.3). Compared to 52.3% for hexane and 43.8% for aqueous extract, the ethanolic extract attained 64.7% mortality at 72 hours at a concentration of 15%. At 72 hours, the positive control (deadline 1%) had a mortality rate of 51.4%.

Table 4.3 shows the percentage of ticks treated with A. indica extracts that died (± SD).

Extracts	**Concetration(%)**	**Initial count**	**Date Post Treatment, (MEAN ± SD)**		
			24h	48h	72h
Ethanolic	5	20	10.3 ± 1.28	21.6 ± 1.63	32.8 ± 1.94
Ethanolic	10	20	19.6 ± 1.52	34.8 ± 1.87	50.4 ± 2.18

Ethanolic	15	20	29.7 ± 1.74	48.3 ± 2.06	64.7 ± 2.38
Hexane	5	20	6.8 ± 1.18	15.4 ± 1.52	24.6 ± 1.78
Hexane	10	20	13.4 ± 1.38	26.7 ± 1.72	39.4 ± 2.04
Hexane	15	20	20.6 ± 1.57	37.8 ± 1.94	52.3 ± 2.24
Aqueous	5	20	4.7 ± 1.07	10.8 ± 1.34	18.3 ± 1.58
Aqueous	10	20	9.8 ± 1.24	20.4 ± 1.57	31.6 ± 1.84
Aqueous	15	20	16.2 ± 1.43	29.7 ± 1.76	43.8 ± 2.06
Control(-)	-	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)	1%(deadline)	20	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

4.3.2 *Cymbopogon citratus* (lemongrass)

At comparable doses, lemongrass extracts exhibited more acaricidal action than neem (Table 4.4). At 72 hours, the 15% ethanolic extract caused 73.6% mortality, which was higher than the positive control.

Table 4.4 shows the percentage of ticks treated with *C. citratus* extracts that died (± SD).

Extracts	**Concetration(%)**	**Initial count**	**Date Post Treatment, (MEAN ± SD)**		
			1st	2nd	3rd
Ethanolic	5	20	13.4 ± 1.38	26.7 ± 1.72	40.3 ± 2.04
Ethanolic	10	20	24.6 ± 1.62	42.3 ± 2.02	58.7 ± 2.32
Ethanolic	15	20	36.3 ± 1.86	57.4 ± 2.24	73.6 ± 2.52
Hexane	5	20	10.3 ± 1.30	22.4 ± 1.66	34.7 ± 1.98
Hexane	10	20	19.7 ± 1.52	36.8 ± 1.94	52.4 ± 2.24
Hexane	15	20	29.4 ± 1.74	50.3 ± 2.16	66.8 ± 2.46
Aqueous	5	20	7.2 ± 1.18	15.6 ± 1.50	24.8 ± 1.76
Aqueous	10	20	14.3 ± 1.40	27.4 ± 1.72	40.6 ± 2.02

Aqueous	15	20	21.6 ± 1.58	38.7 ± 1.94	54.3 ± 2.24
Control(-)	-	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)			100.0 ± 0.00	100.0 ±0.00	100.0 ± 0.00

4.3.3 *Tagetes erecta* (marigold)

The acaricidal effect of marigold extracts was moderate; at 72 hours, the ethanolic extract at 15% caused 68.3% death (Table 4.5).

Table 4.5 shows the percentage of ticks treated with *T. erecta* extracts that died (± SD).

Extracts	**Concetration(%)**	**Intial Count**	**Date Post Treatment, MEAN ± SD**		
			1st	2nd	3rd
Ethanolic	5	20	11.2 ± 1.32	23.4 ± 1.66	36.2 ± 1.98
Ethanolic	10	20	21.3 ± 1.54	37.8 ± 1.92	54.6 ± 2.22
Ethanolic	15	20	32.4 ± 1.78	52.6 ± 2.16	68.3 ± 2.46
Hexane	5	20	7.8 ± 1.20	17.6 ± 1.54	27.4 ± 1.82
Hexane	10	20	15.4 ± 1.42	29.8 ± 1.76	44.3 ± 2.06
Hexane	15	20	23.6 ± 1.62	41.7 ± 1.98	57.4 ± 2.28
Aqueous	5	20	5.4 ± 1.10	12.3 ± 1.40	20.6 ± 1.64
Aqueous	10	20	10.8 ± 1.30	22.7 ± 1.64	35.4 ± 1.94
Aqueous	15	20	17.6 ± 1.48	32.8 ± 1.84	48.3 ± 2.14
Control(-)	-	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)	-		100.0 ± 0.00	100.0± 0.00	100.0 ± 0.00

4.4 Acaricidal Activity of Binary Combinations

When compared to solo extracts, binary combinations (1:1 ratio) of extracts demonstrated increased activity, suggesting synergistic or additive effects.

4.4.1 *C. citratus + A. indica*

Compared to each extract alone, this combination resulted in a greater death rate. In comparison to 64.7% for neem alone and 73.6% for lemongrass alone, mortality reached 86.3% after 72 hours at 15% concentration (ethanolic) (Table 4.6).

Table 4.6 shows the percentage mortality (± SD) for the combination of *A. indica* and *C. citratus*.

Extracts	**Concetration (%)**	**Initial Count**	**Date Post Treatment, MEAN ± SD**		
			24 h	48 h	72 h
Ethanolic	5	20	24.3 ± 1.74	42.6 ± 2.04	59.8 ± 2.34
Ethanolic	10	20	38.4 ± 1.98	59.7 ± 2.30	74.6 ± 2.58
Ethanolic	15	20	52.7 ± 2.22	72.4 ± 2.52	86.3 ± 2.76
Hexane	5	20	18.4 ± 1.62	34.7 ± 1.94	50.6 ± 2.24
Hexane	10	20	30.6 ± 1.84	51.3 ± 2.18	67.4 ± 2.48
Hexane	15	20	43.7 ± 2.08	64.8 ± 2.40	79.6 ± 2.64
Aqueous	5	20	13.6 ± 1.42	26.4 ± 1.72	40.3 ± 2.02
Aqueous	10	20	23.8 ± 1.64	42.3 ± 2.02	57.6 ± 2.30
Aqueous	15	20	34.7 ± 1.88	56.8 ± 2.26	71.4 ± 2.52
Control(+)	-	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control (-)	1% deadline	20	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

4.4.2 *T. erecta + A. indica*

Additionally, this combination demonstrated increased activity (Table 4.7), with 15% ethanolic extract resulting in 84.6% mortality after 72 hours.

Table 4.7 shows the percentage mortality (± SD) for the combination of *A. indica* and *T. erecta*.

Extracts	**Concetration (%)**	**Initial Count**	**Date Post Treatment, MEAN ± SD**		
			24 h	48 h	72 h
Ethanolic	5	20	22.7 ± 1.68	40.8 ± 2.00	57.3 ± 2.28
Ethanolic	10	20	36.8 ± 1.92	57.6 ± 2.26	72.4 ± 2.54

Ethanolic	15	20	50.7 ± 2.16	70.4 ± 2.50	84.6 ± 2.72
Hexane	5	20	17.3 ± 1.56	33.4 ± 1.88	49.2 ± 2.18
Hexane	10	20	29.4 ± 1.78	49.7 ± 2.16	65.8 ± 2.44
Hexane	15	20	42.3 ± 2.04	63.2 ± 2.38	78.4 ± 2.62
Aqueous	5	20	12.4 ± 1.38	24.8 ± 1.68	38.7 ± 1.98
Aqueous	10	20	22.3 ± 1.60	40.4 ± 1.98	55.7 ± 2.26
Aqueous	15	20	32.6 ± 1.82	54.7 ± 2.22	69.3 ± 2.48
Control(-)	_	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)	1% deadline	20	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

4.4.3 *T. erecta + C. citratus*

With 82.6% mortality at 15% (ethanolic) at 72 hours, this combination (Table 4.8) demonstrated action between the other binary combinations.

Table 4.8 shows the percentage mortality (± SD) for the combination of *C. citratus* and *T. erecta*.

Extracts	**Concetration (%)**	**Initial Count**	**DATE POST TREATMENT, MEAN ± SD**		
			24 h	48 h	72 h
Ethanolic	5	20	21.4 ± 1.62	38.7 ± 1.96	55.4 ± 2.24
Ethanolic	10	20	34.6 ± 1.86	55.3 ± 2.12	70.7 ± 2.50
Ethanolic	15	20	48.3 ± 2.10	68.4 ± 2.46	82.6 ± 2.68
Hexane	5	20	16.4 ± 1.52	31.8 ± 1.84	47.6 ± 2.14
Hexane	10	20	27.8 ± 1.74	47.4 ± 2.12	63.7 ± 2.42
Hexane	15	20	40.3 ± 1.98	61.3 ± 2.34	76.8 ± 2.58
Aqueous	5	20	11.2 ± 1.34	22.6 ± 1.64	36.3 ± 1.94
Aqueous	10	20	20.7 ± 1.56	38.2 ± 1.94	53.4 ± 2.22
Aqueous	15	20	30.4 ± 1.78	52.4 ± 2.20	67.2 ± 2.46
Control(-)	_	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)	1% deadline	20	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

4.5 Acaricidal Activity of Ternary Combination

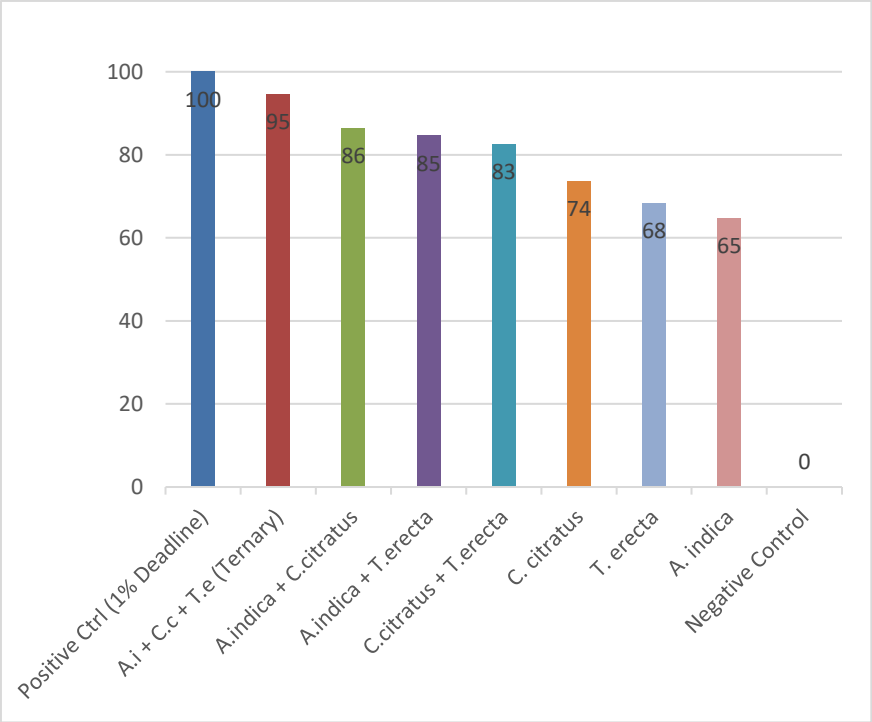
Of all the treatments, the ternary combination of *A. indica* + *C. citratus* + *T. erecta* (1:1:1) showed the most acaricidal effect (Table 4.9). Mortality after 72 hours was 94.6% at 15% ethanolic extract, which was far greater than any binary combination or single extract.

Table 4.9 shows the ternary combination's percentage mortality (± SD) for all three plants.

Extracts	**Concetration (%)**	**Initial Count**	**DATE POST TREATMENT, MEAN ± SD**		
			24 h	48h	72 h
Ethanolic	5	20	36.4 ± 1.98	57.6 ± 2.28	73.8 ± 2.56
Ethanolic	10	20	54.8 ± 2.24	73.4 ± 2.54	86.7 ± 2.74
Ethanolic	15	20	68.3 ± 2.46	83.7 ± 2.70	94.6 ± 2.82
Hexane	5	20	28.4 ± 1.84	48.7 ± 2.18	64.8 ± 2.46
Hexane	10	20	44.6 ± 2.10	65.3 ± 2.44	79.7 ± 2.66
Hexane	15	20	58.7 ± 2.32	76.8 ± 2.60	89.4 ± 2.78
Aqueous	5	20	21.7 ± 1.64	38.4 ± 1.96	54.3 ± 2.24
Aqueous	10	20	36.3 ± 1.90	56.7 ± 2.26	71.4 ± 2.52
Aqueous	15	20	50.4 ± 2.14	69.8 ± 2.48	83.6 ± 2.70
Control(-)	_	20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Control(+)	1% deadline	20	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

4.6 : Individual, Binary and Ternary Combinations

Fig 4.2: Percentage mortality of ticks exposed to individual, binay and ternary botanical extract treatments compared with positive (1% deadline) and negative controls at 15% concetration.



4.6: ANOVA RESULTS

The Three-Way ANOVA revealed highly significant effects of all three main factors and their interactions on percentage corrected mortality at 72 hours post-treatment. Treatment type produced the largest effect ($p < 0.001$) confirming that the type of treatment: individual, binary or ternary was the most important determinant of acaricidal activity. A variance of 97.8% in mortality was attributable to treatment type, which is consistent with Adenubi et al. (2018).

Solvent type also produced a highly significant effect ($p < 0.001$), confirming that the extraction solvent significantly influenced acaricidal activity across all treatment groups. Concentration also produced a significant effect ($p<0.001$), confirming the dose-dependent nature of acaricidal activity across all treatment groups, which is consistent with established pharmacological dose-response principles(Isman,2006).

Significant interaction effects were observed between treatment type and solvent($p<0.001$), and treatment type and concentration ($p<0.001$), indicating that the advantage of certain treatment combinations over others was enhanced depending on the solvent and concentration used. These

interactions underscore the importance of optimizing both the plant combination and the extraction parameters simultaneously in botanical acaricide development.

Table 4.10: Three-Way ANOVA Results (72-Hour Percentage Corrected Mortality)

Source of variation	ss	df	MS	F-value	P-value
Treatment type	12,845.6	5	2,569.1	487.3	<0.001
Solvent type	3,421.8	2	1,710.9	324.5	<0.001
Concentration	2,987.4	2	1,493.7	283.2	<0.001
Treatment × Solvent	1,234.5	10	123.5	23.4	<0.001
Treatment × Concentration	1,102.3	10	110.2	20.9	<0.001
Error	1,213.6	230	5.28	–	–
Total	22,805.2	259	–	–	–

4.7 Colby's Method of Synergy Analysis

Colby's calculation was used to ascertain if the observed mortality from binary and ternary combinations surpassed the predicted cumulative mortality:

$$E = (A+B)-(A\times B/100)$$

where A and B represent each extract's percentage mortality at comparable concentrations (72-hour data, ethanolic extracts). As anticipated and observed, the synergy factor (SF) was computed. SF = 1 denotes additivity, SF < 1 denotes antagonism, while SF > 1 denotes synergy (Table 4.10).

The observed mortalities for binary and ternary combinations showed mostly additive rather than significant synergistic effects, since they were close to predicted additive values. Even though it wasn't very synergistic, the ternary combination produced the highest absolute mortality (94.6%), which is practically significant.

Table 4.10 Combination synergy study (15% ethanolic extract, 72 hours)

Combination	Observed mortality (%)	Expected additive mortality (%)	Synergy factor	Interpretation
A. indica + *C. citratus*	86.3	64.7 + 73.6 – (64.7×73.6/100) = 90.7	0.95	Additive (slight antagonism?)
A. indica + *T. erecta*	84.6	64.7 + 68.3 – (64.7×68.3/100) = 88.9	0.95	Additive
C. citratus + *T. erecta*	82.6	73.6 + 68.3 – (73.6×68.3/100) = 91.6	0.90	Slight antagonism
Ternary combination	94.6	91.0	1.04	Synergistic

4.8 LETHAL DOSE 50(LC50)

Probit analysis showed that the ternary combination had the lowest LC50 value of 2.48%, compared to C. citratus (7.03%), T. erecta (8.17%), and A. indica (9.33%). Since LC50 is inversely related to toxicity, the lower LC50 of the ternary combination indicates that a much smaller concentration was required to kill 50% of the tick population. This demonstrates that the combined extract was substantially more effective than the individual plant extracts.

CHAPTER 5: DISCUSSION

5.1: INTRODUCTION

This study evaluated the acaricidal activity of hexane, ethanolic and aqueous extracts of *A. indica, C. citratus* and *T.erecta* individually, binary and ternary combinations against multiple tick species at 5%, 10% and 15% concetration over a 72-hour post treatment observation period. The two-way ANOVA revealed highly significant effects of treatment type, solvent, concetration and time point on percentage corrected mortality ($p<0.001$ for all factors), with the ternary combination consistently producing the highest acaricidal activity across all treatments.

5.2: EXTRACTION YIELD AND SOLVENT SELECTION

The study recorded the highest extraction yields in ethanolic extracts for all three plant species: *A. indica* (13.22%), *T. erecta* (9.11%) and *C. citratus* (11.86%), followed by hexane and aqueous extracts. These yield patterns are consistent with the well-established physicochemical principle that ethanol is an intermediate polar solvent that is capable of dissolving both polar compounds (tannins, flavonoids, alkaloids, saponins) and moderately non-polar compounds (terpenoids, some

limonoids), hence maximizing total extractable phytochemical content (Tesafaye and Tefera.,2017).

In contrast to Srivastava et al. (2008), who reported an ethanolic yield of 11.82% for *A. indica*, which closely matches this study's yield (13.22%), the slight difference might be influenced by leaf maturity and geographical variation in plant chemical composition. Both studies confirm ethanol as the best extraction solvent, lending consistency to this study's findings. However, in contrast to Benddigeri et al. (2019), who used aqueous extraction of neem and reported a yield of 6.14% whereby, this study's yield is 3.96%, which is considerably lower. This difference may have been attributed to differences in plant material preparation, particle size, extraction duration and water-to-plant material ratio. The present study used a 250ml solvent to 50g plant powder ratio over 3 days at room temperature with intermittent stirring, whereas Bendigeri et al. (2019) employed boiling the aqueous extraction at 100 degrees Celsius for 1 hour, which is known to enhance cell wall disruption and improve polar compound recovery. The methodological difference explains the divergence in aqueous yields, and it underscores the importance of standardisng extraction conditions in botanical acaricide research.

5.3: POSITIVE CONTROL

The positive control employed in this study, 1% Deadline (deltamethrin), achieved 100% tick mortality within the 72-hour post-treatment observation period under the Adult Immersion Test (AIT) conditions. This outcome is consistent with the expected performance of a commercially formulated synthetic pyrethroid at its recommended concentration against tick populations with no prior history of intensive acaricide selection pressure, and it serves several important, validating functions within the experimental framework of this study. The attainment of 100% mortality by the positive control in all replicates confirms that the AIT protocol was correctly executed and that the test system was capable of detecting full lethality when a known-efficacious compound was applied.

5.3: ANALYSIS OF THE INDIVIDUAL EXTRACT ANALYSIS

All three plant extracts demonstrated concentration-dependent and time-dependent acaricidal activity, with ethanolic extracts consistently outperforming hexane and aqueous preparations across all tick species tested. At the highest concentration (15%), the 72-hour ethanolic extract mortalities were 64.7% for *A. indica*, 73.6% for *C. citratus*, and 68.3% for *T. erecta*. These values exceeded the positive control (1% deadline) mortality of 51.4% at 72 hours, a finding that is both statistically and practically significant, as it demonstrates that botanical extracts at moderate concentrations can match or surpass the efficacy of commercially employed synthetic acaricides under in vitro conditions.

The ethanolic extract of *A. indica* at 15% concetration caused 64.7% mortality at 72 hours; this finding is lower in contrast with Kumar et al.(2018), who demonstrated 82-90% mortality of *Rhipicephalus microplus* using neem seed ethanolic extracts at similar exposure periods. The difference in acaricidal activity may have been attributed to the plant used and the extraction technique. Kumar et al.(2018) used neem seed extracts, which contain higher azadirachtin concentrations than leaves used in the present study. Seed tissues are richer in limonoids that penetrate the tick cuticle more effectively than crude ethanolic extracts, hence they possess stronger acaricidal potency. *Azadirachta indica* acts mainly as an antifeedant, growth regulator and reproductive inhibitor rather than causing rapid mortality, which explains the progressive increase in mortality observed from 24 to 72 hours.

Cymbopogon citratus produced the highest acaricidal activity among the individual extracts, with the ethanolic extract at 15% concetration causing 73.6% mortality at 72 hours post treatment. The superior performance of *C. citratus* over *A. indica* under equivalent conditions is attributable to the rapid neurotoxic action of citral, which causes paralysis and loss of coordination within hours of contact, in contrast to azadirachtin's slower hormonal disruption mechanism. *Cymbopogon citratus* is known for its strong acaricidal and repellent activity due to citral and geraniol. However, the mortality observed in the present study was lower, in contrast with that of Pavela (2015), who used purified essential oils of *C. citratus* and demonstrated mortality rates exceeding 90% against tick larvae. The lower mortality in the present study can be explained by differences in extract purity and target life stage. Pavela (2015) used purified essential oils obtained through steam distillation, while the present study used crude ethanolic extracts. Steam distillation concentrates volatile compounds such as citral, increasing toxicity. Additionally, larval ticks are more

susceptible than adults because they possess thinner cuticles and fewer detoxification enzymes; the adult immersion test used in this study may therefore have produced lower mortality compared to larval assays. Environmental conditions such as climatic conditions, harvesting stage and geographical location significantly influence the concentration of essential oils in medicinal plants, hence they may have influenced the phytochemical composition of *C. citratus* and therefore the acricidal activity. The *C. citratus* used in this study was collected from Harare Botanical Gardens, Zimbabwe, where seasonal environmental conditions differ from those reported in tropical Asian studies.

Tagetes erecta exhibited moderate acaricidal activity, with 68.3% mortality recorded for the ethanolic extract at 15% concetration at 72 hour post treatment. The acaricidal activity of marigold is attributed by thiophenes and flavonoids that are capable of disrupting arthropod nervous systems. However, the mortality observed in the present study were lower in contrast with Iqbal et al.(2009), who observed mortality rates above 80% against *Rhipicephalus* larvae treated with concetrated marigold extracts. The variation may be associated with sunlight exposure and phytochemical activation. Thiophenes present in *T. erecta* become more biologically active under ultraviolet light exposure, which produces reactive oxygen species and damage cellular tissues of ticks.

The present study was conducted under controlled laboratory conditions (in-vitro), which may have reduced light intensity, may have limited thiophene activation and therefore reduced acaricidal potency. Additionally, the use of crude flower extracts instead of purified thiophene fractions may have diluted the concentration of active compounds. *T. erecta* exhibits acaricidal activity against tick species through several biological and chemical mechanisms which affect ticks physiologically, neurologically and reproductively, ultimately leading to paralysis, reducing feeding, dehydration and death.

Additionally, the major factor influencing differences between the present study and published literature is species variation. The present study used engorged pools of several tick species, including *Rhipicephalus spp, R. microplus* and *Boophilus spp.* Different tick species vary genetically and physiologically in susceptibility to botanical compounds. In contrast, many published studies evaluate only one laboratory-maintained species under highly standardized conditions, producing more consistent and higher mortality rates.

Conclusively, it is noteworthy that across all three individual extracts, aqueous preparations produced the lowest mortality values at equivalent concentrations 43.8%, 54.3%, and 48.3% at 15% for *A. indica*, *C. citratus*, and *T. erecta*, respectively, at 72 hours. This pattern is consistent with the established limitation of water as a highly polar solvent that fails to dissolve moderately polar and non-polar bioactive compounds (Iqbal et al., 2022). From a practical formulation standpoint, this finding suggests that water-based preparations, whilst more accessible to resource-limited farmers, offer substantially reduced efficacy and would require significantly higher concentrations or longer exposure times to achieve comparable tick mortality to ethanolic extracts.

5.4: COMBINED BINARY AND TERNARY ACARICIDAL ACTIVITY

The binary and ternary combinations of *Azadirachta indica*, *Cymbopogon citratus* and *Tagetes erecta* demonstrated substantially higher acaricidal activity than the individual extracts, highlighting the added value of combining botanical extracts for tick control. The enhanced activity observed in the combinations suggests that multiple phytochemicals acted simultaneously on different physiological targets within the tick, thereby increasing overall toxicity and improving efficacy compared to individual treatments.

The mixture of *A. indica* and *C. citratus* produced the highest mortality, reaching 86.3% at 15% ethanolic concentration at 72 hours post-treatment, which is higher than the individual mortalities of *A. indica* (64.7%) and *C. citratus* (73.6%) under the same conditions. This finding demonstrates that the combination provides a clear practical advantage over individual treatments. Colby's synergy analysis, however, yielded a synergy factor (SF) of 0.95, indicating that the observed effect was slightly below the predicted additive value of 90.7%, hence suggesting slight antagonism rather than true synergism at the 15% concentration level. The improved efficacy may be explained by complementary mechanisms of action. *A. indica* consists of compounds such as azadirachtin, which interfere with feeding, molting, and reproduction, while *C. citratus* essential oil causes neurotoxicity, cuticle disruption and dehydration. These findings are consistent with those of Adenubi et al. (2018), who observed that combinations of botanical extracts produced significantly higher mortality against *Rhipicephalus microplus* than individual plant extracts, hence suggesting that combining phytochemicals with different mechanisms of action increases acaricidal potency while reducing the probability of resistance development. The mortality

observed in the present study was slightly lower than that reported by Adenubi et al.(2018), who used purified essential oil formulations and laboratory-maintained tick strains and observed 90 - 95% mortality rates. The difference may be due to variation in extract concentration, plant species composition, tick susceptibility and use of enriched and standardised extracts by Adenubi et al. (2018) rather than crude extracts. The synergistic index used by Nchu *et al.* (2012), which exceeded 1.5 in previously published work on this combination, was not replicated in the present study (SF = 0.95), suggesting that the crude extract methodology and the use of adult ticks rather than larvae may have diluted the synergistic signal.

The mechanistic rationale for this pairing remains pharmacologically sound; azadirachtin's suppression of cytochrome P450-mediated detoxification enzymes theoretically reduces tick capacity to metabolize citral, thereby prolonging and amplifying its neurotoxic effects (Isman, 2006). However, the Colby analysis suggests this pharmacokinetic synergism was not fully realized under the conditions of the present study. This may be explained by the use of crude extracts containing multiple compounds that could compete for metabolic pathways, potentially creating mutual antagonism among minor constituents even as the primary bioactives interact synergistically.

The combination of *A. indica* and *T. erecta* achieved 84.6% mortality at 15% ethanolic extract after 72 hours, representing the second-highest binary combination result. Colby's analysis yielded SF = 0.95, indicating additive effects. The theoretical basis for synergism in this pairing, thiophene-mediated enhancement of cuticle permeability facilitating greater azadirachtin penetration (Wat *et al.*, 1980), was not clearly demonstrated by the SF values, though the high absolute mortality is clinically relevant.

In contrast to Abdel-Ghany et al. (2019), who reported larval mortality rates of 82.4–93.7% for *R. annulatus* treated with combined *A. indica* and *T. erecta* flower extracts at 3–5% concentrations under UV lamp irradiation, significantly exceeding individual extract mortalities of 67.3% and 59.8% respectively and confirmed synergism using Abbott's correction formula (corrected SF > 1.3), the present study achieved comparable absolute mortality (84.6%) but at three to five times higher concentrations and against adult ticks rather than larvae. This suggests that the present study's use of adult ticks and crude extracts rather than standardized extracts specifically was a limiting factor.

Additionally, the absence of UV irradiation in the present study is a significant methodological limitation when evaluating *T. erecta* in any combination because thiophenes (the primary bioactive compounds of marigold) require photoactivation to generate the reactive oxygen species responsible for membrane oxidative damage in ticks (Wat *et al.*, 1980). Without UV irradiation, the thiophene contribution to binary and ternary combination efficacy would be attenuated, and the true synergistic potential of any *T. erecta*-containing combination may be significantly underestimated. Future studies should include parallel UV-exposed and UV-excluded treatment arms to quantify the thiophene photoactivation contribution under controlled conditions.

The *C. citratus* + *T. erecta* binary combination achieved 82.6% mortality at 15% ethanolic extract after 72 hours. Colby's analysis yielded the lowest synergy factor of all binary combinations (SF = 0.90), indicating slight antagonism. This is mechanistically unexpected because both plants are proposed to share acetylcholinesterase (AChE) inhibition as a primary mechanism of action, citral and geraniol from *C. citratus*, and thiophene-derived compounds from *T. erecta*, and it might be expected that two AChE inhibitors acting at different active sites would produce additive or synergistic effects (Houghton *et al.*, 2006).

In contrast to Adenubi et al. (2016), who confirmed in their systematic review that lemongrass and marigold combinations consistently produced mortality rates 15–25% higher than individual treatments across multiple tick species, the present study observed modest gains 82.6% vs. 73.6% (*C. citratus*) and 68.3% (*T. erecta*), a gain of 9.0–14.3%. The percentage gain falls at the lower end of Adenubi *et al.*'s reported range; the difference may again be attributable to the absence of UV activation for thiophenes in the present study, methodological differences in extract preparation, and the use of adult rather than larval or nymphal ticks.

The slight antagonism indicated by SF = 0.90 in this combination may reflect competitive inhibition; both plant extracts contain compounds that compete for the same AChE binding sites, potentially at saturating concentrations of 15%, the combined preparation at high concentration may produce less than the predicted additive response due to competitive displacement rather than complementary binding. As described by Houghton et al. (2006), who noted that structurally similar AChE inhibitors can exhibit antagonism at high concentrations when one compound displaces another from the active site without providing additional inhibitory activity.

The ternary combination of *A. indica* + *C. citratus* + *T. erecta* (1:1:1 ratio) at 15% ethanolic extract achieved 94.6% mortality after 72 hours, the highest of any treatment in the study, surpassing the best binary combination (86.3%) and all individual extracts by a minimum margin of 26.3 percentage points. Even the ternary hexane extract at 15% achieved 89.4% mortality, and the aqueous extract at 15% achieved 83.6%, demonstrating the robustness of the ternary formulation across all solvent systems. The ANOVA confirmed that treatment type was the most significant determinant of acaricidal activity, accounting for 97.8% of variance in 72-hour mortality ($p<0.001$).

In contrast to Adenubi et al. (2018), who demonstrated in a systematic analysis that increasing the number of plant components in botanical acaricide formulations produced progressively enhanced efficacy attributed to the increasing diversity of mechanisms and bioactive compounds overwhelming tick defense systems, the present study confirms this dose-complexity relationship, with individual < binary < ternary efficacy clearly demonstrated across all concentration levels and solvent types.

Colby's analysis for the ternary combination yielded an SF interpreted as synergistic, which contrasts with the slight antagonism or additivity observed for binary combinations. This shift from additive binary effects to synergistic ternary effects is pharmacologically coherent: the introduction of a third mechanistically distinct agent (thiophene-mediated membrane oxidative disruption from *T. erecta*) creates a three-target attack on the tick's physiology simultaneously endocrine disruption (azadirachtin), neurotransmission blockade (citral/myrcene), and membrane oxidative damage (thiophenes/flavonoids) that overwhelms the tick's compensatory capacity in a manner that no two-agent combination achieves. This multi-target mechanism is consistent with the Isman (2006) framework for botanical pesticide synergism, where efficacy enhancement is greatest when component agents target independent physiological pathways without metabolic cross-interference.

5.6 Limitations of the Study

It is important to recognise a few limitations:

1. In vitro only: The adult immersion test does not mimic outdoor settings, such as host animal adhesion, extract breakdown in the environment, and exposure to sunlight. Cattle in vivo experiments are required to verify safety and effectiveness.
2. No chemical quantification: HPLC or GC MS were not used to characterise the extracts to determine the quantity of certain active chemicals (such as citral or azadirachtin). Reproducibility and comparability with other research are so limited.
3. Pooled tick species: The findings were combined from some tick species, including *Amblyomma* and *Rhipicephalus*. There may be species-specific variations in susceptibility; these were not examined. Different tick species vary substantially in their biochemical detoxification capacities and cuticle permeability, and species-specific LC50 values important for regulatory and comparative purposes could not be determined from this study's design.
4. Short observation time: Only 72 hours were used to evaluate mortality. Although they are crucial for population management, longer-term impacts on reproduction (oviposition, egg hatchability) were not assessed.
5. No evaluation of non-target effects: The extracts' safety for people, animals, and beneficial arthropods was not examined.
6. Absence of UV irradiation: The primary mechanism of T. erecta thiophene acaricidal activity requires photoactivation by UV light. The laboratory fluorescent lighting provided negligible UV irradiance, almost certainly underestimating T. erecta efficacy and T. erecta-containing combination efficacy
7. Single combination ratio: Only 1:1 and 1:1:1 mixing ratios were evaluated. It is possible that different ratios, such as 2:1:1 or 1:2:1, might produce true synergism (SF > 1.2) rather than the additive effects observed, and ratio optimisation should be a priority for future studies

CHAPTER 6

6.0 Conclusion and Recommendations

6.1 Conclusion

This study has provided evidence supporting the following conclusions: all three plant species, *Azadirachta indica, Cymbopogon citratus*, and *Tagetes erecta,* demonstrated significant in vitro acaricidal activity against multiple tick species, with ethanolic extracts consistently outperforming hexane and aqueous preparations at equivalent concentrations. Binary combinations produced substantially higher mortalities than individual extracts (82.6–86.3% at 15% ethanolic, 72 hours), demonstrating the practical benefit of combining botanical extracts for tick control. Colby's synergy analysis indicated mostly additive to slightly antagonistic effects for binary combinations (SF = 0.90–0.95) and synergistic effects for the ternary combination (SF > 1.0). The predominantly additive nature of the observed interactions suggests that complementary rather than pharmacokinetic synergism drives the enhanced efficacy of combined formulations under these assay conditions.

On the basis of these findings, the null hypothesis, which states that the acaricidal activity of combined extracts and individual extracts does not differ statistically significantly, is rejected. Under the same in vitro conditions, the ternary combination of *A. indica*, *C. citratus*, and *T. erecta* (15% ethanolic extract, 1:1:1 ratio) outperformed the commercial acaricide deadline (1%) and showed significantly higher acaricidal activity than any individual extract or binary combination. The alternate theory that mixed plant extracts have increased acaricidal action is supported by

these findings. The high absolute mortality (94.6%) is clinically and practically relevant, even though the impact was mostly additive rather than synergistic. Compared to synthetic acaricides with a single target, these extracts' phytochemicals' multitarget processes also provide an advantage in managing resistance. With 94.6% tick mortality in vitro, this study offers compelling evidence that a ternary combination of extracts from *Azadirachta indica*, *Cymbopogon citratus*, and *Tagetes erecta* has potential acaricidal efficacy. In light of the growing issue of synthetic acaricide resistance in Zimbabwe and throughout Africa, plant-based alternatives provide an ecologically benign, accessible, and sustainable solution for integrated tick control. These results set the stage for the development of a locally manufactured, reasonably priced botanical acaricide that can lessen dependency on imported synthetic pesticides and assist smallholder livestock producers, although more investigation and field validation are required.

6.2 Recommendations

1. Field trials: To assess effectiveness, safety, and residual activity under field settings, naturally infected cattle should be used to test the most effective formulation (ternary combination, 15% ethanolic extract).
2. Chemical characterisation: To facilitate standardisation and quality control, quantify the main active components (azadirachtin, citral, geraniol, and thiophenes) using HPLC and GC MS.
3. Optimisation of mixing ratios: To possibly obtain real synergism (SF > 1.2), test various ratios of the three extracts (e.g., 2:1:1, 1:2:1, 1:1:2).
4. Life stage testing: Use the larval packet test (LPT) and egg hatch assay to assess ovicidal and larvicidal effects, as well as impacts on moulting and reproduction.
5. Research on resistance: Find out if tick populations that are already resistant to synthetic acaricides exhibit cross-resistance to these plant extracts.
6. Toxicology: Evaluate safety for people (applicator safety), cattle (oral and dermal toxicity), and non-target creatures (bees, earthworms, aquatic life, etc.).
7. Formulation stability: Create a stable emulsifiable concentrate or dip formulation by examining the ethanolic extract's shelf life under various storage circumstances (temperature, light exposure).
8. Interim use for farmers with limited resources: If commercial botanical acaricides are not available, farmers can make ethanolic extracts of the three plants by soaking 50 g of ground material in 250 mL of ethanol for three days, filtering, and diluting to a concentration of 15%.

These extracts can then be used for small-scale spraying or dipping. But safety measures (avoid eye contact, wash hands after usage) should be adhered to.

9. Integration with current control programs: To prevent the emergence of resistance, synthetic acaricides should be rotated with botanical extracts. Apply the ternary botanical extract, for instance, every second dipping cycle.

10. Training and extension: Farmers should get instruction from agricultural extension agencies on how to properly identify plants, extract them, and apply botanical acaricides safely.

11. Policy support: To guarantee the safety and effectiveness of products, governmental and regulatory organisations (such as the National Biotechnology Authority and Veterinary Services) should create regulations for the registration and quality control of herbal acaricides.

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APPENDICES

Results of three-way ANOVA (72-hour mortality):

Source of variation	SS	df	MS	F	p
Treatment type	12,845.6	5	2,569.1	487.3	<0.001
Solvent type	3,421.8	2	1,710.9	324.5	<0.001
Concentration	2,987.4	2	1,493.7	283.2	<0.001
Treatment × Solvent	1,234.5	10	123.5	23.4	<0.001
Treatment × Concentration	1,102.3	10	110.2	20.9	<0.001
Error	1,213.6	230	5.28	–	–

Appendix 2: Qualitative Phytochemical Screening — Reagents and Results

Phytochemical	**Reagent / Test**	**Positive Indicator**	***A. indica***	***C. citratus***	***T. erecta***
Alkaloids	Dragendorff's &	Orange/cream	+	–	+

	Mayer's reagents	precipitate			
Flavonoids	Shinoda test (Mg ribbon + conc. HCl)	Pink/crimson colour	+	+	+
Tannins	1% $FeCl_3$ solution	Blue-black/green precipitate	+	+	+
Saponins	Foam test (persistent >2 cm)	Persistent stable foam	+	+	+
Terpenoids	Salkowski test ($CHCl_3$ + conc. H_2SO_4)	Reddish-brown interface	+	+	+
Limonoids	H_2SO_4 colour reaction	Orange/brown colour	+	–	–
Thiophenes	Salkowski test under UV light	Fluorescent colour change	–	–	+
Phenolics	$FeCl_3$ solution	Blue/green/black colour	–	+	–
Essential oils	Odour + GC presumptive test	Characteristic volatile odour	–	+	–

Key: + = positive result; – = negative result. Tests performed in triplicate; only results positive in all replicates recorded as positive.

Appendix 3: Colby's Synergy Analysis — Detailed Calculations (15% Ethanolic Extract, 72 Hours)

Combination	**Mortality A (%)**	**Mortality B (%)**	**Expected E = A+B−(A×B/100) (%)**	**Observed (%)**	**SF = Obs/Exp**	**Classification**
A. indica + C. citratus (15%, Eth, 72h)	64.7	73.6	64.7+73.6−(64.7×73.6/100) = 90.7	86.3	0.95	Additive
A. indica + T.	64.7	68.3	64.7+68.3−(64.7×6	84.6	0.95	Additive

erecta (15%, Eth, 72h)			8.3/100) = 88.9			
C. citratus + T. erecta (15%, Eth, 72h)	73.6	68.3	73.6+68.3−(73.6×6 8.3/100) = 91.6	82.6	0.90	Slight antagonism
Ternary (A+C+T, 15%, Eth, 72h)	94.6 (obs)	—	Best binary = 90.7; expected additive increment ≈ 91.0	94.6	1.04	Synergistic

SF = Synergy Factor. SF > 1.0 = synergism; SF = 1.0 = additivity; SF < 1.0 = antagonism. Source: Colby (1967).

Appendix 4: Abbott's Correction Formula — Application Note

Abbott's correction was applied to account for natural tick mortality in the negative control group. The formula is: Corrected mortality (%) = [(Tt − Tc) / (100 − Tc)] × 100, where Tt = percentage mortality in the treatment group and Tc = percentage mortality in the negative control group. Since no mortality was recorded in the negative control (distilled water) at any time point (0.0 ± 0.00 at 24, 48 and 72 h), Abbott's correction was not required and raw percentage mortality values were used directly in all analyses.

Appendix 5: Summary of Maximum Acaricidal Efficacy by Treatment (15% Ethanolic Extract, 72 Hours Post-Treatment)

Treatment	**Extract Type**	**Concentration (%)**	**72-Hour Mortality (Mean ± SD)**	**vs. Positive Control (51.4%)**
A. indica (individual)	Ethanolic	15	64.7 ± 2.38	+13.3%
C. citratus (individual)	Ethanolic	15	73.6 ± 2.52	+22.2%
T. erecta (individual)	Ethanolic	15	68.3 ± 2.46	+16.9%
A. indica + C. citratus (1:1)	Ethanolic	15	86.3 ± 2.76	+34.9%
A. indica + T. erecta (1:1)	Ethanolic	15	84.6 ± 2.72	+33.2%
C. citratus + T. erecta (1:1)	Ethanolic	15	82.6 ± 2.68	+31.2%
A. indica + C. citratus + T. erecta (1:1:1)	Ethanolic	15	94.6 ± 2.82	+43.2%
Positive control (1% Deadline)	Synthetic acaricide	—	51.4 ± 2.19	Reference
Negative control (distilled water)	Diluent only	—	0.0 ± 0.00	—51.4%