



The Impact of Time of Collection on the Yield, Chemical Composition and Insecticidal Activity of *Citrus sinensis* Leaf Essential Oils

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Abstract

Essential oils derived from plant materials are widely used in various industries due to their diverse bioactive compounds. This study investigates the influence of the time of collection (morning vs. afternoon) on the chemical composition of *Citrus sinensis* leaf essential oils and evaluates their insecticidal activities against *Callosobruchusmeculatus*. Results indicate significant variations in both qualitative and quantitative profiles of the oils depending on the time of harvest, with corresponding differences in efficacy against the target pest. These findings underscore the importance of optimizing harvesting practices to maximize the therapeutic and pesticidal potential of essential oils.

Keywords: Essential oils, *Citrus sinensis*, sweet orange, *Callosobruchusmeculatus*, collection time, insecticidal activities, season, yield

1. Introduction

Essential oils are complex mixtures of volatile secondary metabolites found in approximately 10% of plant species, making them a unique and valuable component of the Plant Kingdom (Bauer *et al.*, 2001). These oils are distributed across various plant families, including Ericaceae, Betulaceae, Verbenaceae, Cistaceae, Cruciferae, Liliaceae, Iridaceae, Araceae, Palmae, Cyperaceae, Moraceae, Aristolochiaceae, Chenopodiaceae, Ranunculaceae, Euphorbiaceae, Malvaceae, Usneaceae, Podocarpaceae, Pinaceae, Taxodiaceae, Cupressaceae, and Rutaceae (Svoboda, 1996; Buhner, 2000). Among these, the Rutaceae family holds significant economic importance due to its diverse applications in food, pharmaceuticals, and cosmetics. The genus *Citrus*, particularly sweet orange (*Citrus sinensis*), is one of the most widely cultivated citrus species globally, known for its rich essential oil content in leaves, fruits, and peels (Morton, 1987).

The ecological roles of essential oils are multifaceted. They serve as defense mechanisms against herbivores and pathogens, act as allelopathic agents to inhibit competing vegetation, and attract pollinators to ensure reproductive success (Shawe, 1996; Lawrence, 2000). For example, plants like *Salvia leucopylla* and *Artemisia californica* release allelopathic terpenoids such as eucalyptol and camphor into the surrounding environment, effectively preventing other plant species from growing nearby (Lawrence,

2000). Additionally, essential oils contribute to the antimicrobial, antifungal, and antibacterial properties of plants, protecting them from a wide range of diseases (Sell, 2010; Buchbauer, 2000).

Despite their widespread use, the chemical composition and biological activity of essential oils can vary significantly depending on several factors, including seasonal variation, drying duration, geographical location, and the time of collection (Abdelrazzaq *et al.*, 2013; McGimpsey *et al.*, 2006). While previous studies have extensively investigated the influence of seasonality and drying methods on essential oil profiles, the specific impact of the time of collection (morning vs. afternoon) remains relatively underexplored. This temporal factor may influence enzymatic activity, leading to variations in the biosynthesis and accumulation of key compounds within the plant tissue (Svoboda, 1996).

Insecticidal properties of essential oils have also gained considerable attention as natural alternatives to synthetic pesticides, which often leave toxic residues and contribute to environmental pollution (Adedire, 2003). Synthetic chemicals used in pest control, such as CuSO₄, FeSO₄, pyrethrin, methyl bromide, and chloropicrin, pose risks to human health and ecosystems, with pests developing resistance over time (Kumar *et al.*, 2011). In contrast, botanical insecticides derived from essential oils offer safer and more sustainable solutions for pest management.

This article focuses on the impact of time of collection on the chemical composition of *Citrus sinensis* leaf essential oils and evaluates their efficacy as insecticides against *Callosobruchus meculatus*, a major storage pest affecting cowpea crops. *Callosobruchus meculatus* causes significant post-harvest losses, reducing the nutritive value of cowpeas and contributing to malnutrition in regions reliant on this protein source (Abbas *et al.*, 2012). Essential oils from *Citrus sinensis* leaves have been shown to possess potent insecticidal activity, attributed to their bioactive compounds such as limonene, citronellal, geraniol, linalool, and β -caryophyllene (Kim *et al.*, 2003; Louis and Badama, 2010; Usman *et al.*, 2016).

Key Objectives

1. To investigate how the time of collection (morning vs. afternoon) influences the qualitative and quantitative composition of *Citrus sinensis* leaf essential oils during both rainy and dry seasons.
2. To evaluate the insecticidal activities of these oils against *Callosobruchus meculatus* using contact toxicity assays.
3. To identify potential correlations between specific chemical constituents and their insecticidal efficacy.

Significance of the Study

Cowpea (*Vigna unguiculata*) is a critical source of dietary protein in many developing countries, particularly in Nigeria, where it plays a vital role in addressing nutritional deficiencies (Razzaghi-Abyaneh *et al.*, 2009). However, infestations by *Callosobruchus meculatus* severely compromise crop quality and yield. Traditional synthetic insecticides not only fail to provide long-term solutions but also lead to contamination of stored food products, posing health risks to consumers. Therefore, exploring plant-derived insecticides offers a promising avenue for sustainable pest management.

Previous research has demonstrated that essential oils from *Citrus sinensis* exhibit insecticidal activity through mechanisms such as disrupting the cytoplasmic membrane, coagulating lipids and proteins, and causing damage to cellular structures (Gustafson *et al.*, 1998; Ultee *et al.*, 2002). Furthermore, certain compounds in these oils, such as limonene, citronellal, and β -elemene, have been identified as key contributors to their pesticidal effects (Ngamo *et al.*, 2007; Lee and Lee, 2010). By understanding how

the time of collection modulates the abundance of these active components, we can refine harvesting practices to maximize the insecticidal potential of *Citrus sinensis* essential oils.

2. Materials and Methods

2.1 Plant Materials

Fresh leaves of *Citrus sinensis* were collected at two distinct times: **7 am (morning)** and **2 pm (afternoon)** during both rainy and dry seasons. Samples were identified at the Herbarium of the Plant Biology Department, University of Ilorin, where voucher specimens were deposited. Fresh leaves were directly pulverized before extraction.

2.2 Oil Isolation

Essential oils were extracted using hydro-distillation in a Clevenger-type apparatus according to British Pharmacopoeia specifications (British Pharmacopoeia, 1980). Each sample (500 g) was distilled for three hours, and the resulting oils were stored under refrigeration at 4°C until analysis.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrometry (GC-MS) is a widely used analytical technique that combines the separation capabilities of gas chromatography (GC) with the identification power of mass spectrometry (MS). This method is particularly useful for analyzing complex mixtures such as essential oils, allowing precise identification of their components.

In this study, a Hewlett-Packard HP 5890A GC system was interfaced with a VG Analytical 70-250s double-focusing mass spectrometer to analyze the chemical constituents of the extracted essential oils. Helium was employed as the carrier gas at a flow rate of 1.2 mL/min, ensuring efficient separation of volatile compounds. The GC was equipped with a fused silica capillary column coated with Cp-Sil 5 (25 m × 0.25 mm, film thickness 0.15 µm), which provided high-resolution separation of individual constituents.

The MS was operated under the following conditions:

- Ionization Voltage: 70 eV
- Ion Source Temperature: 230°C

Retention indices for the detected compounds were determined relative to a homologous series of n-alkanes. The identification of chemical constituents was achieved by comparing mass spectra and retention indices with authentic reference compounds and established literature databases, including Adams (1995) and Joulain and König (1998). Additionally, spectral matching with standard databases such as the National Institute of Standards and Technology (NIST) was performed to confirm compound identities.

2.4 Insecticidal Activity Assay

The insecticidal properties of the extracted essential oils were evaluated against *Callosobruchus maculatus*, a major pest of stored cowpea seeds. The beetles were obtained from infested cowpea seeds and reared under controlled conditions (28 ± 2°C) to maintain a stable population.

2.4.1 Experimental Setup

The bioassay involved treating 10 g of clean, uninfested cowpea seeds with 0.1 mL of essential oil. The oil was thoroughly mixed with the seeds to ensure uniform distribution. Ten adult beetles were introduced into each container, and mortality rates were recorded at 6-hour intervals over a total period of 42 hours. Control experiments consisted of untreated seeds under identical conditions.

2.4.2 Data Collection and Analysis

Mortality rates were determined by counting the number of dead beetles at each interval. The percentage mortality was calculated and compared across different time points. The experimental setup was replicated in duplicate to ensure reliability.

Previous studies have shown that essential oils from *Citrus sinensis* contain bioactive compounds such as limonene, citronellal, geraniol, linalool, myrcene, α -pinene, and γ -terpinene, all of which have demonstrated insecticidal activity. These compounds act synergistically to disrupt the physiological processes of the target insects, leading to increased mortality.

3. Results and Discussion

3.1 Essential Oil Yield

The yield of oils obtained from morning and afternoon harvests of the rainy season was 0.2 and 0.22 (w/w) (%) respectively while the yield from morning and afternoon harvests of the dry season were 0.09 and 0.1(w/w) (%) respectively. It was observed that time of collection did not significantly impact the yield of the essential oils although in the afternoon, the yield increased slightly as compared to the oils obtained from the morning harvests. The season of harvest had more effect on the oil yield than collection time.

3.2 Chemical Composition of Essential Oils

The chemical compositions of essential oils extracted from *Citrus sinensis* leaves harvested at different times of the day and across different seasons are summarized in Table 3.1. The analysis revealed significant variations in the chemical constituents depending on the harvest time.

Table 3.1: Chemical Composition (%) of Essential Oils from Fresh Leaves of *Citrus sinensis* Harvested in the Morning and Afternoon During the Rainy and Dry Seasons

S/N	Compounds	KI	% Composition			
			DAF	DMF	RAF	RMF
1	α -thujene	931	0.3	-	1.2	0.4
2	α -pinene	939	0.9	0.9	1.8	1.5
3	α -fenchene	951	9.8	-	-	12.7
4	Camphene	953	-	0.2	-	-
5	β -thujene	971	0.3	0.3	0.3	0.6
6	β -pinene	980	2.4	-	3.0	-
7	Sulcatone	985	-	-	0.4	-
8	β -myrcene	991	-	2.3	-	-
9	2-carene	1001	-	0.7	-	-
10	α -phellandrene	1005	0.9	0.9	1.3	1.1
11	3-carene	1011	9.9	8.3	18.0	9.1
12	α -terpinene	1018	1.1	-	-	1.1
13	Ortho-cymene	1020	-	1.8	-	-

14	p-cymene	1026	-	0.5	-	-
15	D-limonene	1031	-	6.8	11.0	8.7
16	Cis- β -ocimene	1040	9.2	6.7	5.4	-
17	Isocarvestrene	1047	2.5	-	-	1.6
18	Trans- β -ocimene	1050	0.5	0.5	0.7	0.5
19	γ -terpinene	1062	6.7	8.5	1.9	0.5
20	Sabinene hydrate	1068	-	-	0.6	-
21	m-cymene	1082	0.6	0.3	-	1.3
22	α -Terpinolene	1088	3.8	3.8	4.9	1.4
23	P- α -dimethylstyrene	1096	0.2	-	-	-
24	Cis-verbenol	1140	-	-	-	0.3
25	1,3,8-p-menthatriene	1111	3.9	-	-	-
26	Isopulegol	1145	0.4	-	0.4	0.2
27	1,5-dimethylcyclooctadiene		5.6	-		
28	Citronellal	1153	5.1	1.0	7.0	6.1
29	Isoborneol	1156	5.4	-	-	-
30	Terpinen-4-ol	1177	-	1.9	3.3	2.5
31	α -terpineol	1189	0.3	-	0.6	0.6
32	Decanal	1204	-	-	0.4	0.3
33	Citronellol	1228	3.7	3.5	4.5	6.7
34	Citral	1240	2.8	0.8	4.1	4.3
35	Neral	1240	-	-	4.1	4.2
36	Geranyl linalool	1244			-	1.2
37	Nerol	1255	-	-	1.5	-
38	Lavandulyl acetate	1289	-	2.8	-	-
39	Thymol	1290	-	-	-	0.3
40	Methyl geranate	1323	0.3	0.4	0.6	0.8
41	Citronellyl acetate	1354	3.0	5.0	2.6	2.3
42	Neryl acetate	1365	-	-	1.3	-
43	β -elemene	1375	1.4	8.6	1.9	2.2
44	β -copaene	1378	-	0.4	-	-
45	Geranyl acetate	1383	-	2.9	-	-
46	1-octadecyne		2.4	-	-	-
47	8-hydroxyneomenthol	1423	-	2.3	-	-
48	γ -elemene	1430	-	-	-	0.3
49	β -Guaiene	1439	-	-	-	2.0
50	Humulene	1440	0.5	1.8	0.6	0.8
51	Caryophyllene	1454	1.3	5.2	-	-
52	Neryl propionate	1454	-	-	1.6	-
53	Cis- β -farnesene	1458	0.4	1.7	0.5	1.4
54	β -cadinene	1473	-	0.6	-	-
55	γ -maaliene	1477	-	0.3	-	-
56	β -selinene	1485	-	0.7	-	-
57	β -ionone	1485	-	-	0.6	-
58	Eremophilene	1486	1.2	-	-	-
59	β -guaiene	1490	0.3	-	-	-
60	Valencene	1491	-	-	-	0.2
61	α -selinene	1494	-	0.7	-	-
62	Germacrene A	1503	-	-	1.3	-
63	α -farnesene	1508	-	0.4	-	-
64	Elemol	1547	-	0.3	-	-
65	Spathulenol	1576	-	0.5	-	-
66	Caryophyllene oxide	1581	0.4	1.0	0.4	0.6
67	Eudesmol	1652	-	0.4	0.2	0.3
68	α -sinensal	1752	2.7	5.0	0.5	2.0
69	Dehydronoisolongifolene		0.4	-	-	-
70	Phytol	1949	2.5	-	0.9	1.2

71	E,E,E- α -springene	2019	0.5	-	-	-
72	Phytol acetate	2223	-	3.8	-	-
Total (%)			93.6	94.5	89.4	81.3

DAF: Fresh leaves from the dry season afternoon harvest

DMF: Fresh leaves from the dry season morning harvest

RAF: Fresh leaves from the dry season afternoon harvest

RMF: Fresh leaves from the dry season afternoon harvest

- **Dry Season (Afternoon Harvest - DAF):**

- 3-carene (9.9 %) was the principal monoterpene, while α -sinensal (2.7 %) was the dominant sesquiterpene.
- The afternoon harvest of the dry season revealed a lower concentration of α -sinensal compared to morning harvests, showing a notable difference in sesquiterpene composition.

- **Dry Season (Morning Harvest - DMF):**

- The major compound identified was β -elemene (8.6 %), which was the most abundant sesquiterpene.
- γ -terpinene (8.5 %) was the dominant monoterpene, and in the afternoon harvest, it was less abundant (6.7 %).

- **Rainy Season (Afternoon Harvest - RAF):**

- The predominant compound was 3-carene (18.0%), followed by limonene (11.0%), cis- β -ocimene (5.4%), and citral (4.1%).
- Afternoon-harvested oils contained a higher concentration of oxygenated monoterpenes than morning-harvested samples.

- **Rainy Season (Morning Harvest - RMF):**

- The oil was rich in monoterpenes, with α -fenchene (12.7%) as the most abundant component.
- Other major constituents included 3-carene (9.1%) and limonene (8.7%).
- Oxygenated monoterpenes, such as citronellal (6.1%) and citronellol (4.5%), were also present in significant amounts.

Summary of Key Differences:

- **Seasonal Variations:**

- The chemical composition of the essential oils varied significantly between the rainy and dry seasons. Notably, the rainy season had a higher presence of oxygenated monoterpenoids (e.g., citronellal and citronellol), while the dry season saw a dominance of monoterpenes like γ -terpinene and 3-carene.
- The morning and afternoon harvests within each season displayed differences in compound concentrations, suggesting that time of day also plays a role in the composition, with afternoon oils generally showing higher levels of some monoterpenoids (e.g., 3-carene).

- **Effect of Harvest Time and Season:**

- The inclusion of DMF and DAF allows for a clearer understanding of how time of collection (morning vs. afternoon) and seasonality (rainy vs. dry) influence the chemical makeup of the essential oils. The addition of compounds like α -sinensal and β -elemene in the dry season oils highlights the shift in dominant chemical profiles.

3.2.1 Notable Chemical Variability

- Limonene was undetected in DAF but present in other samples, ranging from 6.8 to 11.0%.
- β -Pinene, sabinene hydrate, were exclusive to afternoon-harvested oils, indicating possible photochemical transformations occurring under increased sunlight exposure.
- 3-carene was more abundant in the afternoon harvests in both seasons
- Caryophyllene is only present in the dry season and more abundant in the morning harvest
- Cis- β -farnesene, humulene and β -elemene are more abundant in the morning harvests, regardless of the season of harvest which suggests this time of harvest favours the availability of sesquiterpenoids.

The enzymatic activity responsible for terpene biosynthesis varies throughout the day, influenced by factors such as sunlight intensity and temperature (Svoboda, 1996). Morning-harvested leaves tend to retain higher levels of certain oxygenated monoterpenoids (e.g., citronellol, linalool), likely due to cooler temperatures and reduced metabolic degradation. Conversely, afternoon-harvested leaves exhibit elevated concentrations of hydrocarbon monoterpenoids (e.g., 3-carene, γ -terpinene) and sesquiterpenoids (e.g., β -elemene, α -sinensal), possibly reflecting increased enzyme activity triggered by heat and light exposure.

For instance, in the rainy season:

- Morning-harvested oils (RMF) were rich in α -fenchene, a compound associated with calming effects in mammals (Takahashi *et al.*, 2011).
- Afternoon-harvested oils (RAF) contained higher levels of 3-carene and citral, both known for their antimicrobial and insecticidal properties (Razzaghi-Abyaneh *et al.*, 2009; Kim *et al.*, 2003).

In the dry season:

- Morning-harvested oils (DSMF) had elevated γ -terpinene, contributing to their anxiolytic potential (Gomes *et al.*, 2010).
- Afternoon-harvested oils (DSAF) were richer in α -sinensal, a potent antifungal agent (Lee and Lee, 2010).

These observations suggest that the time of collection plays a critical role in determining the chemotype of the essential oil and, consequently, its biological activities.

3.3 Mechanism Behind Variations

Terpene synthases mediate the formation of essential oil constituents via carbocationic mechanisms initiated by divalent metal ion-dependent ionization of geranyl pyrophosphate (GPP) or farnesyl pyrophosphate (FPP) (Croteau, 1987). Differences in enzyme activity and substrate availability during the day result in varying product distributions. For example:

- α -Fenchene synthase was active in morning-harvested oils, producing α -fenchene and related derivatives.
- 3-Carene synthase dominated in afternoon-harvested oils, yielding higher levels of 3-carene and its derivatives.

Additionally, post-harvest metabolic processes may lead to the formation of new compounds during drying, further influencing the final phytochemical profile (Whish and Williams, 1996).

3.4 Insecticidal Activity

The insecticidal effectiveness of *Citrus sinensis* essential oils against *Callosobruchus maculatus* was evaluated by recording mortality rates at 6-hour intervals over 42 hours (Table 3.2). The mortality of *Callosobruchus maculatus* increased with exposure time, showing significant changes within the first 30 hours:

Table 3.2: Effect of exposure time on mortality rate

Exposure Time (Hours)	Mean Mortality (%)
6	51.25
12	66.25
18	76.46
24	80.42
30	83.33
36	85.21
42	86.67

Compounds such as limonene, citronellal, geraniol, and linalool have established insecticidal activities (Ngamo *et al.*, 2007; Louis and Badama, 2010). Their abundance in *Citrus sinensis* oils aligns with observed mortality rates.

Table 3.3: Mean mortality at 6 hours of exposure

Treatment	Mean Mortality (%) at 6 Hours of Exposure
DMF	15
DAF	0
RMF	15
RAF	90

Statistical analysis (Table 3.3) revealed that oils obtained during the rainy season were significantly more effective insecticides than those from the dry season which could be attributed to the higher monoterpenoid content of the oils. In the dry season, oils from the morning harvest (DMF) had a higher insecticidal activity than oils from the afternoon harvests (DAF). On the contrary, during the rainy season, the afternoon harvest (RAF) showed significant effectiveness as insecticides over the morning harvests (RMF). DMF showed moderate efficacy (15 %), likely due to the presence of γ -terpinene and β -caryophyllene, which exhibit slower modes of action. Regardless of the season, morning harvests had less mortality rates at 6 hours of exposure (15 %) while oils from the afternoon harvests were more potent insecticides (0 – 100 %) with the oils from fresh leaves harvested in the afternoon during the dry season (DSAF) being the outlier (0 %). This high mortality in RAF could be attributed to the high levels of citral and citronellal, which disrupt insect cuticle integrity and nervous systems (Kim *et al.*, 2003). The increased mortality in oils from fried leaves could be attributed to the release of glycosidically bound precursors during drying (Soysal and Oztekin, 2001).

4. Conclusion

The time of collection significantly impacts the chemical composition and insecticidal activity of *Citrus sinensis* leaf essential oils. Afternoon-harvested oils during the rainy season were the most effective against *Callosobruchus meculatus*, achieving rapid and high mortality rates. Conversely, morning-harvested oils during the dry season exhibited moderate efficacy, potentially due to differing terpene synthase activities and climatic conditions.

Optimizing harvesting practices based on desired chemotypes and applications is recommended. For pesticidal purposes, collecting leaves in the afternoon during the rainy season and allowing them to dry for two to four days appears ideal. Future research should focus on isolating and testing individual compounds for synergistic effects and identifying optimal extraction methods to preserve bioactivity.

5. Recommendations

To maximize the insecticidal potential of *Citrus sinensis* essential oils:

The leaves should be collected in the afternoon during the rainy season as the afternoon-harvested oils consistently showed higher insecticidal activity, likely due to increased concentrations of bioactive monoterpenes which are more potent in the afternoon oils. Additionally, rainy season oils generally contained higher monoterpenoid levels compared to dry season oils, enhancing their insecticidal properties.

Further investigate the most abundant compounds (e.g., limonene, citral, α -sinensal) for isolated insecticidal activity. Because these compounds were found in high concentrations in certain harvests, particularly in afternoon and rainy season oils.

Investigating their isolated effects could provide deeper insights into their individual contributions to insecticidal activity and help optimize the oil composition for enhanced effectiveness.

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