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Efficacy of azadirachtin on *Cannabis sativa* pest *Tetranychus urticae*

Master´s thesis

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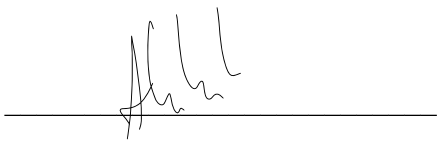
Declaration

I hereby declare that I have authored this master's thesis carrying the name "Efficacy of azadirachtin on *Cannabis* pest *Tetranychus urticae*" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 18.04.2024 

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Efficacy of azadirachtin on *Cannabis* pest *Tetranychus urticae*

Summary:

Non-synthetic pesticides are becoming more and more convenient in global crop production. Safely produced crops are in high demand and *Cannabis sativa* is no different. The two-spotted spider mite, *Tetranychus urticae* is an important *Cannabis* pest as it is for many other crops. In this study the efficacy of azadirachtin is measured for the control of spider mites by the effects on mortality, repellence and the LDI score of foliar damage on a scale of 0-5. Neem oil (0.3% azadirachtin) formulations at two different concentrations are compared with commercially available synthetic acaricide Omite against spider mites on *Cannabis* plants. High concentration neem oil (NOH) formulation, at 10 ml/l was shown to be more effective in protecting *Cannabis* plants against spider mites than Omite at the recommended concentration of 1 ml/l. NOH treatment controlled 100% of the mite population by either killing or repelling the pest whereas Omite treatment controlled 97.8%. The low concentration neem oil (NOL) formulation, at 5 ml/l controlled 76.6% of the mite population. LDI scores for the treated plants were significantly lower than the control, with more foliar damage observed in the control plants. Azadirachtin containing neem oil was shown to be a potential alternative to synthetic acaricide Omite for the control of *T. urticae* on *Cannabis* plants.

Keywords:

Formulation, botanical pesticide, neem, mortality, repellence, foliar damage

Content

1 Introduction

In recent years, the agricultural industry has witnessed a growing awareness and concern regarding the environmental and health impacts of synthetic pesticides. As a result, there has been a renewed interest in exploring sustainable and eco-friendly alternatives to conventional and often chemical pest control methods (Tudi, 2021). One particularly promising method involves the use of botanical pesticides

Botanical pesticides are derived from naturally occurring substances found in various plants, such as neem, pyrethrum and sabadilla, among others. The common bioactive compounds in botanical pesticides are majorly secondary metabolites such as alkaloids, steroids, phenolic compounds, flavonoids, terpenes, tannins and resins that have biocidal properties (Geraldin, 2020). These compounds have been used for centuries in traditional agricultural practices and are gaining traction as viable alternatives in modern integrated pest management strategies. The resurgence of interest in botanical pesticides is driven by their perceived benefits, including reduced environmental impact, minimal harm to non-target organisms, and potentially lower risks to human health. Unlike synthetic chemicals that often leave residues on crops and may contribute to the development of pesticide-resistant pests, botanical pesticides generally break down more rapidly in the environment, offering a more sustainable approach to pest control. Additionally, botanical pesticides often exhibit multiple modes of action, making it more challenging for pests to develop resistance (Acheuk, 2022).

The demand for safely produced agricultural crops has been increasing especially after the turn of the millennium, due to a rising awareness of consumers, producers and policymakers on hazards posed by many of the conventional ways of farming (Giampieri, 2022). In that, the global landscape surrounding *Cannabis* has undergone a significant transformation as well, with an increasing number of regions recognizing its therapeutic potential and legalizing its use for medical and, in some cases, recreational purposes. As the *Cannabis* industry continues to expand, one paramount concern is brought forth – the safety of *Cannabis* products. Safely produced cannabis is a multifaceted concept that encompasses various aspects of cultivation, processing, and distribution, all aimed at ensuring that consumers have access to high-quality, contaminant-free *Cannabis* products. The significance of this issue cannot be overstated, as it directly impacts public health, regulatory compliance, and the overall credibility of the growing *Cannabis* industry (Zheng, 2021). Understanding the importance of safe cannabis production involves acknowledging the potential risks associated with contaminants such as pesticides, heavy metals, and microbial pathogens (Davidson, 2018).

According to Pulkoski (2023), *Tetranychus urticae* (twospotted spider mite), *Aculops cannabicola* (hemp russet mite), *Polyphagotarsonemus latus* (broad mites), and *Phorodon cannabis* (cannabis aphids) are considered the most important pests in greenhouse grown *Cannabis*. Spider mites, belonging to the Acari family Tetranychidae, pose a significant and worldwide challenge in agriculture. Notably, the common red spider mites (*Tetranychus cannabarius*) and the two-spotted spider mites (*Tetranychus urticae*) emerge as major pests affecting most fruit trees, as well as greenhouse and field grown crops (Mansour and Ascher, 1984). The economic impact of these mites continues to escalate due to their remarkable ability to develop resistance to commercially available acaricides and their persistency following the application of non-selective synthetic pesticides. The use of such pesticides not only fails to control the mite population effectively but also harms potential natural enemies (Mansour and Ascher, 1984). As an example, the application of synthetic pyrethroids against tomato aphids has inadvertently contributed to the increased frequency of mite outbreaks (Halle, 1985).

Among the different plant species that have been studied for insecticidal properties, neem, *Azadirachta indica* has been the most commercially exploited for pest management. Azadirachtin, a tetranortriterpenoid, was reported active over nearly 550 insect and arthropod species, acting as an antifeedant and disrupting growth cycles by blocking the release of hormones (Krzyzaniak, 2016).

2 Scientific hypothesis and aims of the thesis

This study aims to analyze the effect of neem-based pesticide against *T. urticae* on *Cannabis* plants. The chosen source of azadirachtin is neem oil 0.3%. The efficacy of different dose of neem oil formulations will be compared with that of commercially available acaricide Omite. The effective concentration of neem oil formulations against *T. urticae* will be determined.

3 Literature research

3.1 Two spotted spider mite, *Tetranychus urticae* (Prostigmata: Tetranichydae)

The polyphagous spider mite is one of the most important pests in commercial crop production. Veerman (1998) describes the pest as a single undivided species whereas Takafuji (2000) reports two distinct species of spider mites, as morphological and ecological differences are observed. Many common names are found in various literature describing the species, including red spider mites, carmine spider mites, two-spotted spider mites, spinning mites, greenhouse (or glasshouse) spider mites. To aggravate the confusion, more than 60 scientific names exist for the taxonomy of the two species (Navajas, 2000). This insidious species is considered as the most destructive pest of controlled environment grown *Cannabis* and the two most frequented taxonomic names found in *Cannabis* literature are the two-spotted spider mite, *Tetranychus urticae* and the Carmine spider mite, *Tetranychus cinnabarinus* (Pulkoski, 2023). These arthropods belong to the the taxon Acari, that contains mites and ticks and to the Tetranychidae family which includes more than 1200 species. *T. urticae* is the most common and most widely distributed species in *Tetranychus* genus.

The species is characterised by the presence of two spots found on their dorsal, green or brown colouration with white or yellow coloured legs. Sexual dimorphism is observed with males being smaller than females. The web structure formed by the spider mites on the plant gives them their name and is the medium for development, growth and protection (Navajas, 2000).

3.1.1 Biology and Physiology

All members of the family Tetranychidae have a life cycle that includes the stages of egg, larvae, nymph and adult, with usually a dormant state between stages. Optimum temperature for the development of two spotted spider mites is between 26°C and 30°C and they thrive in low relative humidity (Gerson, 1992). *T. urticae* eggs are spherical about 0,15 mm in diameter, initially translucent to white and turn yellow before hatching, they can hatch in as little as three days. The hatching larvae are spherical to oval in shape, have pale green colour with three pairs of legs and are slightly larger than the eggs, about 0,2 mm in length. The larvae undergo a short quiescence to pronymphs, which molt to deutonymphs and within 2 days the deutonymphs go through another period of quiescence before the adults emerge. The nymphs are close to 1 mm, develop one more pair of legs and start changing their colour from green to yellow. As adults feed on chlorophyll rich tissue, two brown-black spots appear on their dorsal, males are about 1 mm and their dorsal spots may not be as evident as females that can reach above 1 mm in length (Navajas, 2000). In winter the *T. urticae* change their colour to bright orange or red, this

makes them difficult to distinguish them from the Carmine spider mites or even other predatory mite species (Parmagnani, 2023).

Two-spotted spider mites are equipped with a pair of needle-like stylets that they use to pierce and rupture the leaf cells and suck up the content. They exhibit year-round feeding and breeding, except in extremely cold weather during winter when they enter a dormant state, hibernating on the ground, under leaves, in cracks, crevices, and other sheltered places. The mite population tends to peak in hot, dry weather, often declining after periods of rain (White, 2018). Adult *T. urticae* overwinter and emerge in the spring. The species reaches adult stage in a matter of days and sexual maturity in as little as five days, females lay their eggs on the underside of leaves or in the web structure and can lay as many as 200 eggs during their life. The life cycle from egg to adult takes 10 to 14 days under favourable conditions and extremely high humidities and low temperatures can induce diapause. Likewise, shorter photoperiod was shown to induce a reproductive diapause where females stop feeding and turn orange-red, gather in clusters then hibernate at tips of leaves and flowering tops. Clusters of diapausing spider mites may contain several thousands of individuals and grow quite large (Gotoh, 2014).

The prolificacy of spider mite plays a key role in their population increase, which is determined by factors such as rate and duration of oviparity, hatchability, rate of development, sex ratio, host plant condition and other abiotic factors. A high intrinsic rate of natural increase causes a rapid growth of population with high numbers of annual generations that Rioja (2017) attributes to the ability of spider mites to successfully adapt to an originally unsuitable habitat, where they can produce a large number of offspring under challenging conditions.

3.1.2 Host range

The two-spotted spider mites are known to have one of the largest host range among common pest species and is the most polyphagous member of the Tetranychids. These highly adaptable mites, constitute substantial threats as major pests in various settings. They infest vegetables, ornamentals in greenhouses, and field crops, while also being prevalent in forest nurseries (Fry, 1989). Around 4000 host species were described worldwide with more than 200 species of economical value (Migeon, 2010).

The feeding behavior of spider mites is particularly detrimental to host plants, as they primarily target mature leaves by piercing and feeding beneath the epidermal layer of leaf tissue. Their ability to remove cellular content leads to cell destruction and a subsequent reduction in photosynthesis (Chen, 2022). Severe infestations are easily identifiable by the presence of a fine webbing (Grbić, 2011).

3.1.3 Feeding and plant response

In most cases, the infestation starts in lower leaves and as the population grows, the rest of the plant is colonised. *Tetranychus urticae* mostly feed on the fluids inside the leaf tissue, usually sucking it up from the underside of the leaf (Cazaux, 2014). Individuals feed on the mesophyll cells and therefore cause mechanical damage to cells that results in chlorosis on the feeding site (Attia, 2013). Continuous feeding causes the damaged organs to change color and shape, which often turn yellow, gray, or brown, according to the level of infestation and number of necrotic cells (Sivritepe, 2009). High infestations can stress plants to the extent of complete mesophyll collapse, resulting in leaf necrosis. Beside the appearance of the plant, a spider mite infestation affects both the physiological and biochemical changes in plant tissue and alters cell composition, resulting in lower concentrations of nutrients such as nitrogen and phosphorus and proteins (Grgi'c, 2011). When cell physiology is disrupted, changes will include reduced photosynthetic activity, the abscission of leaves and the injection of phytotoxic compounds. These changes then cause a reduction in yield and negatively affect the quality of plants (Gomez, 2004).

Plant responses to mite infestations vary from host to host and are useful to understand the chemical and ecological processes influencing plant–herbivore interactions. The resistance of a host plant can reduce initial infestations or a higher emigration rate of pests compared with their susceptible counterparts (Dent, 2000). The mechanisms of plant resistance are antixenosis, antibiosis and tolerance (Sarfraz, 2006). Antixenosis, or non-preference, can be considered as the first line of defense in plants in which plant characteristics present a physical or chemical barrier that repel or deter potential pests. Morphological factors such as presence of trichomes, waxes or pigments as well as chemical factors such as secretion of secondary metabolites, enzymes or other volatile compounds adversely affect the action of pests and prompt them to find alternative host plants (Ongaratto, 2021). Antibiosis is a mechanism of pest resistance in which the host plant produces a detrimental effect on the pest, usually through plant biochemicals such as free amino acids, fatty acids, terpenoids and flavonoids thereby impairing pest growth and development (Pachú, 2023). Antixenosis and antibiosis involve both plant and pest characteristics. Tolerance is a plant response to injury, referring to the recovery following an attack, usually measured as greater yield and quality of one plant over another at same levels of infestation (Agut, 2018).

To draw away herbivores, plants generally have two lines of defense, constitutive and induced. Constitutive defenses are always present in the plant, while induced defenses are produced or mobilized to the site where a plant is injured (War, 2012). Induced defense can be classified into direct, where all plant traits that help in the resistance of the host directly impact the physiology or behavior of the pest and indirect, where plant traits impact other organisms in the environment that deter the pest, such as secondary metabolites released by the host plant that attract natural predators (Martel, 2015). In crop production one very important way to regulate insect herbivore populations is this induced response. Inducible defenses not only include the

biosynthesis of toxic secondary metabolites but also of specific enzymes and proteins that have anti-nutritive properties (War, 2012). If the plant response occurs during early stages of an infestation, the defense may reduce future attacks and colonisations. However, in his study, Bensoussan (2016) reports that *T. urticae* prefer to penetrate the leaf with its stylet through the stomata as they suffer damage when piercing through the epidermal pavement cells to feed on the mesophyll cells. This creates a delayed response in the plant as the detection of the attack on the leaf surface is reduced, thereby resulting in altered physiological and biochemical traits of the plant.

3.1.4 Dispersal mechanism

Spider mites have fairly sophisticated dispersal mechanisms, allowing them to colonize host plants that are widely separated, expanding over large areas. *Tetranychus urticae* can crawl over the soil surface to infest nearby plants. Wind plays a role in their dispersal as well and helps them move from plant to plant, particularly with a specific posture that spider mites have adopted that involves elevating the forelegs upright. All active stages, excluding adult males, engage in dispersal posturing, as documented by Halle and Sabelis (1985).

In conditions of high population density, there is a shift in the behavior pattern of spider mites, prompting dispersal from the host plant. Hussey (1969) detailed three distinct methods of spreading observed in *T. urticae*: migration of females to reproduction sites, migration by dropping off of heavily infested crops, and migration guided by the plane of polarized light, the latter dispersal phase is characterized by positive phototaxis. These mechanisms contribute to the remarkable adaptability and widespread distribution of spider mites.
The extensive feeding of spider mites on the foliage not only cause food shortage and desiccation but deeply affects the microclimate of the plant and significantly lowers relative humidity, which initiates their dispersal. The positive phototactic response drives mites to higher regions of the plant and they concentrate around tips and the periphery of the host plant. Presumably, this positioning makes them more exposed to winds which facilitates their aerial dispersal (Clotuche, 2013).
In their study, Clotuche and Mailleux (2011) have observed that mites go up the host plant as they feed on leaf tissue and begin abandoning the host when most apical foliage is damaged. They noted that dispersing mites form clusters at apices of leaves before they drop into their web and reach the ground, where they crawl in search for another host. Charnie (1998) emphasized the role of wind in the dispersal of spider mites, highlighting its significance in their movement between plants. Consequently, various crops, wild plants or weeds can act as potential hosts. The intricate interplay of environmental cues and mite behavior during the dispersal phase underscores the adaptability and survival strategies employed by *T. urticae*, emphasizing the need to consider dispersal patterns when addressing pest management strategies.

3.1.5 Resistance of *T. urticae*

The resistance of *T. urticae* to acaricides has been described in numerous studies (Mansour and Ascher, 1984; Hall and Thacker, 1993; Li, 2002; Sugimoto, 2014; Chen, 2022). In early 1950s the first serious and widespread chemical control failure was documented and a development of resistance to organophosphate acaricides was observed. These included parathion and tetraethyl pyrophosphate at first but subsequently the resistance was reported to many other organophosphates (Saito, 1983). According to Busvine findings (1983), resistance of *T. urticae* to acaricides include funthion, tetradifon, dicofol, binapacryl, carbamates, quinomethionate, and cyhexatine. The multi-resistance of *T. urticae* is also shown by Hall and Thacker (1993) in their study on comparing different permethrin formulations and their lethal effect, which is stated to be low.

One other phase of the resistance is highlighted by Charnie (1998), the prevalence of spider mites on the lower leaf surfaces, protected by their webbing presents yet another challenge in controlling the infestation. In this way, mites are able to shelter from the spraying acaricides, resulting in insufficient doses reaching the mites. The insufficient treatment may be enough to control the more susceptible mites and eliminate them, but this leads to the domination of mites that are not killed by the treatment, giving rise to a resistant population. An acaricide-resistant population is likely to have developed cross-resistance to similar chemical compounds (Charnie, 1998).

The escalating issue of resistance in phytophagous mites is a continuous concern in various settings, including fields, greenhouses, and orchards (Zhou, 2018). The collective evidence underlines the pressing need for sustainable and diversified pest management strategies to address the evolving challenge of spider mite resistance in agricultural settings.

3.2 Use of botanical pesticides

In the last decades, there has been considerable efforts in finding alternatives to synthetic pesticides. One promising way is the use of phytochemicals as active ingredient in pesticide formulations. Botanical pesticides are sought after because of their selective toxicity, natural origin and non-bioaccumulative effects (Ngegba, 2022). Among different plant species, neem, *Azadirachta indica* has been the most studied for its insecticidal properties. Azadirachtin is a mixture of several structurally related tetranortriterpenoids isolated from parts of the plant, including seed, kernel, leaf and fruit (Ascher, 2002). The most commonly used neem-based insecticides are neem seed kernel extract (NSKE), neem oil and neem cake and all act directly

on the insect reproduction, as antifeedants and have physiological effects that reduce growth, increase mortality and delay moulting (Mordue, 2000; Liang, 2023).

3.3 Neem, *Azadirachta indica* (Sapindales: Meliaceae)

Azadirachta indica, neem is a member of the mahogany family, Meliaceae. In earlier literature neem has been known by several other names, from which *Melia indica* and *Melia azadirachta* are the most frequented (Schmutterer, 1990). These botanic names have sometimes created confusion and some botanists formerly related neem with at least one of its relatives, *Melia azedarach,* a West Asian species in the same family, commonly known as Persian lilac (Cui, 2023). The taxonomy of all these closely related species is complex and some botanists have recognized as many as 15 species, others, as few as 2.

Neem is a fast-growing tree, generally 15 to 20 m tall and sometimes reaching 40 m, with a crown diameter of up to 20 m. It is native to the seasonally dry, tropical woodlands of north-east India but has been documented in various parts of South Asia, notably Pakistan, Sri Lanka, Malaysia, Thailand and Indonesia (Akhtar, 2000). The tree has been introduced to Africa in the 20th century and now can be seen in regions of subsaharan countries. A neem tree normally begins bearing fruit after 3 to 5 years, becomes fully productive in 10 years and produces up to 50 kg of fruits annually. In favourable conditions it may live for more than two centuries.

3.3.1 Neem composition

Fascinatingly both wild neem trees and neem groves generally are known to be pest free. This is due to the chemical composition of the tree that helps it protect itself from a multitude of pest species by using its endogenous biocidal ingredients. These compounds are phytochemicals that belong to the triterpenoid class and more specifically to limonoids. At least nine neem limonoids were identified to be active against pests by affecting their growth cycle, but four of them were shown to be the most significant ones, namely azadirachtin, salannin, meliantriol and nimbin (National Research Council, 1992). Propyl disulphide is one of the active compounds in neem seed and is widely used as a potent pesticide in grain storage.

While bioactive compounds can be located throughout the entire tree, the highest concentrations and accessibility are observed in the seed kernels, that comprise 40% neem oil (Isman, 1991). These compounds are extracted through various methods from the kernels and, to a lesser extent, from the press cake. Despite their limited solubility in water, the active ingredients have

a high solubility in organic solvents like hydrocarbons, alcohols, ketones or ethers. The most common extraction processes include water extraction, hexane or pentane extractions and alcohol extraction, that form suspensions from which azadirachtin can be isolated (Fernandes, 2019). Moreover, the neem cake obtained during the neem oil extraction process serves a dual purpose as an important organic fertilizer, adding nitrogen and phosphorus to the soil and as a pesticide in common farming methods. Neem leaves have been utilized as repellents to combat stored grain pests (Koul, 1990).

As a whole, every component of the neem plant is recognized for generating by-products that naturally provide internal chemical defense, making the neem tree resistant to pest attacks. This inherent quality can be exploited to formulate an effective pest control strategy. Furthermore, the functional constituents of neem, including neem oil, bark, leaves, and their refined biochemicals, have been well-documented for their therapeutic significance, demonstrating anticancer and antimicrobial properties (Paul, 2011).

3.3.2 Neem oil

Cold-pressed neem oil, extracted from the seed kernels of the neem tree, proves highly effective against soft-bodied insects and mites (Benelli, 2017). The significant bioactivity of neem oil is also attributed to the presence of disulphide. Remarkably, neem oil is non-toxic to mammals, birds, and fish, and its multiple modes of action on pests reduce the likelihood of developing resistance. Various formulations of neem seed oil demonstrate antifeedant, ovicidal, larvicidal, insect growth regulatory and repellent activities against insect and arachnid pests. The larvicidal potential of neem oil against mosquitoes has also been a subject of long-standing research (Dua, 2009).

Dua (2009) evaluated neem oil formulations against two common mosquito species, *Anopheles* and *Culix*, known for transmitting malaria. Results indicated a mortality rate of 98,1% and 95,5% in *Anopheles* and *Culix* respectively on the first day of treatment and thereafter by day 7, larvae was controlled with a 100% rate. The observed larval effects were attributed to the antiecdysteroidal activity of azadirachtin present in neem oil that inhibits their normal growth. Dua (2009) also reported the efficacy of neem oil against *Sarcoptes scabiei*, an ectoparasitic mite that causes zoonotic infections. The study showed that the acaricidal effects of neem oil after 4.5 hours of exposure were at a 100 %, however the long-term effects of neem oil were not discussed. Neem oil has also been subject of fungicidal studies, Hirose (2001) compared its effects against two entomopathogenic species, *Metarhizium anisopliae* and *Beauveria bassiana* with three different fungicides and found that the neem oil had one of the most significant negative effect on germination, conidium production and vegetative growth. In another research, a similar comparative analysis was conducted on the mango leafhopper, *Idioscopus clypealis.* Three synthetic insecticides were used along with neem oil, that was reported to have comparable efficacy with the most efficient insecticide

(Adnan, 2014). The authors also emphasized on the importance of neem oil for the incorporation of eco-friendly pest management programs for the control of the leafhopper.

In addition to its ability to impede growth, neem oil also substantially delays the reproductive cycle of pests. It induces lethal toxicity during the pupal stage, resulting in diverse morphological abnormalities such as deformed adults, incomplete ecdysis, and blocked molting, thereby prolonging and preventing the formation of mature adults (Boulahbel, 2015). In their research, Kraiss and Cullen (2008) studied the growth regulating role of neem oil on soybean pest, *Aphis glycines* and its predator *Harmonia axyridis.* The neem oil spray formulations showed significant results on the mortality of nymphs and a delayed development time of the surviving adults. However on assessing the deterrence in fecundity, the formulations showed no significant effects and the mortality rate was not immediate. Furthermore a non-target effect was observed on the natural predator of the pest, *H. axyridis,* which requires further investigation.

Recent findings indicate that neem oil, despite its pest-deterring qualities, induces malformations in the growth and survival of a non-target predator, *Podisus nigrispinus*. This predator is commonly used as part of biological pest control. As the concentration of neem oil increases, there is a noticeable rise in morphological deformities observed in the wings, legs, and scutellum of *P. nigrispinus*, coupled with an increase in mortality. Therefore, it becomes crucial to carefully assess the azadirachtin concentration of the formulations for the impact of neem-based pesticides on non-target predators (Zanuncio, 2016).

3.3.3 Neem in IPM

Integrated Pest Management (IPM) requires the farmer's understanding of the identities and functions of beneficial insects and other biological control agents. It also involves awareness of the roles and potential drawbacks of pesticide use and misuse, along with a diverse range of cultural and crop sanitation practices aimed at reducing pest incidence. Two IPM strategies, one with synthetic insecticide and one with neem seed kernel extract NSKE 5%, were compared on insect pests and diseases in various crop species. The results showed that the two strategies were both effective in suppressing pest species, highlighting the efficacy of neem products as part of IPM programs (Dimetry, 2012). Boiça Jr (2007) investigated the effects of different pest control treatments on two determinant tomato cultivars. Their findings suggested that the three treatments, conventional treatment that included use of methamidophos, acephate and permethrin and two IPM treatments, one with imidacloprid and one with neem oil, showed significant and similar effects on the control of the pest. They noted as well that the number of sprayings was reduced by 77% with the two IPM treatments, when compared to the conventional treatment. The use of predatory mites as part of biological control of *T. urticae*

has increased substantially since early trials of Bravenboer and Dosse (1962). The predatory mite, *Phytoseiulus persimilis*, native to South America, has consistently proven to be efficient in both greenhouse and field control of spider mites (Opit, 2004). However *P. persimilis* is described to be susceptible to synthetic pesticides, including organophosphates and pyrethroids, which complicates its introduction in IPM programs (Kim, 2016). While extensive studies exist regarding the impact of neem products on various pests, the research on the influence on natural enemies such as predatory mites needs further investigation.

3.3.4 Azadirachtin

The tetranortriterpenoid azadirachtin was one of the initial active components identified in neem and was shown to be responsible for about 90 percent of its impact on the majority of pests. Research conducted over the last decades has demonstrated the exceptional action of azadirachtin as a growth regulator and its deterrent effects on the feeding and oviposition of plant-feeding pests, including herbivorous insects, arachnids and some nematodes (Schmutterer, 1988; Mordue, 2000; Kilani-Morakchi, 2021).

3.3.5 Pesticidal effects of azadirachtin

3.3.5.1 Growth regulator effect

Azadirachtin has structural similarities to the insect hormone ecdysone that plays a crucial role in guiding the stages of metamorphosis. It primarily impacts the corpus cardiacum responsible for hormone secretion. The smooth progression of metamorphosis relies on the accurate synchronization of multiple hormones and physiological changes. Azadirachtin hinders the production and release of essential hormones in insects, acting as an ecdysone blocker and leading to the disruption of their molting process and consequently, their life cycle (Kubo, 1982).

Azadirachtin has been shown to exhibit acaricidal effects on Tetranychus urticae. The application of neem oil resulted in the repellence of between 70-90% of female spider mites from treated leaf discs (Mansour and Ascher, 1984). The authors also noted that when 24-hour-old eggs of Tetranychus urticae were subjected to a methanolic neem seed kernel extract (NSKE) solution, not only was post-embryonic development significantly delayed, but mortality also occurred progressively. Nevertheless, fewer than 30% of the mites managed to survive and reach adulthood 20 days after the treatment.

3.3.5.2 Antifeedant effect

The feeding patterns of insects and arachnids heavily rely on the neural signals received from their sensors, such as taste receptors located in their mouthparts. These sensors process a “sensory code” transmitted to the central nervous system. Azadirachtin induces antifeedant effects by activating deterrent cells within these chemoreceptors and by inhibiting the stimulation of feeding through the suppression of "sugar" receptor cells (Mordue, 1998). Isman (1990) noted that azadirachtin offers immense antifeedant properties due to its efficacy in suppressing the feeding sensation in insects, at concentrations even less than 1 part per million.

Apart from inducing antifeedant effects, the injection of azadirachtin also triggers physiological changes in the insect's midgut, resulting in a decrease in post-ingestion digestive efficiency. This diminished efficiency, also called "secondary" antifeedancy, stems from disruptions in both hormonal and physiological systems. These disturbances damage the gut lining, creating obstacles for normal food passage through the insect's midgut and suppress the digestive enzyme production (Diabate, 2014).

3.3.5.3 Oviparity effect

Fecundity-reducing effects of azadirachtin have been subject yo numerous studies (Schmutterer, 1988; Riba, 2003; Ferdenache, 2019). Koul (1990) reported adverse effects on ovarian development, fecundity, fertility and egg viability induced by azadirachtin. In an early study, azadirachtin was found active in the inhibition of oogenesis and ovarian ecdysteroid synthesis in *Locusta migratoria* (Rembold and Sieber, 1981). Mordue and Nisbet (2000) studied the effects of azadirachtin-containing diets on *Myzus personae*, a green aphid and found that at a concentration between 10 and 100 ppm azadirachitin, aphids produced nymphs at less than half the rate of those on the control diet. Additionally, a concentration of 10-20 ppm azadirachtin in the diet for longer periods resulted in more than 20 times fewer nymphs compared to aphids feeding on the control diet.

While most studies have primarily focused on the impact of azadirachtin on reducing the reproductive potential of female pests, notable effects have also been observed in males. The spermatozoa maturation in *Oncopeltus fasciatus*, a hemipteran was found to be comparable in both azadirachtin-induced and control treatments, suggesting that this process is mostly independent of the production of morphogenetic hormones. Although spermatogenesis in adult male *Oncopeltus* was found to be azadirachtin-independent, a dose of 0,125 mg was enough to cause improper erection and usage of the aedeagus (Linton, 1997)

Even though approximately 75% of azadirachtin is rapidly eliminated after being applied to *Locusta migratoria* and *Schistocerca gregaria*, a notable portion binds to the ovaries, testes, and accessory glands. Nisbet (1995) demonstrated the presence of specific binding sites for azadirachtin in the developing sperm tails of *S. gregaria.* Ongoing research aims to determine

the impact of azadirachtin and the existence of these particular binding sites throughout the entire process of spermatogenesis, including meiosis (Nisbet, 1996).

3.4 *Cannabis sativa*

Cultivation of *Cannabis* dates back as early as 10 000 years, to the dawn of agricultural farming. The versatile plant has been used for diverse agricultural and industrial purposes ranging from production of paper, wood and fiber to its use in medicinal and pharmaceutical sectors (Schluttenhofer, 2017). From early 1900s, the cultivation and exploitation of *Cannabis* for recreational, medical and industrial use were strictly banned, which resulted in limited scientific work in the field. Rapidly changing jurisdictions in recent years, reopened the gate for research and development, encouraging the exploration of the potential benefits of the plant. Following legal regulations, extensive research on the chemodiversity of *Cannabis* constituents revealed more than one thousand compounds with potential clinical value, including 278 cannabinoids, 174 terpenes, 221 terpenoids, 19 flavonoids, 63 flavonoid glycosides, 46 polyphenols and 92 steroids. The major cannabinoids found in *Cannabis* are tetrahydrocannabinol (THC), cannabidiol (CBD), cannabichromene or CBC and their precursor cannabigerol (CBG) and cannabinol (CBN). Currently, 10 CBN-type, 17 CBG-type, 8 CBD-type, and 18 THC-type cannabinoids have been identified. Notably, cannabigerolic acid (CBGA), a CBG-type cannabinoid, serves as the central precursor for the biosynthesis of psychoactive THC, non-psychoactive CBD, and CBC (Gerra, 2010).

Cannabinoids contribute to the *Cannabis* plant's defense mechanism against various pathogens such as insects, fungi, viruses, and bacteria (McPartland, 2000). These distinctive compounds exhibit an impact on mammalian cells by interacting with membrane receptors of the endocannabinoid system. Specifically, they bind with cannabinoid receptors CB1 and CB2. CB1 is predominantly found in cells of the central and peripheral nervous system, while CB2 is primarily located in immune cells (Mackie, 2008). While eastern cultures have long acknowledged the benefits of *Cannabis*, it is only in the past century that western medicine and its scientific community have taken an interest. In recent years, its medicinal applications have significantly expanded. Medicinal *Cannabis* is globally administered to patients with various ailments including cancer, multiple sclerosis, Parkinson's disease, Crohn's disease, psychiatric disorders, and more (Hill, 2015).

Numerous studies have demonstrated the importance of spider mites in *Cannabis* production (McPartland, 2000; Cranshaw, 2019; Pulkoski, 2023). The two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), poses a significant threat to *Cannabis* cultivation, particularly during the indoor/greenhouse cultivation phase but can attack plants at all stages of production (Cranshaw, 2019). In *Cannabis* production, this pest can cause considerable damage to mother plants utilized for clonal propagation and their prodigy during indoor cultivation

before transplantation. Wainwright-Evans (2017) reported however, that once plants are moved to fields, the populations of two-spotted spider mites do not persist at high levels. Additionally, the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsenomidae) and the eriophyid hemp russet mite, *Aculops cannibicola* (Acari: Eriophyidae) are recognized as important pests in indoor Cannabis cultivation in the United States (Lago, 1989). The damage caused by the hemp russet mite is less obvious compared to that caused by the twospotted spider mite, and its effects have not been thoroughly documented. Leaves heavily infested with hemp russet mites often exhibit subtle changes such as a faint grayish or bronzed discoloration. Some growers have reported instances where these mites have led to plant death during indoor propagation. Additionally, in certain *Cannabis* cultivars, there may be a slight upward curling of leaf edges, although this symptom is not consistent across all varieties and some strains naturally exhibit similar leaf curling even without mite infestation (McPartland, 2003).

4 Methodology

The experiments were carried out in growbox conditions at the Department of Food Science, CZU Prague. CBD strain *Cannabis* plants (THC < 0.03%) were grown as host for *T. urticae,* neem oil and synthetic acaricide Omite were used in the control of the infestation.

4.1 Growbox experiment

4.1.1 *Cannabis* growth

Cannabis cuttings were obtained from stock mother plants maintained in growrooms of the faculty and placed in rock wool cubes using root stimulating powder and kept for two weeks at 25°C and 60% RH. At the end of the two weeks the twelve best clones were chosen for transplanting into the growbox in 9 x 7 cm round mesh pots with rock wool as substrate. BC Northern Light Growbox is an automated deep water culture hydroponic system, 140 x 80 cm of growing space for a maximum of 18 plants and uses two 250W HPS digital ballasts, that were on an 18-hour photoperiod for the entirety of the experiment.

The box was placed in the department's building and the indoor temperature and humidity levels affected the conditions inside the box, where a humidifier was placed when relative humidity dropped significantly. The humidity level was kept between 30% and 40% and the temperature between 24°C and 32°C. Two fans operated for air ventilation, no CO2 regulator was used. The plants were given a two-part base nutrient twice a week for 45 days, Cali Pro A/B by Emerald Harvest, pH was adjusted with Plagron pH Regulator Minus and was kept below 6.5, EC was between 0.8 and 1.2 mS/cm.

4.1.2 Spider mites

The spider mites were provided by the Department of Plant Protection and collected from infested leaves of *Maranta arundinaceae* grown as ornamental. The genus was identified in the entomology laboratory as *Tetranychus*, but the species was not specified. The mites were reared on the same plant for one week before they were transferred inside the growbox.

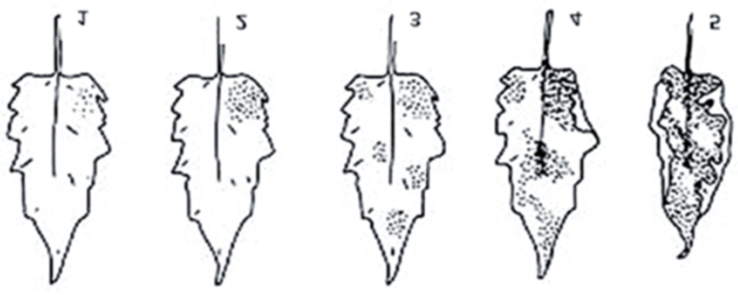
4.1.3 Inoculation

Mite-infested *M. arundinaeae* leaves were segmented into smaller pieces and placed onto *Cannabis* plants using plastic clips. Each plant received two leaf segments that were clipped on side branches of the plant close to lower leaves. The segments were left untouched for two days and the spider mites allowed to migrate on to *Cannabis* plants. Two heavily infested node sections of the plant were placed in the center of the box, ensuring a sufficient level of mite population to establish for the experiment.

4.2 LDI, Leaf Damage Index

At the end of two days, plants were assessed visually for spider mite occurrence and effect before receiving the treatments. The assessment followed the scoring principles on the basis of foliar damage and Leaf Damage Index (LDI) described by Halle and Sabelis (1985).
LDI was observed for 10 leaves on each plant and their average score was assessed based on a 0 to 5 scale as follows:

0 - no damage
1 - small feeding zones
2 - feeding zones < 25% leaf area
3 - feeding zones > 25% leaf area
4 - mostly covered but still green
5 - necrosis



(Drabo, 2022)

LDI was assessed on three occasions for two weeks. First assessment was prior to the first treatment application, second assessment was one week later prior to the second treatment application, third and last assessment was one week after the second treatment.
Halle and Sabelis (1985) reported that on a scale of 0-5 leaf damage, an index of 2.0 was equivalent to approximately 30% damage of the photosynthetic area of the leaves. Scores are shown in Table 4 and Fig 3.

4.3 Formulations

4.3.1 Pesticide formulations

Material used for the formulations are as follows; pure cold-pressed neem oil azadirachtin 3000 ppm by Original Organic, non-ionic polysorbate surfactant Tween 80 by Research Products International and synthetic acaricide Omite Propagite 57% EC by Uniroyal Chemical were used for the treatments and the dilutions were prepared. All three products were purchased at Chládek Zahradnické centrum in Prague.

4.3.2 Dilutions

For the neem oil formulations, Choupanian (2017) was referenced.
Neem oil was diluted to two concentrations, low and high concentrations were at 0,5% and 1% and Tween 80 at 0,1% and 0,2% respectively. The low concentration was prepared by mixing neem oil and Tween 80 polysorbate at 5 ml/l and 1 ml/l respectively, in magnetic stirrer at 700 rpm for 3 minutes. High concentration formulation was prepared in the same way with 10 ml/l neem oil and 2 ml/l Tween 80. The prepared compositions were left for several minutes to obtain equilibrium. Afterwards, water was added to obtain 100 ml solutions. All components were weighed with the use of an analytical balance.

Omite was diluted according to the instructions on the label. The dilutions are shown in Table1. Each treatment had three replications, making a total of 12 observations including the control. The control consisted of infested and untreated plants.

Table 1

Formulation	Concentration
Neem oil low c°	5 ml/l
Neem oil high c°	10 ml/l
Omite	1 ml/l

4.3.3 Treatment application

The 12 plants were arranged randomly and labelled NOL for low concentration neem oil, NOH for high concentration neem oil, O for Omite and C for the control treatments. Each experiment was repeated three times for each of the four treatments.

Products to be tested were prepared prior to the application and sprayed using a pressurized spray bottle. Spraying was performed individually, isolating each plant for a vigorous spraying on all parts of the plant, including underside of leaves and roots and allowing a 20 minute drying before placing them back in the box. Same application was repeated once a week for two weeks.

4.4 Mortality and repellence

Mortality was assessed 6, 12, 24 and 48 hours after each treatment by monitoring mite movements using a 14x hand magnifier. Unresponsive mites to a gentle prodding with a soft brush were reported dead. The dead and living mite numbers gave a mortality percentage for each treatment. Three apical leaves were marked on each plant where the mite count was carried out. This procedure followed the principles of counting mites adapted from Hoddle (1998). The underside of leaves was examined starting from the petiole and following the stronger veins, where mites are likely to be found, mites were counted and mortality rate was assessed. Four treatments with three plants each and three leaves from each plant gave a total of 36 leaves to be examined.

The sum of the number of mites on each leaf represents the mite population per plant, and the sum of the number of mites on each replicate represents the mite population per treatment.
Pre-treatment mite count was recorded for each plant and values were added for each treatment, shown in Table 2. Repellence was determined by the difference between the initial mite count (pre-treatment) and the mite count after treatment for each time interval. Mortality and repellence values are gathered in Table 3, Fig. 1 and Fig. 2

4.4.1 Data analysis

The mortality and repellence values were compared by ANOVA followed by Tukey's HSD test to show statistical difference. The average foliar damage score (LDI score) for each treatment was plotted over two weeks and the progress curve for each treatment was obtained, shown in Fig. 3. To statistically show if there was a significant difference between treatments, the scorings were compared by ANOVA with post hoc Tukey's HSD test at $P < 0.05$ and presented in Table 4.

5 Results

5.1 Mortality and repellence

The mortality and repellence response of *T. urticae* to different treatments are presented in Table 3, Fig. 1 and Fig. 2. The results indicate a significant difference between the three foliar treatments and the control. High concentration neem oil (NOH) had higher mortality and repellence percent than low concentration neem oil (NOL) and NOH treatment effects were comparable to the effects of synthetic acaricide Omite. NOL had lower values compared to NOH and Omite but was significantly higher than the control.

48 hours after the first treatment application, NOH resulted in total control of the mite population on the plants, with the combined effects on mortality and repellence. The effects of NOH and Omite treatments had comparable results. Although the highest mortality of 61.4% was achieved with Omite, the combined results of mortality and repellence were the highest in NOH treatment, where the entire mite population was controlled and no living mites were present on the plants (Table 3d). The highest repellence was observed with NOH treatment at 42.7%, this effect combined with the mortality caused by NOH of 57.3% resulted in the 100% control of the mite population. Omite caused a repellence of 36.4% and controlled 97.8% of the mite population. NOL treatment was effective to control 76.6% of the mite population by either killing or repelling (Table 3d).

Table 2 Initial mite count for each treatment

Treatment	Mite count (pre-treatment)	Mean ± SD (per plant)
NOH	89	26.9 ± 9.46
NOL	81	27 ± 9.62
O	96	32 ± 3.74
C	103	34.3 ± 9.74

Pre-treatment mite count is the sum of the mite numbers counted before application on each replicate of the treatments.

Table 3a
Mite population and percent mortality and repellence of *T. urticae* after **6 hours** of exposure to treatment

Treatment	Total mite count	Living mite count	Dead mite count	**Mortality %**	Mortality Mean ± SD	Repelled mite count	**Repellence %**	Repellence Mean ± SD
NOH	86	72	14	**15.7**	16.5 ± 3.3	3	**3.4**	3.3 ± 2.6
NOL	80	72	8	**9.8**	9.4 ± 3.1	1	**1.2**	0.8 ± 1.3
O	92	69	23	**23.9**	24.1 ± 4.1	4	**4.1**	4.3 ± 2.2
C	103	103	0	**0**	0	0	**0**	0

Table 3b
Mite population and percent mortality and repellence of *T. urticae* after **12 hours** of exposure to treatment

Treatment	Total mite count	Living mite count	Dead mite count	**Mortality %**	Mortality Mean ± SD	Repelled mite count	**Repellence %**	Repellence Mean ± SD
NOH	71	39	32	**36**	34,7 ± 14.3	18	**20**	19.3 ± 7.2
NOL	72	54	18	**22**	23.3 ± 7.7	9	**11**	12.6 ± 3.2
O	83	46	37	**38.5**	36.7 ± 12.8	13	**13.5**	12.3 ± 5.9
C	100	100	0	**0**	0	3	**2.9**	2 ± 1.1

Table 3c
Mite population and percent mortality and repellence of *T. urticae* after **24 hours** of exposure to treatment

Treatment	Total mite count	Living mite count	Dead mite count	**Mortality %**	Mortality Mean ± SD	Repelled mite count	**Repellence %**	Repellence Mean ± SD
NOH	59	12	47	**52.8**	50.3 ± 6.7	30	**33.7**	32.8 ± 4.2
NOL	68	43	25	**30.8**	31.2 ± 4.8	13	**16**	17.2 ± 6.6
O	72	19	53	**55.2**	54.2 ± 6.2	24	**25**	26.4 ± 4.3
C	97	92	5	**4.8**	3.9 ± 2.2	6	**5.8**	4.2 ± 3.2

Table 3d
Mite population and percent mortality and repellence of *T. urticae* after **48 hours** of exposure to treatment

Treatment	Total mite count	Living mite count	Dead mite count	**Mortality %**	Mortality Mean ± SD	Repelled mite count	**Repellence %**	Repellence Mean ± SD
NOH	51	0	51	**57.3**	55.4 ± 7.9	38	**42.7**	43.2 ± 7.8
NOL	57	19	38	**47**	48.2 ± 5.8	24	**29.6**	30.2 ± 6.3
O	61	2	59	**61.4**	60.2 ± 7.3	35	**36.4**	35.3 ± 5.8
C	95	90	5	**4.8**	4.2 ± 2.7	8	**7.7**	7.2 ± 3.2

The ANOVA results indicate a significant difference between the effects of NOH, NOL and the control, as well as a significant difference between effects of Omite, NOL and the control (p-value < 0.05). The results show that there was no significant difference between NOH and Omite effects on mortality and repellence of spider mites 48 hours after the treatment (p-value > 0.05).

Fig. 1a – Percent mortality and repellence of *T. urticae* after **6 hours** of exposure to treatment

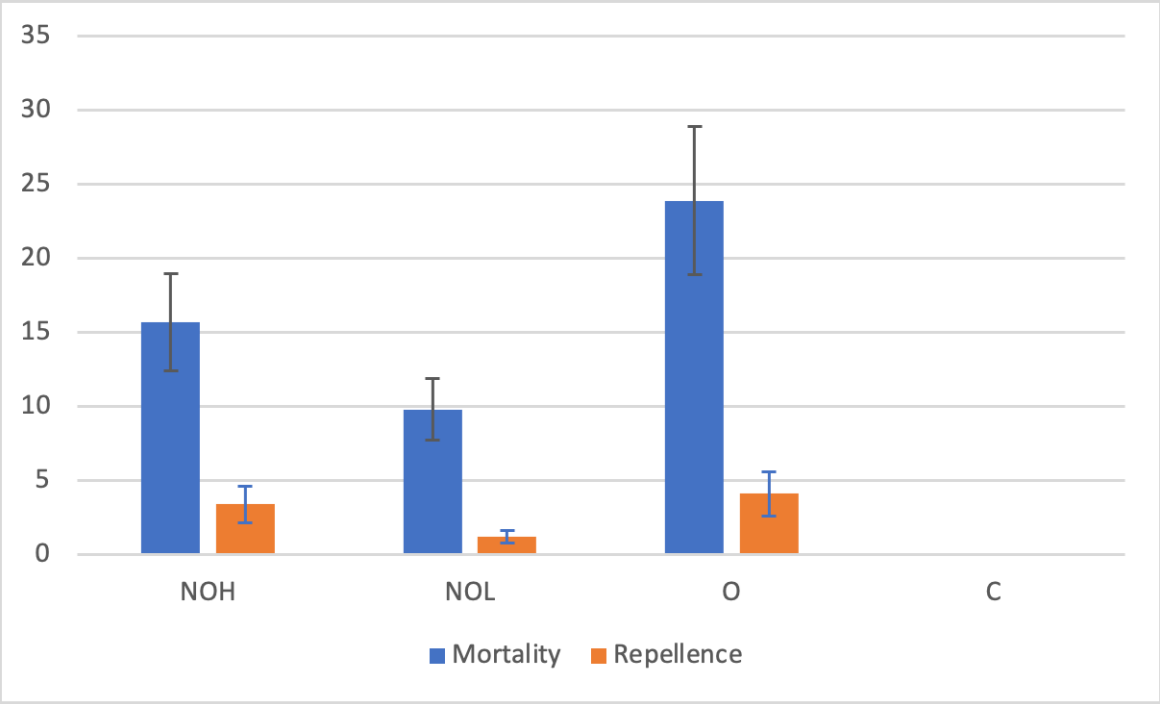


Fig. 1b – Percent mortality and repellence of *T. urticae* after **12 hours** of exposure to treatment

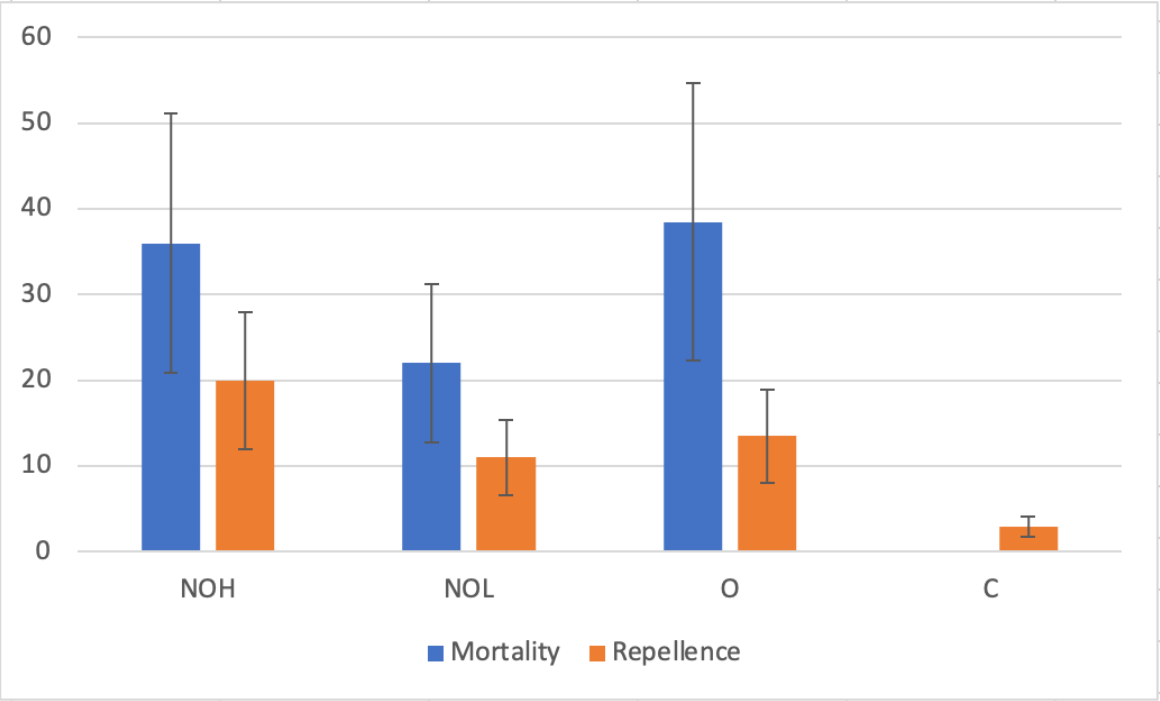


Fig. 1c – Percent mortality and repellence of *T. urticae* after **24 hours** of exposure to treatment

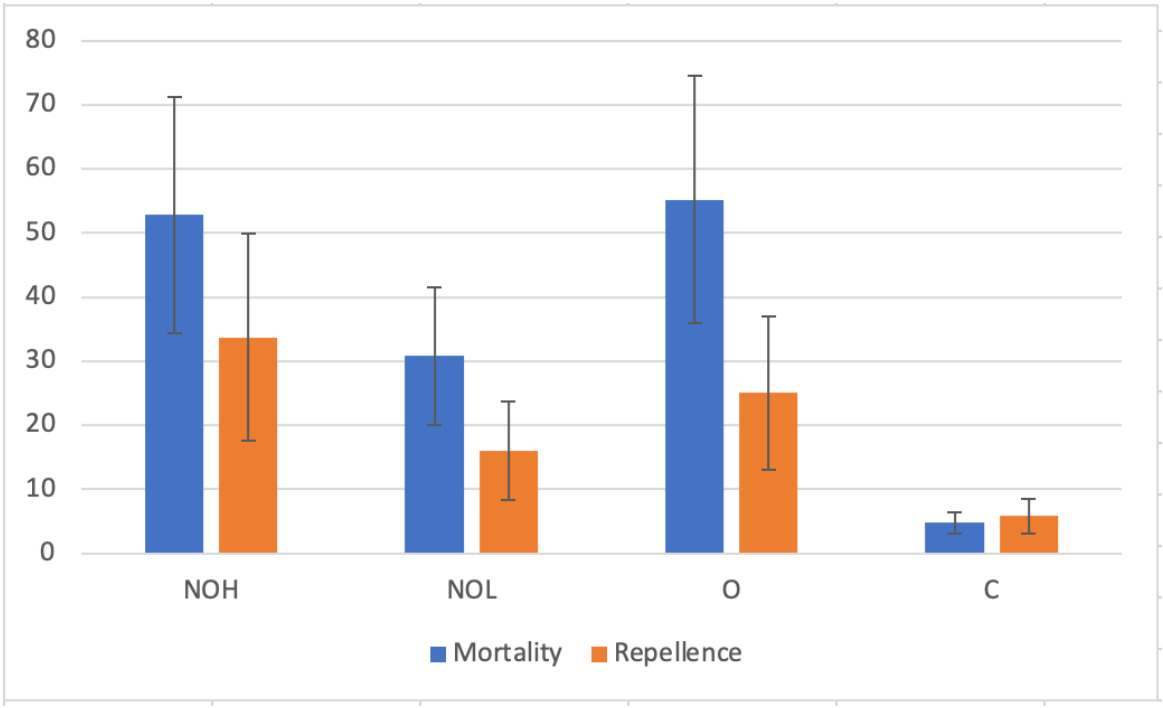


Fig. 1d – Percent mortality and repellence of *T. urticae* after **48 hours** of exposure to treatment

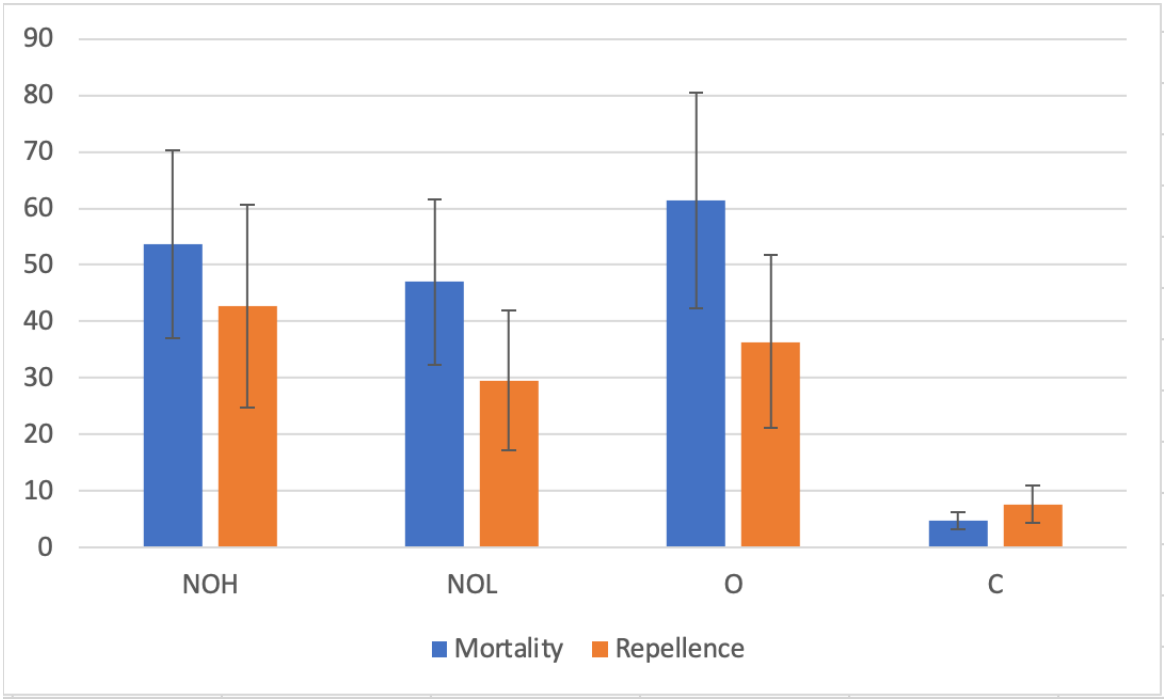


Fig. 2a - Effect of different treatments on percent mortality of *T. urticae* over time (hours)

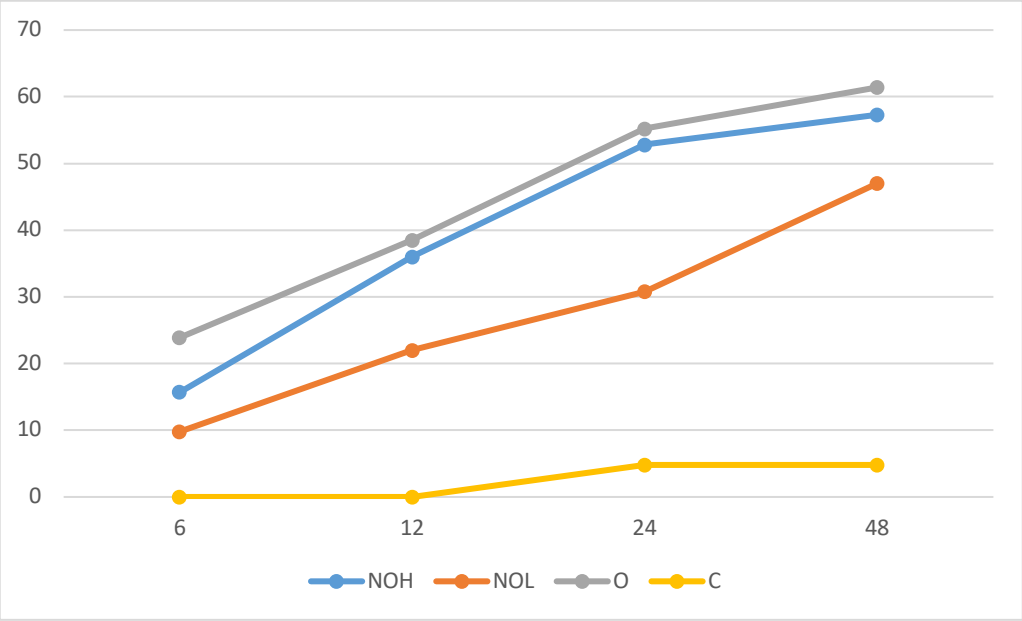
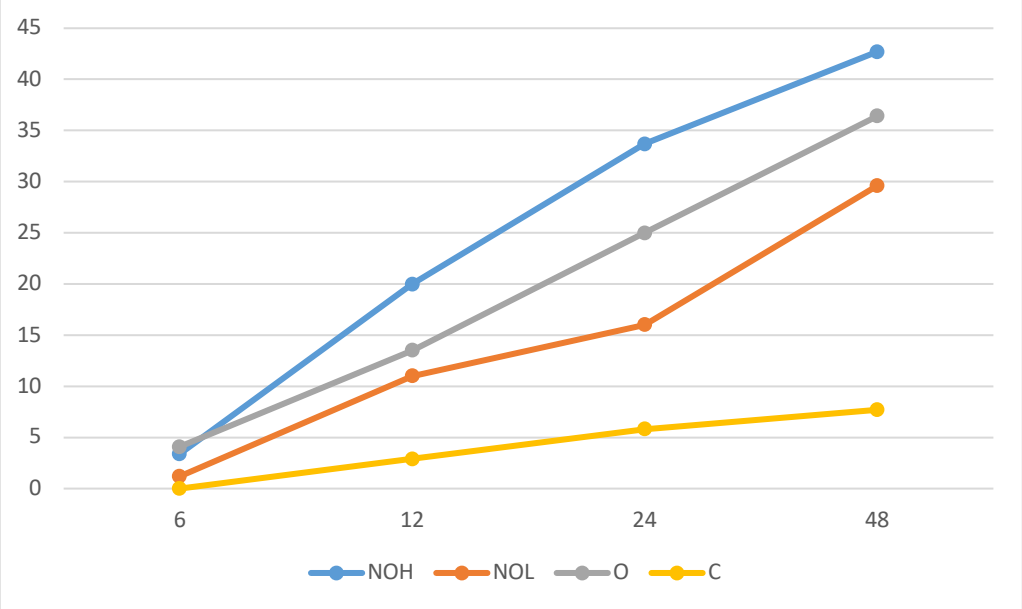


Fig. 2b - Effect of different treatments on percent repellence of *T. urticae* over time (hours)



Mortality

The mortality of *T. urticae* increased with time in all treatments, as shown in Fig. 2a. At 6 hours after exposure to the treatments, all the trials gave higher mortality than the control. Synthetic acaricide Omite gave the highest mortality of mites during the first 6 hours, but after 12 hours of exposure no significant difference was seen between effects of Omite and NOH ($p > 0.05$) (Fig. 2a). NOL had a comparable effect on mortality of mites with that of NOH in the first hours of exposure, but the difference was observed after 12 hours of exposure, where NOL had a significantly lower mortality rate than NOH. In general, there were minor variations in mortality of mites after 12 hours of exposure to NOH and Omite treatments. At the end of 48 hours all three foliar treatments, NOH, NOL and Omite, had comparable mortality rates of 57.3%, 47% and 61.4% respectively, although statistical difference was observed for NOL treatment. At 48 hours the control gave a mortality rate of 4.8%, which was attributed to the proximity of plants and the possible mix up of treatments due to the air flow inside the growbox.

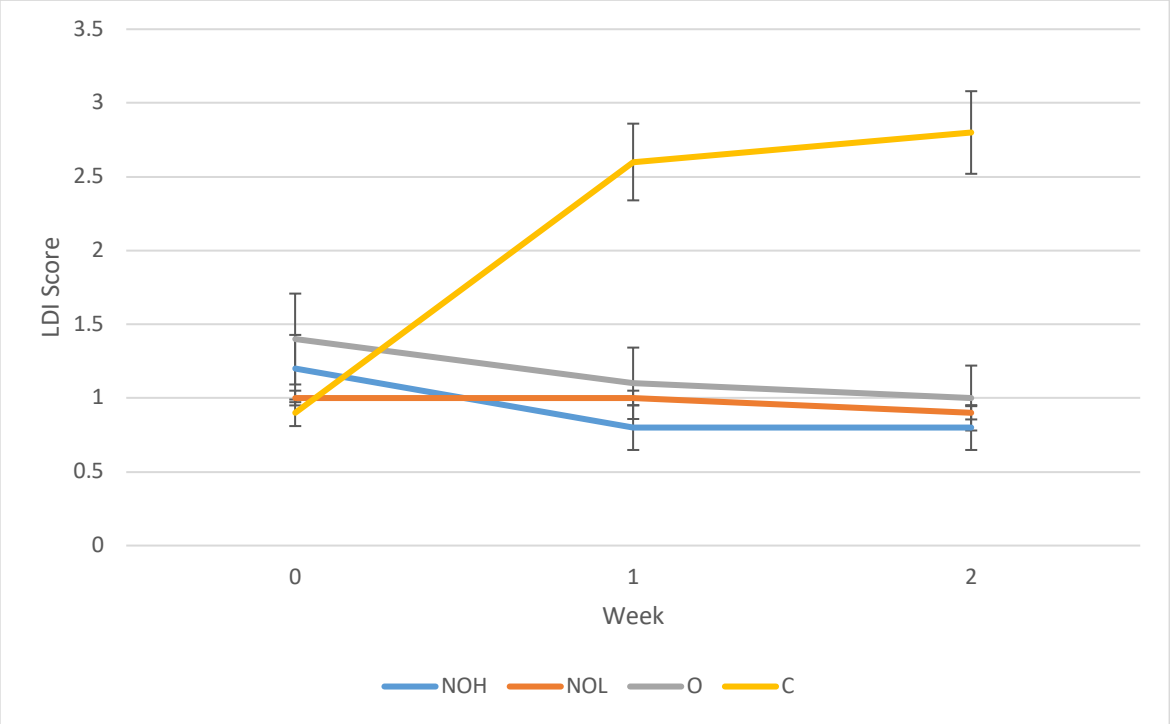
The lethal concentration LC50 was achieved with high concentration neem oil (NOH) and Omite formulations 24 hours after the treatment applications. Low concentration neem oil (NOL) formulation did not reach LC50 suggesting that the concentration of 5 ml/l was not effective to kill 50% of the mite population.

Repellence

The repellence of *T. urticae* increased with time in all treatments, as shown in Fig. 2b. There was no significant difference of repellence between different treatments after 6 hours of exposure or the control. After 12 hours the highest repellence of mites was seen with NOH treatment, which was significantly different from NOL, Omite and the control. At 12 hours, no significant difference was seen between NOL and Omite ($p > 0.05$), but both had higher repellence rates than the control. NOH treatment had the highest effect on mite repellence in all time intervals and reached a repellence rate of 42.7% at 48 hours after exposure, compared with NOL at 29.6% repellence, Omite at 36.4% repellence and the control at 7.7%.

5.2 LDI

Fig. 3 – LDI progress curve over time for foliar damage when treated with the different formulations



Error bars represent relative standard deviation of the mean.

Table 4 – Leaf Damage Index (LDI) per treatment over 2 weeks

Treatments/LDI	Pre-treatment Mean ± SD	1 week later (pre 2nd treatment)	2 weeks later (1 week after 2nd treatment)
NOH	1.2 ± 0.15a	0.8 ± 0.05a	0.8 ± 0.10a
NOL	1.0 ± 0.30a	1.0 ± 0.30a	0.9 ± 0.20a
O	1.4 ± 0.08b	1.1 ± 0.20a	1.0 ± 0.20a
C	0.9 ± 0.05a	2.6 ± 0.40b	2.8 ± 0.30b

Foliar damage scoring: 0 = No damage, 1 = Few mite attacks with small feeding patches, 2 = Large feeding patches < 25% leaf area, 3 = Feeding patches > 25% leaf area, 4 = Entire leaf covered with feeding patches but still green, 5 = Leaf necrosis. Means followed by the same letter within a column are not significantly different from each other at significance level α=0.05 ANOVA followed by Tukey's HSD test.

LDI

The leaf damage index was assessed visually three times for two weeks and no significant difference was seen between the three foliar treatments. In general, during the two weeks a slight decrease in the LDI score was observed for the three foliar treatments, but this decrease was not significant to attribute the foliar damage reduction to the effects of the treatments. A significant difference was shown between the foliar treatments and the control ($p < 0.05$). NOH, NOL and Omite treatments were all efficient in maintaining a low LDI score for the duration of the assessment whereas the control LDI score increased in this duration from 0.9 to 2.8. Omite trials were assessed an LDI score of 1.4 before the treatment application and 1.0 after the two weeks, this was the highest difference of LDI score observed during the two assessment weeks for the foliar treatments.

6 Discussion

The efficacy of azadirachtin against two-spotted spider mites was confirmed with the findings of this study. The high concentration neem oil formulation provided the best protection against the pest by ways of killing and repelling. Isman (1990) attributed this efficacy to the oil content and reported that without the oil content azadirachtin does not provide the same protection against spider mites, as seen with other neem formulations. Same is also confirmed by findings of Knapp (2003), who reported that cold pressed neem oil formulation (3000 ppm azadirachtin) was more effective in controlling spider mites than Neemros that has higher azadirachtin content but no oil.

Various studies attributed the acaricidal effects of neem products to their oil content (Krishna, 2013; Gaber, 2020). Enriched extracts seem to be more effective than aqueous extracts. Knapp (2003) compared the effects of different neem-based pesticides on spider mites and noted that Neemroc formulation, having the smallest azadirachtin content of 0.03% was more effective than other formulations with an azadirachtin content of up tp 2%. The effectiveness of Neemroc was probably enhanced by the oil emulsion rather than the amount of azadirachtin. The findings of the current study are consistent with the observations reported by Knapp (2003). The author added that the low mortality of mites caused by formulations at higher azadirachtin content was due to a low solubility of limonoids, as well as a low oil content. Guettal (2021) also observed that while in enriched extracts azadirachtin exhibits repellent properties, attaining high mortality rates requires higher concentrations. The mortality rates obtained in the current study confirm these observations. The neem oil used in the study contained 0.3% azadirachtin and was prepared into an oil emulsion. Higher mortality and repellence rates might have been achieved with either higher azadirachtin content or different formulation.

LDI

Generally, the LDI scores were too low and differences were difficult to make due to the short amount of time given to mites to establish and infest the plants before receiving the first treatment. Mites were left untouched for two days after the infected *M. arundinaeae* leaves were introduced into the growbox. The infestation could have been more noticeable and the effects more pronounced if mites were given a longer time to establish and LDI scores would have shown bigger differences between the foliar treatments. The LDI scores in this study ranged from 0.8 to 2.8 and highest scores were observed in the control. Halle and Sabelis (1985) reported that on a scale of 0-5 leaf damage, an index of 2.0 was equivalent to approximately 30% damage of the photosynthetic area of the leaves. In the treated plants, highest LDI score was 1.4 before treatment and 1.0 two weeks after the treatment. Stacey (1983) noted that the removal of about 25% of photosynthetic area of the leaf did not affect plant yield. The LDI scores of the treated plants are lower that 1.0 two weeks after the treatment. According to the findings of Halle and Sabelis (1985), the treated plants had a photosynthetic area loss of about 15% which according to Stacey (1983) does not affect plant yield.

Defoliation and curling

Defoliation and leaf curling was observed in all trials and was the highest in the control trials. Treated plants had lower defoliation and leaf curling than the control. These symptoms were the least significant in plants treated with NOH and Omite. NOL treated plants showed similar leaf curling to the control but defoliation was lower than the control.

Other pests

All plants showed symptoms of pest attacks after the first month of the experiment, which were not only attributed to the mite outbreak. Sciarid flies were present in the growbox, and root damage caused by the larvae was observed. In some of the plants, root damage was serious enough to cause limited growth and development of the plant. This had an impact on the LDI scoring with signs of foliar damage, leaf curling and defoliation caused by the flies and their

larvae. Both the neem formulations and the synthetic acaricide appeared to affect the infection of sciarids, however plants treated with NOH and NOL were more resistant to root damage and had healthier root systems compared to untreated plants and Omite treated plants. This shows the wide range of pesticidal effects of neem. Drobnjaković (2019) confirmed the efficacy of neem oil against Sciaridae flies. Kilani-Morakchi (2021) found that neem-based pesticides prevented different pest outbreaks more efficiently than synthetic pesticides that were tested during the study. Another pest was noticed which was not identified and thought to be in the Hemiptera order, but no symptoms were attributed to these bugs due to the very low number seen on and around the plants.

It is also thought that some pests came into the growbox at the very beginning when clones were brought in. The mother plants from which cuttings were taken possibly already had some of the pests seen in the growbox. The conditions in the growbox were maintained at levels that spider mites tend to grow the best in, making the environment suitable for other pests to establish and thrive. The hydroponic state of the growing system also creates a pest-susceptible environment when adequate air flow and oxygen supply are limited and the deep-water culture becomes a the medium of disease transmission. The nutrient pump used for irrigation, circulating the water solution up to the nozzles ran for 15 minutes every day and no air pump was used. This method resulted in a limited oxygen supply for the roots, which then affected growth and development of the plants.

Second treatment

The first foliar treatment was applied two days after the introduction of mites into the growbox. The second foliar treatment was applied one week later in the same way as the first, as explained in the methodology. The symptoms mentioned above exacerbated before the second foliar treatment could be applied. Defoliation and leaf curling rendered an LDI assessment and mite monitoring difficult to perform. Thus, a proper mite count and foliar damage assessment were impossible to be precisely made to examine the effects of a second treatment.

7 Conclusion

The findings of this study suggest that neem oil has a potential as a pest control agent against *Tetranychus urticae* on *Cannabis* plants.

High concentration neem oil (NOH) and synthetic acaricide Omite provided similar protection against the pest. The effects of NOH on mite mortality and repellence were even slightly better than those of Omite, where 100% of mites were either killed or repelled by NOH and 97.8% by Omite. The commercial acaricide Omite had the highest mortality rate of 61.4% compared to 57.3% with NOH. When both mortality and repellence are measured, NOH was more efficient than Omite in controlling the pest with a repellence rate of 42.7% and 36.4% respectively. Low concentration neem oil (NOL) formulation provided some protection and controlled 76.6% of the mites by either killing or repelling.

The leaf damage index, LDI ranged from 0.8 to 2.8 with the highest scores seen in the control. All three foliar treatments maintained a low LDI score during the assessment time, and no significant difference was observed between them. The significant difference ($p<0.05$) was noted between the control and the foliar treatments, all control plants scored higher than treated plants. The highest LDI score reported for the treated plants was 1.1 with Omite treatment, the lowest was seen in plants treated with NOH. NOL treated plants scored an average LDI value of 1.0 during the assessments.

The results reported in this study confirm that neem oil is a promising alternative to synthetic acaricide Omite against *T. urticae* on *Cannabis* plants. The efficacy of azadirachtin was tested with cold press neem oil containing 0.3% active ingredient and was found to be more effective at concentration of 10ml/l than synthetic acaricide Omite at the recommended concentration of 1ml/l.

Further evaluation of these trials in different field settings or controlled greenhouse environments is recommended to verify the efficacy of azadirachtin formulations across varying conditions.

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