SÃO PAULO STATE UNIVERSITY
SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES
JABOTICABAL CAMPUS

ENHANCING ACARICIDE EFFICACY WITH ADJUVANTS FOR INTEGRATED MANAGEMENT OF THE LITCHI ERINOSE MITE

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Dedicate

With heartfelt gratitude, I dedicate this modest work to my dear parents, the source of my life, love, and affection. May God grant you good health and a long life, so that I, in turn, may bring you joy.
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SUMMARY

AUMENTANDO A EFICÁCIA DE ACARICIDAS COM ADJUVANTES PARA MANEJO INTEGRADO DO ÁCARO-DA-ERINOSE-DA-LICHIA

RESUMO

O ácaro-da-erinose-da-litchi (*Aceria litchii*) é a principal praga de plantas de lichia em vários países, causando perdas de produtividade de até 80%. A alimentação do ácaro acarreta em formação de sintomas nas folhas conhecidos como erinose, que protege os ácaros de diversos fatores adversos, incluindo o contato com acaricidas. O estudo teve como objetivo avaliar o efeito de adjuvantes nas propriedades das soluções acaricidas e seu impacto sobre *A. litchii*. Apresenta-se uma ampla revisão bibliográfica sobre o tema, que inclui a importância da produção de lichias no mundo, sintomas e danos causados pelo ácaro-da-erinose, aspectos ecológicos e biológicos do ácaro e por fim uma abordagem geral sobre o manejo do ácaro e o papel dos adjuvantes. Foram selecionados nove adjuvantes (Aureo®, Break-Thru®, Fighter®, Fixer®, Iharaguen-S®, MSO Spray®, Orix AD®, Silwet L-77® e Wetcit Gold®) e dois acaricidas [Sanmite® (pyridaben) e Ortus® (fenpyroximate)] para avaliação. Inicialmente, foram medidas propriedades-chave das soluções — tensão superficial, ângulo de contato e pH — em caldas contendo apenas acaricidas e naquelas misturadas com adjuvantes. Posteriormente, foram conduzidos dois experimentos em laboratório utilizando uma torre de Potter para avaliar a sobrevivência dos ácaros após a aplicação dos acaricidas isolados e em combinação com os adjuvantes. Entre os acaricidas testados, o pyridaben foi o mais eficaz, resultando em menor número de ácaros sobreviventes e deixando o erinea. Além disso, os adjuvantes Break-Thru®, Fighter®, Iharaguen-S®, Silwet L-77® e Wetcit Gold® aumentaram a eficácia dos acaricidas, especialmente quando combinados com pyridaben Deles, Silwet L.77® se destacou como aquele que proporcionou a maior mortalidade dos ácaros.

Palavras-chave: *Aceria litchi*, *Litchi chinensis*, erinose, adjuvantes, tensão superficial e ângulo e contato.

ENHANCING ACARICIDE EFFICACY WITH ADJUVANTS FOR INTEGRATED MANAGEMENT OF THE LITCHI ERINOSE MITE

ABSTRACT

Litchi erinose mite (*Aceria litchii*) is the primary pest of litchi trees in several countries, causing yield losses of up to 80%. Mite infestations lead to the formation of a protective layer on leaves, known as erinose, which shields mites from various adverse factors, including contact with applied acaricides. This study aimed to evaluate the effect of adjuvants on the properties of acaricide solutions and their impact on *A. litchii*. An extensive literature review on the subject, which includes the importance of litchi production worldwide, symptoms and damage caused by the erinose mite, ecological and biological aspects of the mite, and finally, an overview of mite management and the role of adjuvants. Nine adjuvants (Aureo®, Break-Thru®, Fighter®, Fixer®, Iharaguen-S®, MSO Spray®, Orix AD®, Silwet L-77®, and Wetcit Gold®) and two acaricides [Sanmite® (pyridaben) and Ortus® (fenpyroximate)] were selected for investigation. Initially, key solution properties—surface tension, contact angle, and pH—were measured for sprays containing only acaricides and those mixed with adjuvants. Subsequently, two laboratory experiments were conducted using a Potter tower to assess mite survival after applying acaricides alone and in combination with adjuvants. Among the tested acaricides, pyridaben was the most effective, resulting in fewer mites surviving and leaving the erineum. Additionally, the adjuvants Break-Thru®, Fighter®, Iharaguen-S®, Silwet L-77®, and Wetcit Gold® enhanced acaricide efficacy, particularly when combined with pyridaben. Of these, Silwet L-77® stood out as the one provided the highest mite mortality.

Keywords: *Aceria litchi***,** *Litchi chinensis,* erinose, surfactants, surface tension, and contact angle.

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1 - GENERAL CONSIDERATIONS

Lychee *Litchi chinensis* Sonn. (Sapindaceae) is a commercially important fruit crop native to Asia and northern Vietnam, cultivated in several countries in tropical and subtropical regions of the world, such as Australia, Indonesia, the Philippines, Thailand, Taiwan, Pakistan, Israel, Madagascar, Mauritius, South Africa, Brazil, and the United States (Menzel 2002). Lychee (*Litchi chinensis*) is a fruit tree likely originating in southern China (Martins et al. 2001). In many regions around the world, lychee fruits are highly valued commercially, primarily due to their distinctive flavor and notable nutritional properties (Septembre-Malaterre et al. 2016). Brazilian lychee production is concentrated mainly in the states of São Paulo, Minas Gerais and Paraná (Suguino 2006).

Lychee production faces a global threat represented by the litchi erinose mite, *Aceria litchii* Keifer (Acari: Eriophyidae). This invasive mite is the most damaging pest of lychee, causing an estimated 80% reduction in fruit production and 20% increase in production costs (Prasad and Singh 1981; Navia et al. 2013). Litchi erinose mite feeding induces the formation of abnormal structures called "erinea", characterized by the proliferation of hypertrophic hairs on the plant's epidermal cells. This infestation impacts leaves, twigs, flowers, and fruits (Nishida and Holdaway 1955; Castro et al. 2018; Carrillo et al. 2020). When mites infest inflorescences and fruit, it directly jeopardizes the yield, rendering it unsuitable for consumption (Keifer et al. 1982; Westphal and Manson 1996; Waite and Hwang 2002).

Controlling *A. litchii* has been a considerable challenge for growers worldwide. Various methods have been attempted, including cultural practices like removing and burning infested branches from lychee trees (Menzel and Waite 2005; Castro et al. 2014). Severe pruning appears to remove the mites from a tree effectively. Unfortunately, the mites often return when the plant produces new shoots. Chemical control involving repeated pesticide applications has been the most used alternative to protect new plant growth from mite reinfestation (Nishida and Holdaway 1955; Waite and Hwang 2002).

The lack of knowledge about fundamental aspects of this mite-plant interaction system is one of the primary reasons for the unsuccessful control of *A. litchii*. The mites are microscopic and have a cryptic lifestyle, not being able to survive for long outside the erinea (Azevedo et al. 2013; Revynthi et al. 2020). Because of this unique lifestyle, little is known about their biology and behavior. One hypothesis suggests that as the mite population grows, erinea become denser, possibly serving as a protective environment for the mites against biological and environmental stressors.

2 LITERATURE REVIEW

2.1. The lychee plant

The lychee tree is widely cultivated in many countries, standing out for its economic importance, nutritional value and commercial appeal. Native to the southern region of China, where it has been cultivated for over two thousand years, the plant has also adapted to other areas of the Asian continent (Bishayee et al. 2024). It was later introduced to other regions of the world, including Brazil in 1810 and United States (Florida), where it has found favorable climatic conditions for its development in specific areas (Castro et al. 2014; Revynthi et al. 2022).

Lichee represents a promising crop, especially in market niches that value high-quality exotic fruits. The fruit has high commercial value both fresh and in processed forms, such as juices, jams, preserves, and sweets (Vinha et al. 2022). It also holds significant value in gastronomy and the cosmetics industry due to its antioxidant properties and its high content of vitamin C, potassium, and phenolic compounds (Chen et al. 2021; Ataide et al. 2024).

Lychee plants are characterized as medium to large trees, reaching up to 12 meters in height. They have a very dense and rounded crown, with compound, leathery, dark green leaves and panicle-type inflorescences containing small, yellow or greenish flowers, which are mainly pollinated by insects (Ataide et al. 2024) (Figure 1). Fruiting occurs mainly during the summer, and the fruits, gathered in bunches, are of the drupe type, reddish in color when ripe, with a rough and brittle epicarp, the pulp, white in color, and juicy in texture, is translucent, with a sweet and slightly acidic flavor, containing a single seed of dark brown color, shiny and oblong in shape (Chen et al. 2021).

Figure 1. Lychee plant (*Litchi chinensis*) during its reproductive phase (flowering). Note the developing fruits in the lower right corner. Taquaritinga, São Paulo, Brazil

Lychee cultivation requires specific climatic conditions, since the plant is sensitive to frost and requires a period of mild cold during the winter to induce flowering. In addition, it prefers well-drained, deep, and fertile soils with good availability of organic matter. Propagation can be carried out by seeds, although this practice does not guarantee the genetic fidelity of the mother plant. Therefore, vegetative multiplication, such as air layering, is more recommended, as it allows for the production of plants with desirable and productive agronomic characteristics (Castro et al. 2014).

In Brazil, the area dedicated to lychee production is still small compared to other tropical fruit crops. However, lychee production has been gradually increasing, mainly in the states of São Paulo, Minas Gerais, Paraná, and Bahia. Adaptation to Brazilian soil and climate conditions has been made possible through agronomic studies focused on cultivar selection, phytosanitary management, pruning techniques, irrigation, and plant nutrition. The main variety cultivated in Brazil is Bengal, known for its good productivity and the organoleptic quality of its fruits (Salomão et al. 2006; Amaral et al. 2020).

2.2. The litchi erinose mite

2.2.1. Damages

Several pests and diseases can affect lychee plants and compromise fruit production and quality (Chen et al. 2021). However, among the pests, the litchi erinose mite *Aceria litchii* (Keifer) (Acari: Eriophyidae) stands out and is considered the main pest of the crop in several countries (Moraes et al. 2024). To feed, this mite uses specialized mouthparts called stylets, which pierce the epidermal cells of the leaves. The directly affected cells die, while adjacent ones undergo structural changes, leading to hyperplasia of the leaf trichomes, structures commonly known as erineia, which become dense and branched (Karioti et al. 2011; Azevedo et al. 2016; Ataide et al. 2024) (Figure 2).

Figure 2. Damage caused by *Aceria litchii* on lychee plants of the Bengal cultivar. Taquaritinga, São Paulo, Brazil.

The emergence of erineas generally begins on the underside of new leaves, appearing white or translucent and causing distortion of the leaf tissue (Arantes et al.

2017). Although this change occurs primarily on the underside, discoloration on the upper surface can also be observed. As the mite population increases, the erineas undergo changes in both color and density, shifting from white to amber tones and eventually to dark brown or black (Ataide et al. 2024). This color progression indicates the level of mite activity: light and sparse erineas contain fewer individuals, while amber-colored ones indicate heavier infestations. In contrast, dark and thick structures tend to harbor few or no mites, suggesting that the exploitation phase of that leaf has ended and the individuals have already migrated to new areas of the plant (Azevedo et al. 2013; Ataide et al. 2024). In addition to leaves, these structures can develop on petioles, stems, shoots, flower buds, and even fruits, varying in size, shape, and color (Menzel and Waite 2005; Ranjan et al. 2018).

Aceria litchii is a host-specific mite, as it exclusively infests lychee (*L. chinensis*) plants. Although a report from Thailand suggests its occurrence on *Dimocarpus longan* Lour. (longan), this is believed to be a misidentification, possibly confused with *Aceria dimocarpi* (Kuang) (Acari: Eriophyidae). Litchi erinose mite prefers humid environments and mild temperatures, being more active during the plant's budding periods. This mite lives and feeds on the young structures of the lychee tree, where it finds ideal conditions for its development and reproduction (Azevedo et al. 2013).

Young lychee plants tend to be more vulnerable to mite attack. Although some cultivars may exhibit varying levels of sensitivity, all varieties have demonstrated susceptibility to infestation (Amaral et al. 2020). Severe infestations can cause premature leaf and flower drop, as well as fruit abortion. In extreme cases, young plants may experience severely stunted growth, while in mature plants, repeated infestations can reduce both longevity and productivity. Because of its cryptic behavior and minute size, the mite often remains undetected until visible symptoms appear, making preventive management challenging (Revynthi et al. 2020).

Raga et al. (2010) reported that *A. litchii* may transmit the alga *Cephaleuros virescens* Kunze, which in turn induces the formation of erineia. This suggests a possible symbiotic relationship between the mite and the alga, although the nature of this interaction remains poorly understood. According to Somchoudhury et al. (1989) and Sharma and Thakur (1991), *C. virescens* spores present in the environment may colonize lychee leaves through perforations made by the mites, possibly facilitated by toxins they inject. It has been proposed that the erineia might actually be algal filaments, since lychee leaves naturally lack trichomes. However, this hypothesis remains unconfirmed, and there are still many uncertainties surrounding this association.

2.2.2. Biological aspects and behavior

Amaral et al. (2020) described *A. litchii* as a microscopic mite with a vermiform body and a whitish to yellowish coloration, measuring approximately 0.2 mm in length. With an approximate body length of 150 micrometers, it is not visible to the naked eye, requiring magnification equipment such as microscopes for observation (Figure 3). Females are parthenogenetic for most of their life cycle and lay their eggs in protected areas of the plant, such as young shoots and floral or vegetative buds, where the nymphs develop until reaching the adult stage (Song et al. 2024).

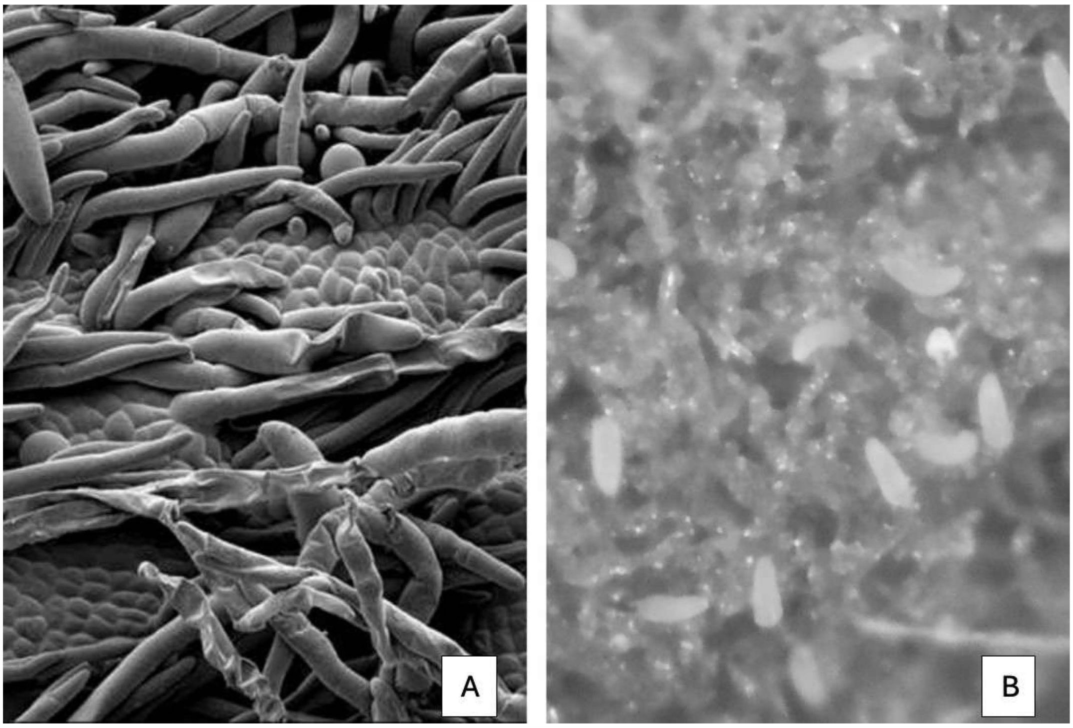


Figure 3. *Aceria litchii* (A- The photo of the mites and eggs was taken by scanning electronic microscopy (Foto: Dr Ronald Ochoa – USDA). B- photo of the mites on the surface of the leaf with symptoms (Foto: Adalton Raga,2010)).

The biological cycle of *A. litchii* ranges from 10 to 14 days. It comprises the stages of egg, protonymph (first instar), deutonymph (second instar), and adult (Alam and Wadud 1963). The eggs are translucent, approximately 0.032 mm in diameter, and are laid singly, primarily at the base of the erineia on leaves, branches, and fruits (Butani 1977). The immature stages and adults exhibit a vermiform body shape, with coloration ranging from straw-white to yellowish (Butani 1977). The high degree of morphological and biological specialization enables the litchi erinose mite to inhabit extremely confined spaces, such as within erineia and galls (Manson and Oldfield 1996).

Dispersal of the litchi erinose mite occurs primarily through air currents, active crawling, and the movement of infested plant material, particularly by human activity. In addition to these means, the mite is efficiently dispersed via phoresy with the assistance of insects (Waite and McAlpine 1992). These authors reported the presence of *A. litchii* on more than 23% of bees that frequently visited infested lychee trees. Mites were frequently found attached to the bees' legs, a site initially considered disadvantageous due to grooming behavior; however, it proved to be an effective means of transport to new leaf surfaces (Sabelis and Bruin 1996). A thorough understanding of the biology, ecology, and the mechanisms of damage and dispersal of the mite is crucial for the implementation of effective control strategies that safeguard crop health and ensure high fruit quality (Ranjan et al. 2018).

2.2.3. Management of the litchi erinose mite

Management of *A. litchii* begins with the acquisition of healthy, mite-free seedlings produced in certified and reliable nurseries. This measure is essential to prevent the early introduction of the pest. Cleaning and disinfecting agricultural tools, restricting the movement of people and equipment between orchards, and adopting physical barriers can all contribute significantly to containing the mite (Song et al. 2024). In general, managing this pest is challenging and requires the integrated use of control strategies, including frequent monitoring of shoots, pruning of infested branches, and the judicious application of acaricides (Amaral et al. 2020; Carrillo et al. 2020; Ataide et al. 2024).

For efficient management of the litchi erinose mite, frequent and systematic monitoring is recommended, especially during budding periods. These structures, being more tender and susceptible, are the first to show symptoms caused by erinose (Song et al. 2024). Visual inspection should preferably be carried out using a hand lens or portable microscope, focusing on the youngest leaves, where erinose symptoms initially appear (Carrillo et al. 2020).

Chemical control combined with pruning is currently the most widely used strategy for managing *A. litchii* in many countries. Several acaricides have proven effective against this pest, but the highest efficacy is generally observed when the product is applied at the beginning of shoot development, to protect the leaves and newly formed structures (Carrillo et al. 2020; Revynthi et al. 2020). It is important to emphasize that pruning alone is not recommended as a control measure, since it may actually favor mite proliferation. Cultural management through phytosanitary pruning, integrated with chemical control to protect new shoots, has yielded the best results in managing the lichi erinose mite (Castro et al. 2018; Song et al. 2024).

Lychee harvesting is carried out by removing the peduncles that contain the fruits, which inherently involves pruning. After this procedure, further pruning of the trees is recommended to control plant size, promote uniform light distribution throughout the canopy, standardize fruiting, synchronize vegetative growth, and facilitate the implementation of agricultural practices such as fertilization and pest control (Amaral et al. 2020). However, it is strongly recommended to protect the new shoots with acaricides to prevent population outbreaks of *A. litchii* on the plants (Ataide et al. 2024).

Acaricides should be applied at the appropriate time, that is, at the onset of infestation, which generally coincides with the emergence of shoots. Applications should be avoided during dormancy or fruiting periods in order to preserve pollinators and other beneficial organisms. Products based on sulfur, abamectin, pyridaben, fenpyroximate, and mineral oils have demonstrated effectiveness in controlling *Aceria litchii* (Azevedo et al. 2013; Revynthi et al. 2022).

In addition to chemical control, biological control has proven to be a promising strategy, although it remains underexplored for this particular pest (Ranjan et al. 2018). Recent studies have investigated the potential of natural predators, such as phytoseiid mites, and the use of entomopathogenic fungi, including *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokin, as biocontrol agents for eriophyid mites. Moreover, the use of plant extracts with acaricidal properties has been evaluated as a viable alternative for management in organic and agroecological systems (Ataide et al. 2024).

Inadequate management of the lichii erinose mite poses a significant risk to the production and health of *L. chinensis* plants. The absence of proper control measures, particularly in regions where the pest is already established, can lead to severe impacts on both plant physiology and the commercial productivity of the crop (Ranjan et al. 2018).

The primary consequence of poor mite management is a decline in the plant's ability to carry out photosynthesis. This occurs because infested leaves become deformed, thickened, and may develop necrotic areas, which impair their normal function (Ataide et al. 2024). As a result, the tree's vegetative growth and fruit development are negatively affected, leading to reduced yield and lower fruit quality. Additionally, prolonged and intense infestations weaken the plant, increasing its vulnerability to other diseases and environmental stresses such as drought and high temperatures (Carrillo et al. 2020).

Furthermore, the inappropriate use of chemical product, whether due to the incorrect selection of active ingredients, inadequate dosing, or application under unfavorable weather condition can lead to phytotoxicity, pest resistance, ecological imbalances, and harm to agroecosystem biodiversity. Consequently, negligent or improper management of *A. litchii* results not only in agronomic and economic losses but also threatens the sustainability and long-term viability of lychee cultivation (Ferraz et al. 2024).

Proper management disrupts the mite's life cycle and prevents the formation of erineia, which are abnormal structures that develop as a result of its feeding. This helps preserve the integrity of the leaves and ensures the proper functioning of key physiological processes such as photosynthesis, respiration, and transpiration. As a result, the plant maintains healthy vegetative growth and produces fruits in greater quantity and with improved quality (Vinha et al. 2022). Furthermore, protecting the young tissues of the plant, which are the most vulnerable to infestation, helps preserve the flowering and fruiting stages that are critical for crop productivity. From an economic perspective, effective management can be the determining factor between a profitable harvest and a production severely affected by losses (Ferraz et al. 2024).

2.3. Adjuvants in the management of the litchii erinose mite

Adjuvants are substances added to spray solutions to modify their physicochemical properties and enhance the performance of active ingredients on the biological target (Castro et al. 2018; Ferreira e Matuo 2024). They play a fundamental role in improving the efficiency of pesticide applications, whether the products are of chemical or biological origin. These products can improve characteristics such as spread ability, adhesion, penetration, stability, leaf coverage, and resistance to wash-off by rain. They are essential allies in pest management, particularly in fruit trees with dense canopies and difficult spray coverage, such as lychee (Castro et al. 2018).

Due to the dense architecture of the lychee canopy and the cryptic behavior of *A. litchii*, which primarily inhabits new shoots, buds, and protected leaf structures, the use of adjuvants is a strategic approach to ensure that the active ingredients effectively reach the target site. Adjuvants such as spreader-stickers and non-ionic surfactants are commonly employed to enhance the product's contact with the plant surface and facilitate its absorption, thereby improving control efficacy (Revynthi et al. 2022).

Adjuvants are valuable technical tools in lychee cultivation, playing a crucial role in the effective control of *A. litchii*. The successful management of *A. litchii* requires an integrated approach that encompasses biological, ecological, technical, and socioeconomic factors, with the goal of preserving crop productivity and promoting the sustainability of the agricultural system.

3 - ADJUVANT-ENHANCED ACARICIDAL SOLUTIONS TO OVERCOME FOLIAR HYDROPHOBICITY FOR CONTROLLING THE LITCHI ERINOSE MITE

ABSTRACT

Litchi erinose mite (*Aceria litchii*) is the primary pest of litchi trees in several countries, causing yield losses of up to 80%. Mite infestations lead to the formation of a protective layer on leaves, known as erinose, which shields mites from various adverse factors, including contact with applied acaricides. This study aimed to evaluate the effect of adjuvants on the properties of acaricide solutions and their impact on *A. litchii*. Nine adjuvants (Aureo®, Break-Thru®, Fighter®, Fixer®, Iharaguen-S®, MSO Spray®, Orix AD®, Silwet L-77®, and Wetcit Gold®) and two acaricides [Sanmite® (pyridaben) and Ortus® (fenpyroximate)] were selected for investigation. Initially, key solution properties—surface tension, contact angle, and pH—were measured for sprays containing only acaricides and those mixed with adjuvants. Subsequently, two laboratory experiments were conducted using a Potter tower to assess mite survival after applying acaricides alone and in combination with adjuvants. Among the tested acaricides, pyridaben was the most effective, resulting in fewer mites surviving and leaving the erineum. Additionally, the adjuvants Break-Thru®, Fighter®, Iharaguen-S®, Silwet L-77®, and Wetcit Gold® enhanced acaricide efficacy, particularly when combined with pyridaben.

Keywords: *Aceria litchi*, *Litchi chinensis,* erinose, surfactants, surface tension, and contact angle.

3.1. Introduction

Lychee (*Litchi chinensis* Sonn.; Sapindaceae) is grown in several tropical and subtropical regions due to its high commercial and nutritional values (Zhao et al. 2020; Bangar et al. 2021). This fruit tree likely originated in southern China and northern Vietnam (Menzel 2001). Today, leading lychee-producing countries include China, Taiwan, Thailand, India, South Africa, Madagascar, Mauritius, Brazil, USA and Australia (Bangar et al. 2021). Lychees are mostly consumed fresh, while a smaller portion is processed as frozen, canned, or dried fruit, or used in beverage and jam production (Bangar et al. 2021; Bishayee et al. 2024).

Among lychee pests, the lychee erinose mite *Aceria litchii* (Keifer) (Acari: Eriophyidae) is the most significant, posing a major threat to production in several countries. In India and Brazil, infestations have led to yield losses of up to 80% and a 20% increase in production costs (Prasad and Singh 1981; Navia et al. 2013; Carrillo et al. 2020). This mite species is classified as an invasive pest in several regions. It was first reported in Brazil in 2008 (Raga et al. 2010) and in the United States (Florida) in 2018 (Carrillo et al. 2020).

This pest primarily infests the abaxial surface of young leaflets, inducing the formation of galls known as erinose (erineum), which are characterized by trichome hyperplasia (Karioti et al. 2011; Azevedo et al. 2016; Ataide et al. 2024). Similar symptoms occur on young branches, flower buds, and fruit (Menzel and Waite 2005; Waite and McAlpine 1992; Azevedo et al. 2014). Early infestation causes young leaflets to twist and develop white, transparent, or hyaline hairy masses, which darken to brown as infestation progresses, ultimately leading to tissue death (Carrillo et al. 2020; Ataide et al. 2024).

Gall-like protuberances often form on the adaxial surface of leaflets (Arantes et al. 2017). Erinose obstructs stomatal openings, disrupting photosynthesis and leading to leaf drop, poor inflorescence development, and premature fruit fall (Carrillo et al. 2020). Some studies have suggested that erinose may not be caused solely by *A. litchii* but rather by an association between this mite and the parasitic alga *Cephaleuros virescens* Kunze. However, this potential coevolutionary relationship remains unclear (Saha et al. 1996; Song et al. 2024).

Managing *A. litchii* remains a significant challenge for lychee production worldwide. Cultural practices, such as pruning and burning symptomatic branches, combined with synthetic acaricide applications, have been recommended for management of *A. litchii* (Castro et al. 2018). However, these measures substantially increase production costs and, in some cases, render cultivation economically unviable. Furthermore, mite populations continuously re-emerge on young leaflets, requiring successive acaricide applications to protect developing tissues during bud formation (Waite and Hwang 2002; Revynthi et al. 2022b).

Due to their cryptic behavior and small size, *A.litchii* and its interactions with lychee plants remain poorly studied, partly explaining the challenges associated with their management (Azevedo et al. 2014; Revynthi et al. 2022b). As mite populations increase, the erinose becomes denser, forming a protective barrier that shields mites from biological and environmental stressors (Ataide et al. 2024). Additionally, the erinose alters leaf surface properties, changing leaflet wettability from hydrophobic to superhydrophobic (Song et al. 2024). These modifications can impact acaricide efficacy by influencing spray solution coverage and deposition (Li et al. 2020; Song et al. 2024).

Adjuvants are non-biocide additives incorporated into formulations or phytosanitary spray solutions to optimize physicochemical properties, thereby improving application performance and treatment efficacy under suboptimal conditions (Ferreira and Matuo, 2024). Some adjuvants, for instance, can reduce environmental impact by improving cuticular penetration (Queiroz 2008; Castro et al. 2014), facilitating wetting on water-repellent surfaces, and promoting better contact between the spray solution and the cuticle—particularly on surfaces covered with fine hairs that suspend droplets (Castro et al. 2014; Santos et al. 2021).

Certain adjuvants, such as organosilicon compounds, are known to reduce surface tension and improve spray coverage (Knoche 1994). Selecting the appropriate adjuvant can lower pesticide application volumes while enhancing pest and disease control across various crops (Gaskin et al. 2004). However, the use of an unsuitable adjuvant may reduce pesticide efficacy.

Therefore, this study aimed to identify adjuvants capable of mitigating the super-hydrophobicity of erinose on lychee leaves, thereby improving acaricide effectiveness in controlling *A. litchii*.

3.2. Methods

3.2.1. Acaricides and adjuvants

The acaricides pyridaben (Sanmite® 200 EC, Iharabras S.A., Indústrias Químicas, Sorocaba/SP) and fenpyroximate (Ortus® SC, Nichino do Brasil Agroquímicos LTDA, Barueri/SP) were studied. These acaricides were selected based on previous studies demonstrating their efficacy in controlling *A. litchii* (Azevedo et al. 2013; Revynthi et al. 2022a). Adjuvants were chosen based on their characteristics and functions, mainly those that enhance droplet spread and wettability (Table 1).

3.2.2. Surface tension and contact angle

This stage of the study was conducted at the Center for Study and Development in Application Technology (NEDTA) laboratory, Department of Plant Health at the College of Agricultural and Veterinary Sciences, São Paulo State University (FCAV-Unesp), Jaboticabal campus, SP, between December 2023 and March 2024.

The surface tension and contact angle of the acaricides pyridaben and fenpyroximate were evaluated individually and when combined with the adjuvants listed in Table 1. Two experiments were conducted: Experiment 1 assessed pyridaben, while Experiment 2 evaluated fenpyroximate.

Each experiment consisted of ten treatments and four replicates. The treatments were as follows: 1) Acaricide; 2) Acaricide + Aureo (250 mL c.p./100 L); 3) Acaricide + Break-Thru (100 mL c.p./100 L); 4) Acaricide + Fighter (100 mL c.p./100 L); 5) Acaricide + Fixer AP (200 mL c.p./100 L); 6) Acaricide + Iharaguen-S (10 mL c.p./100 L); 7) Acaricide + MSO Spray (200 mL c.p./100 L); 8) Acaricide + Orix AD (1000 mL c.p./100 L); 9) Acaricide + Silwet L-77 (75 mL c.p./100 L); and 10) Acaricide + Wetcit Gold (150 mL c.p./100 L).

The nine adjuvants used were selected based on their composition and also on their function, since the objective is to improve the spread of the spray over the leaf surface even in the presence of erionose.

The only difference between the two experiments was the acaricide. Both acaricides were assessed at a concentration of 100 mL of commercial product (c.p.) per 100 L, based on previous studies (Revynthi et al. 2022a). Solutions were prepared using deionized water, and adjuvant concentrations were determined based on manufacturer recommendations.

An automatic tensiometer (OCA 15-plus, DataPhysics Instruments GmbH, Filderstadt, Germany) was used to evaluate surface tension and contact angle. This instrument was equipped with a high-speed digital camera and the SCA20® software for image processing and automation.

Table 1. Adjuvants evaluated in combination with acaricides for control of lychee erinose mite (*Aceria litchi*).

Adjuvant	Manufacturer (Brazil)	Main active agents	Function (Product label)
Aureo® EC	Bayer S.A.	Soybean oil methyl ester (72.0 % m/v)	Spreader adjuvant
Break-Thru® SL	Evonik Degussa	Polyether-polymethyl siloxane copolymer (100% m/v)	Non-ionic adhesive spreader/penetrating agent from the silicone chemical group
Fighter®	De Sangosse Agroquímica	Polyoxyethylene lauryl ether (955)	Performance enhancer
Fixer® AP	De Sangosse Agroquímica	Plant oil	Humectant and spreader
Iharaguen-S®	Iharabras S. A.	Polyoxyethylene alkyl phenol ether + inert ingredients	Nonionic adhesive spreader
MSO spray®	Norac Concepts	Methylated soybean oil	Emulsifiers and surfactants
Orix AD®	Oxiquímica Agrociência	Mineral oil + inert ingredients	Performance enhancer
Silwet L-77	Momentive	Polyether silicone copolymer	Humectant and spreader
Wetcit Gold®	Rovensa Next	Orange peel oil	Spraying process optimizer

Surface tension was determined using the hanging drop method by applying 2.5 µL of solution. Drops were formed using a 500 µL Hamilton® precision syringe with a 0.52 mm needle. A CCD camera with high temporal resolution captured the image of the suspended drop, and the SCA20® software analyzed its shape based on axis asymmetry. Surface tension (γ) was calculated using the Young-Laplace equation: ΔP=2"R; where ΔP is the pressure variation between surfaces, γ is the surface tension, and R is the drop's radius of curvature. Sixty images per minute were captured for analysis (Santos et al. 2021).

Drops were deposited on the abaxial surface of lychee leaflet sections (1.6 cm² rectangles: 0.4 cm × 4 cm) from the Bengal cultivar, obtained from an organic orchard at FCAV-Unesp. The contact angle was recorded every second for 60 seconds after droplet deposition. The 30-second mark was used as the reference value for surface tension and contact angle characterization, as the values tended to stabilize within this interval. Droplet spread on a surface is inversely proportional to the contact angle: the smaller the angle, the greater the spreading, and vice versa (Ferreira and Matuo, 2024).

A completely randomized design was used for both experiments. For each acaricide there were 10 treatments with four replicates. In this type of experiment, four replicates are generally used due to the precision of the equipment. The values obtained are either very similar or differ very small amounts.

Data normality was assessed using the Shapiro-Wilk test, while Levene's test was used to check the homogeneity of variance. As the data met ANOVA assumptions, treatments were analyzed using Tukey's test ($p < 0.05$). Statistical analyses were performed using R 4.2.0 (R Core Team, 2022) and AgroEstat (Barbosa and Maldonado Júnior, 2015) software.

3.2.3 Efficiency of acaricide and adjuvant combinations

This stage was conducted between April and June 2024 at the Acarology Laboratory (AcaroLab), Department of Plant Health, FCAV-Unesp. Five adjuvants were selected to assess their effectiveness in controlling *A. litchii*. The acaricides pyridaben and fenpyroximate were evaluated along with the adjuvants Break-Thru, Fighter, Iharaguen-S, Silwet L-77, and Wetcit Gold.

Lychee leaflets of the Bengal cultivar showing intermediate-stage symptoms of *A. litchii* infestation (amber-colored trichomes) (Ataide et al. 2023) were collected from an orchard at FCAV-Unesp. The leaflets were placed in Styrofoam boxes and immediately transported to the laboratory. The amber stage was chosen due to its association with the highest mite densities (Ataide et al. 2023).

Approximately 20 mite specimens, morphologically resembling *A. litchii*, were transferred from the leaflets to microscope slides and mounted in a modified Berlese medium. The slides were placed in an oven at 75 °C for 7 days. Afterward, they were sealed with colorless nail polish and examined under a phase contrast microscope (Zeiss Axioplan 2 Imaging) for species identification, based on the characters described in Carrillo et al. (2020).

Leaflets were examined under a stereoscopic microscope (Olympus SZ61, 60× magnification) to select those harboring live mites for the experiments. Leafdiscs (2.0 cm in diameter) were excised from leaflet regions displaying *A. litchii* symptoms using a metal punch. Individual arenas (leafdiscs) were placed in the center of black polypropylene plastic rectangles (25.4 mm × 76.2 mm × 0.25 mm thick) with double-sided adhesive tape (1.25 cm-wide) applied along the edges to trap live mites leaving the arenas as they dry, following methods adapted from Azevedo et al. (2013) and Revynthi et al. (2022b). All *A. litchii* mites that left the arenas 96 hours post-treatment, when the arenas dry, were considered uncontrolled by acaricide application, following the methodology of Revynthi et al. (2022).

The experiment followed a completely randomized design with 13 treatments and 20 replicates per treatment, considering the high variability in mite populations within the arenas. The treatments evaluated were: 1) Pyridaben + Break-Thru; 2) Pyridaben + Fighter; 3) Pyridaben + Iharaguen-S; 4) Pyridaben + Silwet L-77; 5) Pyridaben + Wetcit Gold; 6) Fenpyroximate + Break-Thru; 7) Fenpyroximate + Fighter ; 8) Fenpyroximate + Iharaguen S; 9) Fenpyroximate + Silwet L-77; 10) Fenpyroximate + Wetcit Gold; 11) Pyridaben; 12) Fenpyroximate and 13) distilled water (control).

For statistical analysis, data for pyridaben and fenpyroximate were analyzed separately, with the water control serving as a reference for both acaricides. The experiment was replicated twice (Experiments 3 and 4) to confirm the results obtained.

The acaricide and adjuvant concentrations were the same as those in the contact angle and surface tension experiments. Before application, the pH of each solution was measured using a pH meter (QUIMIS® Q400AS) at room temperature (~22 °C), with three measurements per treatment. Treatments were applied using a Potter tower (Burkard Manufacturing, Rickmansworth, England) calibrated to 34.5 kPa (10 lb./in^2), ensuring an average deposition of 1.56 mg/cm^2.

The numbers of mites that left the arenas were subjected to Shapiro-Wilk and Levene's tests to assess normality and homogeneity of variances, respectively. When the data from both experiments did not meet ANOVA assumptions, the mite count data underwent a Box-Cox transformation, with the lambda parameter estimated using the maximum likelihood method. Once assumptions were met, an ANOVA was performed, followed by Tukey's test ($p < 0.05$) for treatment comparisons.

Statistical analyses were conducted using R 4.2.0 (R Core Team, 2022) and AgroEstat (Barbosa and Maldonado Júnior, 2015). Plots and figures were generated using ChatGPT version 4.0.

3.3. Results

3.3.1. Surface tension and contact angle

3.3.1.1. Pyridaben (Experiment 1)

The addition of adjuvants to pyridaben significantly affected spray surface tension (F-test = 110.9, $p < 0.0001$, Coefficient of Variation (C.V.) = 6.6%) (Figure 1A).
All treatments combining pyribaden with adjuvants differed significantly from the pyridaben-only treatment. The lowest surface tension values were observed in treatments containing Iharaguen-S, MSO Spray, and Silwet L-77. Conversely, Wetcit Gold resulted in the highest surface tension. The other adjuvants (Aureo, Break-Thru, Fighter, Fixer AP, and Orix AD) reduced surface tension to intermediate levels (Figure 1A).

Significant differences were also observed in contact angle between the acaricide-alone treatment and those with adjuvants; all treatments differed from the pyridaben-only treatment, except those containing Fighter and Wetcit Gold (F-test = 123.3, $p < 0.0001$, C.V. = 10.4%) (Figure 1B).

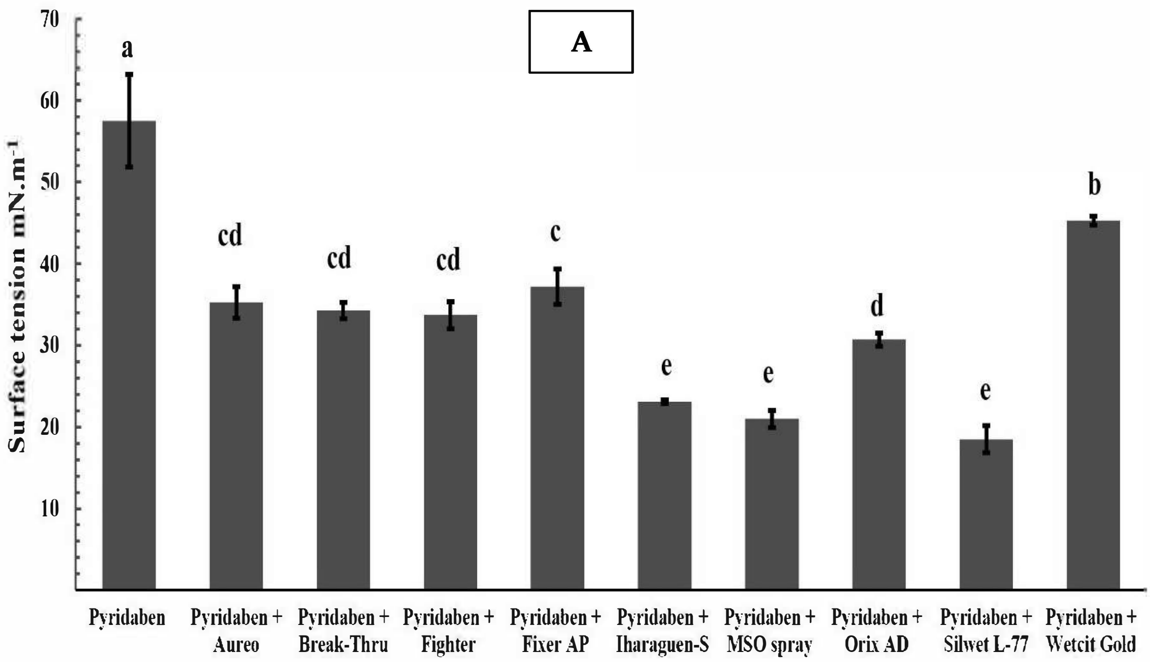
The lowest contact angle values were recorded in treatments with Iharaguen-S, MSO Spray, and Silwet L-77. Notably, Silwet L-77 resulted in a zero contact angle, significantly differing from all other treatments except MSO Spray (Figure 1B). Treatments with Aureo, Break-Thru, and Orix AD exhibited contact angles close to 100° and did not differ significantly. Additionally, Fixer AP differed from all other treatments except Break-Thru and Orix AD (Figure 1B).

3.3.1.2. Fenpyroximate (Experiment 2)

Experiment 2 assessed surface tension and contact angle in sprays containing fenpyroximate. All treatments with adjuvants significantly differed from the fenpyroximate-only treatment, except for Wetcit Gold (F-test = 43.1, $p < 0.0001$, C.V. = 11.2%) (Figure 2A).

The treatments with Iharaguen-S, MSO Spray, and Silwet L-77 resulted in the lowest surface tension values, with no significant differences. In contrast, Wetcit Gold produced the highest surface tension value among the adjuvant treatments and did not significantly differ from the fenpyroximate-only treatment (Figure 2A).

The addition of adjuvants to the acaricide spray significantly affected the contact angle (F-test = 143.2, $p < 0.0001$, C.V. = 8.1%) (Figure 2B). Silwet L-77 had the largest impact on this parameter, followed by MSO Spray. Treatments with Aureo, Break-Thru, Fighter, Fixer AP, Iharaguen-S, and Wetcit Gold did not significantly differ from one another, whereas Orix AD differed from all treatments except Fighter (Figure 2B).



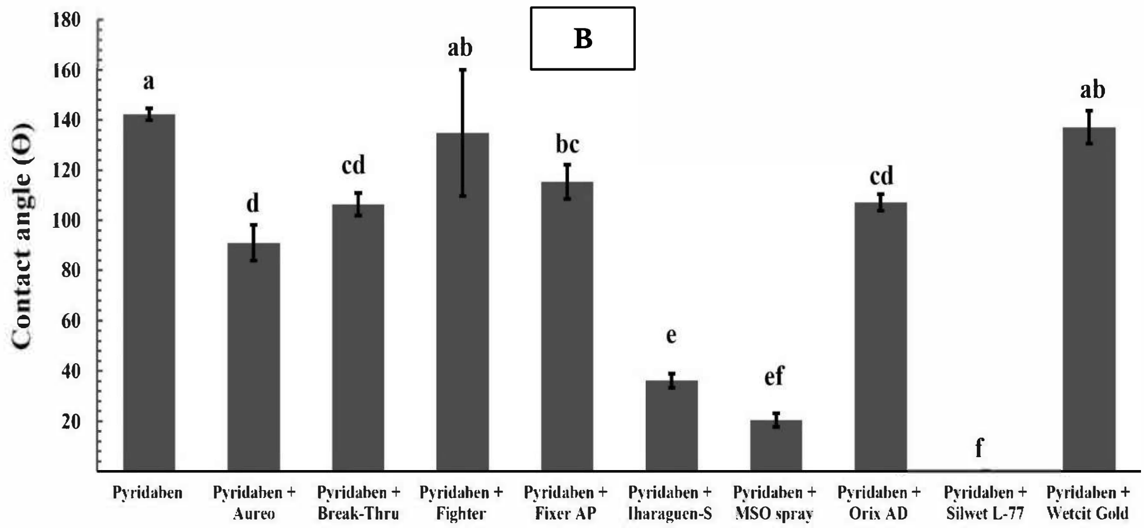
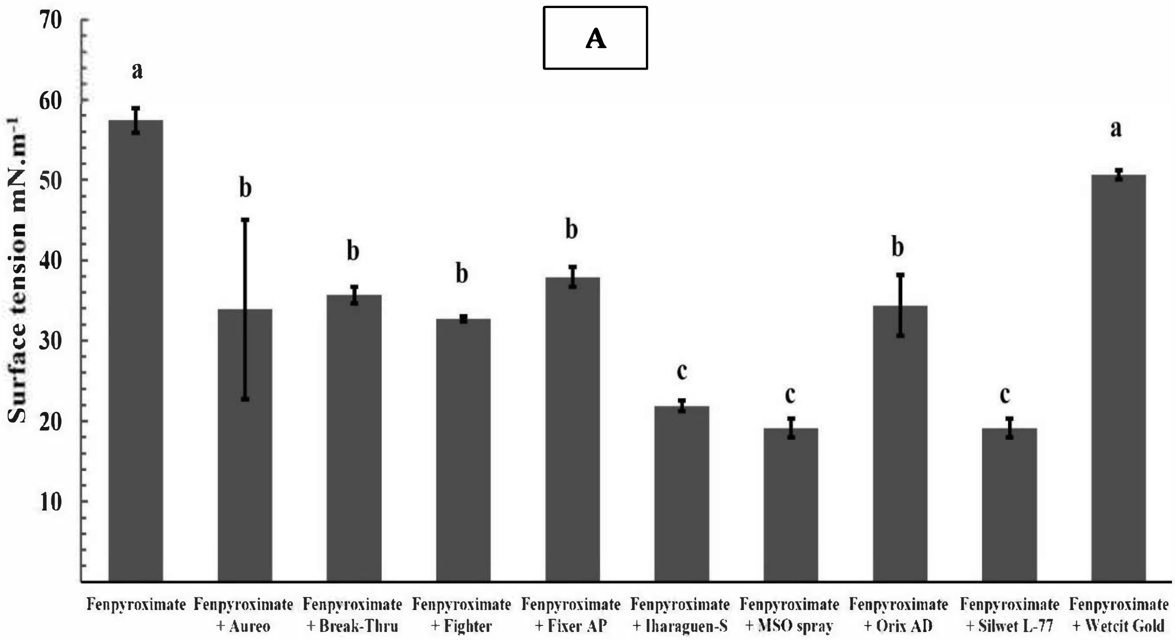


Figure 4. Averages of surface tension (A) and contact angle (B) in the experiment assessing adjuvant combinations with the acaricide pyridaben (Sanmite®). *Averages followed by the same lowercase letters do not differentiate from each other by Tukey's test ($p < 0.05$). **Concentrations of acaricide and adjuvants: pyridaben (100 mL commercial product (c.p.)/ 100 L), Aureo (250 mL c.p./ 100L), Break-Thru (100 mL c.p./ 100L), Fighter (100 mL c.p./ 100L), Fixer AP (200 mL c.p./ 100L L), MSO spray (200 mL c.p./ 100L), Orix AD (1000 mL c.p./ 100L), Silwet L-77 (75 mL c.p./ 100L), Wetcit Gold (150 mL c.p./ 100 L).



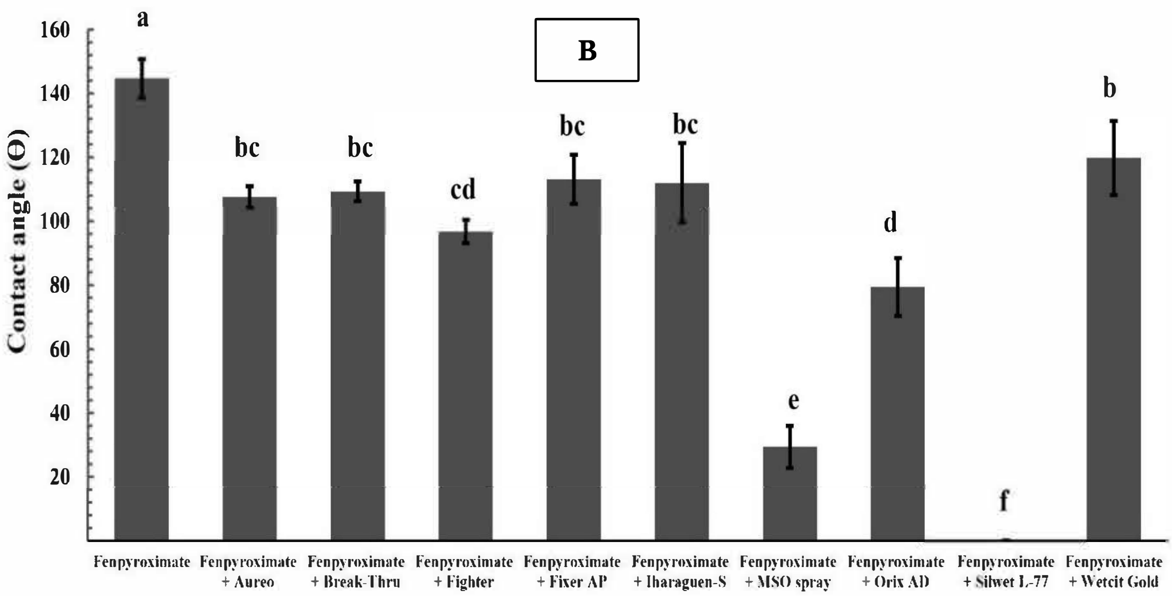


Figure 5. Averages of surface tension (A) and contact angle (B) in the experiment assessing adjuvant combinations with the acaricide fenpyroximate (Ortus®). *Averages followed by the same lowercase letters do not differ from each other by Tukey ($p < 0.05$). **Concentrations of acaricide and adjuvants: fenpyroximate (100 mL commercial product (c.p.)/ 100 L), Aureo (250 mL c.p./ 100L), Break-Thru (100 mL c.p./ 100L), Fighter (100 mL c.p./ 100L), Fixer AP (200 mL c.p./ 100L L), Iharaguen-S (10 mL c.p./ 100L), MSO spray (200 mL c.p./ 100L), Orix AD (1000 mL c.p./ 100L), Silwet L-77 (75 mL c.p./ 100L), Wetcit Gold (150 mL c.p./ 100 L).

3.3.2. Effect of treatments on *Aceria litchii*

The pH of the solutions, ranging from 8.5 to 8.2, did not differ significantly between Experiments 3 and 4.

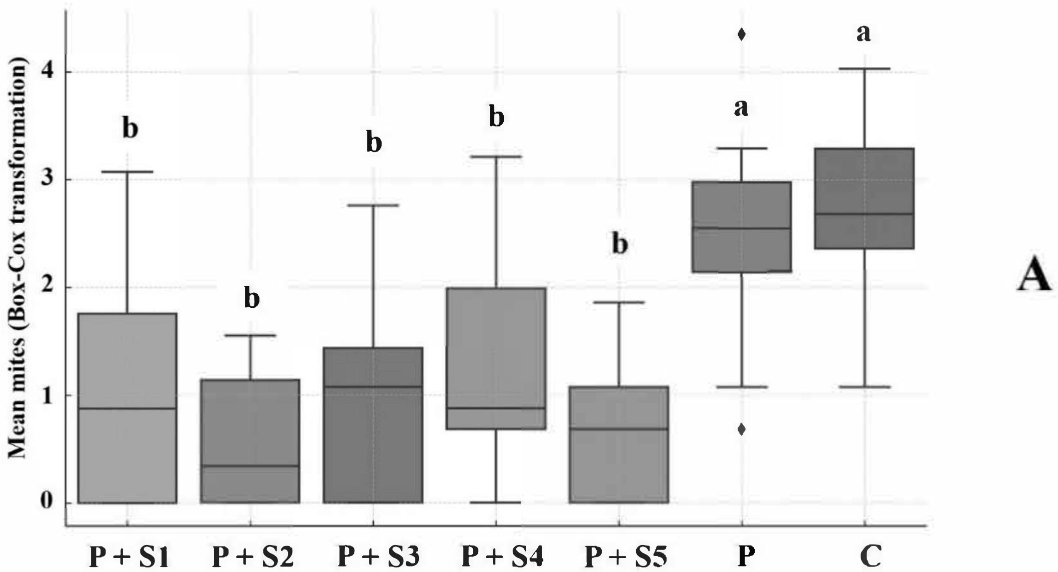
3.3.3. Experiment 3

In Experiment 3, the addition of adjuvants to pyridaben significantly influenced the number of surviving mites that left the leaf arena after application. The mean mite count in treatments with adjuvants was lower compared to the pyridaben-only treatment and the water control (F-test = 10.0, $p < 0.0001$) (Figure 3A). In contrast, fenpyroximate applications failed to control *A. litchii*, as the mite counts in treatments with adjuvants were equal to or even higher than those observed in the water control (F-test = 19.0, $p < 0.0001$) (Figure 3B).

3.3.4. Experiment 4

Overall, the results of Experiment 4 were consistent with those of Experiment 3. Adding adjuvants to pyridaben decreased the number of mites leaving the leaf arena, indicating greater control efficiency in these treatments. Notably, the treatment with pyridaben + Silwet L-77 stood out, as it resulted in an extremely low number of surviving mites, with an average count close to zero (F-test = 28.2, $p < 0.0001$) (Figure 4A).

Once again, pyridaben proved effective in controlling *A. litchii,* while fenpyroximate did not. The results showed no significant differences among treatments with fenpyroximate + adjuvants, fenpyroximate alone, and the water control (F-test = 1.4NS, $p = 0.2044$) (Figure 4B).



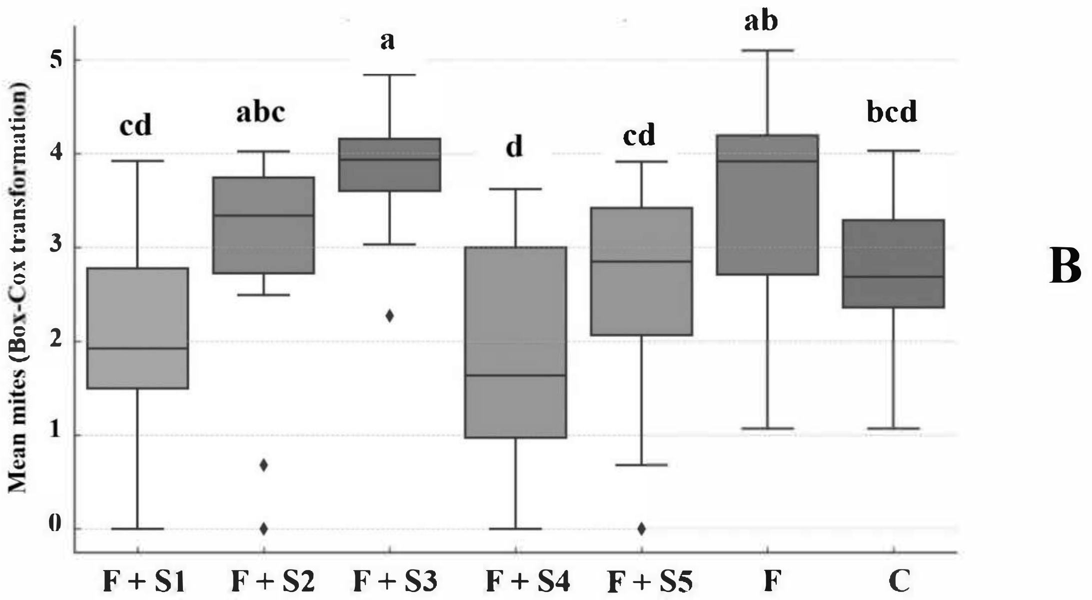


Figure 6. Distribution of mean values for the number of *Aceria litchii* mites that left the arenas in treatments with pyridaben (A) and fenpyroximate (B) during Experiment 3. Pyridaben and fenpyroximate treatments were analyzed separately and compared against the control treatment (water only). Means followed by the same lowercase letter did not significantly differ according to Tukey's test ($p < 0.05$). The horizontal lines within the boxes represent the medians, while the box limits indicate the first and third quartiles. Data points outside the whiskers denote outliers. Treatment Descriptions: Pyridaben treatments (A): P + S1 = Pyridaben + Break-Thru; P + S2 = Pyridaben + Fighter; P + S3 = Pyridaben + Iharaguen-S; P + S4 = Pyridaben + Silwet L-77; P + S5 = Pyridaben + Wetcit Gold; P = Pyridaben alone; C = Control (water only). Fenpyroximate treatments (B): F + S1 = Fenpyroximate + Break-Thru; F + S2 = Fenpyroximate + Fighter; F + S3 = Fenpyroximate + Iharaguen-S; F + S4 = Fenpyroximate + Silwet L-77; F + S5 = Fenpyroximate + Wetcit Gold; P = Fenpyroximate alone; and C = Control (water only).

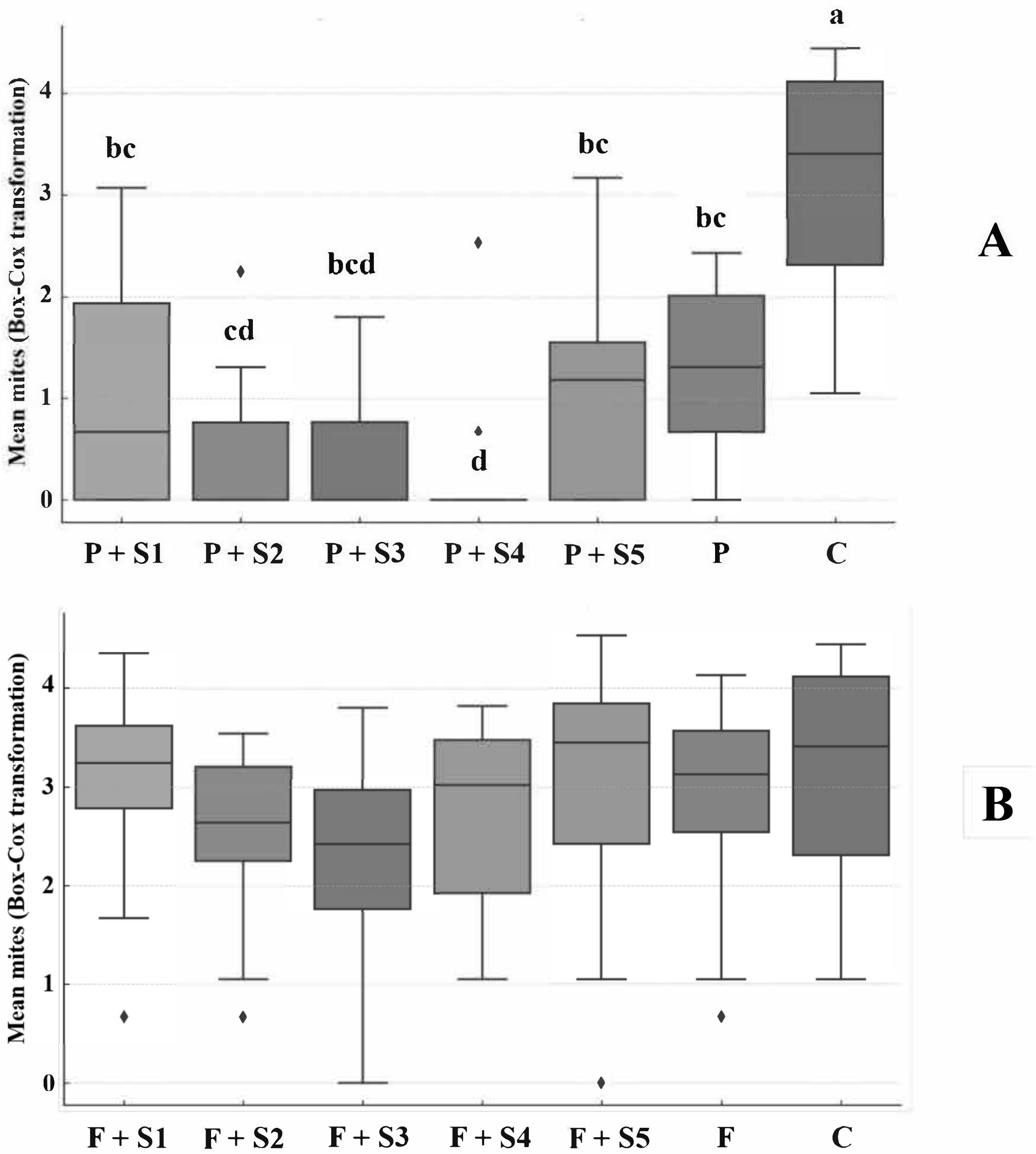


Figure 7. Distribution of mean values for the number of *Aceria litchii* mites that left the arenas in treatments with pyridaben (A) and fenpyroximate (B) during Experiment 4. Pyridaben and fenpyroximate treatments were analyzed separately and compared against the control treatment (water only). Means followed by the same letter did not significantly differ according to Tukey's test ($p < 0.05$). The horizontal lines within the boxes represent the medians, while the box limits indicate the first and third quartiles. Data points outside the whiskers denote outliers. Treatment Descriptions: Pyridaben treatments (A): P + S1 = Pyridaben + Break-Thru; P + S2 = Pyridaben + Fighter; P + S3 = Pyridaben + Iharaguen-S; P + S4 = Pyridaben + Silwet L-77; P + S5 = Pyridaben + Wetcit Gold; P = Pyridaben alone; C = Control (water only). Fenpyroximate treatments (B): F + S1 = Fenpyroximate + Break-Thru; F + S2 = Fenpyroximate + Fighter; F + S3 = Fenpyroximate + Iharaguen-S; F + S4 = Fenpyroximate + Silwet L-77; F + S5 = Fenpyroximate + Wetcit Gold; P = Fenpyroximate alone; and C = Control (water only).

3.4. Discussion

The use of adjuvants in pesticide spray mixtures offers several benefits, including enhanced product efficiency and improved physicochemical properties of the spray solution. Adjuvants are designed for various functions, such as increasing droplet spreading, enhancing pesticide penetration, reducing surface tension, and minimizing drift (Stevens, 1993; Hazen, 2000; Milanowski et al. 2022). However, certain adjuvants may interact with active ingredients, affecting their stability and leading to phase separation, sedimentation, precipitation, or flocculation. These effects can compromise spray system performance, disrupt equipment function, and reduce product efficacy (Gent et al. 2003; Petter et al. 2013).

Our study revealed significant changes in the surface tension and contact angle of acaricide solutions due to adjuvant addition, regardless of the acaricide evaluated. Iharaguen-S, MSO Spray, and Silwet L-77 were the most effective in reducing surface tension in combinations with pyridaben and fenpyroximate. Lower surface tension improves the spreading and surface coverage of spray droplets (Basu et al. 2002). A higher surface tension requires greater force to break droplets, leading to larger droplets and reduced coverage (Mota and Antuniassi, 2013). Conversely, reducing surface tension enhances contact with the leaf epidermis, potentially improving the effectiveness of the active ingredient.

Reducing spray solution surface tension is particularly useful for controlling pests that take refuge within plant structures, such as the lychee erinose mite (*Aceria litchii*). The physical barrier formed by erinose protects mites from direct acaricide exposure, reducing their susceptibility to treatment (Azevedo et al. 2013; Revynthi et al. 2022b).

Among the adjuvants evaluated, Silwet L-77 provided the most significant reduction in contact angle, followed by MSO Spray, which significantly decreased the contact angle in both experiments. According to Santos et al. (2021), the contact angle of a spray droplet on a plant surface determines its wetting capacity. A surface is considered hydrophilic when the contact angle is less than 90 degrees, whereas a contact angle of 90 degrees or greater indicates a hydrophobic surface.

The contact angle of a spray droplet depends not only on the physicochemical properties of the spray solution but also on the surface characteristics of the plant (Santos et al. 2021). In our pyridaben experiment, the adjuvants Iharaguen-S, MSO

Spray, and Silwet L-77 resulted in contact angles below 90 degrees, indicating high wettability and classifying the surface as hydrophilic. Similarly, in the fenpyroximate experiment, the adjuvants MSO Spray, Orix, and Silwet L-77 produced high wetting conditions (contact angle < 90 degrees), indicating hydrophilicity.

When considering both experiments, MSO Spray and Silwet L-77 were the most effective in reducing the contact angle and improving droplet spread. Other researchers have reported similar findings, showing that certain adjuvants significantly reduce the contact angle of spray droplets, thereby enhancing their interaction with plant surfaces (Cunha et al. 2017; Santos et al. 2021).

Song et al. (2024) reported structural and surface property changes in lychee leaves following infestation by *A. litchii*. Microscopic analysis revealed marked differences in trichome height and distribution at various infestation stages. Additionally, the study found that the contact angle increased significantly on the lower (abaxial) surface of infested leaves while Surface Free Energy (SFE) decreased drastically.

This result suggests that the formation of erinose on the abaxial surface transforms leaf wettability from hydrophobic to superhydrophobic, making the surface highly resistant to wetting and reducing droplet adhesion. As a result, acaricide penetration is hindered, potentially reducing its effectiveness. Therefore, for effective pest control, it is crucial to consider the presence and infestation level of *A. litchii* when selecting spray application strategies.

The results of this study demonstrated that pyridaben was more effective than fenpyroximate, as fewer mites survived and left the arenas 96 hours post treatments containing pyridaben. Azevedo et al. (2013) reported divergent results, showing 100% mortality of *A. litchii* mites within 48 hours following fenpyroximate application under laboratory conditions. However, Azevedo et al. (2013) applied fenpyroximate to exposed mites without the protection conferred by the erinea. Moreover, fenpyroximate was classified as harmful to the predatory mite *Phytoseius intermedius* Evans & MacFarlane (Acari: Phytoseiidae).

Conversely, Revynthi et al. (2022a) obtained results similar to ours, demonstrating that pyridaben can be used as a "preventive" acaricide, effectively reducing new infestations by the lychee erinose mite. Revynthi et al. (2022b) developed a method to assess the effect of abamectin (Agri-Mek®), applied alone or in combination with a nonionic organosilicon adjuvant (DyneAmic®), for *A. litchii* control in laboratory conditions. Their findings indicated that none of the treatments effectively controlled mites within the erinea or protected new shoots. They noted that the adjuvant did not enhance abamectin's efficacy, concluding that abamectin either failed to penetrate erinose sufficiently or had no effect on mite eggs, leading to rapid re-infestation.

In many countries, the management of *A. litchii* has traditionally relied on elemental sulfur as a prophylactic treatment to protect new growth (Nishida and Holdaway, 1955; Waite and Hwang, 2002). However, sulfur applications have proven ineffective in controlling *A. litchii* mites protected within the erinea (Revynthi et al. 2022a). Additionally, elemental sulfur presents several limitations, including incompatibility with other pesticides and potential phytotoxicity when applied under high temperatures. An alternative approach is to combine pyridaben with an appropriate adjuvant, which may enhance acaricide penetration and efficacy against existing *A. litchii* infestations. This strategy offers a more effective and compatible control method and can be integrated into sustainable lychee pest management programs

4. Conclusions

Based on the results obtained, it can be concluded that:

- Pyridaben was more effective than fenpyroximate in controlling of *A. litchii.*

- Pyridaben in combination with Silwet was the most effective in controlling *A. litchii* in laboratory condition.

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