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Fumigation activity of lemon grass oil against some medicinal and aromatic plants pests and susceptibility index of fennel seeds in two Egyptian governorates

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Abstract
The objective of this research was to control the presence of Drugstore beetle (*Stegobium paniceum*), Cigarette beetle (*Laisoderma serricorne*), and Lesser grain borer (*Rhizoperth dominica*) in adults. The aim was to investigate how infestation by these insects affects the rate of weight loss and susceptibility index in fennel seeds. Furthermore, the study aimed to identify the volatile organic compounds (VOCs) that are produced when fennel seeds are infested with S. paniceum insects and/or treated with lemongrass (Cymbopogon citratus (DC.). Fumigation with essential oils was considered as a potential and effective method for managing insects due to its proven insecticidal properties and minimal harm to the environment.

Keywords: Fumigation activity, lemon grass oil, *Stegobium paniceum, Laisoderma, serricorne, Rhizoperth dominica*

Introduction

Plant essential oils (EOs) are recognized globally as a valuable natural source of pesticides. The worldwide EO market is estimated to be worth around $700 million, and the overall production makes up approximately 45,000 tons [1]. According to predictions, the botanicals industry is expected to undergo significant growth, with the potential to rise from 1-2% to approximately 7% of the total market share by 2025 [2]. Soon before harvest, it is possible to use essential oil materials on food crops without leaving excessive residues. Essential oils (EOs) are made up of naturally occurring compounds that provide flavor and fragrance, including monoterpenes, sesquiterpenes, and aliphatic compounds, which give them their unique scents. Terpenes, in particular, have gained attention for their potential as agents for insect control. Numerous studies have illustrated that these compounds derived from plants, both monoterpenes and sesquiterpenes, have toxicity against various insects. As a result, there has been significant interest in investigating EOs and their constituents as alternatives for insect control, replacing chemical-based options that might pose greater harm to humans and the environment [3-5]. Their ability to fumigate insects is a crucial attribute of essential oils regarding pest management. This implies that they can be utilized effectively in controlling pests in storage due to their extensive efficacy against insects, minimal harm to mammals, rapid degradation in the environment, and easy local availability [6]. In addition, essential oils have the ability to function as insecticides derived from natural sources, and they have a quick effectiveness in combating pests. They can achieve this not only by coming into direct contact with the pests but also through fumigation. This characteristic has been applied in safeguarding stored items from being infested by pests [7]. Essential oils (EOs) have the potential to be utilized in Integrated Pest Management (IPM) programs for pests found in stored grains due to their strong volatility and fumigant properties. Therefore, the aim of this study was to evaluate the efficacy of Lemongrass essential oils as a fumigant in controlling drugstore beetles (*Stegobium paniceum* L.), cigarette beetles (*L. serricornein*), and lesser grain borers (*Rhyzopertha dominica*) in stored fennel seeds. Fennel (*Foeniculum vulgare*) is a commonly used herb for culinary purposes and as a medicinal plant that contains various nutrients including carbohydrates, proteins, as well as essential minerals such as calcium, iron, zinc, and phosphorus. The cigarette beetle (L. serricorne) is a significant pest that infests a wide range of stored commodities including tobacco, cigarettes, turmeric, ginger, castor beans, wheat, coconut meal, pepper, cardamom, mustard, chilli, fennel, cumin, and opium leaves [9-11]. The *Stegobium paniceum* (L.), also known as the drugstore beetle, poses a significant issue in warehouses where medicinal and aromatic plants are kept, resulting in considerable damages and losses [12]. *Rhyzopertha dominica* (F.), commonly referred to as the lesser grain borer, is a significant worldwide nuisance in regards to stored wheat [13]. Early detection and diagnosis of infestations of insects in stored-grain is of utmost importance to ensure successful implementation of pest control measures and safeguard the stored grains. To prevent insect damage in stored food grains, one viable and economical method is the utilization of plant-based products as grain protectants. By employing gas chromatography-mass spectrometry (GC-MS), the volatile compounds existing in stored grains can be effectively isolated and identified, an approach that has been extensively utilized [14]. In order to ensure the safety of grain products in the future, insect monitoring in stored grain can be achieved by employing headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS). This technique allows for the identification of biomarker compounds. [15] Over the last few years, researchers have been using a combination of headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) to examine volatile compounds found in grain-dwelling insects [16]. [17] Conducted research which showed that the quality attributes of plant-based food could be affected by the presence of insects, leading to alterations in their chemical composition. Moreover, to identify ongoing spoilage caused by insects, SPME-GCMS has been utilized to detect specific volatile organic compounds (VOCs) that insects release for communication with their own species. [18]. The objective of this research was to analyze different samples to determine if there were specific compounds exclusive to healthy fennel seeds, infested fennel seeds, and the contaminating insects. This analysis would help to determine if HS-SPME-GCMS could be employed to detect infestations of particular insects in fennel seeds and potentially evaluate the quality of stored products. As part of this investigation, the toxicity of C. citratus was examined to assess its lethal and sublethal concentrations, along with the biological and biochemical effects of lemongrass essential oil on specific insects found on fennel seeds in a controlled laboratory environment. This could potentially offer a safe alternative to chemical insecticides for integrated pest management programs. Previous studies have shown promising outcomes using natural plant products as protective measures for grains [20, 21]. It is crucial to detect and diagnose infestations of stored-grain insects early in order to implement effective pest control measures and protect the stored grains. One environmentally friendly and cost-effective approach to prevent insect damage in stored food grains is to use plant products as grain protectants. Gas chromatography-mass spectrometry (GC–MS) has been widely employed to isolate and identify the volatile compounds present in stored grains [14]. By utilizing headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC–MS), the biomarker compounds that have been identified can be used for insect monitoring in stored grain, ensuring the safety of our grain products in the future [15]. In the past few years, the combination of headspace solid phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS) has been utilized to analyze volatile compounds from stored product insects in grain [16]. A study conducted by [17] demonstrated that the presence of insects could impact the quality characteristics of plant-based food and result in changes to their chemical composition. Additionally, SPME–GCMS has been employed to identify continuing spoilage through the detection of distinct volatile organic compounds (VOCs) that insects produce for communication within their species [18]. The aim of this study was to examine various samples in order to determine if there were specific compounds that were unique to healthy fennel seeds, infested fennel seeds, and the contaminating insects. This would indicate whether HS-SPME-GCMS could be utilized to identify infestations of certain insects in fennel seeds and potentially assess the quality of stored products. As part of this investigation, the lethal and sublethal concentrations of *C. citratus* were studied to assess its toxicity, as well as the biological and biochemical activities of lemongrass essential oil on certain insects on fennel seeds in a controlled laboratory setting. This could potentially serve as a safe alternative to chemical insecticides for integrated pest control programs. Some studies have shown positive results using natural plant products as protectants for grains [20, 21].

Material and methods

Insect rearing and seeds infestation Plant essential oils are well recognized as a valuable natural resource for pesticides. The global market for these oils is worth approximately $700 million, with a production of about 45,000 tons. It is expected that the botanicals sector will experience significant growth, potentially capturing around 7% of the overall market share by 2025. Essential oil materials can be used on food crops shortly before harvest without leaving excessive residues. These oils contain natural compounds that give them their distinct smells, such as monoterpenes, sesquiterpenes, and aliphatic compounds. Terpenes, in particular, have gained attention for their potential as insect control agents. Multiple studies have shown that both monoterpenes and sesquiterpenes derived from plants have toxicity against various insects. As a result, there is a growing interest in exploring essential oils and their constituents as alternatives to chemical-based insect control options that may be more harmful to humans and the environment. One important characteristic of essential oils is their ability to fumigate insects, making them effective in controlling pests in storage. They have a wide range of efficacy against insects, low toxicity to mammals, rapid degradation in the environment, and local availability. Essential oils act as natural insecticides, working not only through direct contact but also through fumigation. This property has been utilized in protecting stored products against pests. Due to their high volatility and fumigant activity, essential oils have the potential to be used in Integrated Pest Management programs for pests in stored grains. Therefore, this study aimed to evaluate the effectiveness of Lemongrass essential oils as a fumigant in controlling drugstore beetles, cigarette beetles, and lesser grain borers in stored fennel seeds. Fennel, a commonly used culinary herb and medicinal plant, contains various nutrients such as carbohydrates, proteins, and essential minerals like calcium, iron, zinc, and phosphorus. The cigarette beetle is a significant pest that infests a range of stored commodities, including tobacco, turmeric, ginger, castor beans, wheat, and more. The drugstore beetle is a problem in warehouses where medicinal and aromatic plants are stored, resulting in substantial losses. The lesser grain borer, also known as *Rhyzopertha dominica*, is a major global pest of stored wheat. [13] The fennel seeds underwent sterilization at a temperature of 60±5◦C for 8 hours to remove any visible or hidden presence of insects and mites. Following this, the seeds were conditioned in an environmental chamber maintained at a temperature of 30±2 °C and a relative humidity of 75% for a minimum of one week to increase their moisture content. To establish a population of the tested insect, mature adults of *Stegobium paniceum* (L.), *Laisoderma serricorne*, and *Rhyzopertha dominica* (F.) were collected. A uniform group of adult insects of a similar age was obtained by introducing around 100 adults into jars containing seeds for egg-laying, which were then placed in an incubator at a temperature of 30 ± 2 °C and a relative humidity of 65 ± 5%. After three days, all insects were removed from the medium, and the jars were returned to controlled conditions until the emergence of adults [22]. The insect cultivation occurred at the Stored Products and Grains Pests laboratory within the Department of Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.

Bioassay

C. citratus grass essential oil was utilized to treat infestations of adult *Stegobium paniceum* (L.), *Laisoderma serricorne, and Rhyzopertha dominica* (F.) on fennel seeds. Five different concentrations of the essential oil were used (0.01, 0.02, 0.03, 0.04, and 0.05 ml/10 g fennel seeds). Each concentration was replicated three times, with each replication having 20 adults. A control treatment was also conducted without the essential oil. Plastic cups with a capacity of 120 ml were employed, with 20 fresh adult insects placed in each cup. The various concentrations of oils were applied to filter paper, which was then positioned in the cup cover and firmly sealed for fumigation. The cups were maintained at a temperature of 28 ± 2 °C and a relative humidity of 65 ± 5%. After 2 days of treatment, the contents of each cup were sifted through a 2 mm sieve to separate and eliminate the deceased insects from the fennel seeds. The quantities of living and deceased adults were documented at the end of each inspection interval, and the mortality percentages were subsequently calculated. Following each inspection, the contents of each cup were diligently returned to the cup. The cups were covered with muslin cloth and secured with rubber bands, and left under the same conditions. After 30 days, the cups were examined again to record the number of offspring that had hatched.

Susceptibility index (SI)

The study was performed on two distinct sets of fennel seeds collected from Menia and Gharbia governorate, revealing infestation by *Stegobium paniceum* insects.The susceptibility indices were calculated by following the procedure specified in references 23 and 24, which relies on the duration of the immature stages.To calculate the susceptibility index (SI), the formula Log s/T *100 was utilized. In this formula, S represents the percentage of adult emergence (F1), and T represents the developmental period in days.

Weight loss %

Weight loss percentages were assessed for two sets of preserved fennel seeds collected from the Menia and Gharbia governorates, which were both affected by *Stegobium paniceum* pests. The weight loss percentage was calculated using the procedure outlined in [25], which necessitates subtracting the initial dry weight from the final dry weight and dividing the outcome by the initial dry weight. The result is then multiplied by 100. This can be represented by the formula: Weight loss percentage = (treated weight - untreated weight) / untreated weight * 100.

GC/ MS analysis

In order to determine the composition of various samples, such as fennel seed, lemon grass oil, *S.paniceum* adult insects, and a blend of fennel seed and *S.paniceum* adult insects, different analyses were conducted using gas chromatography-mass spectrometry (GC-MS). The samples for the assay consisted of 10g of fennel seeds, 1g of insect adults, and 1g of lemon grass oil. The GC-MS system used in the analysis was located at the Central Laboratories Network, National Research Centre in Cairo, Egypt, and consisted of an Agilent Technologies gas chromatograph (7890B), mass spectrometer detector (5977A), and headspace sampler (7697A). The temperature for the headspace was set at 90 °C for 20 minutes, with the loop and transfer line temperatures at 100 °C and 110 °C, respectively. Hydrogen was used as the carrier gas, flowing at a rate of 3 ml/min. The GC was equipped with a DB-624 column, measuring 30 m in length, 0.32 mm in internal diameter, and with a film thickness of 1.8 µm. The analysis followed a temperature program that started at 40 °C for 1 minute, followed by a temperature increase of 7 °C/min until reaching 250 °C, where it was maintained for 5 minutes. Both the injector and detector temperatures were set at 250 °C. Mass spectra were obtained through electron ionization at 70 eV, using a spectral range of m/z 30-550, and a solvent delay of 3 minutes. The identification of the different constituents was accomplished by comparing the fragmentation patterns of the spectrum to those stored in the Wiley and NIST Mass Spectral Library data.

Statistical analysis

The LC50 values for lemon grass oil against the chosen stored grain pests were estimated using the Ld-p line program. The analysis was conducted using Graph pad.

Results and Discussion

In Table (1), the mortality percentages of *S. paniceum*, *L. serricorne*, and *R. dominica* adults were displayed when they were exposed to varying concentrations (0.01, 0.02, 0.03, 0.04, and 0.05 ml/10 g fennel seeds) of essential oil as fumigants. When the concentration used was 0.01 ml/10 g fennel seeds, the mortality rates for *S. paniceum*, *L. serricorne*, and *R. dominica* adults were 33.3%, 25%, and 26.66%, respectively. The reduction rates were 0.0%, 52.54%, and 54.22% for each insect, and the number of F1 progeny was 4.0, 10.33, and 10.66, respectively. Likewise, at a concentration of 0.05 ml/10 g fennel seeds, the mortality rates for *S. paniceum*, *L. serricorne*, and *R. dominica* adults were 100%, 100%, and 96.66% respectively. The reduction rates were 100%, 13.53%, and 8.44% respectively, and the number of F1 progeny was 0.0, 1.66, and 2.66 respectively. These results were obtained when grass oil was tested on these three insects associated with stored medicinal and aromatic plants.

Table 1: The mortality percentages of *S. paniceum*, *L. serricorne*, and *R. dominica*

Insect	***S.paniceum***			***L.serricorne***			***R.dominica***		
Conc. (ml/10 g seeds) in 120 ml.	**Mortality %**	**No. of F_1 progeny**	**Reduction %**	**Mortality %**	**No. of F_1 progeny**	**Reduction %**	**Mortality %**	**No. of F_1 progeny**	**Reduction %**
0.01	33.3	4	0	25	10.33	52.54	26.66	10.66	54.22
0.02	59.65	2	50	36.66	8.66	44.04	30	9.66	49.12
0.03	65	0.5	87.5	57	5.33	27.11	53.66	6.33	32.19
0.04	100	0	100	83.33	4	20.34	75	4.33	22.02
0.05	100	0	100	100	2.66	13.53	96.66	1.66	8.44
Control	0	4	-	1.60	19.66	-	1.60	19.66	-

20 insect/10 g fennel seeds

Tables (2) presented the probit statistics, estimate of LC50, LC90, and the slope of regression lines for the tested oils after fumigation. The results of the probit analysis indicated that lemongrass oil was the most toxic oil, with LC50 values against *S. paniceum* of 0.017, 0.013, and 0.020. This was followed by the most toxic oil against *L. serricorne*, with LC50 values of 0.023, 0.020, and 0.026. Similarly, *R. dominica* had LC50 values of 0.026, 0.020, and 0.033.

Table 2: Toxicity values (g/ml) and slope of regression line lemongrass oil fumigant against *S. paniceum*, *L. serricorne* and *R. dominica* adults

Parameter	*Stegobium paniceum*	*Laisoderma serricorne*	*Rhizoperth dominica*
LC_{25} (95% Confidence limit)	0.006 (0.003-0.009)	0.011 (0.009 – 0.014)	0.015 (0.006-0.021)
LC_{50} (95% Confidence limit)	0.017 (0.013-0.020)	0.023 (0.020- 0.026)	0.026 (0.020-0.033)
LC_{95} (95% Confidence limit)	0.14 (0.07-0.64)	0.122 (0.091 – 0.190)	0.053 (0.044-0.072)
Slope	1.76 ±0.37	2.28±0.23	3.22±0.36

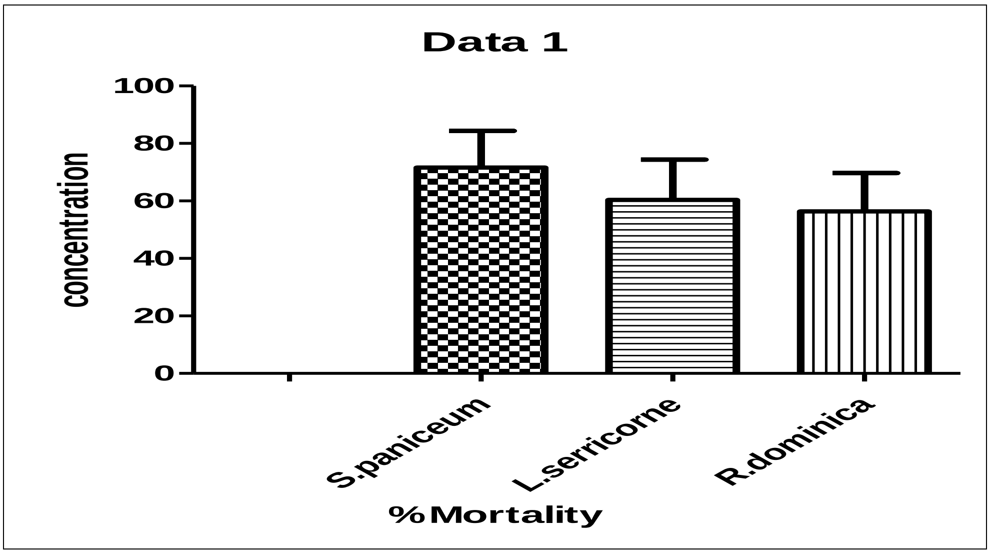

Fig 1: The % mortality for fumigation by Lemon grass oil against *S. paniceum*, *L. serricorne* and *R. dominica* adults.

The data presented in table (3) displays the susceptibility index and percentage of weight loss for fennel seeds infested with the *S. paniceum* insect in two different regions. In the Menia governorate, the duration of progeny was 70 days, while in the Gharbia governorate, it was 46 days. The infestation susceptibility in the Gharbia governorate was considerably higher than in the Menia governorate. Additionally, the weight loss in the Menia governorate was significantly greater than in the Gharbia governorate.

Table 3: Susceptibility index and weight losses % of fennel seeds from two governorates infested with *S.paniceum* insect

	Duration of progney (day)	No. of F_1 progeny	Susceptibility index	Weight loss %
Menia governorate	70	3.66	0.57	1.58
Gharbia governorate	46	8	1.8	1.33

20 insects /25 g fennel seeds.

The information presented in Fig (2) indicates that the retention time (RT) for peak 8 was 11.892 for fenchone, the RT for peak 10 was 12.777 for trans limonene oxide, and the RT for peak 14 was 14.783 for Fenchyl acetate.

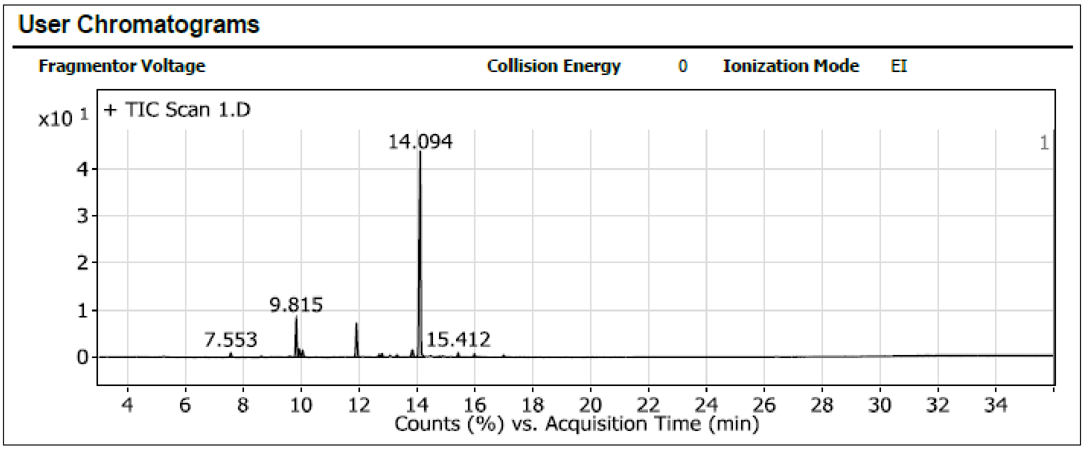

Fig 2: Analysis by GC mass on Fennel seed only

The information presented in Fig. (3) indicates that the retention time (RT) for peak 5 was recorded as 10.046, whereas for peak 8 it was 14.053 (Estragole), and for peak 10 it was 15.982 (Eciral).

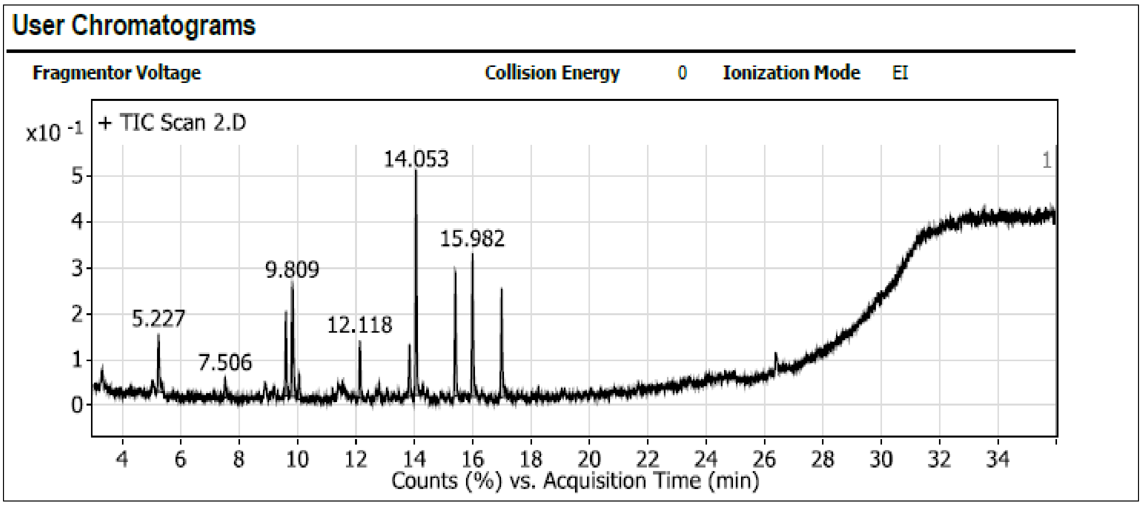

Fig 3: Analysis by GC mass on adult insects of *s. paniceum* only

According to the information presented in Fig. (4), the retention time (RT) for peak 8 was 10.492 for delta-3-carene, the RT for peak 10 was 12.688 for cis-limonene oxide, and the RT for peak 14 was 14.1 for Estragol.

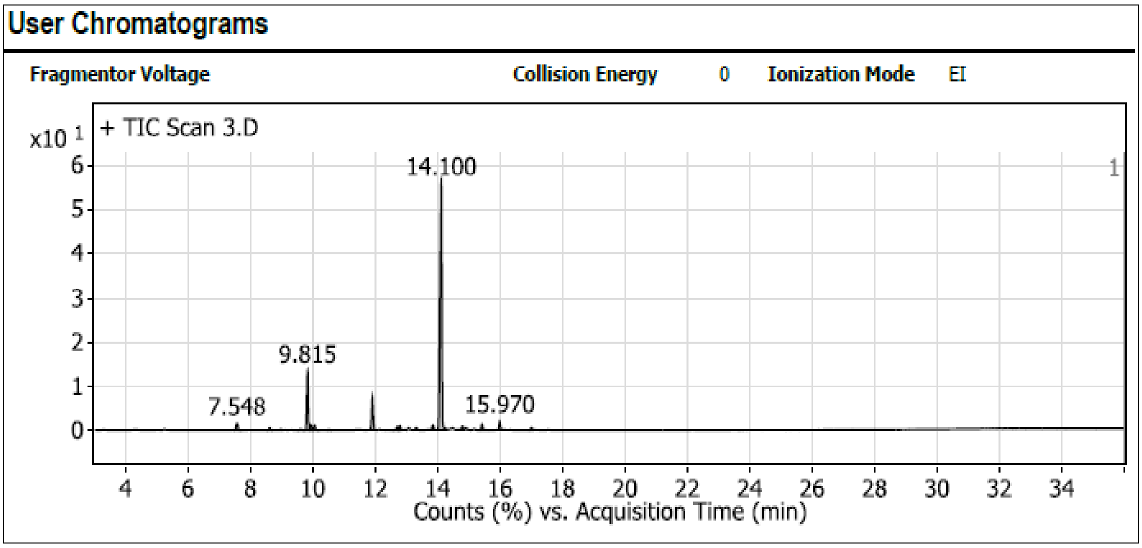

Fig 4: Analysis by GC mass on Fennel seeds infested with *S. paniceum*

The information depicted in Fig. (5) indicates that the retention time (RT) for peak 8 was 10.492 for delta-3-carene, the RT for peak 10 was 12.682 for cis-limonene oxide, and the RT for peak 14 was 14.95 for Estragol.

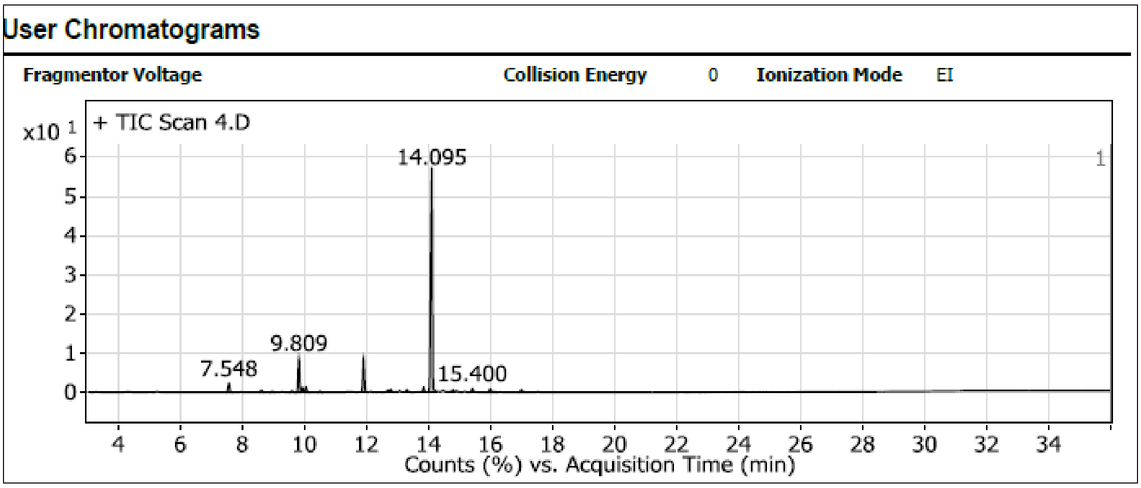

Fig 5: Analysis by GC mass on Fennel seeds infested with *s. paniceum* and fumigated by 0.05 conc. lemon grass oil.

According to the data presented in Fig. (6), peak 8 had a retention time (RT) of 10.486 for delta-3-carene, peak 10 had an RT of 12.694 for cis-limonene oxide, and peak 14 had an RT of 14.088 for Estragol.

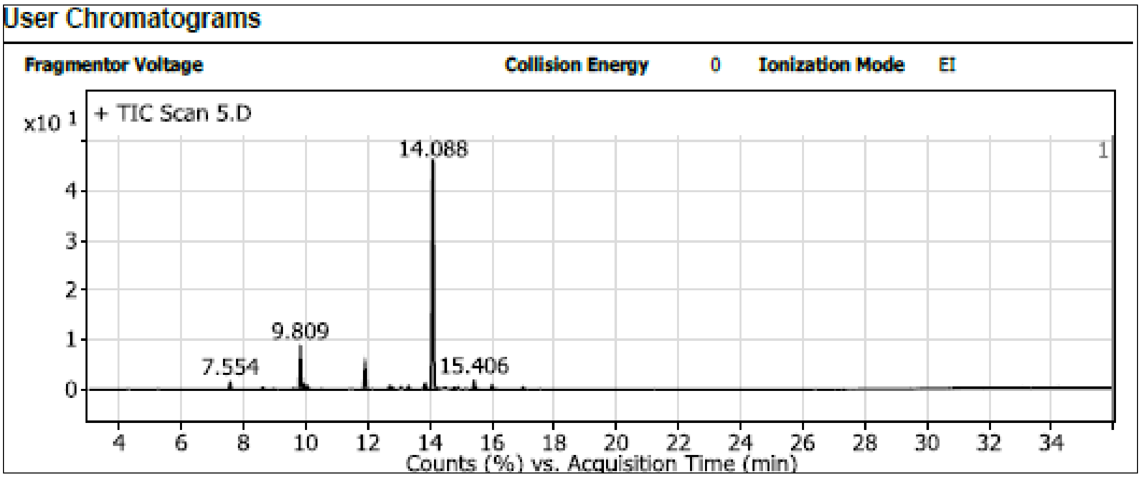

Fig 6: Analysis by GC mass on Fennel seeds fumigated by 0.05% conc. lemon grass oil.

The information presented in Fig. (7) indicates that the retention time (RT) for peak 9 was 10.504 and this peak corresponded to delta-3-carene. Similarly, peak 10 had a retention time of 10.997 and corresponded to 1,6-octadine. Lastly, peak 16 had a retention time of 13.229 and was associated with citronellal.

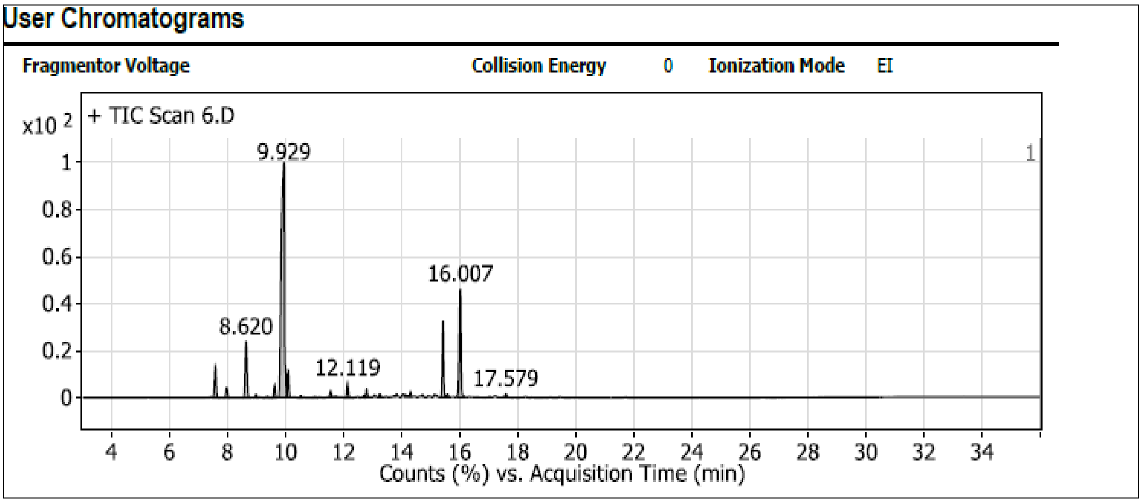

Fig 7: Analysis by GC mass on lemon grass oil only.

Discussion

In this study, the identification of certain compounds like Trans-Limonene oxide, Levomenthol, and Fenchyl acetate was achieved using GC-MS solely in Fennel seeds. The compounds in infested Fennel seeds were found to be distinct compared to non-infested ones. Furthermore, unique compounds including CIS-LIMONENE OXIDE, Camphor, and Estragole were detected in Fennel seeds infested with *stegobium paniceum*. The Fig. (4) displays essential compounds present in fennel infested with *S. Paniceum*, such as CIS-LIMONENE OXIDE and Sesh Estragole. Similarly, Fig. (5) sshows the presence of compounds like CIS-LIMONENE OXIDE and Estragole in Fennel seeds infested with *S. paniceum* and treated with 0.05 conc. lemon grass fumigation. Hence, the use of GC-MS can differentiate between fennel seeds, *S. paniceum* adults, and lemon grass oil by detecting compounds like Estragole and SIS- LEMONENE OXIDE. These two compounds have the potential to determine the infestation of *S. paniceum* in fennel seeds.

Previous studies that used filter paper as a traditional method, as described by [26], found that plant oils showed fumigant toxicity against insects that infest stored grains. [27] In their research, it was discovered that the fumigant derived from Mentha spicata essential oil had the highest toxicity, resulting in a mortality rate of 97.76% for *S. paniceum*. Following treatment, Lemongrass oil had significant effects on mortality, developmental duration, and the expression level of CAT and lipid peroxidase after 96 hours [28]. On the other hand, the treated larvae showed a decrease in activity of detoxification enzymes compared to the control group. [29-33] observed that lemon peel essential oil (LPEO) had varying effects on the biological parameters of Agrotis ipsilon. The tested concentrations of (LPEO) and (LPEO-NPs) significantly increased the duration of the larvae compared to the control group. At high concentrations (75 mg/mL), both the essential oil and nano essential oil resulted in mortality rates of 86.67+8.82% and 93.33+3.33%, respectively. Several studies [34-37] have demonstrated the effectiveness of native and exotic lemon grass essential oils in causing mortality in maize weevils.

The findings from [38-41] support the idea that plant essential oils can affect behavior, have fumigant activity, and can interact with *L. serricorne* adults. In particular, the essential oil showed the most effective fumigation activity against *L. serricorne* adults. Lastly, [42] used essential oils from Melicope pteleifolia to combat insects that infest stored products and found that the oils were highly effective against *Lasioderma serricorne* and *Lasioderma bostrychophila*.

Conclusion

Lemon peel essential oil (LPEO) had varying impacts on different types of insect pests like *S. paniceum*, *L. serricorne*, and *R. dominica* adults during fumigation. Therefore, it is suggested for farmers to employ LPEO in order to control these insect pests in storage places. The strong insecticidal characteristics, cost-effectiveness, and simple preparation method of LPEO make it a promising option as a biological insecticide product.

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