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Acaricidal Activity of *Azadirachta indica* (Neem Tree) and *Carica papaya* (Papaya) Leaf Extracts against *Rhipicephalus sanguineus* (Dog Ticks) Using Spray Method

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Abstract: Ticks pose a serious threat to dogs, with synthetic acaricides leading to environmental impact and resistance concerns. While *Azadirachta indica* and *Carica papaya* have shown acaricidal potential against *Rhipicephalus sanguineus*, their efficacy against dog ticks has not been thoroughly investigated. This study determined the acaricidal activity of *A. indica* and *C. papaya* leaf extract against *R. sanguineus*, both individually and combined, to develop a botanical bio-agent that can be utilized as a substitute to the commercially available Permethrin. Each plant specimen was air-dried, pulverized, and extracted using 95% ethyl alcohol. Phytochemical screening tests were conducted to determine the bioactive components present in each plant extract. The analysis showed that the ethanolic extract of *A. indica* contains moderate levels of tannins, saponins, flavonoids, alkaloids, and glycosides, with a lower concentration of phenolic compounds. In contrast, the ethanolic extract of *C. papaya* has moderate amounts of tannins, flavonoids, and alkaloids, while saponins, glycosides, and phenolic compounds are present in lower concentrations. The spray method of acaricidal activity determination revealed that each plant extract has acaricidal activity, but a better effect against *R. sanguineus* was obtained if both extracts were combined. The *A. indica* and *C. papaya* combined leaf extract at 100% can be used as an acaricidal bio-agent against dogs' ticks. For future directions of similar studies, it is recommended to prioritize refining bioassay methodologies and conducting in-depth chemical profiling. Exploring variations in extraction techniques, solvent types, and concentration gradients may offer valuable insights into the most effective formulations for tick control. In addition, the identification and quantification of major bioactive compounds that are responsible for acaricidal activity could be facilitated by the use of advanced analytical techniques, including Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). This integrated approach would deepen our understanding of the mode of action, enhance efficacy, and contribute to the development of sustainable alternatives to synthetic acaricides.

Keywords: Acaricide, *Carica papaya*, *Azadirachta indica*, Phytochemicals, Ticks

Introduction

Acaricides are indispensable means of managing ectoparasite infestation and safeguarding human and animal health. Nevertheless, the misuse of synthetic acaricides has led to environmental pollution, resistance development, and indiscriminate toxicity. Because of these and their high cost, there is a growing demand for sustainable alternatives made from natural resources.

Among the most important carriers of diseases are ectoparasites, most especially ticks (Ullah *et al*., 2023). They latch onto their hosts, feeding on blood and potentially transmitting various species of protozoa, bacteria, viruses, and nematodes (Jamil *et al*., 2021). Hard ticks, or Ixodidae, are a family of arthropods that are exclusively parasitic. Rhipicephalus is one of the 12 species of Ixodidae (Scharf *et al*., 2020) and one of the most threatening species. This species has been documented to transmit several pathogenic bacteria (Nava *et al*., 2022) and protozoan diseases (Senbill *et al*., 2018).

The brown dog tick, also known as *R. sanguineus*, is a parasite that primarily affects canines. However, it has the potential to infect other hosts, including humans (Lord, 1969). This tick is the main vector responsible for the spread of zoonotic agents and diseases such as *Ehrlichia canis*, the main agent of ehrlichiosis in dogs, *Rickettsia rickettsii*, the cause of spotted fever in humans, *Coxiella burnetii*, the causative agent of Q fever, *Rickettsia conorii* subsp. *israelensis*, the causative agent of Mediterranean spotted fever, and *Candidatus neoehrlichia mikurensis*, an emerging human pathogen (Agwunobi, 2021).

Current tick management strategies utilize a diverse array of compounds that are readily accessible, including arsenicals, chlorinated hydrocarbons, carbamates, macrocyclic lactones, organophosphates, formamidines, pyrethroids, fluazuron, and fipronil (Selles *et al*., 2021). However, in addition to their exorbitant prices, these acaricides have the potential to be hazardous and may have an impact on the health of both humans and animals, as well as contaminate the environment with residues that are detrimental to both humans and animals (Szabó *et al*., 2018).

These issues have motivated scientists and investigators to search for alternative ecologically friendly tick control strategies with less negative consequences. Plant extracts and essential oils are among the natural products that have been demonstrated to have economically significant tick-repelling efficacy. These botanicals have been discovered to contain a variety of active compounds that can hinder or prevent the development of resistance to chemical acaricides.

Furthermore, substances that occur naturally offer a more affordable substitute for synthetic acaricides, and their degradability is another widely recognized advantage of employing them (Shakya *et al*., 2024).

Researchers have advocated using plant extracts as biological agents since plants inherently have phytochemicals. These chemicals serve as plants' protective mechanisms against the action of predators. The phytoextracts exhibit acaricidal effects through a variety of mechanisms, including the inhibition of egg development, molting, fecundity, and viability, as well as the death of adult ticks and a reduction in tick feeding (Kemal *et al*., 2020).

Additionally, Molina (2020) posits that the effects of plant extracts are the result of the aggregation of secondary metabolites in plants, including alkaloids, steroids, tannins, and phenolic compounds, which are considered potential sources of pesticides and herbicides. Domingo (2022) also mentioned, citing Corpuz *et al*. (2021), that these effects were caused by compounds produced in the plant's secondary metabolism. These secondary metabolites in plants are crucial raw materials for the creation of novel bio-agents (Corpuz, 2020).

Bañez and Castor (2011) determined that the tubers of *Cyperus rotundus* could be made into an effective pesticide mainly due to the presence of phytochemicals in the tubers. The authors assert that it is nearly as effective as Organophosphate and is even more effective than Carbamate. Other studies (Bañez and Castor, 2013; Bañez, 2016) claimed that *Syngoniun podophyllum*, *Pachyrhizus erosus*, and *Senseviera trifasciata* plants have insecticidal properties.

Among nature's repositories of bioactive compounds, *A. indica* (neem) and *C. papaya* (papaya) have emerged as potential acaricides for tick management due to their rich phytochemical composition and documented pesticidal activities. *A. indica*, a member of the Meliaceae family, is renowned for its diverse set of secondary metabolites, particularly limonoids, which possess potent insecticidal and acaricidal properties (Saleem *et al.*, 2018). It is a medium-sized, fast-growing tree that stands as high as 20 m and is evergreen in color. Similarly, *C. papaya*, a slender plant with a solitary trunk and a tall (6-20 feet) stature, is a member of the Caricaceae family. It is native to tropical America and has since spread to various tropical countries.

It is celebrated for its enzymatic and phytochemical composition, notably papain, chymopapain, and carpaine, which possess pesticidal properties against a range of pests (Anjula Wijesooriya *et al*., 2019), including mosquito larvae (Corpuz & Savella, 2019). In some places in the Philippines, farmers use the aqueous extract of both leaves to eradicate lice and ticks from carabaos and cows, but this practice has no scientific basis.

While many plant extracts, such as *Eucalyptus*, *Lantana camara*, and *Nicotiana tabacum*, exhibit acaricidal properties, they primarily rely on essential oils, alkaloids, or neurotoxic compounds that disrupt the nervous system (Idrees *et al*., 2016). In contrast, *A. indica* offers a diverse array of bioactive compounds that interfere with multiple physiological functions, including growth and reproduction. Meanwhile, *C. papaya* employs enzymatic mechanisms rather than neurotoxicity, a relatively uncommon trait among plant-derived acaricides (Filgueiras *et al*., 2021). *A. indica* and *C. papaya* are eco-friendly, multifunctional alternatives to synthetic acaricides for the control of ticks due to these unique properties.

While *A. indica* and *C. papaya* have been demonstrated to possess acaricidal properties, there is still a substantial lacuna in our understanding of their efficacy, modes of action, and potential synergistic effects when combined.

Therefore, this research endeavors to bridge this gap by comprehensively investigating the acaricidal activity of these leaf extracts against *R. sanguineus*.

In this investigation, the leaf extracts of two plants, *A. indica* and *C. papaya*, were assessed against *R. sanguineus* both individually and in combination.

With the help of this research, manufacturing companies will be able to create a bio-agent derived from plant sources that can replace hazardous synthetic acaricides. Pet owners will be provided with an alternative agent that is low in toxicity to non-target organisms. Moreover, the result of this study will help minimize or reduce the use of toxic chemicals that can harm the environment. It will offer an affordable, safe, and effective replacement for harmful synthetic insecticides that are safe for the community to use.

Specifically, it aimed to (a) determine the phytochemicals present in the two plant extracts, (b) determine the acaricidal activity of the individual and combined extracts of *A. indica* and *C. papaya* against dog ticks at varying concentrations, and (c) determine if a significant difference exists in the mortality rate of ticks treated with individual and combined extracts of *A. indica* and *C. papaya* against dog ticks at different concentrations.

Materials and Methods

Fresh leaves of *A. indica* and *C. papaya* were collected, rinsed, and air-dried prior to extraction. Ethanol (95%) and distilled water were employed as solvents. *R. sanguineus* was collected from infested canines and subsequently maintained under controlled conditions. Petri dishes, forceps, filter paper, and spray bottles were employed to ensure homogenous exposure during the spray application method. Distilled water served as the negative control, while permethrin solution was employed as the positive control. The systematic evaluation of the acaricidal activity of *A. indica* and *C. papaya* leaf extracts was facilitated by these materials.

Research Design

This study utilized the experimental research design. The experimental group included four concentrations (25, 50, 75, and 100 mg/mL) of the *A. indica* and *C. papaya* leaf extracts used individually and combined. The positive control is the Permethrin solution, and the negative control is distilled water. Permethrin is selected over other insecticides as the positive control because it is more effective against mites and ticks, aligns better with real-world applications, has lower non-target toxicity, and provides more stable and reproducible results in acaricidal bioassays (Obaid *et al*., 2022; Davidson *et al*., 2024; Stará *et al*., 2011). *R. sanguineus* (dog ticks) in their larval stage were the test organisms used because they are more sensitive, uniform, cost-effective, and easier to handle, making them ideal for standardized acaricidal bioassays (Jongejan *et al*., 2024).

Study Locale

The research was conducted at the College of Health Sciences Laboratory, University of Northern Philippines, Vigan City.

Methods and Procedures

Plant Collection, Authentication, and *Preparation*

The mature leaves of the plants were collected from the Barang-ay Demo Farm in San Juan, Ilocos Sur. They were washed separately with tap water and subsequently rinsed with distilled water. The plant samples were subjected to air drying for a minimum of 5-7 days. Afterward, the leaves were pulverized manually.

The plants were verified and authenticated by the Bureau of Plant Industry of the Department of Agriculture, Manila.

Plant Extraction

Extraction of the pulverized samples utilized 95% ethanol using the Soxhlet's apparatus. Each plant yielded 35 mL and 34 mL, respectively, for *A. indica* and *C. papaya*, representing 300 g of each plant sample.

Preparation of the Various Concentrations of the Plant Extracts

The researchers prepared the different concentrations of the *A. indica* and *C. papaya* extracts just before the experiment. Furthermore, the *A. indica – C. papaya* combined extract was prepared by mixing equal volumes of the concentrated extracts of each plant. The result served as the 100% solution where the other concentrations were prepared. To prepare the 25% extract, 2.5 mL of the 100% extract was placed in the graduated cylinder and diluted with distilled water up to the 10 mL mark. The researchers prepared the rest of the solutions similarly.

Collection of Ticks

The dogs were caged and quarantined for one week before ticks were collected. They were given regular food and water but were not allowed to take a bath.

The researchers classified the ticks based on their developmental stage after collection. Only the larval ticks were utilized in this study.

Acaricidal Bioassay

The in-vitro spray method was employed to ascertain the acaricidal activity of the two plant extracts. This method was utilized because it mimics how acaricides are typically applied in agricultural and veterinary settings, making the result more applicable to real-world use. It also lowers the risk of drowning or the stress effect on the test ticks, which might interfere with the accurate assessment of acaricidal properties (Baran *et al*, 2020; Obaid *et al*., 2022).

Six (6) large glass Petri dishes were set up for each plant extract. The Petri dishes were labeled according to the different treatments. Each Petri dish contained fifty (50) ticks, which were sprayed with three full sprays of the extract at varying concentrations every hour.

The researchers regularly observed ticks for changes in behavior and mortality. Every hour for six hours, the researchers counted the number of dead ticks to monitor the effects of the plant extracts. The experiment was conducted in three replications.

Statistical Treatment of Data

The Arithmetic Mean, Percentages, and Two-way ANOVA were utilized to treat the data gathered from the experiment.

Results and Discussion

Phytochemicals Present in A. indica and C. papaya Extracts

Table (1) presents the phytochemicals in the *A. indica* and *C. papaya* leaf extracts. The semi-quantitative phytochemical analysis of the extracts revealed the presence of six substances that are believed to have acaricidal effects on the ticks. These substances can exert their effect either individually or in combination to produce a synergistic effect.

Both extracts have moderate concentrations of tannins, alkaloids, flavonoids, and a low concentration of phenolic compounds. Furthermore, *A. indica* leaf extract has moderate concentrations of saponins and glycosides, while *C. papaya* extract has low concentrations of these phytochemicals.

Table 1: Phytochemicals present in the plant extracts

Phytochemicals	*A. indica*	*C. papaya*
Tannins	(++)	(++)
Flavonoids	(++)	(++)
Saponins	(++)	(+)
Glycosides	(++)	(+)
Alkaloids	(++)	(++)
Phenolic compounds	(+)	(+)

The acaricidal properties of plants are attributed to the presence of phytochemicals (Oyagbemi *et al*., 2019). Flavonoids, tannins, phenols, alkaloids, and terpenoids are among the secondary metabolites that are abundant in plant extracts. They are known for their ovicidal, acaricidal, repellent, and anti-nutrition qualities, making them suitable for use as natural insecticides or acaricides (Guneidy *et al*., 2021). Plants synthesize tannins which are the most abundant secondary metabolites. The number of eggs laid and the larval hatching rate of ticks are significantly influenced by tannins (Quadros *et al*., 2020). Oyagbemi *et al*. (2019) found that *N. tabacum* demonstrated observable acaricidal activity against the adult and larval dog tick *R. sanguineus*. The phytochemical examination of *N. tabacum* leaf extract showed a significant percentage of terpenoids along with saponins, tannins, alkaloids, flavonoids, and anthraquinones. Zara and Ridwan (2022) yielded the same findings when they subjected *A. indica* to qualitative and quantitative phytochemical analysis. The phytochemical screening indicated the presence of glycosides, terpenoids, alkaloids, saponins, and tannins in the extract. Quantitative analysis further indicated that the leaf extract contained elevated levels of flavonoids and total phenolic compounds, highlighting its rich bioactive potential.

Likewise, an analysis of the *C. papaya* leaf extracts using qualitative phytochemical screening showed that the *C. papaya* Linn extracts contained proteins, carbohydrates, glycosides, alkaloids, flavonoids, tannins, and phenolic substances (Hussain *et al*., 2018).

Negash *et al*. (2024) assert that tannins disrupt protein synthesis and enzyme function, which affects the development and survival of ticks. Conversely, alkaloids and phenolic compounds are neurotoxic substances that induce acute toxicity in ticks. These phytochemicals act independently or synergistically.

Acaricidal Activity of the Individual and the A. indica - C. papaya Combined Extracts against R. sanguineus at Different Concentrations

The acaricidal activities of the different concentrations of the *A. indica* and *C. papaya* extracts are presented in Table (2).

At 100% concentration of the *A. indica* extract, 16 of the 50 ticks (32%) died at the 3rd h, and all the 50 ticks died at the end of the 6th h. The same pattern is observed for the 75% concentration, except that more ticks died in the 100% extract than in 75%. In the latter, 46 ticks out of the 50 (92%) died at the 6th h. This finding indicates that the higher the concentration of the extract, the greater its acaricidal effects. For the 25 and 50% concentrations, only 24 and 36% of the ticks died after 6 hours of observation.

Table 2: Acaricidal activity of the individual concentrations of the *A. indica*, *C. papaya*, and the *A. indica* - *C. papaya* combined leaf extracts against the *R. sanguineus*

Treatment	Initial Count	Number of dead ticks within the 6-h observation time						Total mortality
		1st h	2nd h	3rd h	4th h	5th h	6th h	
A. indica leaf extract								
25%	50	0	0	0	0	5 (10%)	7 (14%)	12 (24%)
50%	50	0	0	0	0	8 (16%)	10 (20%)	18 (36%)
75%	50	0	0	8 (16%)	14 (28%)	12 (24%)	12 (24%)	46 (92%)
100%	50	0	0	16 (32%)	10 (20%)	12 (24%)	12 (24%)	50 (100%)
C. papaya leaf extract								
25%	50	0	0	0	0	3 (6%)	6 (12%)	9 (18%)
50%	50	0	0	0	0	6 (12%)	8 (16%)	14 (28%)
75%	50	0	0	0	8 (16%)	22 (44%)	12 (24%)	42 (84%)
100%	50	0	0	12 (24%)	13 (26%)	17 (34%)	8 (16%)	50 (100%)
A. indica – *C. papaya* combined leaf extract								
100-100%	50	2 (4%)	25 (50%)	15 (30%)	8 (16%)	0	0	50 (100%)
75-25%	50	0	3 (6%)	14 (28%)	17 (34%)	16 (32%)	0	50 (100%)
50-50%	50	0	0	7 (14%)	14 (28%)	16 (32%)	6 (12%)	50 (100%)
25-75%	50	0	1 (2%)	7 (14%)	16 (32%)	18 (36%)	4 (8%)	46 (92%)
Distilled water (-) Control	50	0	0	0	0	0	0	0
1% Permethrin (+) Control	50	13 (26%)	17 (34%)	20 (40%)	0	0	0	50 (100%)

On the other hand, the 100% *C. papaya* extract exhibited a 100% mortality rate after the 6th h observation time, although 24% mortality was observed by the 3rd h. The 75% *C. papaya* extract killed 84% of the ticks, while 28 and 18% were killed for the 50 and 25% concentrations, respectively, after the 6th h observation time.

The same pattern is exhibited when the ticks are exposed to different concentrations of *C. papaya*. Thus, the researchers generalized that as the concentration increases, so does the mortality rate of the *R. sanguineus*.

Concerning the controls, as expected, the distilled water (negative control) exhibited no acaricidal activity. In contrast, the 1% Permethrin (positive control) has a very significant effect, killing 26% of the ticks in the 1st h, 34% in the 2nd h, and 40% in the 3rd h. All 50 ticks died after 3 h.

The experiment results show that the *A. indica* leaf extract is more effective in killing the ticks than the *C. papaya* extract. However, it is not as effective as the 1% Permethrin solution.

Adenubi *et al*. (2016) reviewed medicinal plants that have tick-repellent or acaricidal effects in vitro on both immature and adult ticks. *A. indica* and *C. papaya* were among the plants that exhibited good acaricidal and larvicidal activity, with 90-100% effectiveness, which is comparable to the effectiveness of the currently used acaricides. *A. indica* (8 mg/mL) leaves, bark, and stem caused an 80% mortality rate and a 34.0 mg decrease in egg mass, while *C. papaya* (100 mg/mL) seeds caused an 82.2% larvicidal mortality rate and 100% inhibition of oviposition, and eclosion of eggs.

The proteolytic activity of *C. papaya* Linn. fruit latex on the cuticle of the ticks was found to have an acaricidal effect on the larvae and engorged females of *R. microplus*. Females treated with the latex were observed to have reduced weight of egg mass. When compared to the other treatment and control groups, the females treated with the highest concentration of latex had the lowest weight of egg mass. Similarly, there was a significant mean mortality of the tick larvae at all concentrations of the active latex (Filgueiras *et al*., 2021).

On the same table is presented the acaricidal activity of the different concentrations of the *A. indica* – *C. papaya* combined leaf extracts.

The 100% *A. indica* - 100% *C. papaya* combination exhibited the most effective killing effect among the four preparations. At the 1st h, 4% of the ticks are dead, and at the 2nd h, 50% are dead. At the end of the 4th h, all the ticks are dead. Although the 100% *A. indica* - 100% *C. papaya* combination is effective, the 1% Permethrin solution is still better. In the latter, 13% of the ticks were dead at the 1st h, and all 50 ticks died at the end of the 3rd h.

The 25-75% combination is the least effective of the four preparations, killing only 92% of the ticks after the six-hour observation period. This was followed by the 50-50% and 75-25% *A. indica* – *C. papaya* combinations, respectively.

When the effect of the individual extract is compared to the effect of the combined extract, the latter is found to be more effective. This result is a manifestation that the extracts have a synergistic effect. Synergism is the interaction of two or more substances to produce a combined effect more significant than the sum of their separate effects (Roell *et al*, 2017; Calzetta *et al*. 2024).

The synergistic effect between *A. indica* and *C. papaya* extracts in acaricidal activity can be explained by analyzing their chemical interactions and how their bioactive compounds work together to enhance toxicity against ticks. Both extracts contain different classes of bioactive compounds that target physiological pathways in ticks, leading to enhanced toxicity. *A. indica* contains azadirachtin, salannin, and other limonoids that disrupt tick growth, molting, and feeding by interfering with their hormonal and neural systems (Al-Rajhy *et al*, 2003). *C. papaya*, on the other hand, contains papain (proteolytic enzyme), carpaine (alkaloid) (Khaoiam *et al.* 2024), and flavonoids, which degrade cuticular proteins and interfere with tick metabolism (Filgueiras *et al.*, 2021). Together, these compounds amplify the toxic effects on ticks by attacking different pathways simultaneously.

The line graph below (Fig. 1) illustrates the ticks' mortality trend when exposed to the *A. indica* leaf extract at different observation times.

The acaricidal effect of *A. indica* leaf extract against *R. sanguineus* is demonstrated in the line graph over a six-h period. This effect is concentration-dependent. The 75% concentration demonstrated a slightly delayed response, with 46 ticks killed by the 6th h, while the 100% concentration exhibited the most potent and rapid effect, killing 50 ticks by the 4th h. In contrast, the 50% and 25% concentrations exhibited weaker effects, with tick mortality occurring more gradually and reaching only 18 and 12 ticks, respectively, by the end of the investigation. The negative control group did not exhibit any tick mortality, and this confirms that the fatalities were exclusively caused by the neem extract. These findings imply that *A. indica* is significantly more effective in eliminating ticks at higher concentrations, which suggests that it has the potential to serve as a natural alternative to synthetic acaricides.

The result aligns with the findings of Punia *et al*. (2022), where the acaricidal efficacy of the leaves, seeds, and bark of *A. indica* was tested against the cattle tick *R. microplus*. The *A. indica* seeds, leaves, and bark in methanolic extracts have the highest efficacy at 70% concentration which affected 54%, 56.5% and 36% tick mortality, respectively, in the Larval Packet Test after 24 hour of treatment. Larval mortality increased when neem methanol extract concentrations were increased.

Lima de Souza *et al*. (2017) noted the incidence of significant histochemical and morphological alterations in *R. sanguineus*, especially in females subjected to increased azadirachtin-enriched neem oil concentrations. This illustrates the chemical's dose-dependent activity. A reduction in the thickness of the cuticle was noted in conjunction with a shift in the epithelial cell distribution. The cells exhibited highly vacuolated cytoplasm and pyknotic, fragmented nuclei. These features suggest that these cells are going through a dying phase.

Similar to (Fig. 1), Figure 2 presents the trend of the acaricidal activity of the different concentrations of the *C. papaya* leaf extract against *R. sanguineus* as observed in 6th h. As gleaned from the graph, Permethrin (positive control) exerted the most effective acaricidal activity. This was followed by the 100, 75, 50 and 25% respectively. In general, as the concentration of the leaf extract increases, so does its acaricidal activity.

Figure 3 depicts the mortality trend of *R. sanguineus* exposed to the different combinations of the two plant extracts used in this study. While it is true that the 1% Permethrin solution is the most effective among the test solutions, the 100% combination of the two plant extracts is comparable. Thus, 100% combined extract can be used as a substitute for the Permethrin pesticide.

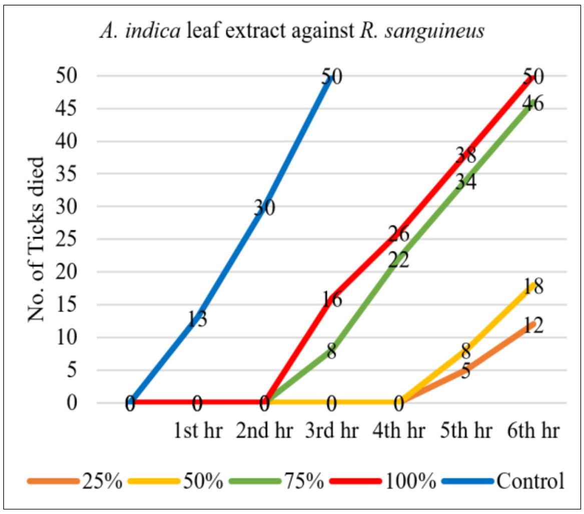

Fig. 1: Line graph showing the mortality trend of *R. sanguineus* exposed to the different concentrations of the *A. indica* leaf extract as observed in 6 h

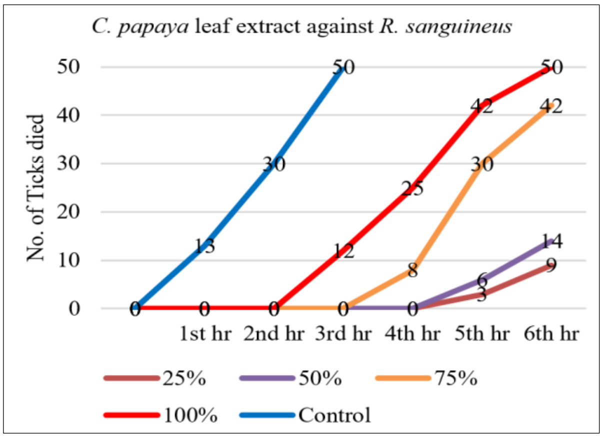

Fig. 2: Line graph showing the mortality trend of *R. sanguineus* exposed to the different concentrations of the *C. papaya* leaf extract as observed in 6 h

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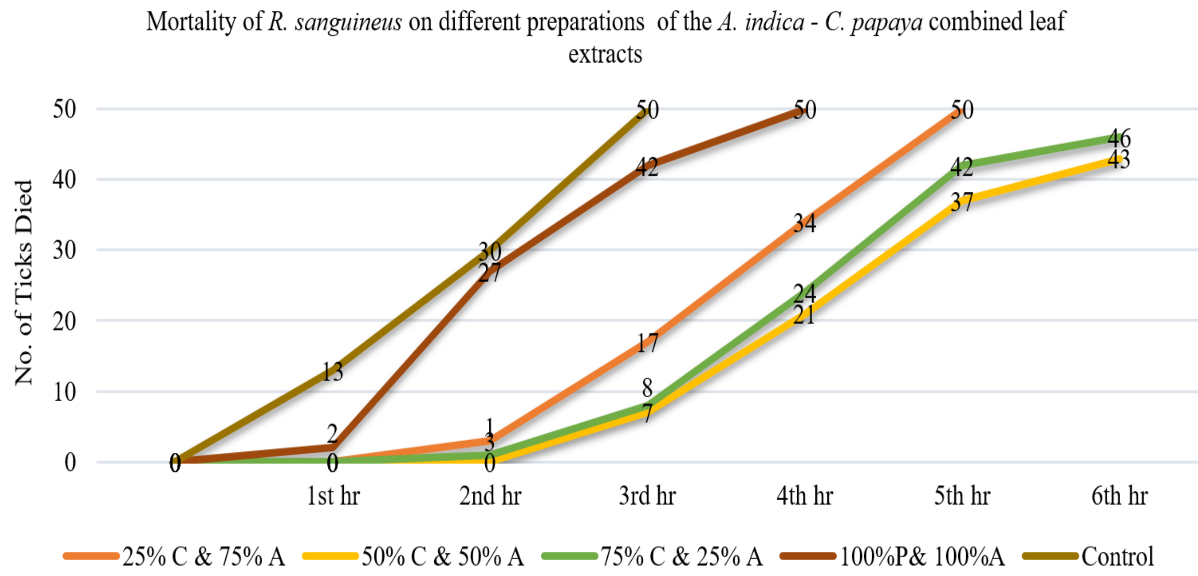

Fig. 3: Line graph showing the mortality of *R. sanguineus* exposed to the different combinations of the *A. indica* - *C. papaya* leaf extract as observed in 6 h

Particularly, the acaricidal effect was most rapid and potent when the 100% papaya and 100% neem extract were combined, resulting in complete tick mortality (50 ticks) by the third hour. The 50% papaya and 25% neem mixtures exhibited a more gradual but still significant increase in tick mortality, reaching 46 and 43 dead ticks, respectively, by the 6^{th} h. This is consistent with the 25% papaya and 75% neem combination, which reached full mortality by the 4^{th} h. The absence of tick fatalities in the negative control group serves as confirmation that the observed mortality was caused by the plant extracts. These results indicate that the acaricidal activity of neem extract is significantly increased when used in conjunction with papaya, which makes these natural extracts a promising alternative for tick control.

The synergistic acaricidal potential of *Andropogon citrates*, *Cymbopogon citratus*, *Ocimum sanctum*, *Pinus longifoia*, *Calotropis procera*, *Datura stramonium*, *Aegle marmelos*, *Ricinus communis*, *A. indica*, *Allium sativum*, *C. papaya*, *Annona squamosa* and *Pongamia glabra* was assessed against tick infestation in cattle by the formulation of a polyherbal spray. Twenty randomly chosen tick-infested cattle were treated with a single application of a polyherbal spray. Results showed a significant reduction in the mean tick count from three days to 21 days after treatment. A highly significant increase in total erythrocyte count and packed cell volume was observed compared to pre-treatment values, indicating the reduction in blood loss due to heavy tick infestation before treatment, suggestive of improvement in hematological and clinical conditions (Bhikane *et al*., 2018).

Significant Difference in the Mortality Rate of Ticks when Treated with each of the A. Indica And C. Papaya Extracts and when Grouped According to Concentration

Table (3) shows the variation in the mortality rate of ticks treated with *A. indica* and *C. papaya* at different concentrations of leaf extracts.

The level of concentrations shows a significant difference in the mortality rate of ticks with a p-value of 0.000, as shown in Table (3). The result means that the different levels of concentrations have different degrees of killing ticks. Generally, as the concentration of the extract increases, the tick's mortality likewise increases.

The interaction effect was also significant, $F = 4.778$, $p = 0.015$ (treatment and Concentration level), indicating that the mortality of ticks is significantly higher in *A. indica* extract with a 25%-75% level of concentration compared to the *C. papaya* extract. The 100% level of concentration shows no significant difference in both plant extracts.

Moreover, the treatment effect is significant, indicating that different plant extracts or combinations have varying levels of acaricidal activity. Similarly, the concentration effect is highly significant, meaning that increasing the dosage of the extracts leads to significant differences in tick mortality. The significant interaction (Treatment* Concentration) implies that the acaricidal activity is not solely due to individual extracts but is influenced by their combined effect at different concentrations. This supports the possibility of synergism, where the combined extracts enhance efficacy beyond what would be expected from their individual effects.

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Table 3: Differences in the effect of *A. indica* and *C. papaya* against the mortality of *R. sanguineus*

Variables	F-Statistics	Sig (P)	Analysis
Treatment	40.333	0.000	Significant
Concentration	2083.889	0.000	Significant
Treatment*concentration	4.778	0.015	Significant

Table 4: Differences in the effect of *A. indica* and *C. papaya* with their different level concentrations against *R. sanguineus*

Comparison	Mean Diff.	P	Analysis
25 vs 50%	-5.5	0	Significant
25 vs 75%	-33.5	0	Significant
25 vs 100%	-39.5	0	Significant
25 vs. positive control	-39.5	0	Significant
50 vs 75%	-28	0	Significant
50 vs 100%	-34	0	Significant
50% vs positive control	-34	0	Significant
75 vs 100%	-6	0	Significant
75 vs. positive control	-6	0	Significant
100% vs positive control	0	0	Significant

Tables (3-4) reveal that the main effect for level concentration was significant, with a value of $F = 2083$ and significance of $p = 0.000$, such that the different levels of concentration had significant differences in the mortality of ticks for 25% ($M = 10.5$, $SD = 2.074$), 50% ($M = 16.00$, $SD = 2.280$), 75% ($M = 44$, $SD = 2.53$) and 100% ($M = 50.00$, $SD = 0.00$). The result means that the 100% concentrations in both plants have the highest significant variation in the effect of the plants against the ticks.

The same result was observed in the study of Shyma *et al*. (2014), wherein cattle tick larvae treated with all the tested concentrations of *A. indica*, *C. procera*, *D. stramonium*, *A. sativum*, and *C. papaya* extracts by larval packet test showed significant mortality ($p<0.001$) at the highest concentration.

Zaman *et al*. (2012) combined aqueous herbal extracts of *A. indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers, and *Trachyspermum ammi* seeds and evaluated their efficacy against cattle ticks using an adult immersion test, larval packet test, and ear bag methods. The herbal extracts exerted dose-dependent and time-dependent responses against all the developmental stages of the *Rhipicephalus* (Boophilus) *microplus*. The same result was observed in the present study, which signifies that the higher the extract's concentration, the higher the level of tick mortality and the shorter the time required to kill the ticks.

The differences in mortality rates caused by the activity of the *A. indica* and *C. papaya* extracts directly influence the selection, formulation, and application of plant extracts for tick control. More effective extracts provide opportunities for optimized, cost-efficient, and environmentally sustainable acaricidal strategies while also helping to manage tick resistance and non-target effects.

Conclusion

Ticks are major carriers of diseases to humans and animals. Anti-tick agents prepared from synthetic materials are commercially available in the market and are widely used to eradicate these disease carriers. However, these compounds are detrimental to the environment, humans, and animals. Acaricidal resistance also arises because of repeated exposure to these chemical acaricides. Hence, plant-based acaricides are strongly recommended as alternatives since they exhibit no negative effects on host organisms and the environment.

Extracts of *A. indica* and *C. papaya* were tested against *R. sanguineus* for their acaricidal effect at different concentrations and time intervals. The findings suggest that the extract of the plants contains secondary metabolites with high acaricidal activity properties. The efficacy of the extracts increases with increasing concentration and exposure time. Therefore, the 100% *A. indica* - 100% *C. papaya* combined leaf extracts can be used as an alternative bio-agent against dog ticks. This finding presents a solution to the growing problem of finding alternative environmentally friendly methods of controlling parasites that have fewer adverse effects.

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Author’s Contributions

Alfredo Vita Corpuz: Conceived the study, participated in all experiments, analyzed the data, and contributed to the manuscript.

Carmela Montoya Florentino: Contributed to the writing of the manuscript and analysis of data.

Paul Erman Tabuloc Quilana: Took part in sample collection.

Amelia Jane Pasion Reotutar: Participated in the experiment.

Rods Anthony Tacal Reyes: Participated in the experiment.

Ethics

This article is entirely original. The corresponding author certifies that all other authors have read and accepted the work and that there are no ethical contradictions.

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