Repellency and Phytoxicity Assessment of Plant Extracts, Essential Oils and Nanoparticles on *Tribolium castaneum* Herbst Beetles Infesting Wheat Grains (*Triticum estivum* L.)

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ABSTRACT

Plant extracts and nanoparticles were tested for Tribolium castaneum Herbst Beetle repellency and wheat grain phytotoxicity. Mugwort plant extract had 100% repellency after 3 hours for 10%, 15%, and 20% concentrations, 85% after 2 hours at 20%, and 82% at 15%. The lowest significant repellency values for Mugwort (Artemisia annua) plant extract and EOs after 1 hour were 42% and 54% at 5% concentration. After 3 hours, plant extract and essential oil at 10%, 15%, and 20% concentrations repelled black seeds (Nigella sativa) 100%. Black seeds plant extract had 93% repellency at 20% after 2 hours and 85% at 5% after 3 hours, compared to 50% for EOs. The lowest significant repellency values for plant extract and EOs after 1 hour were 50% and 52% at 5% concentration. SiNPs and AgNPs have similar repellency values across exposure times. SiNPs and AgNPs had 40% repellency at 100 ppm after 1 hour compared to the control. After 1 hour of exposure, SiNPs had 2% repellency and AgNPs 4% at 100 ppm. Phytotoxicity tests showed that wheat grains did not affect seed germination characteristics or biological measurements. In treated wheat grains, most germination and biological indices increased. This shows that these treatments did not kill the wheat embryo. These findings suggest that these treatments could protect grains, particularly wheat, for human consumption, animal feed, or agricultural cultivation.

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1. Introduction

Grains play a critical role in global agriculture and serve as essential sources of carbohydrates and minerals for human consumption, making them crucial for ensuring food security. After the harvest, grain crops are vulnerable to insect-related harm, leading to a reduction in quantity and quality [1]–[4]. In Iraq, the exact magnitude of grain loss during storage remains uncertain; nevertheless, the FAO estimated this loss to fall within 10 to 20%. Wheat holds significant importance as a staple food in numerous nations, including Iraq. According to the Central Statistical Organization (CSO), wheat cultivation in Iraq surpassed a particular threshold during the 2019/2020 season [5], [6]. Unfortunately, due to drought and insufficient water supply affecting grain growth, the Iraqi Ministry of Agriculture agricultural activity in irrigated regions by 50% in 2022, leading to a notable decline in wheat output. Projections for the 2022/23 period anticipate wheat production to reach a mere 3.25 million metric tons, signifying a substantial decrease from previous years (USDA, 2023). Several studies concentrate on elevating the crop quality by decreasing the damages caused by pests and reduced microorganisms during storage and distribution [6–8]. Grains, particularly wheat and its derivatives, remain susceptible to deterioration and losses during storage due to a range of factors. Notably, pests like flour beetles are a notable source of impairment within storage facilities, contributing to an estimated annual grain loss of roughly 36 million tons (FAO) [4] identified the economic detriments stemming from flour beetles, encompassing weight reduction, alterations in color, pathogen transmission, and contamination from chemical residues [6]. Coleopterans are the most prominent insects that are commonly infesting grains and their stored products; more than 600 species in order Coleoptera have been identified as stored products pests [11-12]. Among these, *Tribolium castaneum* Herbst (Red flour beetle) is considered as one of most broad cosmopolitan pests, posing considerable economic harm wide range of food items [9]. The feeding activities of *T. castaneum* adults and larvae can lead to substantial damage to stored products, both directly through consumption and indirectly through the deposition of eggs and the secretion of toxic compounds with highly volatilized secretions “benzoquinones1’ [3]. Furthermore, the ability of the adults as strong fliers caused an easy distribution among the storage facilities and between field and storage [2-4] [13-14]. Despite there is potential for utilizing plant-based natural repellents, including powders, essential oils, and extracts, as substitutes for synthetic compounds. The primary approach remains limited to creating unfavorable conditions for insect development, such as temperature and

humidity control [15]. Alternative solutions derived from natural sources are being considered to protect stored food products from insect infestations. Recent reports suggest that using plant extracts as natural insecticides to control stored grain insects provides several advantages compared to chemical compounds. These advantages include enhanced biodegradability, reduced human toxicity, and increased insecticidal effectiveness while maintaining the integrity of the storage environment [3].The phytotoxicity of essential oils (EOs) to the wheat grains has been extensively examined. Recent years have witnessed extensive exploration of the phytotoxic attributes of essential oils (EOs) concerning wheat grains. Some EOs have demonstrated phytotoxic impacts on wheat grains, while others have exhibited negligible effects. A recent inquiry delved into the phytotoxicity of lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), and peppermint (*Mentha piperita*) EOs on wheat grains. The study of [10] identified mild phytotoxicity in lavender and rosemary EOs, while peppermint EO yielded no such effects. Similarly, another investigation assessed oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris* L.) EOs for their phytotoxic impact on wheat grains. Outcomes indicated phytotoxic effects of both EOs, with thyme EO demonstrating greater efficacy compared to oregano EO (Papachristos et al., 2020). Likewise, a study examined thyme (*Thymus vulgaris*) and savory (*Satureja thymbra* L.) EOs in terms of their phytotoxic influence on wheat grains, revealing phytotoxicity in both cases, with thyme EO being more potent [11]. Conversely, another research endeavor scrutinized lemon (*Citrus limon* (L.) Osbeck) and eucalyptus (*Eucalyptus globulus* Labill.) EOs for their phytotoxic effects on wheat grains. The findings demonstrated the absence of phytotoxic impacts from both EOs [12]. Overall, the phytotoxicity stemming from EOs in relation to wheat grains hinges on the specific EO type and its concentration. Further exploration is warranted to comprehend the intricacies of EOs' phytotoxic effects on wheat grains and their potential utility in safeguarding crops [8]. The phytotoxic attributes of nanoparticles (NPs) predominantly hinge on their dimensions, structure, and surface composition ([13]. In general, smaller NPs tend to manifest heightened toxicity owing to their expanded active surface area, which promotes greater interaction with the adjacent environment [14]. Additionally, their reduced size facilitates easier infiltration into the inner recesses of plant cells, potentially causing harm. It's worth noting that the pores of plant cell walls are typically quite narrow, with a diameter spanning approximately 3 to 20 nanometers, thereby permitting passage exclusively for select particles [15]. Nonetheless, research has indicated that larger NPs possess the capability to widen the pores within the cell wall, thereby facilitating their entry into the interior of the cell [16]. These disturbances

can impinge on critical cellular processes including photosynthesis, respiration, and nutrient absorption [17]. Phytotoxic nanoparticles hold the potential to disrupt plant growth and development, leading to inhibited seed germination, hampered root elongation, stunted shoot growth, and diminished biomass accumulation. The extent of these effects can be contingent upon factors such as the nanoparticle's type, size, concentration, and surface characteristics [18,19].A comprehensive understanding of the key features of NPs is crucial for interpreting their interactions with organisms. Unfortunately, some studies investigating the toxicity of specific nanoparticles (NPs) on various organisms, including plants, frequently neglect to provide precise information about the characteristics of these NPs. Additionally, the morphology and structure of the plant itself play a pivotal role in evaluating the toxicity of NPs. Presently, a notable gap exists in terms of comprehensive studies that delve into how plants, being globally significant autotrophic entities positioned at the forefront of trophic chains, react to NPs. Considering the ongoing and dynamic release of NPs into the environment, the assessment of risks stemming from the presence of nanomaterials assumes paramount importance. This is especially pertinent for newly synthesized products with commercial potential [20, 21]. The present study has been performed to evaluate the phytotoxic effect of some plant extracts; essential oils and nanoparticles on the viability and chemical compositions of wheat grains.

2. Materials and Methods

2.1 Test Insect

The red flour beetles *T. castaneum* were obtained from the Entomology laboratory of Plant Protection Department, College of Agriculture, University of Basrah, Iraq. The colony was maintained in (1000 ml) sterilized glass jars filled with wheat grains and covered with gauze and held on with rubber bands [22] at 28℃ ± 2 and 65 ± 5% relative humidity in a growth chamber (Binder, Germany) [23].

2.2 Plant Extracts Preparation

The powders of plant material of Mugworts, *Artemisia annua* (Asterales: Asteraceae), and the seeds of the Black seeds, *Nigella sativa* (Ranunculales: Ranunculaceae) were obtained using a grinding machine. The resulting dried powder (50 g) was soaked in 500 mL of hot distilled water and stirred magnetically for 2 hours. Subsequently filtered using sterilized medical gauze, followed by three rounds of filtration using 9 cm Whatman Nº 1 filter papers. The filtrate was centrifuged

at 3000 rpm for 10 minutes, and the clarified solution was transferred to another centrifuge tube for sterilization using a syringe filter (45 µL). The solvent was evaporated using a rotary evaporator at 60 ºC under reduced pressure, resulting in the crude water extract. Stock solutions were prepared by dissolving the crude extract in distilled water to obtain different concentrations of each plant extract. The selected concentrations (5, 10, 15, 20% v/v) were determined based on preliminary tests. The crude plant extracts were stored at 4 ºC. To formulate the solutions, the food-grade emulsifier TWEEN® 20 (97%) from Sigma-Aldrich, Steinheim, Germany, was used [24-26].

2.3 Essential Oil Isolation

The essential oils (EOs) derived from the plant materials of Mugworts and Black seeds were acquired using the hydro-distillation technique employing a Clevenger apparatus, following the procedure outlined by Evergetis et al., (2016). The resultant extract was concentrated in a hot air oven set at 40 ℃. The obtained yield of all the EOs was stored at a temperature of 4 ºC for subsequent utilization, as per [28].

2.4 Nanoparticles preparations and Characterization

Silicon nanoparticles (SiNPs) and silver nanoparticles (AgNPs) were procured from Prof. Dr. Mazin Auny Mahdi at the nanotechnology Lab, Department of Physics, College of Science, University of Basrah, Iraq. These nanoparticles had a particle size of less than 100 nm and were synthesized following the method elucidated by [29]. For the preparation of nanoparticles via pulsed laser ablation (PLA), a second harmonic pulsed Nd:YAG laser (with a wavelength of 532 nm, power ranging from 130 to 140 mJ/pulse, and pulse width of about 5 ns) was focused using a single glass lens onto a Silicon/Silver substrate (with an inner diameter of 10 mm). The resultant solution containing the free nanoparticles was examined using the laser beam and stored at 4℃ until further use [29].

2.5 Repellent Bioassay (Filter Paper Disc Bioassay)

A bioassay method was employed to assess the efficacy of the investigated botanical extracts and essential oils (EOs) using the area preference technique [33]. Each Petri dish was coated with Whatman filter paper No.1. The Whatman filter paper disc was split into two halves; one of these halves was sprayed with 0.5 mL of acetonic solutions containing the studied plant extracts and EOs, while the other half (control) received an equal amount of acetone. The examined plant

extract and EOs were tested in five duplicates for concentrations of 5, 10, 15, and 20% (v/v), relative to the control treatment, on the second half of the filter paper. After approximately 60 minutes of solvent evaporation, the treated and control filter papers were combined and affixed to the base of the Petri dish using double-sided adhesive tape. Around 20 sexually indistinct adult insects aged between 3 and 7 days were introduced at the center of both halves, followed by immediate placement of a second Petri dish to prevent insect escape. The experiment was conducted under dark conditions at 28 ±2 ℃ and 65 ±5 %. Repellency percentage was documented at intervals of 1, 2, 3, 4, and 5 hours, based on the count of insects present on both treated and untreated halves. The repellency percentage was computed using the subsequent formula:

$$PR = [(Nc - Nt) /Nc] \times 100 \quad .....1$$

where Nc = the number of insects on the untreated half, and Nt = the number of insects on the treated half, after the time exposure.

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

he chemical composition of the essential oils extracted from Mugwort and Black seeds was analyzed using a GC-MS system (Agilent HP-5973) with a Shimadzu QP-500 Mass Spectrometer, following the method outlined by Abbasipour et al. (2011). The essential oil samples were diluted using acetone in a 1:25 ratio, and 1µl of the sample was utilized for the GC-MS analysis. A fused silica column coated with polydimethyl siloxane, measuring 30m in length, 25nm in diameter, and with a film thickness of 0.25 µm, was employed. Helium gas served as the carrier gas, flowing at a rate of 1 ml/min. The injector temperature was set at 250° C, while the oven temperature was initially set at 60° C for 1 minute, subsequently increasing gradually at a rate of 2° C/min until it reached 225° C. Following the steady rise in column temperature, it was maintained at 225°C for 5 minutes. The ionization voltage of 70eV with a 1:25 split rate was employed. Components were deemed major constituents if they comprised more than 1% of the oil. All percentages of essential oil constituents are expressed as % (v/v). The identification of the essential oil's composition was accomplished by comparing retention times, as described by [30]

2.7 HPLC analysis

A standard curve was generated using commercial standards sourced from Sigma (St. Louis, Missouri, USA) for compounds like daidzein, genistein, kaempferol, myricetin, and quercetin. To

assess the crude plant extract obtained from Mugworts and Black seeds, a modified High-Performance Liquid Chromatography (HPLC) technique was employed, following the procedure established by Selin et al. (2017). The aromatic water was directly introduced into the system which encompassed a diode array detector (DAD) functioning at 278nm, an autosampler (SIL – 10AD vp), a vacuum degasser (DGU-14A), system control (SCL-10Avp), and a pump (LC-10ADvp). Compound separation was achieved using an Agilent Eclipse XDB-C18 column (250x4.60 mm, 5 µm) maintained at a column temperature of 30°C. The mobile phase utilized was a mixture of 3% acetic acid and methanol (50:50) flowing at a rate of 0.8 units. Each sample and standard were injected in 20µL volumes. The analysis spanned 30 minutes, and detection was set at a wavelength of 280 nm to identify flavonoids like quercetin, kaempferol, myricetin, and others. This specific wavelength is commonly employed as it corresponds to the absorption peak of numerous flavonoids [31]. To ensure accuracy, the injection was replicated twice, and data collection involved using the average of two injections from each extraction, as outlined by [32].

2.8 The germination indices of treated wheat Seeds

Wheat seeds were combined with varying concentrations of six different substances: Mugworts plant extract, Mugwort essential oil, Blackseeds plant extract, Blackseeds essential oil, silver nanoparticles, and Silicon nanoparticles. The concentrations used were 5, 10, 15, and 20% v/v. In each case, three separate lots of ten treated seeds were sown, alongside a control group. The seeds were placed in 9 cm diameter Petri dishes, with No.1 Whatman filter paper moistened with distilled water. The growth experiment took place within a growth chamber set at 25±1° C. To ensure the necessary humidity for germination, the plates were watered daily using sterilized water. Data recording commenced once the root length of the germinated seeds reached 3 mm, signifying successful germination [23] as following:

1. Germination percentage (GP) = (n /N) x 100 Where n is the number of germinated seeds and N is the number of Sown seeds in each experimental unit .
2. Germination Initial Time (GIT): The number of days at first germinated seed .
3. Maximum germination time (MGT): Number of days until the highest count of germinated seeds .
4. Germination Duration time (GDT): The interval from GIT to MGT.
5. Mean daily Germination (MDG) = GP/MGT [23].

6. Mean Germination time (MGT): the count days until reached to 50% of germinated seeds. MGT = (Σni x di)/Σni, where n is the number of grains that germinated on the day (i); di is the number of days counted from the beginning of germination [23].
7. Speed of Germination rate (SP): The average of seed numbers germinated in one day. SP = Σ(ni/di) [23].
8. Germination Coefficient (GC) = (N/(Σni x di))x100 [23]
9. Germination value (GV): This combines both SP and GP. GV= PVxMDG, where PV is the Peak value, (PV = highest seed germinated/Number of days).
10. Germination Vigor Index (GVI) = N x SDW (mg), where N is the total germinated seeds and SDW is the shoot dry weight of the seedling (mg) [23].
11. The shoot and root length [23]

2.9 The biological impact of plant extracts, EOs, and nanomaterials treatments on the chemical compositions of Wheat grains

Wheat grains exposed to plant extracts, EOs, and nanomaterials, were obtained from the experiment after 24 hours. The ground wheat of the treated and untreated wheat grains samples (for comparison) was sent to the laboratories of the Ministry of Science and Technology/Environment and Water Department/Pollution Treatment Center for the estimation of Carbohydrate, amino acids, and fatty acids and estimation of gluten protein as follows:

2.9.1 Screening for Sugars

To initiate the test, 1 mL of Essential Oils (EOs) extracted from the chosen plants was combined with 1 mL of Benedict's reagent. This mixture was then subjected to a 2-minute heating session in a boiling water bath. A green-hued solution indicated the presence of reduced sugar. For the subsequent step, both treated and untreated grain powders, each weighing 0.1 g, were mixed with 4% NaOH and subsequently boiled in a water bath for a duration of 2 hours. Following the cooling phase, the resultant mixture was subjected to centrifugation at 3000 rpm for 10 minutes. A volume of 0.5 ml of the resultant solution was then blended with an equal volume of 5% phenol reagent and 2.5 ml of concentrated H_2SO_4. This mixture was allowed to stand at room temperature for 30 minutes. Subsequently, the optical density of the resultant solution was gauged at a wavelength of 490 nm using a Spectronic 21D instrument (Milton Roy Co. USA). The extension coefficient was

ascertained as a percentage of the total weight, employing glucose as a standard for reference, following the methodology described by Dubois et al. (2006).

2.9.2 Screening for Amino Acid

The procedure for digesting and extracting amino acids followed the method adopted by an American company. It commenced with taking 0.2 grams of finely ground wheat sample, to which 12 mL of Agilent acid was added. The resulting mixture underwent filtration through a 0.8-nanometer filter paper, after which it was subjected to an oven at a temperature of 110° C for a period of 24 hours. Subsequent to this incubation, the mixture underwent two rounds of washing with deionized water and was subsequently transferred to a rotary evaporator. Deionized water was introduced, and post-drying the sample, an additional 10 mL of deionized water was added, followed by further drying in the rotary evaporator at a temperature of 50° C. Then, 3.5 mL of HCl (0.02 M) was added, and the acid was neutralized by adding a base. The sample was then injected into an Agilent AAA (amino acid analysis) instrument after adding the reagent (Ortho-Phthalaldehyde reagent) according to the method provided by Agilent (Woodward *et al*., 2007). The analysis was conducted under the following conditions:

- Mobile phase buffer: Solution
- Carrier gas flow rate: 2 mL/minute
- UV detector wavelength: 340 nanometers to 450 nanometers

2.9.3 Screening for Fatty acids

For the extraction of fatty acids, a process was employed as follows: A volume of 0.2 grams of the sample was taken, and to this, 2 mL of CH_3OH (methanol) and 10 mL of KOH (potassium hydroxide) solution were added. The KOH solution was prepared by dissolving 2.244 grams of potassium hydroxide in water. This mixture was then subjected to a water bath shaker set at a temperature of 50°C for a duration of 30 minutes, followed by allowing it to cool down to room temperature. Subsequently, 1 mL of deionized water was introduced into the mixture, after which it was transferred into a centrifuge tube. The tube was then centrifuged at a speed of 10,000 rpm for 10 minutes. Following this, upon separation, the organic layer was extracted and filtered using a microfiber filter (David *et al*., 2009). The extracted methyl esters were analyzed using Gas Chromatography (GC) under the following conditions:

- Separation column: 10 DN
- Mobile phase: $N_2 + H_2 + O_2$
- Carrier gas flow rate: 1 mL/minute
- Temperature: 150° C (heating rate of 1.5°C/minute)
- Detector wavelength: 220 nm
- Pressure: 4 psi

The concentration of the fatty acid in the sample was determined using the formula:

The concentration of fatty acid in the sample = (Area of sample / Area of standard solution) x Concentration of standard fatty acid. …… 2

The concentration of the standard fatty acid was 0.5 parts per million.

2.9.4 Screening for Gluten

The determination of Wet Gluten Protein was carried out following the established standard method as outlined by the American Association of Cereal Chemists (AACC-2000) 39-12A. To begin, grain samples were subjected to grinding using a laboratory electric mill model 7880 from a company of German origin. For each treatment, a weight of 10 grams of flour was measured, and 6 mL of distilled water was introduced to the flour sample. This mixture was meticulously blended to achieve a consistent and uniform dough. Then, the dough was placed on the Perten Glutomatic® sieve, specifically designed for the Glutomatic 2000 instrument, for gluten separation. It was washed with distilled water for 12 minutes, ensuring a rate of 2-3 drops per second. The sample was then transferred to a centrifuge device (Centrifuge 2010) after stopping the flow of water, and the weight of the wet gluten was directly measured [23]. The percentage of wet gluten was calculated using the following equation:

% Wet Gluten = (Weight of Wet Gluten / Weight of Flour Sample) × 100 …… 3

2.10 Data Analysis

The acquired experimental data were subjected to univariate analysis utilizing SPSS software [33]. Statistical analysis was performed to ascertain significant differences. To compare these differences, a two-way analysis of variance (ANOVA) was carried out. Further examination of the

means was undertaken through the utilization of Tukey's test to estimate and pinpoint differences between them.

3. Results and discussion

3.1 Repellence effect of plant extract, essential oils and nanoparticals against *T. castaneum* adult stage

The repellent effect of the plant extract and essential oils of both Mugworts and Black seeds in addition to SiNPs and AgNPs at different concentrations with different exposure period intervals (1, 2 and 3 h) against *T. castaneum* adults was studied. The results of Table (1) illustrated that these plant materials and NPs had a remarkable repellent activity toward this insect. Generally, the repellency percentage in *T. castaneum* adults increased with increasing the concentrations and the exposure period s of the two examined plant extracts and EOs. Accordingly, all the examined EOs were more efficient as repellent than the plant extracts for the same plant species. Regarding, Mugworts EOs results in Table (1), the highest significant repellency of *T. castaneum* was recorded as 100% for both 15 and 20% concentrations after 1 and 2 h compared to control. However, the repellency percentage reached 100% for 10, 15 and 20% concentrations after 3 h compared to control. Hence, Mugworts plant extract results in Table (1), the highest significant repellency of *T. castaneum* was recorded as 100% after 3 h. Thus, these repellency percentages followed by Mugworts plant extract caused 85% at concentration 20% after 2h of exposure period while, the Mugworts EOs caused 82% repellency at concentration 15% after the same exposure period. However, the lowest significant repellency values were 42 and 54% at concentration 5% after 1h of exposure period for Mugworts plant extract and EOs, respectively. For Black seeds (*N. sativa*) EOs, data represented in Table (1), the highest significant repellency of *T. castaneum* was recorded as 100% for both black seeds plant extract and essential oil at concentration 10, 15 and 20% after 3h of exposure period compared to control. However, the repellency percentage of black seeds plant extract reached 93% at concentration 20% after 2h of exposure period compared to control. Hence, black seeds plant extract, reached 85% at concentration 5% after 3h of exposure period compared to EOs recorded 50% at the same exposure period. However, the lowest significant repellency values were 50 and 52% at concentration 5% after 1h of exposure period for both plant extract and EOs, respectively. In general, there was no significant difference between the repellency values and the exposure period of both Silicon and silver nanoparticles. The repellency impact of SiNPs and AgNPs on *T. castaneum* adults presented in Table (1), revealed

that the highest significant repellency of both was recorded as 40% concentrations 100 ppm after 1h compared to control. However, the lowest significant repellency values were 2 and 4% at concentration 100 ppm after 1h of exposure period for SiNPs and AgNPs, respectively. The current results of potential repellency of examined plant materials are in accordance with the findings and explanations of [34] when they pointed out the effectiveness of essential oils are largely depending on the volatile constituents. However, Lee *et al*. (2004) found that 1,8-cineole exhibits fumigant toxicity against *S. oryzae*. Similarly, [35] reported that 1,8-cineole demonstrates both repellent and toxic effects against *T. confusum*. Previous studies have shown eugenol which detected in GCMS analysis to be an effective pesticidal agent against various pests (Pohlit *et al*., 2011a, b). Specifically, Monoterpenes have been observed to act as neurotoxicants against different insect species to inhibit both GABA receptors [36] and acetylcholinesterase (AChE) [37, 38]. Therefore, it is possible that the tested materials exert their insecticidal activity through one or more of these modes of action. These results partially agree with [38] who mentioned that the larvicidal activity of seven mugwort species essential oils showed significant effectiveness against *Anopheles sinensis* larvae compared to the control group; no dead larvae were observed. Additionally, all seven oils demonstrated significant repellent activities against adults' females when compared to the negative control. Four of the oils (CQ, HN, SD, and GS) exhibited repellency levels similar to DEET, a common mosquito repellent, with the highest repellency observed from the GS oil [38].

Table 1. The repellent effect of Mugworts EOs, Mugworts PE, Black seeds EOs, Black seeds PE, SiNPs, and AgNPs at different concentrations with different exposure period intervals on *T. castaneum* adults.

Treatments	Repelled individuals (%)											
	1h of Exposure period				2h of Exposure period				3h of Exposure period			
	Concentrations											
	5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%
Mugworts PE	42 ± 1.2	60 ± 1.3	60 ± 1.3	65 ± 0.90	50 ± 0.00	44 ± 1.6	66 ± 1.3	85 ± 0.90	70 ± 0.89	80 ± 0.47	100 ± 0.0	100 ± 0.0
Mugworts EO	54 ± 0.75	60 ± 1.4	79 ± 0.75	100 ± 0.0	54 ± 0.75	60 ± 1.4	82 ± 0.75	100 ± 0.0	75 ± 0.82	100 ± 0.0	100 ± 0.0	100 ± 0.0
Black seeds PE	50 ± 0.00	57 ± 1.1	60 ± 0.89	63 ± 1.10	56 ± 0.49	63 ± 1.1	70 ± 0.89	82 ± 0.75	80 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Black seeds EO	52 ± 1.2	60 ± 1.6	62 ± 0.00	65 ± 0.90	50 ± 0.47	54 ± 1.6	80 ± 0.00	93 ± 0.90	85 ± 0.47	100 ± 0.0	100 ± 0.0	100 ± 0.0

Treatments	Repelled individuals (%)											
	1h of Exposure period				2h of Exposure period				3h of Exposure period			
	Concentrations											
	5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%
Nano-materials	100 ppm	200 ppm	300 ppm	400 ppm	100 ppm	200 ppm	300 ppm	400 ppm	100 ppm	200 ppm	300 ppm	400 ppm
SiNPs	4 ± 0.49	15 ± 1.1	20 ± 0.00	40 ± 1.10	5 ± 0.47	10 ± 1.1	15 ± 0.89	40 ± 1.10	5 ± 0.47	10 ± 1.1	15 ± 0.89	40 ± 1.10
AgNPs	2 ± 0.49	10 ± 1.1	15 ± 0.89	30 ± 1.3	3 ± 0.49	5 ± 1.1	15 ± 0.89	40 ± 1.10	3 ± 0.49	5 ± 1.1	15 ± 0.89	40 ± 1.10

Significant differences between different treatments at $p < 0.05$; LSD = 0. 0.157.

3.2 Preliminary Phytochemical Screening Analysis of Mugworts and Black seeds EOs

The qualitative analysis of the Mugworts and Black seeds essential oils utilized in this study involved the employment of GC-MS and HPLC techniques. Both analyses revealed the presence of numerous bioactive compounds, which are potentially involved in exerting insecticidal effects. In the chromatogram, the peak area (%) and retention time of the chemical constituents in the analyzed oils were determined. Notably, many essential oils contain insecticidal compounds classified as monoterpenoids. Specific examples of these compounds encompass camphor, camphene, 1.8-cineol, α-pinene, linalool, methyl acetate, limonene, menthone, geraniol, citral, citronellal, thymol, carvacrol, eugenol, myristic acid, and trans-anethol. These pesticide compounds derived from plant essential oils have been extensively investigated for their potential as control agents and are recognized for their toxicity towards insects [39].

3.2.1 Black seeds (Nigella sativa) EO

Chemical analysis of *N. sativa* essential oils (EOs) was conducted using GC-MS analysis, and the results were illustrated in Fig.1. The essential oils were found to contain numerous biologically significant compounds, which are listed in Table 2 and known for their insecticidal properties. Notably, the active compound identified in Black seeds was 9, 12 Octadecadienoic acid also known as linoleic acid. Additionally, the seeds contained linoleic acid ethyl ester, and methyl cinnamate as their primary active compounds. However, active compounds found in botanical EOs have some limitations, such as low bioavailability, high volatility, and susceptibility to photodegradation, which can restrict their application under specific circumstances. Based on the phytochemical analysis conducted in this study, the observed insecticidal activity of the studied essential oils

(EOs) derived from *Nigella sativa* could be attributed to the presence of specific compounds such as Linolelaidic acid, linalyl acetate, linalool, and other bioactive constituents. These findings are consistent with prior research, which has demonstrated potent insecticidal and repellent properties of essential oils from plants like *Cuminum cyminum* and *Lavandula angustifolia* against various stored-product insects. Notably, these effects have been associated with the presence of compounds like linalyl acetate and linoleic acid ethyl ester [40,41]. Moreover, the fumigant toxicity of 1,8-cineole and linalool was investigated in separate studies by [42] and [43], respectively. These studies highlighted the effects of these compounds against *Blattella germanica* (L.) and *Oryzaephilus surinamensis*. Additionally, the exposure to substances like linoleic acid ethyl ester and linalool was observed to result in the inhibition of acetylcholinesterase in both *Sitophilus oryzae* adults and *T. castaneum* larvae, as documented by Chaubey (2012b). The effectiveness of the investigated plant essential oils (EOs) can be attributed to the presence of highly toxic components, such as linoleic acid ethyl ester in *N. sativa*, which is consistent with prior research. These significant constituents found in essential oils demonstrate toxic and insecticidal properties, akin to other compounds like limonene, camphor, 1,8-cineole, and γ-terpinene, as documented by studies conducted by [34]. The observed mortality of the tested essential oils towards *S. oryzae* adult pests might be attributed to their neurotoxic effects, akin to findings from earlier investigations. Many medicinal volatile and aromatic wild plants are known to possess essential chemical constituents that serve as inhibitors for *S. oryzae* and various other insects, as established by studies by Pandey (2017), Owolabi et al. (2020) [44].

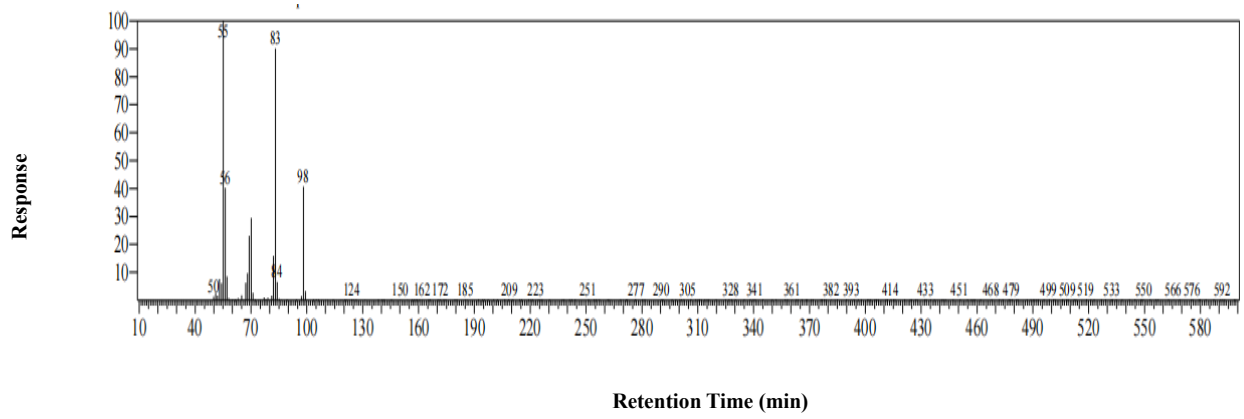

Figure 1. GC-MS chromatogram of Black seeds (*Nigella sativa*) EO.

Table 2: The GC-MS Chemical analysis of Black seeds (*Nigella sativa*) EO.

Peak	R.Time	Area	Area%	Name
1	3.06	832849	0.32	Cyclohexane, methyl-
2	18.442	383539	0.15	Methyl tetradecanoate (myristic acid)
3	20.308	439333	0.17	9-Hexadecenoic acid, methyl ester, (Z)-
4	20.54	47036224	18.25	Hexadecanoic acid, methyl ester
5	20.83	760072	0.29	n-Hexadecanoic acid
6	21.387	1133167	0.44	Tetracosanoic acid, methyl ester
7	21.92	1.48E+08	57.23	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	22.111	1121025	0.43	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
9	22.185	39203051	15.21	Methyl stearate
10	22.392	1264374	0.49	Octadecanoic acid
11	22.536	504783	0.2	9,11-Octadecadienoic acid, methyl ester, (E,E)-
12	22.601	542345	0.21	Hexadecanoic acid, 1,1-dimethylethyl ester
13	23.132	3060123	1.19	9,12-Octadecadienoyl chloride, (Z,Z)-
14	23.248	931276	0.36	Oxiraneoctanoic acid, 3-octyl-, methyl ester
15	23.365	1179453	0.46	cis-13-Eicosenoic acid, methyl ester
16	23.54	1876719	0.73	Eicosanoic acid, methyl ester
17	23.696	551103	0.21	Linoelaidic acid
18	23.743	1084032	0.42	cis-Vaccenic acid

19	24.277	2238653	0.87	2-(4-Benzyl-5-cyclopropyl-4H-[1,2,4]triazol-3-ylsulfanyl)-N-(3-fluorophenyl)acetamide
20	24.889	6112453	2.37	Docosanoic acid, methyl ester
		2.58E+08	100	

3.2.2 Analysis of Mugworts (Artemisia annua) EO.

Chemical analysis of *Artemisia annua* EO was conducted using GC-MS analysis (Figure 2). The EO was found to be characterized by the presence of many biologically important compounds, as listed in Table (3). Among these compounds are linoelaidic acid, Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, methyl ester, linoleic acid ethyl ester, Octamethyl cyclotetrasiloxane and linalyl acetate compounds, which have been associated with insecticide in previous studies. The analyses proved that there are a common components between both EOs such as linoelaidic acid, Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, methyl ester, linoleic acid ethyl ester. In this particular study, Artemisia essential oil was found to contain various monoterpenoids with high evaporation rates. Due to their volatility, these compounds demonstrated fumigant activity, which could be significant for controlling stored-product insects.

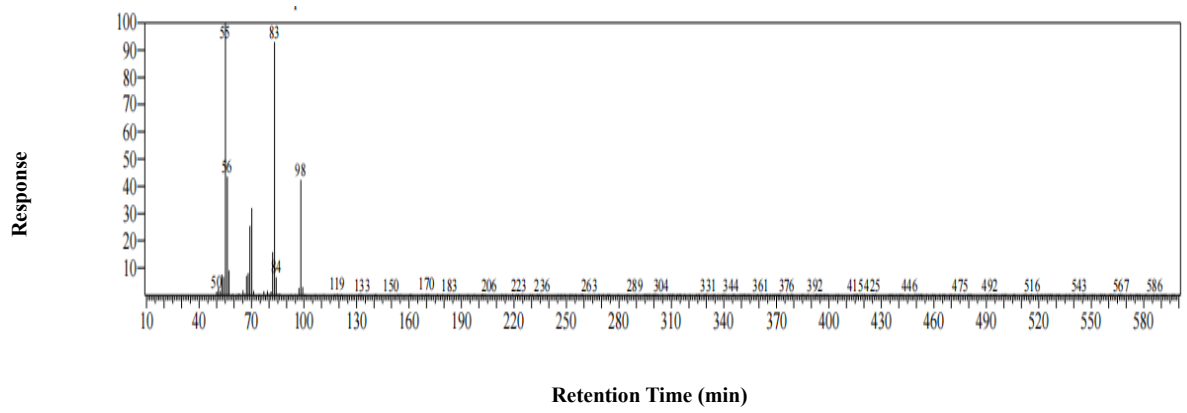

Figure 2: GC-MS chromatogram of Mugworts (*Artemisia annua*) EO.

Table 3: The GC-MS Chemical analysis of Mugworts (*Artemisia annua*) EO.

Peak#	R.Time	Area	Area%	Name
1	3.061	739892	0.32	Cyclohexane, methyl-
2	18.441	281907	0.12	Methyl tetradecanoate
3	20.307	322538	0.14	9-Hexadecenoic acid, methyl ester, (Z)-
4	20.537	33596629	14.71	Hexadecanoic acid, methyl ester
5	20.827	714058	0.31	n-Hexadecanoic acid
6	21.390	163129	0.07	Heptadecanoic acid, methyl ester
7	21.920	149706058	65.53	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	21.958	318211	0.14	5-(5-[5-(5-Cyano-4,4,5-trimethyl-4,5-dihydro-3H-pyrrol-2-ylmethylene)-4,4-dimethyl-py
9	22.109	1758158	0.77	Hexadecane, 1,1-bis(dodecyloxy)-
10	22.182	28027350	12.27	Methyl stearate
11	22.537	281211	0.12	9,12-Octadecadienoic acid, methyl ester
12	22.601	394852	0.17	Hexadecanoic acid, butyl ester
13	23.257	2267562	0.99	i-Propyl tricosanoate
14	23.365	1784582	0.78	Methyl 9-eicosenoate
15	23.541	1558768	0.68	Eicosanoic acid, methyl ester
16	23.697	616899	0.27	Linoelaidic acid
17	23.743	1433558	0.63	trans-9-Octadecenoic acid, pentyl ester
18	24.891	4485988	1.96	Docosanoic acid, methyl ester
		228451350	100.00	

3.3 Flavonoids in Black Seeds (*Nigella sativa*) and Mugwort (*Artemisia annua*) Essential Oils

Flavonoids are a class of polyphenolic compounds that are widely distributed in plants and are known for their antioxidant and anti-inflammatory properties. Black seeds, also known as *N. sativa* or black cumin seeds, contain various bioactive compounds, including flavonoids. Specifically, black seeds are rich in several flavonoids as presented in the analysis, including: Quercetin, Kaempferol, Myricetin, Hesperidin, Rutin. Forty-seven peaks resulted from the HPLC analysis of black seeds' EO (Figure 3). Seventeen Peaks were identified and presented in Table (4), whereas

the rest of the peaks were considered to be unknown. The identified peaks represent gallic acid, Rutin, Kaempferol, caffeic acid, cinnamic, catechol, 4-hydroxy, Qurctin, cinnamaldehyde, hesperidin, Eugenol, naringenin, Lignan, Chlorogenic and nigellone. The HPLC analysis of Mugworts (*A. annua*) results revealed the presence of forty-one peaks of flavonoids of *A. annua*. Seventeen major flavonoid elements are identified and presented in Table (5), whereas the rest of the peaks were considered to be unknown. The identified peaks represent Pyrogallol, gallic acid, Rutin, kaempferol, vanillic, caffeic acid, cinnamic, catechol, 4-hydroxy benzoic acid, Qurcitin, cinnamaldehyde, hesperidin, eugenol, naringenin, lignin, chlorogenic and nigellone (Figure 4). Comparing the flavonoids and with other bioactive compounds presented in the literature of black seeds, it becomes evident that flavonoids play a significant role in the insecticidal potential of black seed extracts or oil [45]. These results agree with [46] that identified six flavonol aglycones derived from *A. annua*. Among them, the primary constituents are quercetagetin 3,6,7-trimethyl ether, 6-OH-kaempferol 3,6,7-trimethyl ether, and small amounts of quercetagetin 3,6,7,3',4'-pentamethyl ether, and kaempferol 3,7-dimethyl ether. Eugenol has been found to exhibit repellent activity against *Ixodes ricinus* [47] Similarly, Estragole, another active compound, demonstrated insecticide activity against stored Vigna pest (*Callosobruchus maculatus*) [48]. The presence of Estragole and t-anethole in essential oils indicated their insecticidal properties, in addition to antimicrobial activity [49]. In particular, Eugenol was identified as the crucial active compound, showing significant effects against *Sitophilus zeamais* and *T. castaneum*. It also demonstrated significant fumigant activity against rice weevils when used in storage rice. These findings suggest that the insecticidal potential of black seeds and mugwort may rely on the fumigation potential of the Eugenol consider as main component. However, further studies are needed to clearly understand their mechanisms of action and insecticidal potential.

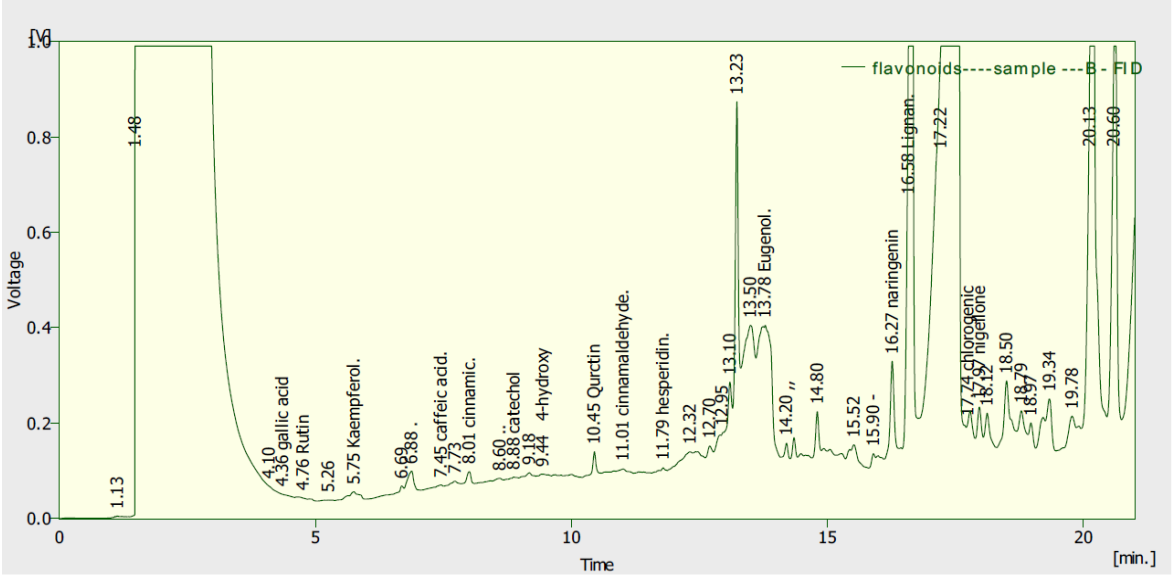

Figure 3: Chromatogram of Black Seeds flavonoids by HPLC.

Table 4. HPLC analysis of Black Seeds flavonoids

Peak	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Identified Compound
5	4.357	495.096	26.089	0.2	0.3	0.40	gallic acid
6	4.760	213.565	15.258	0.1	0.2	0.32	Rutin
8	5.753	428.264	21.382	0.2	0.2	0.33	Kaempferol.
11	7.453	533.985	23.803	0.2	0.2	0.46	caffeic acid.
13	8.007	489.495	47.674	0.2	0.5	0.18	cinnamic.
15	8.877	484.055	30.262	0.2	0.3	0.29	catechol
17	9.440	1496.704	32.387	0.6	0.3	0.91	4-hy droxy

18	10.450	717.340	72.128	0.3	0.7	0.09	Qurctin
19	11.007	1057.164	32.159	0.4	0.3	0.63	cinnamaldehy de.
20	11.790	896.911	28.969	0.4	0.3	0.66	hesperidin.
27	13.783	6282.310	313.361	2.7	3.2	0.33	Eugenol.
32	16.267	1562.146	220.099	0.7	2.2	0.09	naringenin
33	16.583	8142.913	878.118	3.5	8.9	0.14	Lignan.
35	17.740	442.732	77.543	0.2	0.8	0.10	chlorogenic
36	17.967	1326.720	110.150	0.6	1.1	0.11	nigellone

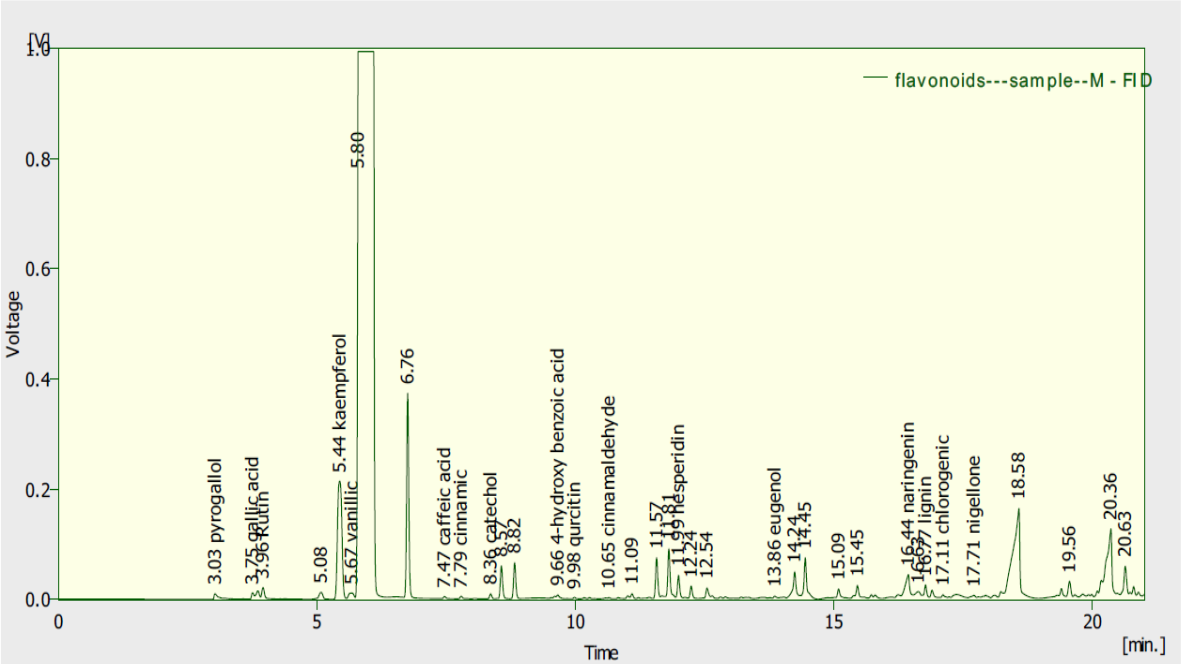

Figure 4: Chromatogram of Mugworts flavonoids by HPLC.

Table 5: HPLC analysis of Mugworts flavonoids.

Peak	Reten.Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Identified Compound
1	3.030	35.662	9.050	0.1	0.3	0.07	pyrogallol
2	3.753	20.406	8.288	0.1	0.3	0.05	gallic acid

3	3.957	54.472	17.563	0.2	0.6	0.05	Rutin
5	5.440	1241.199	212.882	4.0	6.8	0.10	kaempferol
6	5.673	26.585	4.144	0.1	0.1	0.09	Vanillic
9	7.467	13.228	3.870	0.0	0.1	0.05	caffeic acid
10	7.790	11.251	4.095	0.0	0.1	0.05	Cinnamic
11	8.360	16.512	7.090	0.1	0.2	0.04	Catechol
14	9.660	24.977	5.522	0.1	0.2	0.05	4-hydroxy benzoic acid
15	9.980	6.946	2.347	0.0	0.1	0.05	Qurcitin
16	10.647	10.072	1.901	0.0	0.1	0.05	cinnamaldehy de
20	11.993	117.325	40.139	0.4	1.3	0.05	hesperidin
23	13.857	10.803	3.509	0.0	0.1	0.05	Eugenol
28	16.440	244.383	39.451	0.8	1.3	0.09	naringenin
30	16.770	13.076	6.891	0.0	0.2	0.02	Lignin
31	17.110	12.133	4.267	0.0	0.1	0.05	chlorogenic
32	17.707	17.148	3.635	0.1	0.1	0.07	Nigellone

3.4 The Impact of plant extracts, EOs, and nanomaterials treatments on Wheat grains

3.4.1 The Effect of treatments on Sugar content (Oligosaccharides) of wheat grains

The results of Table (6) present the assessment of the sugar content and their retention time of wheat grains treated with several materials. The tested concentrations were, 20% of each mugwort plant extract, mugwort EOs, Black seeds plant extract, Black seeds EOs, and at concentration 400 ppm of Silver NPs and Silicon NPs were compared to the control group. The total sugar content not significantly difference between the treated wheat grains with SiNPs 0.3 g/100 g wheat grains and the control group to 0.3 g/100 g. Additionally, all the other treatments, Mugwort EOs, Black seeds plant extract, Black seeds EOs and AgNPs have high significant effect in increasing the total sugar content of the treated wheat grains (1.4, 1.5, 1.2, 1.9 and 1 g/100 g, respectively) compared to the control group. However, the concentrations of the total sugar content of the wheat grains are

typically expressed as a percentage of the grain's dry weight. On average, wheat grains may contain around 0.5 to 2% sugars. These findings found to be compatible with that found by [23]. Accordingly, [50] mentioned that, the expression levels of essential pathway genes and resulting in a larger biomass associated with soluble sugars were enhanced by SiNPs treatments. Raffinose, stachyose, and verbascose are complex carbohydrates known as oligosaccharides. They are composed of multiple sugar molecules linked together and are commonly found in various plants, including some grains. Thus, these oligosaccharides play an important role in energy storage and other functions, such as protecting seeds from desiccation. Thus, when the treatment caused an increase, it led to improve the other grains germination and biological parameters.

Table 6: The Effect of treatments on Sugar content of Wheat Grains.

Sugar content	Control		Mugwort EO		Mugwort PE		Blackseed EO		Blackseed PE		SiNPs		AgNs	
	RT (min)	Conc. (%)	RT (min)	Conc. (%)	RT (min)	Conc. (%)	RT (min)	Conc. (%)	RT (min)	Conc. (%)	RT (min)	Conc. (%)	RT (min)	Conc. (%)
Raffinose	5.78	0.2	5.78	0.7	5.78	0.7	5.78	0.6	5.78	0.9	5.78	0.2	5.78	0.5
Stachyose	10.46	0	10.64	0.2	10.64	0.2	10.64	0.1	10.64	0.3	10.64	0	10.64	0.1
Verbascose	17.11	0.1	17.11	0.5	17.11	0.6	17.11	0.5	17.11	0.7	17.11	0.1	17.11	0.4
Total	33.35	0.3	33.54	1.4	33.54	1.5	33.54	1.2	33.54	1.9	33.54	0.3	33.54	1

3.4.2 The Effect of treatments on the Amino Acid content of Wheat Grains

The total free amino acids content and their retention time of wheat grains treated with several materials present in the Table 7. The concentration 20% of each mugwort plant extract, mugwort EOs, Black seeds plant extract, Black seeds EOs, and at concentration 400 ppm of Silver NPs and Silicon NPs were compared to the control group. The total amino acid (TAA) content significantly decreased only in the treated wheat grains with mugwort plant extract from 10.00 g/100 g wheat grains in the control group to 7.90 g/100 g, indicating a decrease of -2.1%. Additionally, all the other treatments, Mugwort EOs, Black seeds plant extract, Black seeds EOs, Silver NPs and

Silicon NPs, have no significant effect on the total amino acid content of the treated wheat grains compared to the control group. The concentrations of glutamic acid, serine, histidine and glycine increased approximately more than two-folds in all treatments. On contrary, all the other amino acids significantly decreased, compared in the control group. These findings found to be compatible with that found by [51], that was reported that an aqueous extract of mugwort was applied led to a significant increase in wheat grains amino acid contents. In nature, there are 20 common amino acids that serve as the building blocks of proteins. These are often referred to as the standard or proteinogenic amino acids. In wheat grain, the amino acid profile can vary, but it typically contains a combination of these 20 common amino acids. The precise composition of amino acids in wheat grain depends on factors such as the wheat variety, growing conditions, and processing methods [51]. Some of the major amino acids found in wheat grain include: Glutamic acid, Aspartic acid, Leucine, Alanine, Glycine, Proline, Valine, Isoleucine, Serine, Phenylalanine, Tyrosine, Threonine, Methionine, Cysteine, Lysine, Arginine, Histidine, Tryptophan. It's important to note that the quantity and ratio of these amino acids can vary based on factors such as wheat variety, soil conditions, climate, and agricultural practices. Different wheat cultivars and varieties may have slightly different amino acid profiles. Nonetheless, wheat grain, like most grains, generally contains a diverse array of amino acids that contribute to its nutritional value as a source of protein [51]. However, there is no toxic effect of the different treatments on the treated wheat grains.

Table 7: The Effect of treatments on the Amino Acid content of Wheat Grains.

Amino acids	Control		Mugwort EO		Mugwort PE		Blackseed EO		Blackseed PE		SiNPs		AgNs	
	RT (min)	**Conc. (%)**	**RT (min)**	**Conc. (%)**	**RT (min)**	**Conc. (%)**	**RT (min)**	**Conc. (%)**	**RT (min)**	**Conc. (%)**	**RT (min)**	**Conc. (%)**	**RT (min)**	**Conc. (%)**
Aspartic acid	3.98	0.11	4.07	0.01	4.14	0.03	4.24	0.20	3.94	0.07	4.17	0.17	4.22	0.11
glutamic acid	7.71	0.38	7.70	1.30	7.98	0.64	7.94	0.80	7.86	0.71	7.86	1.44	7.70	1.84
Serine	8.54	0.71	8.57	1.06	8.45	2.82	8.54	2.64	8.61	0.50	8.56	1.01	8.54	1.32
Histidine	8.75	0.24	8.66	0.46	8.69	0.45	0.00	0.00	0.00	0.00	8.70	0.75	8.77	0.52
Glysine	8.92	0.13	8.84	0.45	8.88	0.58	8.83	0.68	9.02	0.18	8.96	0.63	8.95	0.60
Therionine	9.42	0.21	9.38	0.30	9.34	0.20	9.36	0.68	9.39	0.08	9.22	0.56	9.20	0.56

Arginine	9.76	0.26	9.65	0.18	9.75	0.16	9.74	0.30	9.56	0.08	9.66	0.26	9.68	0.46
Alanine	0.00	0.00	9.92	0.51	9.97	0.16	9.94	0.59	10.00	0.24	9.90	0.23	10.02	0.42
Tyrosine	12.11	0.39	10.58	0.54	10.34	0.21	11.20	0.24	11.48	0.29	12.20	0.42	12.44	0.41
Cystine	11.34	0.94	11.13	0.21	11.38	0.30	11.35	0.43	11.98	1.34	11.24	0.64	11.47	0.68
Valine	0.00	0.00	12.10	0.00	12.02	1.04	11.91	0.27	12.42	0.63	11.99	0.95	12.80	0.23
Methionine	12.40	1.03	12.30	0.87	12.36	1.00	12.24	0.97	12.74	2.84	0.00	0.00	0.00	0.00
Phenylalanine	13.80	1.03	12.74	0.27	12.89	0.31	13.04	0.38	12.96	0.45	13.96	1.22	14.73	1.10
Isoleucine	13.01	0.62	12.94	0.59	0.00	0.00	0.00	0.00	13.34	0.74	12.92	0.70	13.02	0.24
Leucine	13.39	0.68	13.34	0.62	13.28	0.32	13.47	0.45	0.00	0.97	13.27	1.01	13.60	0.79
Lysine	14.21	3.27	14.08	0.53	13.87	1.78	13.95	1.39	14.15	0.89	0.00	0.00	14.15	0.73
Total	147.34	10.00	166.00	7.90	153.34	10.00	145.77	10.02	147.45	10.01	142.61	9.99	159.27	10.01

3.4.3 The Effect of the treatments on the Fatty Acid Content of Wheat Grains

Palmitoleic, Palmitoleic, stearic, Oleic, linoleic and linolenic acids are the main fatty acid content in the wheat grains. The results indicated in Table (8) and according to the ANOVA showed a significant decrease in the content of some fatty acids and an increase in others at a significance level of ($P \leq 0.05$). The wheat grains treated with several materials at concentration 20% of each mugwort plant extract, mugwort EOs, Black seeds plant extract, Black seeds EOs, and at concentration 400 ppm of Silver NPs and Silicon NPs were compared to the control group. The total content of fatty acids decreased in mugwort plant extract, black seeds essential oil and SiNOs to 0.08, 0.06 and 0.07 g/100 g of wheat grains after being 0.16 g/100 g of wheat grains in the control group. The total content of fatty acids increased significaly AgNPs to 0.41 of wheat grains. However, the concentration of the total fatty acid content in mugwort EO decreased to 0.13 g/100 g after being 0.16 g/100 g in the control, but this decrease was not statistically significant at ($P \geq 0.05$). The results are similar to a study conducted by [51] assessing AgNPs effects on wheat grains and find that all the biochemical parameters enhanced and the fatty acid contents increased by 4-folds. Accordingly, [50] mentioned that, the expression levels of essential pathway genes and

resulting in a larger biomass associated with fatty acids were enhanced by SiNPs treatments. Additionally, [51] reported that an aqueous extract of mugwort was applied led to a significant increase in wheat grains fatty acid contents.

Table 8: The Effect of the treatments on the Fatty Acid Content of Wheat Grains.

ID	Fatty acids	Control		Mugwort EO		Mugwort PE		Blackseed EO		Blackseed PE		SiNPs		AgNs	
		RT (min)	**Conc. (%)**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**	**RT (min)**	**Area (%)**
1	Palmitic	7.42	0.03	7.98*	0.02	7.98	0.01	6.69**	0.01**	7.56	0.02	7.47	0.01	7.31	0.13
2	Palmitoleic	13.15	0.01	13.04	0.01	13.04	0.01	13.61**	0.00**	13.21*	0.01	13.11	0.01	12.68	0.09
3	Stearic	15.44	0.01	15.27*	0.01	15.27	0.01	14.57**	0.01**	15.40	0.02	15.49	0.01	15.22	0.08
4	Oleic	15.86	0.02	15.83	0.01	16.19	0.02	16.19**	0.01**	15.83	0.05	15.91	0.01	16.97	0.01
5	Linoleic	20.20	0.05	19.84**	0.04	20.10	0.03	20.26**	0.02**	20.22	0.06	19.84	0.01	20.62	0.04
6	Linolenic	21.43	0.04	21.32	0.04	21.85	0.00	21.88**	0.01**	21.55	0.02	21.98	0.02	23.38	0.06
	Total	93.50	0.16	93.28	0.13	94.42	0.08	93.20	0.06	93.78	0.18	93.79	0.07	96.17	0.41

3.4.4 The Effect of treatments on the estimation of Gluten Protein Content of Wheat Grains

The results of the wheat grains treated with several materials at concentration 20% of each mugwort plant extract, mugwort EOs, Black seeds plant extract, Black seeds EOs, and at concentration 400 ppm of Silver NPs and Silicon NPs were compared to the control group. The treatments impact on the fresh gluten content of the exposed wheat grains showed no significant change in gluten protein after treatment with the weight was 2.1 g of fresh gluten in the treatment, and there were no significant differences ($P \geq 0.05$) between the treatment and the untreated control. The percentage of gluten was 10.5%. As for the weight of fresh gluten in the untreated wheat, it was 2.21 g, and the percentage of gluten was 11.05%, as shown in Table (9). This result suggests that all the used materials did not have any negative effect on the gluten content of the treated wheat grains. Gluten is a group of proteins found in wheat and other cereal grains, responsible for giving dough its elasticity and contributing to the texture of baked goods. In this

study, we aim to investigate the impact of the plant extracts, essential oils (EOs) and NPs on the estimation of gluten protein content in wheat grains. The use of these materials has been gaining attention in various fields, including agriculture and food science, due to their insecticidal potential. Furthermore, their effects on specific analytical measurements, such as the estimation of gluten protein content, have not been extensively explored. Thus, the results are similar to a study conducted by [51] assessing AgNPs effects on wheat grains and find that all the biochemical parameters enhanced, and the gluten protein content have no significant differences. Accordingly, [50] mentioned that, the gluten protein contents were enhanced by SiNPs treatments. Additionally, [51] reported that an aqueous extract of mugwort was applied led to a significant increase in wheat grains gluten protein content.

Table 9: The Effect of treatments on the estimation of Gluten Protein Content of Wheat Grains.

Treatments	Fresh weight of gluten (gm) ±SD	Gluten (%)
Control	2.24 ± 0.09	11.01
Mugwort EOs	2.11 ± 0.10	10.71
Mugwort PE	2.10 ± 0.09	10.61
Blackseed EO	2.12 ± 0.10	10.62
Blackseed PE	2.11 ± 0.10	10.53
SiNPs	2.21 ± 0.09	11.02
AgNPs	2.15 ± 0.09	11.01

3.4.5 The Effect of the treatments on wheat seed germination indices

The results of the impact on seed germination indices were presented in Table 10. The wheat grains treated with several materials at concentration 20% of each mugwort plant extract, mugwort EOs, Black seeds plant extract, Black seeds EOs, and at concentration 400 ppm of Silver NPs and Silicon NPs were compared to the control group. The germination percentage at the seeds treated with all the plant materials and NPs indicating no significant difference ($p \geq 0.05$) between the two treatments compared with control treatment. This result suggests that all the treatments did not have any negative effect on seed germination rates s. Furthermore, the treatments resulted in higher

seedling length, coleoptile length, and radicle length, measuring 11.5, 3.82, and 7.67 cm, in Mugworts plant extract respectively, compared to the control group, which were measured 10.6, 3.1, and 7.55 cm, respectively. The increase in these lengths favored the all treatments, and the differences were statistically significant ($p \leq 0.05$) for coleoptile and radicle lengths. The first germination count (MGT), the germination index (GIT) and the Germination Duration time (GDT) were not significantly different ($p \geq 0.05$) between all treatments, with 7.0, 4.0 and 3.0 seeds/day for all treatments, in addition to the control treatment, respectively. However, the mean daily Germination (MDG) was significantly increased ($p \leq 0.05$) for all the treatments, with 5.39, 4.98, 4.90, 5.10, 5.27 and 5.18% for mugwort PE, mugwort EOs, Black seeds PE, Black seeds EOs, and SiNPs and AgNPs respectively, compared to 3.43% for the control treatment. The speed of germination (SP) differences for all treatments were not statistically significant ($p \leq 0.05$) compared to the control treatment. Regarding, germination capacity (GV) for the mugwort PE, mugwort EOs, Black seeds PE, Black seeds EOs, and SiNPs and AgNPs treatments showed higher values, measuring 38.48, 35.57, 33.59, 34.26, 35.35 and 33.32% respectively, compared to 24.49% for the control treatment. However, germination coefficient (GC%) for the same treatments the differences were not statistically significant ($p \geq 0.05$). Whereas, the germination vigor index (GVI) for the mugwort PE, mugwort EOs, Black seeds PE, Black seeds EOs, and SiNPs and AgNPs treatments showed variable values reached 35.30, 34.80, 30.82, 29.42, 29.33 and 27.72% respectively, compared to 31.00% for the control treatment. Overall, the results indicate that there were no significant negative effects on the seed germination parameters of the tested wheat grains. Quite the reverse, it increases most of the germination and the biological parameters of the treated wheat grains. This suggests that the wheat embryo exposed to these treatments remained viable that allowing its potential use as a promising alternative in grain protection, especially for wheat, whether for human consumption, animal feed, or agricultural cultivation purposes. These findings agree with a study conducted by [51], it was reported that an aqueous extract of mugwort was applied led to a significant increase in wheat germination rates and biological measures. Likewise, these results are consistent with what stated with [23]. However, significant variations ($P<0.05$) in wheat seed germination were observed after treatment with some essential oils, indicating the presence of phytotoxic effects. Lemongrass, Palmarosa, and Citronella treatments resulted in complete inhibition of seed germination. Additionally, Lavender treatment showed reduced germination, followed by Rosemary. At concentrations of 0.25% and 0.5%, significant differences were observed between Rosemary and Lavender. Allelopathic interactions in nature, caused by

aromatic plants containing volatile allelochemicals, have been frequently documented in various studies and references . Hence, that supports the findings that the most effective plant material with no significant effects on the germination parameters is the mugwort plant extract. Additionally, in a study conducted by [51], [52], it was observed that mugwort extract had a positive effect on the radicle and mesocotyl length of maize and bean. The obtained results revealed that, there is no significantly difference ($p \geq 0.05$) in the first germination count, the germination index and the Germination Duration time. However, the mean daily Germination (MDG) was significantly increased ($p \leq 0.05$) compared to the control treatment. The speed of germination (SP) differences for all treatments were not statistically significant ($p \leq 0.05$) compared to the control treatment. Regarding, germination capacity and germination coefficient the differences were not statistically significant ($p < 0.05$). In context with [52] investigated the phytostimulatory effect of silver nanoparticles (AgNPs) on seed germination and seedling growth of rice. The results showed that all tested concentrations of AgNPs promoted both shoot and root growth, leading to increased seedling length and biomass. Moreover, exposure to AgNPs resulted in a significant increase in the contents of chlorophyll a and carotenoids in the rice seedlings. Moreover, the study highlights the potential of AgNPs as a phytostimulant that can enhance the growth and health of rice seedlings.

Table 10: The Effect of the treatments on Seed Germination Indices

Parameter	Control ±SD	MPE ±SD	MEO ±SD	BsPE ±SD	BsEO ±SD	SiNPs ±SD	AgNPs ±SD
GIT (day)	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0
MGT (day)	7.00±0.0	7.00±0.0	7.00±0.0	7.00±0.0	7.00±0.0	7.00±0.0	7.00±0.0
MDG %	3.43±0.1	5.39±0.37	4.98±0.23	4.90±0.40	5.10±0.06	5.27±0.37	5.18±0.37
GDT day	3.00±0.0	3.00±0.0	3.00±0.0	3.00±0.0	3.00±0.0	3.00±0.0	3.00±0.0
GC %	100.00±0.0	107.25±0.04	121.41±0.01	121.95±0.01	112.62±0.02	100.28±0.01	101.81±0.02
SP	7.14±1.7	7.14±1.7	7.14±1.7	6.86±1.1	6.71±1.2	6.71±1.3	6.43±1.3

GP %	100.00±0.0	100.00±0.0	100.00±0.0	100.00±0.0	100.00±0.0	100.00±0.0	100.00±0.0
GC	14.29±	14.29±	14.29±	14.88±	15.20±	15.20±	15.87±
PV	7.14±0.03	7.14±0.03	7.14±0.03	6.86±0.07	6.71±0.06	6.71±0.06	6.43±0.03
GV	24.49±2.1	38.48±3.7	35.57±3.3	33.59±2.9	34.26±3.1	35.35±3.3	33.32±3.2
SDW	0.62±	0.71±0.005	0.70±0.013	0.64±0.011	0.63±0.006	0.62±0.005	0.62±0.006
GVI	31.00±2.7	35.30±3.1	34.80±3.1	30.82±2.9	29.42±2.7	29.33±2.7	27.72±2.3
Seedling length cm	10.6± 0.12	11.5±0.12	11.36±0.09	11.36±0.09	10.64± 0.04	10.86±0.06	10.50±0.04
coleoptile length cm	3.1± 0.07	3.82±0.07	4.76±0.04	4.60±0.04	4.00±0.09	4.28±0.04	4.14±0.05
radicle length cm	7.55± 0.08	7.67±0.09	6.62±0.03	6.75±0.03	6.64±0.13	6.58±0.07	6.36±0.03

SD= Standard Division. treated with different tested materials at 20% Conc. for each mugwort plant extract (MPE), mugwort EO (MEO), Black seeds PE (BsPE), Black seeds EO (BsEO) and 400ppm for both SiNPs and AgNPs

4. Conclusions

The effects of plant extracts and nanomaterials on the repellent ability of the red flour beetle (*Tribolium castaneum* Herbst) and their impact on wheat grains have been studied. In the case of Mugwort (*Artemisia annua*) and black seed (*Nigella sativa*) plant extracts, repellent effect was observed to be more effective than the EOs for the same plants during the same exposure period. However, the lowest repellent values were 42% and 54% at a concentration of 5% after one hour for *Artemisia annua* plant extract and essential oils, respectively. Regarding silicon nanoparticles (SiNPs) and silver nanoparticles (AgNPs), there were no significant differences in repellent values across exposure periods. Both SiNPs and AgNPs exhibited a moderate repellent capacity at the same plants during the same exposure period compared to the control group. As for phytoxicity, the results indicated no significant negative effects on the tested seed germination standards and biological characteristics. Instead, an increase in most of the tested grain germination standards and biological characteristics was observed in the treated grains. These results highlight the

potential use of these treatments as promising alternative for protecting grains, especially wheat, intended for human consumption, animal feed, or crop cultivation.

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تقييم التأثير الطارد والسمية النباتية لبعض المستخلصات النباتية والزيوت العطرية والجسيمات النانوية على خنفساء الطحين الحمراء *Tribolium castaneum* Herbst التي تصيب حبوب القمح(*Triticum estivum* L.)

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المستخلص

تمت دراسة تأثيرات مستخلصات النباتات والجسيمات النانوية على قدرة طرد حشرة خنفساء الطحين الحمراء (*Tribolium castaneum* Herbst) وتأثيرها على حبوب الحنطة. في حالة مستخلص نبات الشيح (Mugwort)، تم ملاحظة قدرة طرد بنسبة 100% بعد مرور 3 ساعات عند تراكيز 10% و 15% و 20%، مع قدرة طرد بنسبة 85% عند تركيز 20% بعد مرور 2 ساعة وقدرة طرد بنسبة 82% عند تركيز 15% بعد نفس الفترة. ومع ذلك، كانت أدنى قيم لقدرة الطرد هي 42% و 54% عند تركيز 5% بعد ساعة واحدة لمستخلص نبات الشيح (*Artemisia annua*) والزيوت العطرية على التوالي. أما بالنسبة لبذور الحبة السوداء (*Nigella sativa*)، فقد تم ملاحظة قدرة طرد بنسبة 100% لكل من المستخلص النباتى وزيت العطري عند تراكيز 10% و 15% و 20% بعد مرور 3 ساعات. وقد بلغت قدرة طرد مستخلص بذور الحبة السوداء 93% عند تركيز 20% بعد مرور 2 ساعة، بينما بلغت 85% عند تركيز 5% بعد 3 ساعات مقارنة بالزيوت العطرية، التي أظهرت قدرة طرد بنسبة 50% في نفس الفترة. ومع ذلك، كانت أدنى قيم لقدرة الطرد هي 50% و 52% عند تركيز 5% بعد ساعة واحدة من التعرض لكل من مستخلص النبات والزيوت العطرية. فيما يتعلق بالسيليكون (SiNPs) وجسيمات الفضة النانوية (AgNPs)، لم تظهر هناك فروقات معنوية في قيم الطرد عبر فترات التعرض. لقد أظهرت كل من SiNPs وAgNPs قدرة طرد بنسبة 40% عند تركيز 100 جزء في المليون بعد ساعة واحدة مقارنة بالمجموعة المقارنة. أما أدنى قيم لقدرة الطرد فكانت 2% لـ SiNPs و 4% لـ AgNPs عند تركيز 100 جزء في المليون بعد ساعة واحدة من التعرض. من حيث السمية النباتية، أشارت النتائج إلى عدم وجود تأثيرات سلبية ملحوظة على معايير انبات البذور المختبرة وخصائصها البيولوجية. بدلاً من ذلك، تم ملاحظة زيادة في معظم معايير انبات الحبوب المختبرة وخصائصها البيولوجية في الحبوب المعاملة. وهذا يشير إلى أن حيوية حبوب الحنطة الجنينية تم الحفاظ عليها حتى بعد التعرض لهذه المعالجات. تسلط هذه النتائج الضوء على إمكانية استخدام هذه المعالجات كبديل مشجع لحماية الحبوب، وخاصة الحنطة، المخصصة للاستهلاك البشري أو علف الحيوانات أو زراعة المحاصيل.