

EXTRACTION OF VOLATILE BIOACTIVE COMPOUNDS FROM HELIOTROPIUM DASYCARPUM AND ITS MITICIDAL ACTIVITY AGAINST TWO SPOTTED SPIDER MITES (TETRANYCHUS SSP)

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ARTICLE INFO | ABSTRACT

Received: 14/11/2024.

Accepted: 23 /11/2024.

Published: 26/11/2024.

Keywords:

Heliotropium Dasycarpum,
Tetranychidae Urticate
Adults, Nymphs,

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Citation:

Rukia Akbar, Dua Nizam,
Murad Nizam, Irshad,
Mohammad Fahim, Saqiba, &
Nizam ud Din. (2024).

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SPIDER MITES
(TETRANYCHUS SSP).

Pakistan's Multidisciplinary
Journal for Arts & Science,
5(3), 11–25.

<https://doi.org/10.5281/zenodo.14499209>

DOI:

<https://doi.org/10.5281/zenodo.14499209>

The study on Heliotropium dasycarpum (HD), a plant known for its biologically volatile bioactive constituents, highlighting its potential as a miticidal against two-spotted spider mite (Tetranychidae urticae). The research investigates the effectiveness of these biological volatile constituents in different concentrations, ranging from 1:10 to 1:100,000, applied to the adults, nymphs and eggs of the mite. Sprinkle the biological volatile bioactive compounds of Heliotropium dasycarpum showed significant success in controlling the two-spotted mite. The compounds exhibited notable activity at various time intervals (12, 24, 48, and 72 hours), with marked effects on nymphs and adults compared to the control group. The efficacy of the compounds was found to be concentration-and time-dependent. the study emphasizes the environmental and human health benefits of using these volatile bioactive compounds. unlike synthetic pesticides, which often have harmful side effects on the environment and human health, these compounds appear to offer a safer alternative.

INTRODUCTION.

Medicinal plants:

Every nation-state, regardless of its size or development, has its socio-economic, unique culture values, and standard, yet the use of herblore in medicine remains a shared tradition. Each year, countless plant genera are used in the pharmaceutical industry to create various drugs, as plants are profuse in both biological volatile and non-volatile bioactive compounds with a wide range of healing properties. Due to their production of bioactive constituents, plants are commonly utilized by practitioners for treating various ailments, such as relieving pain, expelling gas, flavouring food, and serving as pathogens, fungicides, pesticides, Killers, food preservations, and key constituents in the perfume industry. (Gavahian et al.,2011).

The Boraginaceae family is a large group with hundreds of genera and around 2,000 species, primarily distributed in hot and temperate regions, particularly the Mediterranean. Key genera include *Heliotropium*, *Cordia*, *Martensia*, *Trichodesma*, and *Arnebia*, *Heliotropium*, the largest genus, comprises 250–300 species globally. The name 'Heliotrope' derives from the Greek words 'helios' (sun) and 'tropein' (to turn), reflecting the plant's sun-oriented behavior. Medicinal plants in this family are widely used for treating various diseases. In the 19th century, over 25% of medicines in developed countries were plant-derived, thanks to advancements in pharmaceutical science (Selvi and Bigazzi, 2001; Sharma et al., 2009).

Heliotropium dasycarpum is abandon plant flourishing dry grasslands, wilderness, sand dense on bending soil and foothills. It can also grow on saline soils and cultivated lands. This herbaceous and perennial plant features a woody structure with a branching system. Its stem appears greenish, measuring approximately 40–50 cm in height. Leaves are 0.1–0.5 cm wide and 1–3 cm long. The plant produces medium-sized yellow flowers in March, characterized by hairy and acute petals, with a corolla longer than the calyx (Akhani and Sendtner, 1994).

Heliotropium dasycarpum ledeb is a traditional Balochi medicinal plant, locally referred to as Sagdaroo. It is widely used in Balochistan, Pakistan, for its therapeutic properties. The plant provides relief from inflammation, gout, menstrual disorders, rheumatism, skin ailments, and insect bites (Kumar et al., 2007). *Heliotropium strigosum* aids in alleviating common colds and serves as a diuretic and laxative (Modak et al., 2003).

Heliotropium dasycarpum ledeb, encompassing 1.92% mass fraction of bioactive compounds, has been integral to traditional medicine. These compounds include pyrrolizidine alkaloids, terpenoids, and flavonoids (Basri et al.). Leaf extracts of this plant have been evaluated for treating eye diseases in animals (Tareen et al., 2010). Its juice, when applied to wounds, prevents bleeding and infection spread (Khurram et al., 2016). Furthermore, the plant exhibits

diverse biological activities, including enzyme inhibition, cytotoxicity, pesticidal, and antifungal properties (Ghaffari et al., 2013; Ghaffari et al., 2016).

Pesticides:

Pesticides are substances or mixtures designed to resist, eliminate, control, or reduce pests. These substances help prevent injury during the manufacture, delivery, wrapping, transportation, and marketing of food, agricultural products, animal feed, and timber. Furthermore, insecticides include substances controlled to animals for the control of insects, arachnids, or other pests affecting them. Pesticides also incorporate substances used as plant growth regulators, desiccants, defoliants, or agents for thinning fruit and preventing premature fruit drop. Additionally, they are applied to crops before or after harvest to protect the products from weakening during wrapping and transportation (FAO, 2007).

Impacts of Synthetic pesticides:

Pesticide use indicates thoughtful environmental problems, with over 95% of herbicides and 98% of insecticides affecting unintended targets and contaminating soil, air, and water. Pesticide drift occurs when floating pesticide particles are carried by wind, dispersal contamination to other areas. Additionally, pesticides contribute to water pollution and, as persistent organic pollutants, they also contaminate the soil. Ideally, pesticides should be degradable or at least break down quickly in the environment. This loss of activity or toxicity is influenced by both the chemical properties of the substances and environmental conditions. The chemical structure of pesticides often slows their breakdown in aerobic environments. Soil properties may delay the degradation of pesticides but can also reduce their bioavailability to microbes (Sims and Cupples, 1999).

Extracts and essential oils from *Heliotropium dasycarpum*, which contain biological volatile aromatic and phenolic bioactive compounds, are gaining recognition for their potential as natural pest control agents. These compounds are effective against weeds, as well as drug-resistant bacteria and fungi that pollute and spoil stored food, damaging crops and leading to momentous economic losses in both quality and quantity. They also help prevent the spread of diseases, which can be particularly injurious to immunocompromised individuals. Commonly used pesticides, such as those based on organophosphates and carbamates, are limited in the USA under the Food Quality Protection Act of 1996 due to their harmful effects on the environment and human health (Isman, M. B. 2000).

MATERIAL AND METHODS



The author is currently conducting investigative research on the extraction of volatile bioactive compounds from *Heliotropium dasycarpum*. It also seeks to assess the efficacy of the extracted volatile bioactive compounds against the two-spotted spider mite.

Plant collection

The aerial parts and stem of *Heliotropium dasycarpum* were gathered from Behncha and Neechara in District Kalat. Freshly harvested *Heliotropium dasycarpum* samples were transported to the Chemistry Department, Organic Research Laboratory, and University of Balochistan. The samples were authenticated with the guidance of Dr. Shazia Saeed, Assistant Professor in the Botany Department at the University of Balochistan, and preserved as specimens in the university's Botanical Garden.



Figher 1: Collection of *Heliotropium Dasycarpum* from Kalat District

Sample Drying and Grinding:

The composed sample was shade-dried for 6 to 12 days, as shown in Figher 2. The dried plant branch was chopped with a prune cutter, and the aerial parts were ground into powder using an electric grinder.



Figher 2: Drying of *Heliotropium Dasycarpum* in shade at Organic Research Laboratory of UoB

Plant sample extraction of volatile bioactive constituents:

A total of 650g of crushed stem pieces and 350g of powdered aerial parts, including leaves of *Heliotropium dasycarpum*, were placed in a glass jar. The sample was soaked in 2.5 liters of absolute ethyl alcohol at approximately $\pm 25^{\circ}\text{C}$ for 15 to 20 days to extract the volatile bioactive constituents, such as alkaloids. After 20 days, the samples were filtered, and the filtrate was collected in a round-bottom flask. The extraction was repeated twice to maximize yield, and the resulting filtrates were stored in separate round-bottom flasks labeled E, F, and G.

Extract of concentration:

After 20 days, the samples were filtered, and the filtrate was collected in a round-bottom flask. The extraction was repeated twice to maximize yield, and the resulting filtrates were stored in separate round-bottom flasks labeled E, F, and G.

Extraction of bioactive volatiles.

The concentrated crude extracts (50-60) were left in a 0.2 M HCL solution for 12 hours to extract volatile bioactive compounds, such as alkaloids. Subsequently twenty four hours, the acidified extract was filtered. Then, two to three grams of motivated charcoal remained added to the remainder, and it was gradually boiled for 20 minutes to remove residual chlorophyll. The mixture was allowed to cool for 3 to 4 minutes before being filtrated again. The filtrate was basified with a 5 M NaOH solution, causing brownish precipitates of alkaloids to form. The solution was filtered, and the alkaloids were collected on filter paper. The alkaloids on the filter paper were dried, resulting in light brown-colored alkaloids. Round about 1.0 gram of alkaloids was weighed from each of the three round-bottom flask extracts. These alkaloids were then used to prepare solutions of varying concentrations, as described below.

Preparation of the obtained alkaloids imitates:

Preparation of 1:10 concentration of alkaloids obtained imitates

A 1:10 concentration sample was prepared by dissolving 1.0 gram of the obtained alkaloids in 10 mL of absolute ethyl alcohol in a 15 mL Falcon tube.

Preparation of 1:100 concentration of alkaloids obtained imitates:

A 1:100 concentration sample was prepared by pipetting 1 mL of the 1:10 sample into a Falcon tube containing 10 mL of absolute ethyl alcohol.



Preparation of 1:1000 concentration of alkaloids obtained imitates:

A 1:1000 concentration sample was prepared by transferring 1 mL of the 1:100 sample into a Falcon tube containing 10 mL of absolute ethyl alcohol.

Preparation of 1:10,000 concentration of alkaloids obtained imitates:

A 1:10,000 concentration sample was prepared by transferring 1 mL of the 1:1000 sample into a Falcon tube containing 10 mL of absolute ethyl alcohol.

Preparation of 1:100,000 concentration of alkaloids obtained imitates

A 1:100,000 concentration sample was prepared by transferring 1 mL of the 1:10,000 sample into a Falcon tube containing 10 mL of absolute ethyl alcohol.

Company and recognition of two-spotted spider mites from scattered plants:

Various plants organize different of two-spotted spider mites, counting eggs, adults, nymphs, were identified using a powerful enhance glass. At around 34°C in June round about 80 eggs, adults, nymphs were company using an aspirator from unscattered from plant in Mastung, zehri, Mungachar, and Kalat and plants leaves in the Botanical Garden of the University of Balochistan. The company mite eggs, nymphs, and adults were sign over into pre-marked petri dishes lined with damp filter paper and newly harvested plant leaves.

The petri dishes containing the mites were carefully sign over to the Organic investigation Laboratory in the Chemistry Department at the University of Baluchistan. the sample were divided into seven groups: one control group and six experimental groups, namely propagate, 1 :10, 1:100, 1:1000, 1:10,000, 1:100,000.each group consisted of 3 petri china, Marked R1, R2, R3, respectively.

Imitates of alkaloids sprinkle on experimental and control groups:

Control group:

This group acted as the impartial control, with no dose applied 1:10 to 1:100,000.

Results:

The biological volatile bioactive compounds of *Heliotropium dasycarpum* demonstrated significant miticidal effects on adults, eggs, and nymphs of two-spotted spider mites across various concentrations.



Egg Period of two-spotted spider Mites:

At 34°C, the control group showed a 94% egg hatching rate. In the propagating group, egg hatching was limited to just 5%. The replicate group at a 1:10 concentration exhibited only 4% egg hatching. The replicate group at a 1:100 concentration showed 6% egg hatching. The replicate group at a 1:1000 concentration exhibited 16% egg hatching. The replicate group at a 1:10,000 concentration displayed 26% egg hatching. The replicate group at a 1:100,000 concentration demonstrated 55% egg hatching.

Nymphs period of two-spotted spider Mites:

At approximately 34°C, 8% of the total eggs in the control group hatched. In the propagating group, all 100 nymphs remained originate deceased. In the 1:10 concentration imitate group, only 49% of nymphs remained detected. In the 1:100 concentration imitate group, 36% of nymphs remained detected. In the 1:1000 concentration imitate group, 28% of nymphs remained detected. In the 1:10,000 concentration imitate group, 20% of nymphs remained detected. In the 1:100,000 concentration imitate group, 9% of the nymphs remained originate deceased.

Adult stages of two-spotted spider Mites:

At approximately 34°C, the control group exhibited a 9% mortality rate in adult mites. In the propagating group, all adult mites (100%) were found dead. In the replicate group with a 1:10 concentration, only 61% of the adult mites were affected. In the replicate group with a 1:100 concentration, 54% of the adult mites were affected. In the replicate group with a 1:1000 concentration, 39% of the adult mites were affected. In the replicate group with a 1:10,000 concentration, 245% of the adult mites were affected. In the replicate group with a 1:100,000 concentration, 16% of the adult mites were found dead.

1:10 concentration imitate sample on Adult, Nymph and Egg and period of two-spotted spider Mites:

At round about 34°C, the control group showed a 93% egg hatching rate, with 5% mortality in nymphs and 9% mortality in adults. In contrast, the propagating group experienced only 3 % egg hatching, with 98% mortality in nymphs and 100% mortality in adults. After applying a 1:100 concentration, the egg hatching rate was reduced to 8%, with 54% mortality in nymphs and 60% mortality in adults.



1:100 concentration imitate sample on Adult, Nymph and Egg period of two-spotted spider Mites:

At 34°C, the control group showed a 93% egg hatching rate, with 5% mortality in nymphs and 9% mortality in adults. In the propagating group, only 4% of eggs hatched, while 98% mortality was observed in nymphs, and 100% mortality was recorded in adults. After applying a 1: 100 concentration, the egg hatching rate dropped to 10%, with 36% mortality in nymphs and 53% mortality in adults.

1:1000 concentration imitate sample on Adult, Nymph and Egg period of two-spotted spider Mites:

At 34°C, the control group had a 93% egg hatching rate, with 5% mortality in nymphs and 9% in adults. In the propagating group, only 4% of the eggs hatched, with 98% mortality in nymphs and 100% mortality in adults. After applying a 1:1000 concentration, egg hatching dead to 28%, while nymph mortality was 36% and adult mortality reached 19%.

1:10,000 concentration imitate sample on Adult, Nymph and Egg period of two-spotted spider Mites:

At 34°C, the control group showed a 93% egg hatching rate, with 5% mortality in nymphs and 9% in adults. In the propagating group, only 4% of eggs hatched, with 98% nymphs mortality and 100% mortality in adults. After applying a 1:10,000 concentration, egg hatching dead to 26% with 20% nymphs mortality and 24% adult mortality.

1:100,000 concentration imitate sample on Adult, Egg and nymphs stage of two-spotted Mites:

At round about 34°C, the control group showed a 93% egg hatching rate, with 5% mortality in nymphs and 9% in adults. In the propagating group, only 4% of the eggs hatched, with 98% mortality in nymphs and 100% in adult mites. After applying a 1:100,000 concentration, 56% of the mite eggs hatched, with 9% mortality in nymphs and 13% in adults.

DISCUSSION

Foodborne illness defined by the World Health Organization as “a disease of infectious or toxic nature, caused by, or thought to be caused by, the consumption of food and water”, is caused by a wide range of agents (Baird-Parker, 2016). Fish, being rich in nutrients and moisture, provides an ideal environment for microbial growth. The number of spoilage microorganisms increases significantly during improper transportation and storage conditions, which is especially problematic given the highly perishable nature of fish. Without proper handling and

storage, microbial contamination can rapidly escalate, leading to spoilage and potential health hazards (Dilmaçunal & Kuleaşan, 2018). To prevent spoilage and ensure the quality and safety of fish products, it's crucial to maintain optimal postharvest conditions, including adequate refrigeration, minimal handling, and strict hygienic practices (Nie et al., 2022).

Coliforms, an important group of the family Enterobacteriaceae are considered as bacterial indicators of sanitary quality of food. *Escherichia coli*, the most significant member of the Enterobacteriaceae family, is a universal resident of the human intestine and primarily a facultative anaerobe. Fecal coliforms are well known to contribute to fish spoilage and cause illness in both humans (Patel et al., 2014). Infections from coliforms are transmitted through contaminated fish, and coliform counts serve as indicators of potential fecal contamination, reflecting the hygiene standards maintained during food preparation. Improper processing, handling, and storage of fish can significantly increase contamination levels, thereby compromising fish quality and safety.

At the time of harvest, the microbial load of the fish normally varies from 10^2 to 10^5 cfu.g⁻¹ (ICMSF, 1986). In present study, total microbial load of about 40.0 – 83.33 % of fish samples collected from each zone showed total plate count in the range of 10^3 - 10^4 cfu.g⁻¹. However, the microbial load of any fish sample did not exceed the permissible limit of 5×10^5 cfu.g⁻¹. The admissible limit for total coliform, fecal coliform and *E. coli* is <100 MPN.g⁻¹, <10 MPN.g⁻¹, and <500 MPN.g⁻¹ respectively (ICMSF, 1986). Our results showed that total coliform count was higher than the permissible limit in 9.30% of the total fish samples.

The presence of these microorganisms reflects poor hygiene practices during post-harvest handling of fish. Fecal coliform was higher than permissible limit of >10 MPN.g⁻¹ in 53.45% of the samples. *E. coli* count did not exceed limit of 500 MPN.g⁻¹ in any of the samples. Higher microbial load of the fish samples collected from the traditional vendors in comparison to superstore indicate improved storage conditions and fish handling at the latter premises. Nevertheless, higher fecal coliform count of the fish sourced from superstore indicate that these market settings also need to improve hygiene practices in the fish supply chain.

Earlier studies on the microbial quality and safety of fish available in the commercial markets of Lahore, Pakistan, are meager. Nevertheless, studies in various regions have indicated the potential of food safety issues for the fish available in the commercial markets. Chatta et al., (Chatta et al., 2018) reported increase in microbial load of fish as it moved from primary to secondary retailers in the supply chain in Lahore and attributed it to poor hygienic conditions in the fish markets. The authors reported that aerobic plate count in samples of *Labeo rohita* and *Ctenopharyngodon idella* collected from retailer shops (2.25×10^6 cfu.g⁻¹ and

4.82×10^6 cfu.g⁻¹) were significantly higher than the samples collected from main fish market (1.22×10^6 cfu.g⁻¹ and 1.12×10^6 cfu.g⁻¹).

Sarkar et al. (2020) determined the bacterial contamination of Nile tilapia samples collected from super shops and local market of Dhaka, Bangladesh. The authors reported that all the fish samples were found to be contaminated with *E. Coli*, *Shigella spp.*, *Salmonella spp.* and *Vibrio spp.* Mitiku et al. (2023) determined the occurrence and microbial load pattern along the fish origin food value chain in upper Blue Nile watershed, Ethiopia. The authors reported an average total coliform count of 1.2×10^2 cfu.g⁻¹ and 5.10×10^4 cfu.g⁻¹ in cooked and raw fish samples, respectively. Alkuraieef et al. (2021) evaluated microbiological quality of fish sold in Saudi Arabian markets and found that microbial load was higher than permissible limit in some of the samples.

The findings of the present study have significant implications for public health and food safety in Lahore. The detection of elevated levels of total coliform, fecal coliform, and the presence of *E. coli* in fish samples from both local markets and superstores indicates the need for improved sanitation and hygiene practices throughout the fish supply chain.

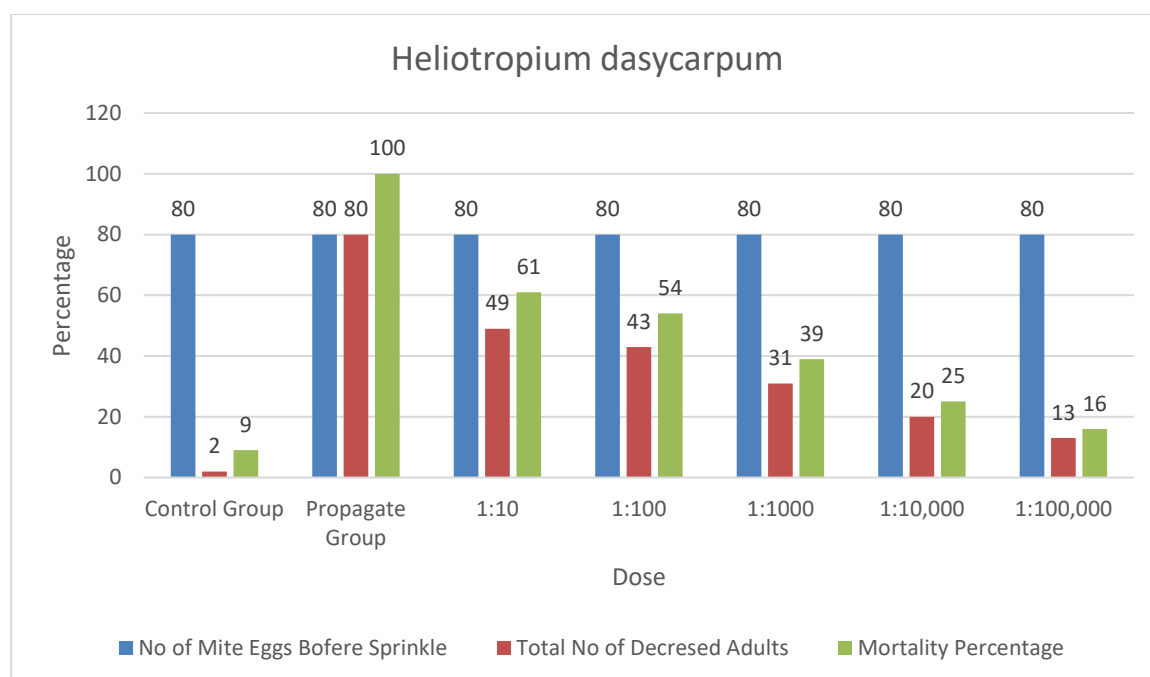
CONCLUSION

Studies have shown that the bioactive compounds in volatile oils are highly effective against all stages of two-spotted spider mites, including adults, eggs, and nymphs. Research highlighting that medicinal plants, containing bioactive components in their essential oils. Can serve as an alternative to synthetic pesticides. As a natural green pesticide, these oils effectively control a variety of pests and insects while being safe for both the environment and humans (Mossa, A. T. H., 2016). The essential oil of *Heliotropium dasycarpum* exhibited pesticidal activity against common plants pests, showing effective results at different concentrations against adults, eggs, and second larvae of *Tuta absoluta*. Antiseptic studies showed that the essential oil of *Heliotropium dasycarpum* was effectual against the eggs, larvae, and adults of *Callosobruchus maculatus*, with LC50 values demonstrated greater pesticidal effectiveness against the eggs (Rastegar et al., 2011). The study found that alkaloids extracted from *Heliotropium dasycarpum* displayed time- and dose-dependency miticidal and pesticidal activity against two-spotted mites. Within the first 12 hours, there was an increase in egg hatching and lower mortality rates in nymphs and adults. However, over the next 24, 48, and 72 hours, a consistent decline in egg hatching and a rise in mortality rates of both adults and nymphs were observed. The results suggests that the effectiveness of these volatile bioactive compounds is time-dependent. Regarding dose-dependency, the alkaloids concentration decreased as the samples were progressively dilution by adding 10 mL of absolute ethyl alcohol, creating replicated samples with concentration ranging from 1:10 to 1:100,000. The

pesticidal, miticidal effects of these samples gradually diminished from the 1:10 sample to the 1:100,000 sample over a period 12 to 72 hours.

Table 1: Biological Volatile Bioactive compounds of *Heliotropium Dasycarpum* tried on Adults of Two - spotted Spider Mite

Dose	No of Mite Adults Previously Sprinkle	Mortality Rate in hours				Total No of Deceased Adults	Mortality percentage
		12	24	48	72		
1:100,000	80	2	2	3	6	13	16%
1:10,000	80	2	3	8	7	20	25%
1:1000	80	4	7	10	11	31	39%
1:100	80	5	6	12	20	43	54%
1:10	80	7	9	12	21	49	61%
Propagate	80	42	28	6	4	80	100%



Figher 3: Biological volatile bioactive compounds of *Heliotropium Dasycarpum* tried on Adults of two Spotted Spider Mite.

Table 2: Volatile Bioactive compounds of *Heliotropium Dasycarpum* tried on Eggs of Two-spotted Spider Mite

Dose	No of Mite Eggs Before Sprinkle	Hatching Rate in hours				Total No of Deceased Nymphs	Mortality percentage
		12	24	48	72		
1:100,000	80	2	9	17	16	44	55%
1:10,000	80	1	4	9	8	22	26%
1:1000	80	1	2	7	4	14	16%
1:100	80	0	3	2	1	29	6%
1:10	80	0	1	2	2	5	4%
Propagate	80	1	1	1	1	4	5%

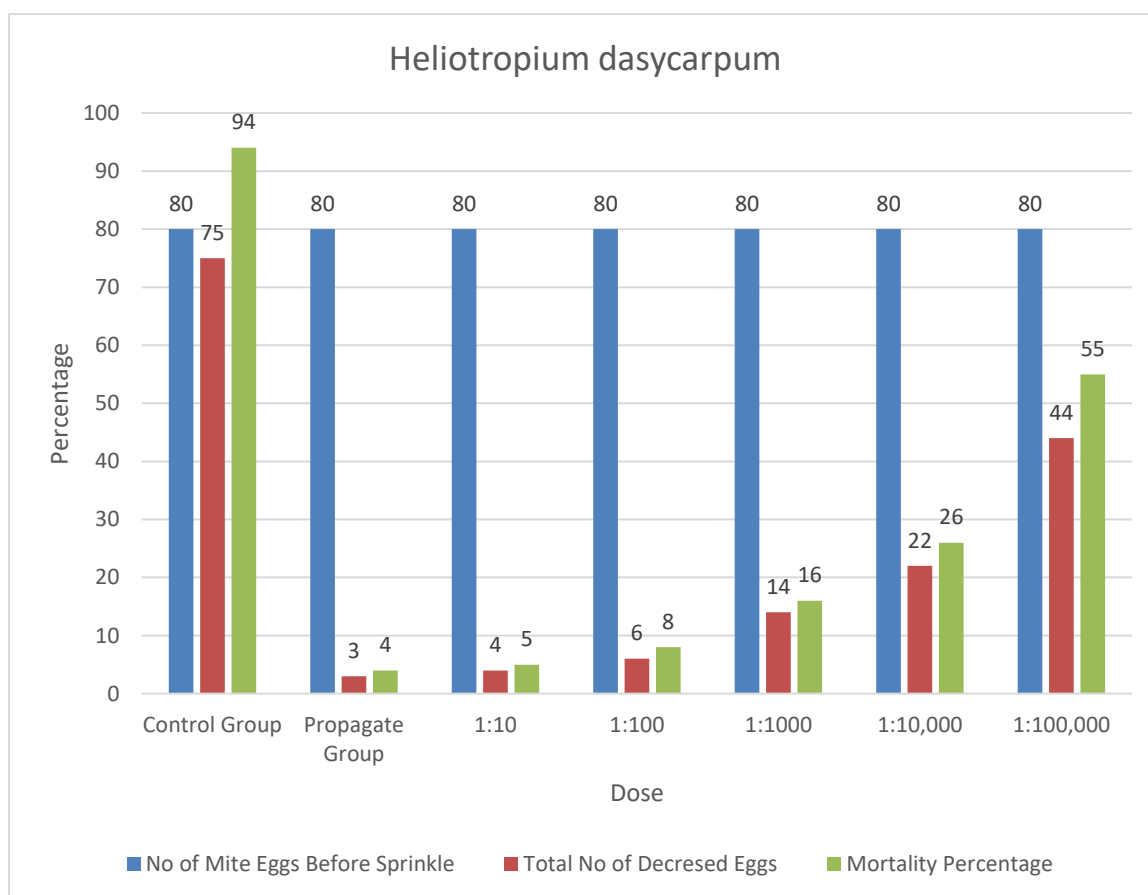
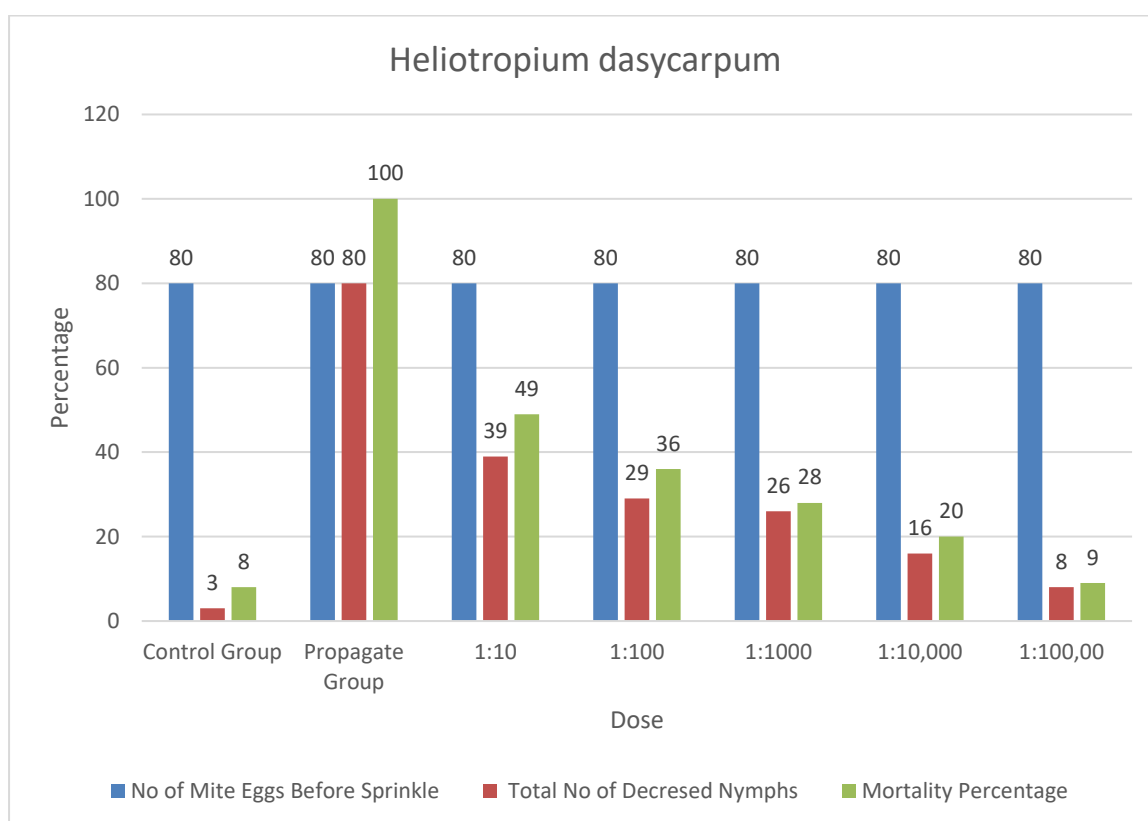


Figure 4: Volatile Bioactive compounds of *Heliotropium Dasycarpum* tried on Eggs of Two-Spotted Spider Mite

Table 3: Biological Volatile Bioactive compounds of *Heliotropium Dasycarpum* tried on Nymphs of Two-spotted Spider Mite

Dose	All of Mite Nymphs Previously Sprinkle	Mortality Rate in hours				Whole No of Deceased Nymphs	Mortality Percentage
		12	24	48	72		
1:100,000	80	0	2	2	3	8	9%
1:10,000	80	2	2	5	7	16	20%
1:1000	80	4	4	6	8	26	28%
1:100	80	3	4	10	12	29	36%
1:10	80	3	7	12	17	39	49%
Propagate	80	44	26	8	2	80	100%
Control Group	80	0	1	1	1	3	8%



Figher 5: Volatile Bioactive compounds of *Heliotropium Dasycarpum* tried on Nymphs of Two-Spotted Spider Mite

CONCLUSION



Notable pesticidal and miticidal effects remained detected from sprinkling biological volatile bioactive compounds extracted from both the uplifted and underground parts. The stem *Heliotropium dasycarpum* contains unique primary and secondary metabolites, including alkaloids, which work for effectual miticides and pesticides against numerous mite and pest species. Although present in small quantities by weight, the volatile bioactive constituents demonstrate significant miticidal activity, effectively targeting the adults, eggs, and nymphs, of two spotted spider mite over time. The miticidal activity of these compounds from *Heliotropium dasycarpum* is both time-and dose-dependency. Over time, their pesticidal and miticidal effects intensify, with higher concentrations proving to be the most effectual. The most effectual pesticidal and miticidal activity of these volatile bioactive compounds is detected at a 1:10 dilution, targeting adults, eggs, and nymphs of two-spotted spider mites. As the dilution increase starting 1:10 to 1:100,000, the miticidal and pesticidal effects against all three stages of the mites decrease.

RECOMMENDATION

Although these biological volatile bioactive compounds are present in small quantities by weight, their volatile nature enhances their effectiveness. Their pesticidal and miticidal activity produces significant and highly effectual results over time against the adults, eggs, and nymphs of two-spotted spider mites. As plant-based and environmentally friendly substances, these biological volatile bioactive compounds can be considered a meaningful substitute synthetic pesticides miticides, which are injurious to human healthiness and the environment.

CONFLICT OF INTEREST

Authors declare no conflict of interest for this study.

FUNDING INFORMATION

This study did not receive funding from any source.

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