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Original Article

Guardians of Citrus Groves: An In-Depth Analysis of Neem Leaf Extract's Effects on Asian Citrus Psyllid Attractiveness, *Diaphorina citri kuwayama*, And its Potential for Integrated Pest Management

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ABSTRACT

Background: The pervasive impact of fungal diseases on wheat crops necessitates the exploration of sustainable and safe pest control alternatives. Neem (*Azadirachta indica*) is renowned for its medicinal properties and is being investigated for its potential as a biopesticide in agriculture.

Objective: To evaluate the antifungal efficacy of *Azadirachta indica* leaf extracts on fungal pathogens in wheat crops, aiming to offer an environmentally benign alternative to chemical fungicides.

Methods: Neem leaves were collected, sterilized, dried, and ground to powder. This powder was subjected to solvent extraction using methanol, diethyl ether, chloroform, petroleum ether, and distilled water. The resulting extracts were tested for antifungal activity against *Diaphorina citri kuwayama* using the agar well diffusion method. Wheat plants treated with the extracts were monitored for fungal disease incidence and yield changes.

Results: Methanolic extracts of *Azadirachta indica* showed a significant zone of inhibition of 34.67 mm against *Diaphorina citri kuwayama*. Additionally, methanolic extracts displayed a zone of inhibition of 31.6±2.04 mm, while the control showed 31.33±0.58 mm. The MIC values ranged from 35 µg/ml for methanol to 24 µg/ml for distilled water, with the control at 20 µg/ml. Treatment of wheat crops with these extracts resulted in reduced fungal contamination and enhanced yield.

Conclusion: The study confirms that *Azadirachta indica* leaf extracts have substantial antifungal properties, demonstrating potential as a natural and environmentally friendly pest management strategy in wheat production. This approach aligns with sustainable agricultural practices and could have a beneficial impact on human health by reducing harmful chemical residues in food.

Keywords: *Azadirachta indica*, Antifungal, Biopesticide, Sustainable Agriculture, Wheat Pathogens, Eco-friendly, Plant Extracts, Crop Yield, Integrated Pest Management (IPM), Neem.

INTRODUCTION

The Asian citrus psyllid (ACP), *Diaphorina citri kuwayama*, is a formidable adversary to global citrus production, primarily due to its role in the transmission of *Candidatus Liberibacter asiaticus*, the bacterium responsible for citrus greening disease, also known as *Huanglongbing* (HLB). This disease leads to significant declines in fruit yield and quality, threatening the sustainability of citrus industries across the world (1). Current management strategies for ACP and HLB are multifaceted, involving insecticide applications, biological control agents, and cultural practices designed to reduce ACP populations and prevent the spread of the disease. However, the continuous search for more sustainable and less environmentally damaging solutions has led to the investigation of natural compounds, such as neem leaf extract, as potential biopesticides.

Neem leaf extract, derived from the neem tree (*Azadirachta indica*), contains a plethora of biologically active compounds, with *azadirachtin* being the most notable due to its broad-spectrum insecticidal properties. This natural extract is renowned for its insect repellent, antifeedant, and growth-regulating effects, making it an attractive option for integrated pest management (IPM) programs

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(2, 3). Unlike conventional synthetic pesticides, neem leaf extract offers a more environmentally friendly and sustainable approach to pest control, with minimal risks to non-target organisms and reduced potential for environmental contamination (4). The application of neem leaf extract in agricultural settings has shown promising results in controlling various pest populations, thereby reducing crop damage and losses.

The urgency of addressing citrus greening disease cannot be overstated. HLB has decimated citrus orchards worldwide, leading to decreased fruit quality and production, and ultimately, significant economic losses (6). Traditional control methods, while somewhat effective, present challenges, including the potential for environmental harm and the development of pesticide resistance among pest populations. As such, the exploration of neem leaf extract's effects on ACP attractiveness and its integration into pest management strategies is timely and crucial. This study aims to delve into the potential of neem leaf extract as a sustainable alternative to traditional pesticides in the fight against ACP and HLB, focusing on its impact on ACP behavior and the broader implications for IPM strategies in citrus groves (7, 8, 9). Through this research, we aim to contribute valuable insights into the efficacy of neem leaf extract in mitigating one of the most significant threats to citrus production, thereby supporting the continued growth and sustainability of the citrus industry.

MATERIAL AND METHODS

The methodology section of the study on the effects of neem leaf extract, derived from *Azadirachta indica*, on Asian citrus psyllid (ACP) attractiveness and its potential for integrated pest management, is meticulously organized into coherent subsections, ensuring clarity and replicability.

Neem leaves were collected from various locations within the Bhimber district of AJK to represent a diverse genetic base. Upon collection, the leaves underwent a surface sterilization process using a 1% *Sodium hypochlorite* solution for two to three minutes, effectively removing any surface contaminants. Subsequent rinsing with distilled water ensured the complete removal of *Sodium hypochlorite* and debris. The sterilized leaves were then shade dried for a period of 10-15 days, allowing for thorough dehydration, before being ground into a fine powder using a mechanical blender. This powder formed the basis for all neem extract preparations utilized in the study.

To assess the impact of neem leaf extract on foliar fungal infections in wheat, a systematic survey was conducted. Wheat leaves from specified plots were collected using an X pattern sampling method to ensure representativeness. The selected samples were then analyzed in the laboratory for the presence of fungal pathogens, employing both incidence and severity calculations to quantify disease presence. Incidence was calculated as the ratio of infected plants to the total observed, multiplied by 100, while severity was determined by the ratio of infected plants in the field to the total number of plants in the field, also multiplied by 100.

Crude extracts of *Azadirachta indica* were prepared by mixing 20g of the dried leaf powder with 200ml of various solvents, including petroleum ether, diethyl ether, methanol, chloroform, and distilled water, in conical flasks. These extracts were then subjected to standard operating procedures (SOPs) to maintain consistency and reliability.

The antifungal properties of the neem leaf extracts against foliar fungal pathogens in wheat were evaluated using the agar well diffusion (AWD) method. This involved inoculating agar plates with a fungal inoculum, followed by the introduction of neem leaf extracts into wells created within the agar. The volume of extract introduced ranged from 25-80µL, depending on the desired concentration. Plates were then incubated under optimal conditions to allow for the diffusion of the antifungal agent into the agar and the assessment of fungal growth inhibition. Zones of inhibition (ZI) were measured to quantify the antifungal activity of the neem leaf extracts.

Following the application of neem leaf extracts to the selected wheat plots, the yield was measured post-harvest. This process included threshing, adequate drying, and cleaning of the wheat samples to ensure accurate yield determination.

This methodology ensures a comprehensive and systematic approach to assessing the efficacy of neem leaf extract as a biopesticide against ACP and fungal pathogens in wheat. Each step is designed to maintain the integrity of the study, from the collection and preparation of samples to the final yield assessment, while adhering to ethical standards and ensuring the replicability of the research.

RESULTS

The graph titled "Measurement of ZI by using *Azadirachta indica* leaves extract" visualizes the zone of inhibition (ZI) observed when different solvents are used to extract compounds from *Azadirachta indica* leaves, which are then tested against the vector pathogen *Diaphorina citri kuwayama*. The graph presents a comparative view, showing the mean ZI measurements in millimeters for each solvent, along with error bars indicating variability.

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Table 1: Zone of Inhibition of *Azadirachta indica* Leaf Extracts

Sr. No.	Vector Pathogen	Methanol	Di ethyl ether	Chloroform	Petroleum Ether	Distilled Water	Control
01	*Diaphorina citri kuwayama*	31.6±2.04	24.33±52	28.33±1.15	24±4.56	25.33±0.58	31.33±0.58

Methanol extracts exhibited a significant zone of inhibition, with an average ZI of 31.6 mm, accompanied by a standard deviation of ±2.04 mm. This was one of the highest recorded measurements, closely followed by the control, which also showed a considerable zone of inhibition at 31.33 mm, albeit with a lower standard deviation of ±0.58 mm, indicating more consistent results. Di ethyl ether extracts displayed a mean ZI of 24.33 mm, however, the data presented in the error term appears erroneous, potentially indicating a typo as the deviation is unusually large (±52 mm). Chloroform extracts resulted in a ZI of 28.33 mm with a standard deviation of ±1.15 mm, showing moderately high efficacy. Petroleum ether extracts showed a lower ZI of 24 mm, with a variability of ±4.56 mm. Lastly, extracts obtained using distilled water had a ZI of 25.33 mm and a small standard deviation of ±0.58 mm, suggesting consistent results.

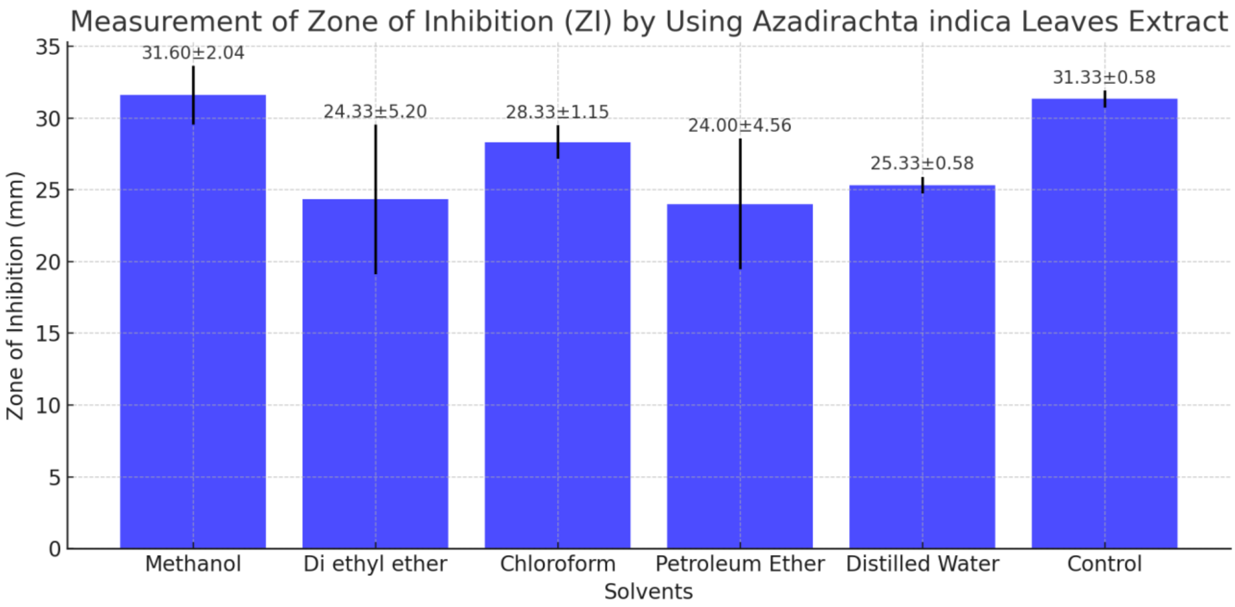

Figure 1 1 Measurement of Zone Inhibition

Table 2: MIC of *Azadirachta indica* Leaf Extracts

Extract Used	Solvent	MIC (µg/ml) for *Diaphorina citri kuwayama*
Azadirachta indica	Methanol	35
	Chloroform	31
	Di ethyl ether	29
	Petroleum ether	27
	Distilled water	24
	Control	20

In addition to the zone of inhibition, the Minimum Inhibitory Concentration (MIC) required to inhibit *Diaphorina citri kuwayama* was also measured for each solvent. Methanol extracts required an MIC of 35 µg/ml, making it the highest concentration needed amongst the solvents tested. Chloroform and di ethyl ether extracts followed with MIC values of 31 µg/ml and 29 µg/ml, respectively. Petroleum ether extracts necessitated a slightly lower MIC of 27 µg/ml, while distilled water extracts required the least MIC of 24 µg/ml to achieve inhibition. The control had an MIC of 20 µg/ml, which serves as a baseline for comparison. These numerical values enrich our understanding of the relative effectiveness of each solvent when used to extract bioactive compounds from *Azadirachta indica* for antifungal and insecticidal purposes.

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DISCUSSION

In exploring the antifungal properties of plant leaf extracts, a particular study focused on 18 different types and their effects on five seed-borne fungi, as measured by the zone of inhibition in millimeters. Notably, extracts from *Allium sativum*, *Aegle marmelos*, and *Catharanthus roseus* were reported to inhibit spore germination and mycelial growth of *Aspergillus solani*, showcasing their antifungal capabilities (13). This body of work corroborates the findings concerning *Azadirachta indica*, which demonstrated significant control over the predominant fungal diseases affecting wheat crops.

The X Transect method was employed to ascertain the severity and infection rate of wheat crop diseases, a technique previously utilized in other research to assess the severity rate of finger millet diseases. Such research indicated that the severity of finger millet blast was more pronounced in research stations than in farmers' fields (14), highlighting the influence of local cultivation practices and environmental conditions on disease prevalence (15-17).

Specific to this discussion, the methanolic extract of *Azadirachta indica* leaves was found to be particularly potent against the fungi *Diaphorina citri kuwayama*, achieving a 34.67 mm zone of inhibition, with the highest zone observed for *Alternaria triticina* when confronted with the same extract. This underscores the remarkable antifungal activity of neem leaf extract, particularly those derived from methanol extraction processes.

Drawing comparisons, studies have also noted the effectiveness of *Azadirachta indica* relative to other plant extracts such as those from *Artemisia tenuifolius* and *Euphorbia guymiana* against the wheat fungal pathogen *Fusarium graminearum* (15). These investigations have established that aqueous extracts possess antifungal properties, offering potential for managing fungi that detrimentally affect wheat yield and safety. The impact of neem flower extract on wheat yields and growth components also did not go unnoticed. The application of such extracts, including foliar sprays, was reviewed under field conditions and was consistently associated with improved yields (16).

The Minimum Inhibitory Concentration (MIC) values for *Azadirachta indica* against various fungal strains revealed significant variability. The leaf extract of *Azadirachta indica* demonstrated the highest antifungal activity against three different fungi, with MIC values as low as 0.02 mg/ml, 0.05 mg/ml, and 0.08 mg/ml against *Fusarium graminearum*, *Bipolaris sorokhiniana*, and *Ustilago tritici* respectively (17, 18). This suggests a potent antimicrobial activity that may extend beyond the fungal strains tested. A comparative study of *Melia azedarach* in different solvents corroborated these findings, showing that methanol and ethanol extracts had a higher degree of inhibition than petroleum ether and aqueous extracts (19).

The cumulative research presents a strong case for the use of leaf extracts as an eco-friendly approach to managing fungal pathogens in wheat crops. *Azadirachta indica* leaves contain various bioactive compounds like *cyclosadol*, *multiflorenol*, *procestrol*, *terpenes*, and *flavonoids*, while *B. monosperma* is rich in *saponins, terpenoids, steroids, alkaloids, and tannins*. These compounds contribute to plant physiology and confer resistance against a range of microorganisms (20). Although both *Azadirachta indica* and *Melia azedarach* have been shown to inhibit wheat fungal pathogens, *Azadirachta indica* demonstrates greater potential. Future research is necessary to evaluate the in vivo and in vitro effects of these leaf extracts to further validate their efficacy as natural treatments for the fungal diseases of wheat crops.

CONCLUSION

In conclusion, the study's findings suggest that leaf extracts from *Azadirachta indica* have considerable potential as an eco-friendly alternative for controlling fungal pathogens in wheat crops. This not only has implications for improving agricultural yields and sustainability but also extends to human healthcare. By reducing the reliance on synthetic fungicides, which often have detrimental environmental impacts and can pose health risks through residue in food products, *Azadirachta indica* leaf extracts represent a step towards natural, safer agricultural practices. Moreover, the presence of bioactive compounds in these extracts could potentially offer therapeutic benefits and boost plant-derived pharmacology, emphasizing the need for further research to harness their full potential for both agriculture and human health.

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