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Research Article

Physiological Effects of Some Stress Factors in *Tribolium castaneum* (Herbst)

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ABSTRACT

Stored-product insects, particularly the red rust flour beetle, *Tribolium castaneum*, cause considerable quantitative and qualitative losses in stored grains and flour products. The present study evaluated the insecticidal efficacy of the essential oils of *Mentha piperita* and *Laurus nobilis*, alone and in combination with elevated temperatures, against adults of the red rust flour beetle. Peppermint oil exhibited significantly higher toxicity than bay oil at all tested concentrations and exposure periods. Adult mortality increased with increasing oil concentration and exposure duration, reaching a maximum of 81.9% after 7 days at 5% peppermint oil, whereas bay oil caused a maximum mortality of 71.9% under the same conditions. The overall mean mortality was 53% for peppermint oil compared with 39.1% for bay oil. Probit analysis confirmed the greater toxicity of peppermint oil, which recorded a lower LC₅₀ value (4832.31 ppm) than bay oil (24,744.23 ppm). The synergistic interaction between essential oils and elevated temperatures further enhanced insect mortality. Exposure to 45°C alone caused 38.6% mortality; however, mortality increased to 85% when peppermint oil was combined with 45°C. Similarly, the combination of bay oil and 45°C resulted in 76.6% mortality. Physiological investigations revealed significant increases in the activities of glutathione-S-transferase, catalase, and peroxidase enzymes under thermal and oil treatments, indicating oxidative stress and activation of detoxification mechanisms in the insects. In conclusion, peppermint oil, particularly when combined with elevated temperatures, demonstrated strong potential as an environmentally sustainable strategy for the management of stored-product insect pests.

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Introduction

The red flour beetle, *Tribolium castaneum*, is one of the most damaging stored-grain insect pests throughout the world causing significant economic losses to grain and grain products annually (Scudder and Cannings, 2005). Larvae and adults consume a variety of stored commodities. There are about 246 different products reported to be infested by this insect pest around the world, such as flour, grain products, dried fruits, dried flowers, spices, oilseeds and medicinal herbs. The

infestation by this insect pest results in considerable quantitative and qualitative losses in stored grains as well as serious damage to grain storage structures and flour mills (Arthur, 2000; Hagstrum and Subramanyam, 2009). A variety of methods have been applied to control stored-grain pests, especially chemical insecticides that continue to be one of the most effective methods. The emergence of insect resistance to chemical pesticides and the negative impact on human health and the environment have led to the search for alternative

insecticides that are safer and environmentally friendly. Of these, plant secondary metabolites and essential oils have been given special focus for being effective agents for pest management (Abdul Hadi and Al-Khazraji, 2021; Al-Ani et al., 2025).

Lashgari et al. (2013) reported fumigant toxicity of peppermint oil extracted from *Mentha piperita* against the stored-product insects. The oil proved to be toxic and lethal to *T. castaneum* (64%) and *Sitophilus oryzae* (82%) after 3 days of exposure. Likewise, Bachrouch et al. (2023) showed that Tunisian pennyroyal oil extracted from *M. pulegium* was found effective against *T. castaneum* causing a mortality of 72.2% during a period of 30 and 60 days of storage. The essential oil also inhibited the activity of acetylcholinesterase in the insect. The chemical characterization showed that the oil was composed mainly of pulegone, a compound which might directly interact with the insect detoxification system, by inhibiting the cytochrome P450 activity (Rossi et al., 2012).

Demirel and Erdoğan (2017) found that the essential oils of rosemary and *Laurus nobilis* were most effective against *Callosobruchus maculatus*, the cowpea weevil, with the mortality rate of 100% and 93%, respectively. The efficacy of these oils was better than the essential oils of Turkish oregano, mountain oregano, Greek oregano and sweet marjoram. The authors found that α -terpinyl acetate (15.33%) and 1,8-cineole (37.84%) are the major bioactive compounds that have insecticidal activity in laurel oil. They found that the essential oils of these plants can be used as alternative insecticides for stored products insect pests.

Insects have antioxidant defense mechanisms which help them to withstand high temperatures. Oxidative enzymes are known to be important for insects against heat induced oxidative damage and are regarded as important biomarkers of oxidative stress (Kang et al., 2017). Miao et al. (2020) found that the malondialdehyde (MDA) content enhanced and the activities of some antioxidant enzymes, including heat shock proteins (HSPs), superoxide dismutase (SOD), glutathione-S-transferase (GST), peroxidase (POD), and catalase (CAT), increased in *Liposcelis bostrychophila* under high-temperature exposure. The highest enzymatic activities were found at 42°C as compared to the control treatment at 27°C. Hence, the current study was undertaken to assess the potential of the selected essential oils and temperature regimes for the management of red flour beetle, *T. castaneum*.

Materials and Methods

Laboratory rearing of the red rust flour beetle

Red rust flour beetles, *T. castaneum*, were collected at different developmental stages from the Insect Laboratory of the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad in 2025. The insects were kept in sanitized and clean plastic containers with 12 cm diameter and 20 cm height. Wheat flour of the Abaa cultivar was used as the food source. The containers were then covered with muslin cloth and tied with rubber bands and were kept in a Binder-type incubator at $65 \pm 5\%$ RH and $30 \pm 2^\circ\text{C}$. The culture was maintained continuously to get a good supply of adults for use in the subsequent experiments.

Preparation of plant essential oils

Fresh leaves of *Laurus nobilis* (Lauraceae) and *Mentha piperita* (Lamiaceae) were obtained from the fields and gardens of Al-Jadriya campus of the University of Baghdad, Iraq. The leaves were gently washed with running tap water, air-dried, and pulverized. The pulverized leaf samples were kept in separate labeled plastic bags for extraction.

To extract essential oils, 50 g of each pulverized leaf sample was added to a glass flask filled with 700 mL of distilled water. A Clevenger apparatus was used to extract essential oils and the extraction was repeated multiple times to collect enough essential oils for the laboratory assays. The extracted oils were stored in cool place in labeled tubes until they were used (Al-Khazraji et al., 2016). All extraction procedures were done in the Central Laboratory of College of Agricultural Engineering Sciences, University of Baghdad.

Subsequent concentrations of 1%, 3%, and 5% of essential oils were made by adding requisite amounts of hexane and the final volumes of 100 mL were made. The concentrations were then evaluated in adult bioassays.

Effect of essential oil fumigation on adults of the red rust flour beetle

The fumigant activity of the volatiles of peppermint essential oil was tested against the newly emerged adults of *T. castaneum* (2-5 days old). Ten adults were added to 1-L plastic containers. A piece of cotton was soaked in 0.5 mL of peppermint essential oil and exposed to air for 30 min to allow volatilization prior to insertion inside the container. For the control treatment, another piece of cotton was impregnated with hexane and then left in the air for 30 min before putting in the container. Afterwards, the containers were firmly

covered for 60 min to assure exposure to the volatiles. The cotton pieces were removed after the exposure, and 25 g of the flour was added as food source. Fine muslin cloth was used to cover the containers to allow aeration and to prevent escape of insects. Each treatment was replicated three times and there were 10 adults per replicate. All the bioassays were carried out at 30°C. Data regarding adult mortality was collected following exposure to essential oil volatiles. The mortality percentages were determined on the 1st, 3rd, 5th and 7th day after exposure to essential oils. Abbott's formula (Abbott, 1925) was used to calculate corrected mortalities as reported by Al-Khazraji et al. (2016). The same process was carried out with the essential oil of bay laurel at the same concentrations.

Estimation of lethal concentration (LC₅₀)

The lethal concentration for 50% of the adults (LC₅₀) was estimated using Probit analysis based on the number of dead adults and mortality percentages 7 days after treatment with concentrations of 0, 1, 3, and 5%. These assessed LC₅₀ values were then used to estimate the synergistic effects of the essential oils and the temperature treatments, and their potential use in the management of *T. castaneum*.

Synergistic effect of different temperatures and essential oils on adult mortality of red rust flour beetle

To study the adult mortality of *T. castaneum*, the temperature regimes of 30, 37 and 45°C were chosen. For each replicate, 50 adults were placed into 1-L containers and exposed to the selected temperature regimes for 1 h. The treated adults were then placed in an incubator at 30°C and mortality was determined after 7 days. Each temperature regime was replicated 5 times.

To study the synergistic effect of temperature regimes and lethal concentrations of essential oil, 50 adults were transferred to 1-L containers having a cotton piece soaked in 0.5 mL of the lethal concentration of peppermint essential oil. For this purpose, the adults were exposed to essential oil vapors for 1 h in tightly closed containers and were individually incubated at 30, 37, and 45°C. Cotton pieces were removed following exposure and the adults were placed in an incubator set at 30°C and percentage mortalities were recorded after 7 days. Each treatment was replicated 5 times.

Likewise, the synergism between various temperature regimes and the lethal concentrations (LC₅₀) of the essential oil of bay laurel was tested, using the same

treatments and replicates. The adults which survived the various treatments were picked up for further physiological studies.

Evaluation of the activity of some oxidative enzymes in adults of red rust flour beetle

Preparation of enzyme extracts from adults

Twenty adults from each temperature regime and essential oil treatment were taken separately for enzyme extraction. Adults were thoroughly homogenized in 0.1 M phosphate buffer (pH = 7.4) in a porcelain mortar. The homogenate was then centrifuged at 7000 rpm for 10 min at 4°C in a refrigerated centrifuge. The precipitation was removed after centrifugation and the supernatant was collected. Supernatant was then placed in 2-mL Eppendorf tubes, marked for each respective treatment, and then kept in the freezer for subsequent use.

Determining the activity of Glutathione S-Transferase (GST)

The activity of glutathione S-transferase (GST) was measured using a modified spectrophotometric method based on the work of Morou et al. (2010). In short, 20 µL of the enzyme extract from the adult insects was added to test tubes containing 25 µL of glutathione solution and 25 µL of the solution of iodoethane. After that, 50 µL of 0.2 M phosphate buffer (pH = 8.2) was added to make up reaction mixture.

The reaction mixtures were softly shaken and incubated at 25°C for 30 min for the enzymatic reaction to occur. Following incubation, 50 µL of 1% starch solution was added and then 100 µL of a stopping solution (HCl and H₂O₂) was added to stop the reaction.

The absorbance of the reaction mixture was measured using a spectrophotometer at 610 nm. Potassium iodide (KI) was used to prepare a standard calibration curve to estimate the activity of GST. Each assay was done three times to assure authenticity and reproducibility of the results.

Estimation of catalase activity

The activity of catalase enzyme was measured by the protocol described by Beer and Sizer (1952) which involves the enzymatic breakdown of hydrogen peroxide (H₂O₂). In short, 20 µL of the enzyme extract of the adult insect was mixed with 50 µL of phosphate-buffered saline (50 mM, pH = 7.0) containing 0.036% hydrogen peroxide as reaction substrate. The reaction mixture was slowly stirred and allowed to incubate in a controlled laboratory environment. Catalase activity was measured by measuring the decrease in absorbance due to the

breakdown of H_2O_2 at 240 nm with a spectrophotometer. Each measurement was repeated three times to ensure accuracy and reproducibility of the assay.

Estimation of peroxidase activity

For the determination of peroxidase activity, the method of Addy and Goodman (1972) was used. In short, 50 μ L of enzyme extract was added to 250 μ L of buffered pyrogallol solution and then 250 μ L of 1% hydrogen peroxide (H_2O_2) was added to start the reaction. The reaction mixture was incubated at the laboratory conditions and the absorbance of the mixture was measured spectrophotometrically at 430 nm. Enzyme activity was measured as the rise in absorbance due to the oxidation of pyrogallol. Three independent replicates were made for all assays.

Protein estimation

For the estimation of protein content, Bradford's method was used (Bradford, 1976). In brief, 40 μ L of insect extract was mixed with 200 μ L of Bradford reagent and absorbance was determined at 595 nm on a spectrophotometer. There were three replicates for all experiments.

Statistical analysis

The experiment was laid out in completely randomized design (CRD). The least significant difference (LSD) test was used to compare treatment means at a 5% probability level. All the data were analyzed using analysis of variance (ANOVA) and all analyses were done in GenStat Discovery Edition 2012.

Results and discussion

Effectiveness of essential oils on the percent adult mortality of red rust flour beetle

The results provided in Table 1 indicated that peppermint oil had significant effect on the adult mortality of red rust flour beetle. Peppermint oil caused an adult mortality of 26.7% after one day when exposed to the concentration of 1%, while the mortality reached 50% during the same exposure period with 5% concentration. After 7 days of exposure, the 5% concentration caused the maximum adult mortality of 81.9%, whereas the mortality was found to be the lowest (54.1%) at a concentration of 1%, recorded after the same treatment period.

Table 1. Effect of different concentrations of essential oils on the percentage mortality of *T. castaneum* adults.

Essential oils	Concentrations	Mortality percentage				Mean
		After 1 day	After 3 days	After 5 days	After 7 days	
<i>M. piperita</i>	1%	26.7	36.7	48.9	54.1	41.6
	3%	46.7	53.3	55.6	68.2	55.9
	5%	50.0	56.7	57.8	81.9	61.6
	Mean	41.1	48.9	54.1	68.0	53.0
<i>L. nobilis</i>	1%	20.0	20.0	30.0	46.7	29.2
	3%	23.3	26.7	46.7	60.7	39.4
	5%	30.0	40.0	53.3	71.9	48.8
	Mean	24.4	28.9	43.3	59.8	39.1
Grand Mean		32.8	38.9	48.7	63.9	46.1

LSD (0.05) for oils = 7.44, Concentrations = 9.11, days = 10.5 and oil $\times$ concentration $\times$ day = 18.23.

Likewise, bay oil showed insecticidal activity against the beetle adults. Percent adult mortality reached 20% and 30% respectively after one day of exposure at concentrations of 1% and 5%. The percent mortality increased gradually with an increase in the treatment period and after 7 days of exposure, it reached 46.7 and 71.9% respectively at concentrations of 1 and 5%. Statistical analysis showed significant variations in mortalities among the tested treatments. Generally, essential oil of peppermint showed more effectiveness as compared to bay oil causing mortalities of 53% and

39.1% respectively.

The toxic and antifeedant effects of *M. piperita* oil are attributed to the volatile compounds such as menthol, pulegone, and menthone, which are monoterpenoid compounds. These compounds possess insecticidal properties because of their ability to penetrate the insect body and interrupt their physiological functions, especially disruption of the nervous system (Bachrouch et al., 2015; Saeidi and Mirfakhraie, 2017).

In Iraq, 38 active compounds were identified in peppermint oil. Among these identified compounds, 2-

Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-, known as (R)-carvone, and D-Limonene, belonging to the monoterpene compound group, were the most prominent active constituents (Mohammed et al., 2025). *M. piperita* oil has, therefore, been identified as a promising bio-insecticide due of its neurotoxic and fumigant effects against a large number of stored-grain insect pests.

In addition, *L. nobilis* oil has been reported to have strong insecticidal activity. In their study, El-Baghazaou et al. (2024) revealed that 70-100% cowpea bruchid adults were killed by laurel oil after 96 h of exposure. The aromatic compounds with high fumigant properties such as linalool and 1,8-cineole are thought to be responsible for the toxic and repellent activity of the oil. The toxicity of oil was attributed mainly to the high binding affinity of 1,8-cineole to the active site of the enzyme acetylcholinesterase, which results in inhibition of the degradation of the neurotransmitter acetylcholine. In the same way, Ali et al., (2025) found that the major active compounds in the extracts of laurel leaves collected from Iraq were eucalyptol (1,8-cineole) and α -terpinyl acetate.

Lethal Concentration (LC₅₀)

Table 2 shows the probit analysis data, which revealed that the adults of the red rust flour beetle were more susceptible to the essential oil of *M. piperita* than to the essential oil of *L. nobilis*. The LC₅₀ value for *M. piperita* oil was 4832.31 ppm (0.48%), whereas *L. nobilis* oil recorded a substantially higher LC₅₀ value of 24,744.23 ppm (2.47%).

The probit regression lines showed that the response of the insect individuals to *M. piperita* oil was more heterogeneous (slope = 0.49) than to *L. nobilis* oil (slope = 0.86). There were no significant differences between observed and expected mortality values with both oils, as the chi-square goodness-of-fit test was not significant for either oil (P = 0.93 and 0.98, respectively). The results demonstrate the suitability of the probit model for the experimental data and the statistical reliability of the values of the estimated LC₅₀. However, the relatively wide fiducial limits indicate that there is some variation within the biological response of the insects tested (Figure 1 and 2).

Table 2. Estimated LC₅₀ values, slope, and 95% fiducial confidence intervals derived from probity analysis of two extracts under different concentration treatments.

Essential oils	Slope	LC50 PPM	95%Fiducial CI		Chi-test (χ^2) Sig
			Lower	Upper	
<i>M. piperita</i>	0.49	4832.306	548.174	42598.14	0.93 N.S
<i>L. nobilis</i>	0.86	24744.23	7306.169	83802.75	0.98 N.S

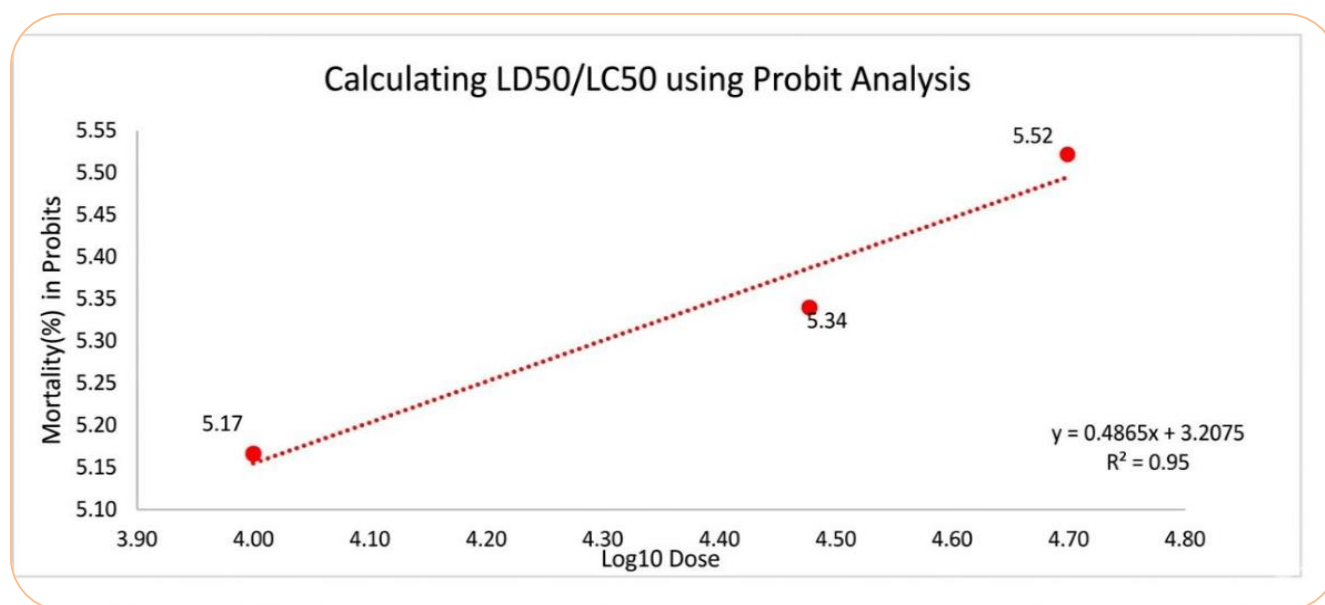


Figure 1. Relationship between Log₁₀ concentration and probity mortality when treated with different concentrations of the essential oil of *M. piperita*.

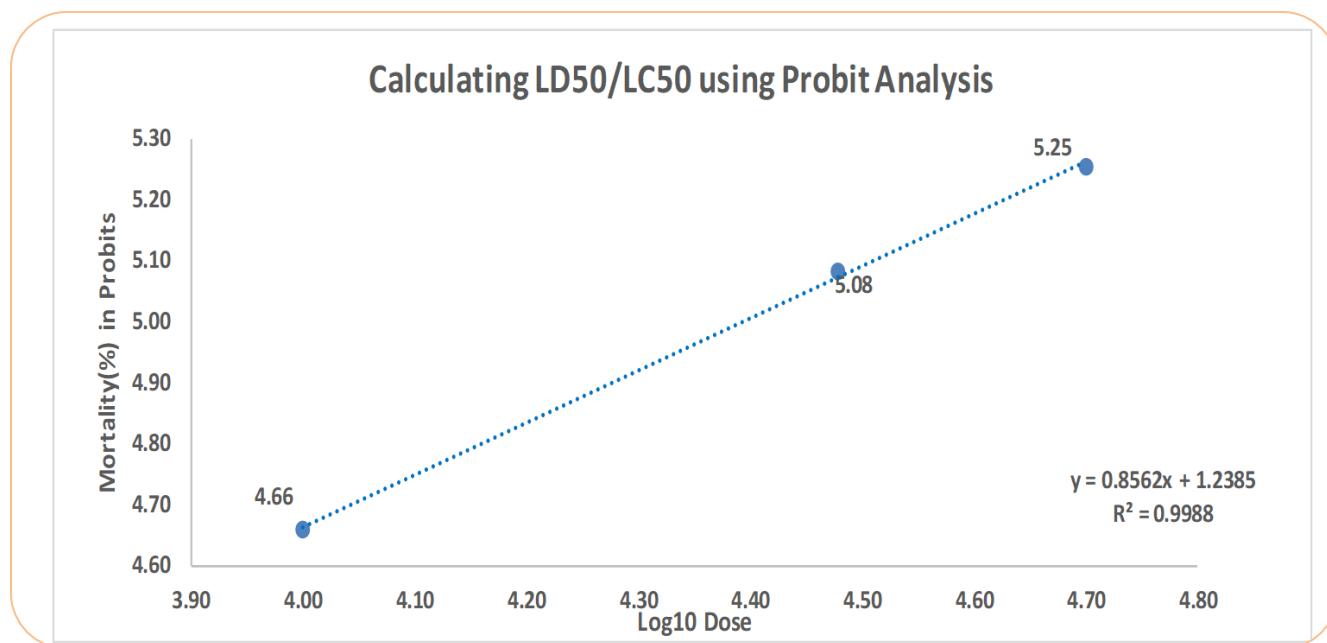


Figure 2. Relationship between Log₁₀ concentration and probity mortality when treated with different concentrations of *L. nobilis*.

Synergistic effect of temperatures and essential oils on the mortality of *T. castaneum* adults

The results provided in Table 3 showed that temperature had significant effect on the adult mortality of *T. castaneum*, and this effect on adult mortality increased as the temperature increased. The mortality rate was 26.6% after exposure to 37°C and rose to 38.6% after exposure to 45°C. The combined treatments of essential oils with elevated temperatures had remarkably higher mortality than the elevated temperatures alone. Of all the tested oils, *M. piperita* oil showed the highest efficacy with mortality of 71.6% at 37°C and 85.0% at 45°C. On the other hand, the combined effects of *L. nobilis* oil and the other treatments caused a mortality of 55.0% at 37°C and 76.6% at 45°C. The results are quite clear that multiple control strategies should be implemented to achieve greater mortality using lower concentrations of plant oils and shorter exposure times to reduce potential harmful effects on stored products.

The use of essential oils to control stored-product insects is regarded as environmentally-benign strategy due to their low persistence and accumulation levels in the atmosphere. The volatile bioactive compounds present in essential oils are fumigant insecticidal agents, which make them ideal for enclosed storage. In addition, higher temperatures can boost the effectiveness of these

oils by accelerating volatilization of active constituents from the oil, and by facilitating permeation of the insect cuticle. Furthermore, heat stress can aggravate the toxicity of these compounds by interfering with detoxification and/or oxidative equilibrium in the insect, resulting in higher death rates.

According to Pointer et al. (2021), the mortality rate of insects exposed to high temperatures can be caused by "severe stress and oxygen deficiency causing increased metabolic respiration", causing a higher mortality rates. Likewise, Oaya and Barde (2024) showed that high temperatures are very effective against stored-product pests. They found that exposure of red rust flour beetle for 15 min to 50°C resulted in 13.2% mortality while 20 and 25 min exposures caused 65% and 100% mortalities respectively. The 100% mortality was reached within 10 min when the sorghum flour was exposed to 70°C without having adverse effect on fine sorghum flour.

In another study, Modi (2025) evaluated the acclimatization capacity of red rust flour beetle under various temperature and humidity levels. The findings revealed a reduction in survival rates of adults after exposure to 45°C as compared to 28°C (control treatment) which showed a survival rate of 95%. The higher oxygen consumption and metabolic rate at higher temperatures was attributed to the higher demand for survival energy under the thermal stress condition.

Table 3. Percentage mortality of *T. castaneum* adults at different temperatures and synergistic treatments.

Temperatures	% mortality in treatments			Mean
	Temperature (°C)	Tem.+ <i>M. piperita</i> oil (LC50)	Tem.+ <i>L. nobilis</i> oil (LC50)	
30°C	0.	50.0	50.0	33.3
37 °C	26.6	71.6	55.0	51.1
45 °C	38.6	85.0	76.6	66.7
Mean	21.7	68.8	66.5	50.4

LSD (0.05) for treatments = 4.33, temperature = 4.33, temperature × treatments = 7.50.

Effect of treatments on the physiological activity of some oxidation enzymes

The oxidative enzyme activity changes in *T. castaneum* adults were significantly affected by the different temperature and lethal concentrations of essential oils as found in the results presented in Table 4. The activity of glutathione-S-transferase (GST) increased with the

highest activity seen in the group treated at 45°C (2.27 unit/mg protein), synergistic treated with *M. piperita* oil (2.42 unit/mg protein) and synergistic treated with *L. nobilis* oil (2.26 unit/mg protein). In the same way, catalase activity also showed significant enhancement under the same treatments, 5.67, 6.67 and 5.33 unit/mg proteins respectively.

Table 4. Physiological effects of temperatures and essential oils on the activity of some enzymes.

Temperatures	Activity of glutathione-S-transferases (unit/mg protein)			Mean
	Temperature	Temperature + <i>M. piperita</i> oil (LC ₅₀)	Temperature + <i>L. nobili</i> oil (LC ₅₀)	
30°C	1.20	1.58	1.45	1.41
37 °C	1.86	2.23	2.00	2.03
45 °C	2.27	2.42	2.26	2.32
Mean	1.78	2.08	1.90	1.92

LSD (0.05) for treatments = 0.16, temperature = 0.16, temperature × treatments = 0.29

Temperatures	Activity of Catalase (unite/mg protein)			Mean
	Temperature	Temperature + <i>M. piperita</i> oil (LC ₅₀)	Temperagure + <i>L. nobili</i> oil (LC ₅₀)	
30°C	2.00	4.33	3.00	3.11
37 °C	2.67	5.33	4.33	4.11
45 °C	5.67	6.67	5.33	5.89
Mean	3.44	5.44	4.22	4.37

LSD (0.05) for treatments = 0.80, temperature = 0.80, temperature × treatments = 1.39

Temperatures	Activity of peroxidases (unite/mg protein)			Mean
	Temperature	Temperature + <i>M. piperita</i> oil (LC ₅₀)	Temperautre + <i>L. nobili</i> oil (LC ₅₀)	
30°C	0.90	1.00	0.93	0.94
37 °C	1.16	1.26	1.16	1.20
45 °C	1.39	1.50	1.40	1.43
Mean	1.15	1.25	1.16	1.17

LSD (0.05) for treatments = 0.28, temperature = 0.28, temperature × treatments = 0.48

There was also an increase in peroxidase activity under thermal stress and when exposed to toxic volatile compounds from essential oils. Generally, higher

temperatures will lead to a greater metabolic rate and hence greater enzymatic reactions as insects strive to keep their physiological levels under stress. Such

investigations are important for understanding the physiological and biochemical responses of insect pests to different stress factors.

Iqbal et al. (2025) found that the activities of various enzymes have significantly altered in *T. castaneum* after the treatment with the clove oil and its nanoemulsion. The catalase, trehalase, glutathione S-transferase and invertase activities of treated adults were increased. The authors hypothesized that high level of catalase activity may be due to the insect's defense against the oxidation stress caused by the exposure to oil, as catalase degrades the reactive oxygen species. The glutathione S-transferase is an important enzyme in the detoxification pathway and in protecting cells from oxidative stress, such as the inactivation of toxic lipid peroxidation products produced under thermal stress. It was also thought that the rise in invertase activity indicated a physiological response to the stress situation.

Conclusion

Based on the present results, it can be concluded that essential oil and higher temperature caused mortality of red rust flour beetle adults; *M. piperita* oil being more effective than *L. nobilis* oil. The tested temperatures were found to be most effective at 45°C. When insects were exposed to increased temperatures and toxic chemicals, oxidative stress was induced and several enzyme activities were significantly changed, suggesting that changes in the activities of these enzymes are physiological defense mechanisms against heat stress. Thus, a combination of pest management methods could be an efficient and environmentally friendly way of controlling stored-product insects.

Author's contributions

The contributed to the conceptualization and design of the study, conducted the laboratory experiments, collected and analyzed the data, interpreted the results, reviewed the relevant literature, and prepared and revised the manuscript for publication.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

Policy Addressed

Plant-based essential oils (*Mentha piperita* and *Laurus nobilis*), especially when combined with heat treatment, should be promoted as eco-friendly alternatives to synthetic insecticides in stored-grain protection. Their inclusion in IPM policies can reduce chemical residues and environmental risks while improving control of *Tribolium castaneum* in storage systems.

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