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Evaluating the efficacy of *Monarda citriodora* essential oil as a biorational against *Rhyzopertha dominica* infestation in stored sorghum and pearl millet

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A R T I C L E I N F O

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A B S T R A C T

Sorghum and pearl millet are vital sources of food and fodder in semi-arid regions, prone to infestation by stored grain insect pests. Essential oil (EO) from the Lamiaceae family plants is gaining attention because of its effectiveness against these pests. We report for the first time on the utilization of essential oil from *Monarda citriodora* (MC-EO) against *Rhyzopertha dominica* (RD), a significant pest prevalent in both stored sorghum and pearl millet. The current work highlights fumigant and contact bioassays (without and with grains), germination ability of post-treated grains, EO residual analysis, and *in vitro* enzyme assays (AChE and GST). The *M. citriodora* exhibits fumigant (FT) and contact (CT) toxicity in 12 h with an LC_{50} of 48.94 μL/L and 0.005 $\mu L/cm^{2,}$ respectively. Moreover, with sorghum and pearl millet for 168 h, FT was 494 μL/L and 272.68 μL/L. The EO treatment showed no residues on grains without any significant change in germination. Inhibition (*in vitro*) of AChE and GST activity was noticed with 10 % EO. Computational studies with the major components of EO (thymol and *o*-cymene) showed the binding affinities towards RD AChE and observed to be −6.1 kcal/mol and −5.5 kcal/mol, respectively. Hence, *M. citriodora* EO can be a good biorational candidate against *R. dominica*.

1. Introduction

Millets are considered super crops because of their resistance to extreme drought conditions, where dominant cereals like rice and wheat have low productivity (Hegde et al., 2025). They belong to the Poaceae family and are mainly cultivated in dry and semi-arid regions in Asia and Africa. Millets are more feasible for cultivation as they are harvested nearly 65 days after sowing and thus serve as a staple for larger populations in middle and lower-income countries (Joshi et al., 2025). Millets are also called as Nutri-cereals because of their super-nutritional content and diverse phytochemical composition. They are grabbing global attention because of their naturally gluten-free nature with higher fibre, various vitamins, minerals, and more unsaturated fatty acid content (Antony Ceasar and Maharajan, 2022). Millet farming can effectively address global warming, carbon credits, and climate change. India, Ethiopia, the USA, China, and Nigeria produce most of the millet in the world.

Millets are classified as major and minor millets. The major class includes sorghum, pearl, and finger millet, whereas the minor class consists of proso, foxtail, barnyard, and little millet. Each millet is specific in its nutrient content. In this study, we focused on sorghum (*Sorghum bicolor* (L.) M) and pearl millet (*Pennisetum glaucum* (L.)). Sorghum is a larger grain millet that can be grown in diverse soil types, including drylands (Madhusudhana et al., 2025). Sorghum, commonly called Jowar in India, is preferred as food amongst other millets. It contains complex carbohydrates and magnesium, which can regulate calcium absorption (Suman and Chandra, 2024). On the other hand, Pearl millet, known as Bajra in India, shares a significant portion of production and is more tolerant than any other millet. It has a smaller grain than sorghum, can grow in low-fertility soils with drylands, and carries higher iron, phosphorus, and fibre content with the lowest glycemic index. Millet grains are considered coarse grains and possess a tougher pericarp. However, during prolonged storage, millets are prone to infestation with stored product insect pests such as *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, and *Oryzaephilus surinamensis* (Chandrashekar and Satyanarayana, 2006). *S. Oryzae* and *R. dominica* cause primary infestation prevalent in the field samples of sorghum and pearl millet (Swamy and Wesley, 2021). *R. dominica* (F.) (Coleoptera: Bostrichidae), commonly identified as the lesser grain borer, attacks stored cereal grains, mainly wheat, rice, soybean,

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sorghum, and pearl millet, by feeding on the germ portion of the grain. Its prominent flying ability makes it the best candidate for infesting grains across a vast geographic area, unlike other stored grain pests (Edde, 2012). Even though other pests attack stored grains, the infestation share *R. dominica* possesses is nearly 40 %, which causes substantial grain loss and leads to nutritional and economic loss altogether (Ren et al., 2023). Grain damage by *R. dominica* serves as the invitation for the secondary pests and microbial load. To overcome the loss caused by *R. dominica* infestation, various methodologies are being followed by the farmers and stakeholders. Curative methods such as fumigation by phosphine and prophylactic solutions of pyrethroid like deltamethrin are extensively used (Ramírez-Cabrera et al., 2024). Improper disinfestation methodologies lead to the development of resistance in insect species towards insecticides. The development of phosphine resistance in *R. dominica* and *T. castaneum* was detected early in the 1970s and was stabilized in the larger population (Daglish et al., 2024). Present disinfestation methodologies are associated with many drawbacks, including toxicity towards humans and other non-target organisms, biomagnification, global warming, and, as mentioned before, the development of resistance, which necessitates the development of novel strategies to combat storage insect pest infestation. Many efforts were made to eradicate insect pest infestation from the commodity, including gamma radiation, ozone, and high CO_2 treatment. Apart from these, many biorationals are gaining attention because of their broad-spectrum action towards insect pests, low non-target toxicity, and low environmental impact. Among these, essential oils became choice because they belong to the Generally Recognized as Safe (GRAS) category of substances. Therefore, their feasibility increased in application towards the stored grains (Rashmi et al., 2024).

The Monarda genus belongs to the Lamiaceae family (Mint family), consisting of around 18 species of flowering plants reported in Mexico and the USA. It is already documented for its antibacterial, antifungal, nematoxic, phytotoxic, antiseborrheic, repellent, antilipase, anti-cancer, antiplasmodial, and antioxidant properties (Ghosh et al., 2020). *Monarda citriodora* Cerv. ex Lag, commonly called lemon beebalm, is an ornamental plant native to the southern US and northern Mexico. In contrast, scientists at the Indian Institute of Integrative Medicine (CSIR), Jammu, introduced it to India from Europe (Meena and Gochar, 2018). It is an annual plant with an average height of 3 ft, with white, pink, and purple-coloured flowers. The species is rich in thymol and *o*-cymene, which give aromatic properties to its essential oil. Because of monoterpenes, it shows prominent insect-repellent and anti-microbial properties (Lu et al., 2011). The phytochemicals from *M. citriodora* are used mainly for culinary and cosmetic purposes, as in perfumery, making it a good candidate for industrial farming (Verma and Chandra, 2013).

Even though the species of Monarda possess many biological activities, a comprehensive study on the biorational potential of *M. citriodora* against *R. dominica*, a primary pest of sorghum and pearl millet, has not been reported. The present study uncovers the role of *M. citriodora* essential oil as a biorational candidate and its importance in mitigating the infestation of *R. dominica* in stored sorghum and pearl millet.

2. Materials and methodology

2.1. Chemicals

The pure commercial-grade *M. citrioda* essential oil was procured from Nishant Aromas, Mumbai (Lot No. MCONAPL0623, GC% Purity – Thymol content minimum 45 %). L-glutathione reduced (G4251-1G) CAS-97-00-7 Lot #SLBZ6732,1-Chloro-2,4-dinitrobenzene (237329-10G) CAS-70-18-8 Lot #BCBN7826V, Acetylthiocholine iodide (A5751-5G) CAS-No:1866-15-5 Lot#BCCJ0961, 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) (D8130-1G) CAS No. 69-78-3 Lot#0000196702 above chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA)., Physostigmine salicylate (RM2173-100 MG) CAS No. 57-64-7 was purchased from HiMedia (Bengaluru, Karnataka, India). , n-Hexane (95 %) HPLC grade from SRL chemicals.

2.2. Insect culture

The insect culture of *R. dominica* used in the experiments had been maintained at 30 ± 2 °C with a relative humidity of 75 ± 5 % and 11:13 (light: dark) photoperiodic conditions in the insect culture room of the Food Protectants and Infestation Control department at CSIR-CFTRI. The tested insects were reared with high-quality sorghum grains sourced from Vasuvamba Food Needs, Mysore. Sequential culturing was carried out, and unsexed adult insects aged 7–10 days were collected for the experiments (Oppert and Morgan, 2013).

2.3. GC-MS analysis of essential oil

M. citriodora essential oil was procured from Nishant Aromas and was analysed using M/s Agilent Technologies, USA Gas Chromatography-Mass Spectrometer Model 8890 GC, 5977C MSD equipment with acquisition software Openlab CDS: version 2.7. The chemical profiling of essential oil was conducted following the method slightly modification to the method as described in Bincy et al. (2023). A total of 10 μL EO was dissolved in 990 μL of HPLC-grade n-Hexane, from which 1 μL of sample with a split ratio of 1:20 was injected into the multimode inlet and kept at 250 °C. Analysis was performed with an HP-5MS column; Part number 19091S-433, 30 m length x 0.25 mm diameter x 0.25 μm film thickness, with a total run time of 38 min. Helium gas was the carrier gas with a 1 ml/min flow rate. The initial oven temperature was maintained at 40^0C, kept for 0 min hold and increased to 120^0C at the rate of 10^0 C/min with a hold of 1 min and further increased to 180^0C at the rate of 3^0 C/min and hold for 2 min and finally increased to 290^0C at the rate of 25^0 C/min and hold for 2.6 min at 290^0C. A solvent delay of 5 min was adopted. The source and quadrupole temperatures were set at 230^0C and 150^0C, respectively, and compounds were scanned between 40 m/z and 400 m/z. The collected data were analysed using NIST 2023 version 3; Mass Spectral Database.

2.4. Fumigant toxicity assay with *M. citriodora* essential oil

The efficacy of essential oil as a fumigant was checked on *R. dominica* using the protocol given by Remesh et al. (2022). The experimental setup consists of a 50 ml glass tube, a 5 cm × 1 cm filter paper strip stuck to the lid of the glass tube, impregnated with different volumes of pure essential oil. The filter paper without essential oil served as the negative control for the experiment. The volumes of essential oils 1 μL, 2 μL, 4 μL, 6 μL, 8 μL, and 10 μL, corresponding to 20 μL/L, 40 μL/L, 80 μL/L, 120 μL/L, 160 μL/L, and 200 μL/L, respectively, were tested against the insects. The time duration of the experiment was kept at 12 h and 24 h for the assay without grains.

The fumigant bioassay with sorghum and pearl millet grains was carried out. For this assay, 10 gm of grains of each millet and 10 mixed-aged male and female (unsexed) individuals of *R. dominica* were taken in a 30 ml glass tube, and the treatment was given on filter paper as suggested above. The Dose-response relationship was established for 5 μL, 10 μL, 15 μL, 20 μL, and 25 μL, corresponding to 166.66 μL/L, 333.33 μL/L, 500 μL/L, 666.66 μL/L, and 833.33 μL/L for both the millets selected. The treatment was observed continuously for 168 h. The dead insects were observed to exhibit total mortality. The insects were examined under the stereomicroscope (Olympus) at fixed intervals. Immobile insects, showing no movement of their legs or antennae and not responding to a light probe, were classified as dead (Hou et al., 2019). The corrected mortality was calculated with the help of the Abbott formula (Abbott, 1925). The differentiation of insects as live and dead was done accordingly. A fumigant toxicity assay was carried out in three replicates and performed three times independently.

2.5. Insecticidal efficacy of a binary combination of *M. citriodora* EO

The insecticidal potential of *M. citriodora* EO combined with different EO and aroma compounds was evaluated using a fumigant bioassay (Ou et al., 2025). The binary combination of *M. citriodora* oil with *Ocimum gratissimum* (OG), lemongrass essential oil, citronella oil, estragole, and geraniol was tested in 75:25, 50:50, and 25:75 ratios. 5 μL corresponds to 100 μL/L of the combination, which was used in binary combination fumigation toxicity with 10 unsexed (male and female) adults of *R. dominica* in a 50 ml glass tube. Mortality was recorded after 12 h of treatment, and corrected mortality was calculated using the Abbott formula (Abbott, 1925).

2.6. Contact toxicity

The *M. citriodora* EO was checked for its contact toxicity towards the *R. dominica* following the methodology given by Kozhissery Sreekrishnakumar et al. (2025). A glass petri plate of 100 mm diameter was used for contact toxicity studies. Whatman filter paper no. 01 was placed inside the petri plate at the bottom, and treatment was given with the help of acetone as a carrier. The doses selected were 1 μL, 2 μL, 4 μL, 6 μL, 8 μL, and 10 μL, corresponding to 0.00318 μL/cm^2, 0.00639 μL/cm^2, 0.012 μL/cm^2, 0.0191 μL/cm^2, 0.0254 μL/cm^2, and 0.0318 μL/cm^{2,} respectively. 10 adult *R. dominica* were released inside the petri plate and sealed with parafilm to make it a closed system. The same assay was performed with both sorghum and pearl millet grains. 10 gm of grains were used in the assay for each millet. The experimental setup was the same as above. Essential oil treatment was used as 5 μL, 10 μL, 15 μL, 20 μL, and 25 μL (0.0159 μL/cm^2, 0.0318 μL/cm^2, 0.0477 μL/cm^2, 0.0636 μL/cm^2, and 0.0796 μL/cm^2). The assay was carried out for 168 h. The total number of dead individuals was counted in the control and treatment groups to get corrected mortality by using the Abbott formula (Abbott, 1925). A contact toxicity assay was carried out in three replicates and experiments were performed three times independently.

2.7. Seed germination assay of treated grains

The effect of essential oil treatment on sorghum and pearl millet grain germination was tested. After 20 days of treatment initiation, grains from the contact bioassay were subjected to a germination test using the method described by Ketoh et al. (2005) and Vendan et al. (2017). Grains were cleaned thoroughly with distilled water, and then 10 randomly selected grains from treated samples were selected and placed on moist Whatman filter paper inside a petri plate and observed till they germinated. The fresh grains were kept as a control for the assay.

2.8. Essential oil residue detection

The grains from the contact bioassay were tested for residual analysis of essential oil (Vendan et al., 2017). The grains were soaked in 10 ml of n-hexane to extract essential oil for 1 h. Then, the contents were filtered through the filter paper, and the filtrate was kept for evaporation for 3 days. After complete evaporation, the content was resuspended in n-hexane for analysis through GC-MS as described in the above methodology.

2.9. In vitro enzyme inhibition assays

In vitro activity of *M. citriodora* essential oil was evaluated against the enzyme extract of *R. dominica* using 1 %, 2.5 %, 5 %, 7.5 %, and 10 % of *M. citriodora* oil with ethanol as a vehicle. To get enzyme extract, 40 adults of *R. dominica* were crushed in ice-cold phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4 °C with a Remi table top cooling centrifuge. The supernatant served as the enzyme source, stored at −20 °C. For assessing the biological activity, the enzyme source was pre-incubated with 5 μL of the treatment at 37 °C for 30 min, and then processed with the individual enzyme assay (Ellman et al., 1961; Singh et al., 2023a; Bhatt et al., 2024).

2.9.1. Acetylcholine esterase inhibition assay

20 μL of 10 mM ATChI (in distilled water) was added to the pre-incubated enzyme solution, and the reaction mixture was incubated at 37 °C for 15 min. Next, 30 μL of 10 mM DTNB (in buffer) was introduced and incubated for 10 min at 37 °C. A yellow-coloured complex was formed at the end of the reaction, which was measured at 412 nm (Ellman et al., 1961) with the help of a Thermo Fisher Varioskan Flash spectral scanning multimode reader.

2.9.2. Glutathione-S-Transferase (GST)

The total reaction volume for the GST assay was 200 μL. The reaction was followed as the addition of substrate, 5 mM reduced Glutathione to the buffer, and 20 μL of 1 mM CDNB (In methanol) was added to start the reaction. As soon as CDNB was added, reading was taken at 314 nm for 15 min at an interval of 30 s with the help of Thermo Fisher Varioskan Flash spectral scanning multimode reader. The GST activity was represented in nmol/ml/min by taking 0.0096 as the molar extinction coefficient (Singh et al., 2023ab)

2.10. Molecular docking analysis

Protein modeling performed using AlfaFold2 software and UCSF Chimerax-daily. exe platform (Jumper et al., 2021; Mirdita et al., 2022; Meng et al., 2023). Thymol and *o*-cymene were selected as the ligands for this computational study. Ligand structures were sourced using PubChem and docked with RD AChE employing AutoDock Vina v1.2.5 (Trott, 2010; Eberhardt et al., 2021). The best alignment of these ligands with the highest binding affinity was visualized in Discovery Studio Visualizer.

2.11. Statistical analysis

All the experiments were performed three times with three different independent sets. All biological and technical triplicate data were processed through the IBM SPSS ver. 26 software. The statistical analysis consists of a one-way analysis of variance (ANOVA) with Tukey's test. Statistical significance between the control and *M. citriodora* EO-treated groups was evaluated at $p < 0.01$ and $p < 0.05$. where *, **, and ns read as significant, very significant, and non-significant. GraphPad Prism (version 9.4.1) software (GraphPad Inc., USA) was used to plot the graphs of a data set.

3. Results

3.1. GC-MS analysis of essential oil

The analysis of *M. citriodora* oil through Gas chromatography mass spectroscopy showed the major compounds in the EO (Fig. 1 and Table 1). Analysis showed the presence of Isothymol (Thymol) (IUPAC name- 2-Isopropyl-4-methylphenol) as a primary compound, from a total % area, it shared 49.71 %, while *o*-cymene accounted for 13.03 %. In contrast, OG showed 41.13 % thymol, lemongrass with 71.5 % citral, and citronella oil with 38 % geraniol, whereas aroma compounds such as geraniol and estragole were confirmed with 96.22 % and 95.47 % total area respectively (Suppl. Table 1).

3.2. Fumigant toxicity assay with *M. citriodora* essential oil

Fumigant bioassay with *M. citriodora* EO on *R. dominica* showed a linear dose-response relationship when tested with 1 μL, 2 μL, 4 μL, 6 μL, 8 μL and 10 μL, corresponding to 20 μL/L, 40 μL/L, 80 μL/L, 120 μL/L, 160 μL/L and 200 μL/L and denoted in graph in Fig. 2a. Which showed

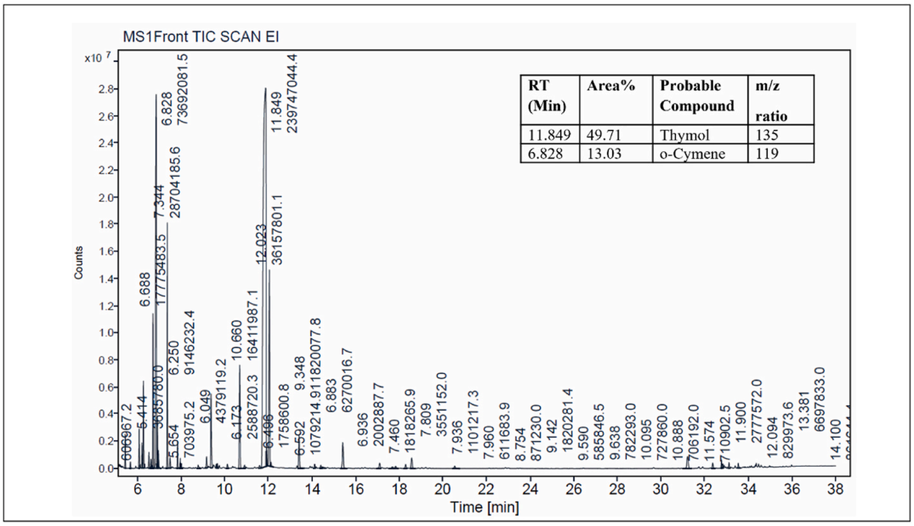

Fig. 1. Chromatogram of Characterisation of *M. citriodora* EO by gas-chromatography mass-spectroscopy.

Table 1
Chemical composition of *M. citriodora* EO detected through GC-MS.

Compound	RT (Min)	Area % ± SD
β-Pinene	6.25	2.12 ± 0.44
α-Terpinene	6.68	4.10 ± 0.81
o-Cymene	6.82	13.03 ± 2.25
γ-Terpinene	7.34	7.66 ± 2.78
Terpinen-4-ol	9.34	2.04 ± 0.43
p-Cymene	10.66	2.50 ± 1.06
Thymol	11.84	49.71 ± 3.04
5-isopropyl-m-cresol	12.02	6.75 ± 0.59

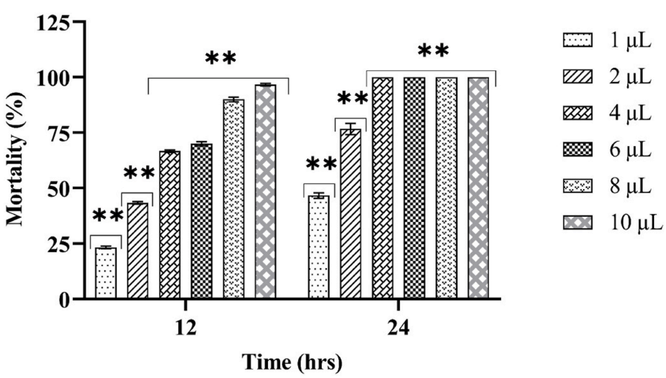

Fig. 2a. Fumigant toxicity of *M. citriodora* EO against *R. dominica* was shown by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, representing significance level respectively compared to control).

23.33 %, 43.33 %, 66.66 %, 70 %, 90 %, and 96.66 % mortality without food after 12 h. The fumigant toxicity after 24 h showed complete mortality with 80 μL/L onwards, whereas 46.66 % and 76.66 % for 20 μL/L and 40 μL/L, respectively. With probit analysis, we got lethal concentrations as 1.08 μL (21.6 μL/L) LC_{25}, 2.44 μL (48.8 μL/L) LC_{50}, 5.51 μL (110.2 μL/L) $LC_{75,}$ and 11.45 μL (229 μL/L) LC_{90} for 12 h.

With food commodity, in sorghum, as seen in Figs. 2b and 5 μL showed no mortality, 10 μL showed 13.33 %, 15 μL and 20 μL showed a maximum of 66.66 % and 70 % mortality, respectively, after 168 h. The most efficient treatment of all was 25 μL. It showed a mortality of 66.33 % at 96 h and reached 90 % at the end of the experiment. The lethal concentrations for 168 h were 11.24 μL (374.66 μL/L) LC_{25}, 14.82 (492 μL/L) LC_{50}, 19.53 (651 μL/L) $LC_{75,}$ and 25.04 (833.33 μL/L) LC_{90}. The response was more efficient with pearl millet, and results can be seen in Fig. 2c, Treatment of lower concentration like 5 μL and 10 μL showed 33.33 % and 76.66 % mortality whereas in 15 μL, 20 μL, and 25 μL, observed mortality was 83.33 %, 90 %, and 93.33 %, respectively. Lethal concentrations for 168 h can be calculated as 3.46 μL (161.74 μL/L) LC_{25}, 6.489 μL (272.6 μL) LC_{50}, 12.15 μL (459.73 μL/L) $LC_{75,}$ and 21.381 μL (735.63 μL) LC_{90}.

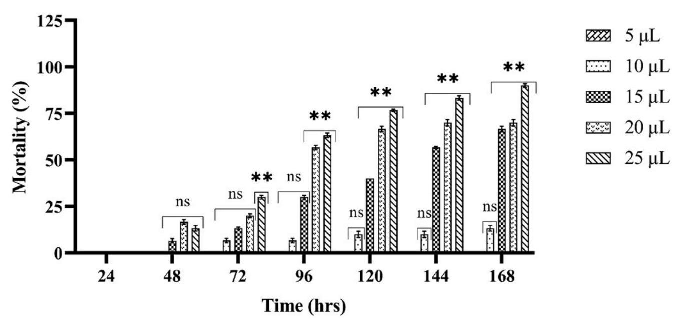

Fig. 2b. Fumigant toxicity of *M. citriodora* EO against *R. dominica* with sorghum showed by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, representing significance level compared to control).

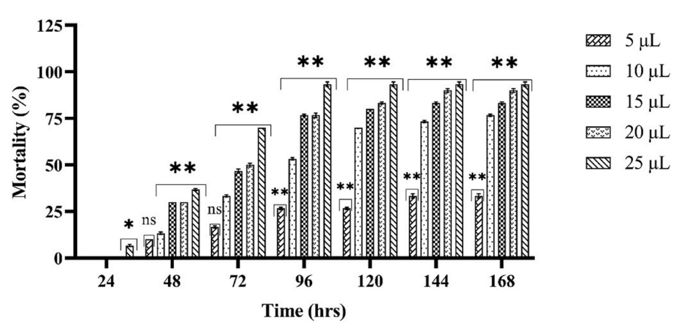

Fig. 2c. Fumigant toxicity of *M. citriodora* EO against *R. dominica* with pearl millet showed by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, representing significance level compared to control).

3.3. Insecticidal efficacy of a binary combination of *M. citriodora* EO

The efficacy of the ***M. citriodora*** EO with other phytochemicals as an insecticide was evaluated, and the results are depicted in Table 2. Three ratios of 3:1, 1:1, and 1:3 ***M. citriodora*** EO were blended with *Ocimum gratissimum*, lemongrass EO, geraniol, citronellol, and estragole. In the bioassay, we found that the ratio of ***M. citriodora*** EO with OG, lemongrass, geraniol, and citronellol acted antagonistically in contrast to the binary combination of ***M. citriodora*** EO with estragole, which exhibited an additive relationship.

3.4. Contact toxicity

Experimental results of contact toxicity of *M. citriodora* towards *R. dominica* can be seen in Fig. 3. It showed more efficient results than fumigant toxicity. Contact toxicity without food with 1 μL, 2 μL, and 4 μL can be seen as 23.33 %, 53.33 %, and 90 % respectively. Whereas 6 μL onwards showed complete mortality of test insects in 12 h with 1.09 μL (LC_{25}), 1.73 μL (LC_{50}), 2.76 μL (LC_{75}), and 4.19 μL (LC_{90}) being the lethal concentrations for 12 h. At the end of 24 h, 4 μL also showed 100 % mortality (Fig. 3a).

Contact toxicity of *M. citriodora* on *R. dominica* with sorghum is shown in Fig. 3b as a food source; early mortality of 46.66 % and 90 % with 5 μL and 10 μL, respectively, and complete mortality with 20 μL at the end of 24 h. After completion of the treatment period, 5 μL gave 79.12 %, and 10 μL gave complete mortality. Fig. 3c showed the contact toxicity of *M. citriodora* EO towards *R. dominica* in stored pearl millet, with completion of 24 h, 5 μL resulted in the least mortality of 6.66 %, and 10 μL, 15 μL, and 20 μL resulted in 73.33 %, 96.66 %, and 100 % mortality, respectively. A gradual increase in contact toxicity can be seen in the treatments, along with 56.66 %, 96.66 %, and 100 % mortality when checked for 5 μL, 10 μL, and 15 μL, respectively, at the end of treatment duration.

3.5. Essential oil residue detection

The residual analysis of *M. citriodora* EO-treated sorghum and pearl millet has been performed, and the results are depicted in the chromatogram (Suppl. Fig. 1 and 2). The GC-MS chromatograms of pure EO were compared with the EO-treated samples. When checked with grains after treatment, the chromatograms showed no significant individual peaks corresponding to the EO or the individual phytochemicals.

Table 2
Fumigant toxicity of binary combinations on *R. dominica*. (MC- *M. citriodora*, OG- *Ocimum gratissimum*, LG-Lemongrass, CT-Citronella oil, E-Estragole, G-Geraniol).

Binary Combinations		Mortality (%)		Chi^2	Effect
		Expected	Observed		
MC:OG	(75:25)	88.67	66.66	5.17	Antagonistic
	(50:50)		40	26.18	Antagonistic
	(25:75)		16.66	57.83	Antagonistic
MC:LG	(75:25)	83.33	46.66	15	Antagonistic
	(50:50)		0	83.33	Antagonistic
	(25:75)		0	83.33	Antagonistic
MC:CT	(75:25)	80	60	5	Antagonistic
	(50:50)		3.33	73.47	Antagonistic
	(25:75)		3.33	73.47	Antagonistic
MC:G	(75:25)	80	50	11.25	Antagonistic
	(50:50)		10	61.25	Antagonistic
	(25:75)		0	80	Antagonistic
MC:E	(75:25)	99.2	93.33	0.34	Additive
	(50:50)		83.33	2.53	Additive
	(25:75)		83.33	2.53	Additive

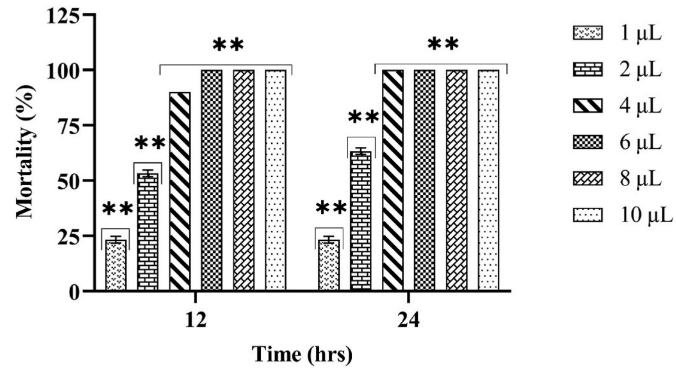

Fig. 3a. Contact toxicity of *M. citriodora* EO against *R. dominica* was shown by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, represented significance level compared to control).

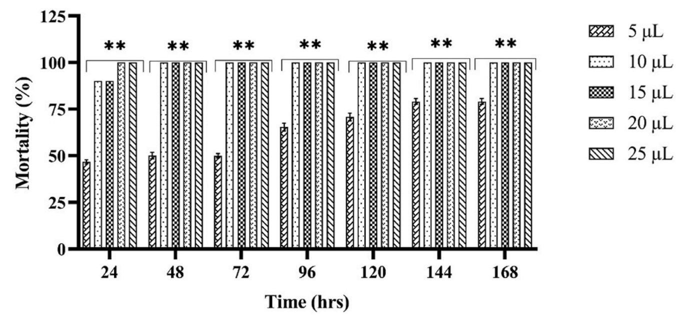

Fig. 3b. Contact toxicity of *M. citriodora* EO against *R. dominica* with sorghum showed by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, represented significance level compared to control).

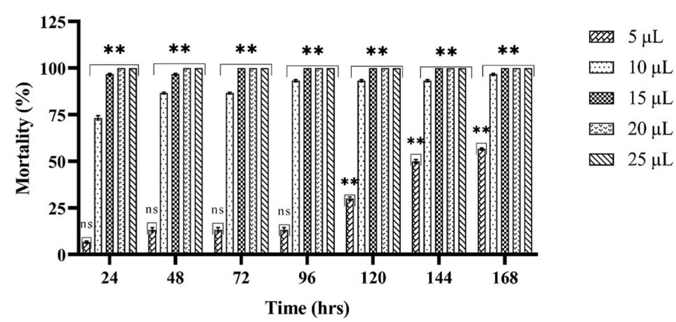

Fig. 3c. Contact toxicity of *M. citriodora* EO against *R. dominica* with pearl millet showed by % Mortality, and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, representing significance level compared to control).

3.6. Seed germination assay of treated grains

The germination capability of ***M. citriodora* EO**-treated sorghum and pearl millet grains is presented in Fig. 4. Fresh grains of sorghum and pearl millet attained a maximum of 90 % germination, whereas ***M. citriodora* EO**-treated grains showed 90 % and 80 % germination. In contrast, a control group showed 70 % and 66.66 % germination, respectively. There is no significant difference in the germination capability of grains after ***M. citriodora*** EO treatment.

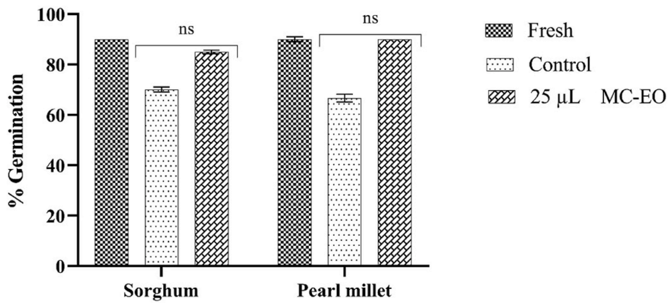

Fig. 4. % Germination of sorghum and pearl millet grains treated with *M. citriodora* EO and a mean value of triplicates is represented, with error bars denoting the mean ± SD. (*, **, and ns for $p < 0.05$, $p < 0.01$, and non-significant, respectively, representing significance level compared to fresh grains).

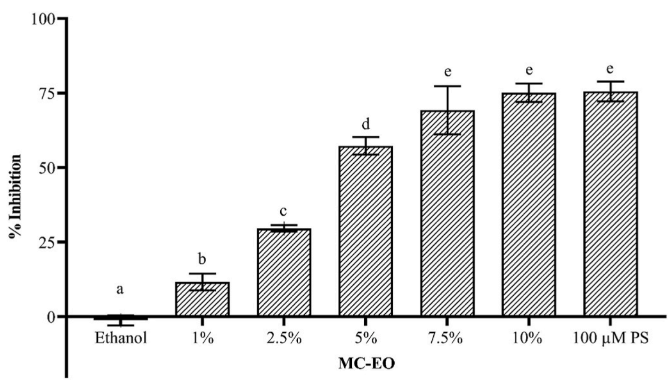

Fig. 5. *In vitro* % inhibition of *R. dominica* AChE by the *M. citriodora* EO and a mean value of triplicates is represented, with error bars denoting the mean ± SD (significant difference shown by different letters by Tukey's test at $p < 0.01$ between the groups).

3.7. Acetylcholine esterase inhibition assay

In the *in vitro* analysis of acetylcholine esterase inhibition, we observed a significant influence of the treatment on the enzyme system. Fig. 5 shows the overall inhibition % of AChE when treated with different doses of ***M. citriodora*** EO. The vehicle ethanol interfered non significantly in the reaction. The dose-dependent % inhibition of AChE was observed; 1 % ***M. citriodora*** EO showed the least inhibition of 11.62 %, doses of 2.5 %, 5 %, and 7.5 % ***M. citriodora*** EO resulted in 29.63 %, 57.305 % and 69.25 % inhibition, respectively, whereas 10 % and standard drug 100 μM physostigmine showed similar 75.14 % and 75.58 % inhibition of AChE.

3.8. Glutathione-S-transferase

Glutathione-S-Transferase, the enzymatic antioxidant system, was evaluated for its activity when treated with different doses of *M. citriodora* EO. *In vitro* investigation of GST indicated a significant decrease in GST activity, as seen in Fig. 6. The vehicle ethanol showed no changes in GST activity. The control reaction showed activity of 30.18 nmol/ml/min. When treated with 1 % *M. citriodora* EO, it reduced to 15.86 nmol/ml/min. The steady decrease can be seen in the consequent doses till the 10 % treatment, with the activity lowest at 9.25 nmol/ml/min. Overall, the treatment of *M. citriodora* EO inhibited GST activity significantly.

3.9. Molecular docking analysis

Thymol had a slightly higher binding energy of −6.1 kcal/mol

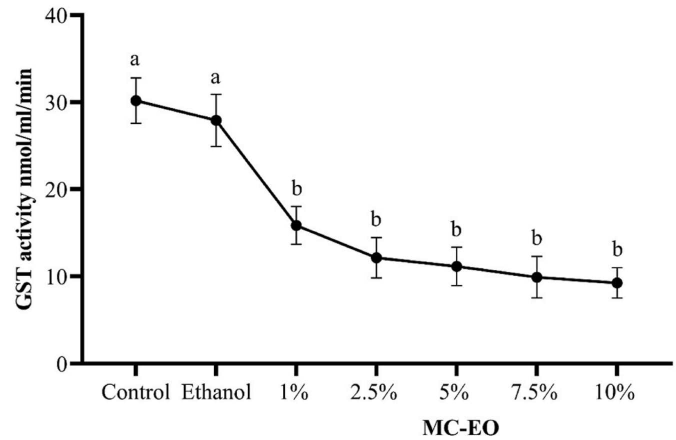

Fig. 6. *R. dominica* GST activity treated with M. citriodora EO *in vitro* and a mean value of triplicates is represented, with error bars denoting the mean ± SD (significant difference shown by different letters by Tukey's test at $p < 0.01$ between the treatments).

between the docked ligands, whereas *o*-cymene had an affinity of −5.5 kcal/mol. Thymol formed hydrogen bonds with LE 355, GLN 468, and Pi-Anion interaction with GLU 357. In contrast, *o*-cymene formed hydrogen bonds and hydrophobic interactions with TYR 467 (Fig. 7). The described protein-ligand interactions and their bond lengths and types can be inferred from the Suppl. Table 2.

4. Discussion

Primary feeders such as *S. oryzae* and *R. dominica* can flourish in stored sorghum and pearl millet. Their attack or any physical damage to the grains can invite secondary insect pests and mold formation, collectively impacting the yield, quality, and quantity (Rajendran and Sriranjini, 2024), leading to the overall reduction in the nutritional profile of these grains. When the choice test was done on different millets with *R. dominica* and *T. castaneum*, the results showed that the insects least preferred little and finger millets. In contrast, the insect species preferred sorghum, pearl, and foxtail millet, and the progeny development was more remarkable than in other millets (Swamy et al., 2023). The work by Mariadoss et al. (2024) checked the feeding preference of *R. dominica* towards the millets and observed that the feeding preference was towards sorghum and barnyard millet. The main contributing factor to these preferences is the higher sugar content than in other millets (Anagha, 2023). The size was the limiting factor for larval development in minor millets, and adults showed lesser attraction (Rajendran and Chayakumari, 2003).

Rhyzopertha dominica causes significant damage to stored wheat, rice, and sorghum grains. Many efforts have been taken to control the *R. dominica* infestation, including the chemical-based phosphine fumigation and deltamethrin treatment. However, because of their tolerance to these treatments, different groups started exploring eco-friendly options, including essential oils, especially from the Lamiaceae family. Other methods include entomopathogenic nematodes, diatomaceous earth, and amorphous silica, which have significant mortality in *R. dominica* (Manivannan and Subramanyam, 2023). Biopesticides such as essential oil and their significant compounds have been studied extensively for stored product insect pests, proving to have the least adverse effects and being eco-friendly towards nature. The Lamiaceae family, consisting of flowering plants, is vastly studied for their phytochemical contents and biological activities (Lokesh et al., 2024). For *R. dominica*, prominent fumigant toxicity was shown by the *Ocimum basilicum* (Estragole), Eucalyptus spp. (Eucalyptol), *Ocimum gratissimum* (Eugenol), *Citrus sinensis* (Limonene), and contact toxicity were shown by *Thymus quinquecostatus* (Anethole), *Trachyspermum ammi*

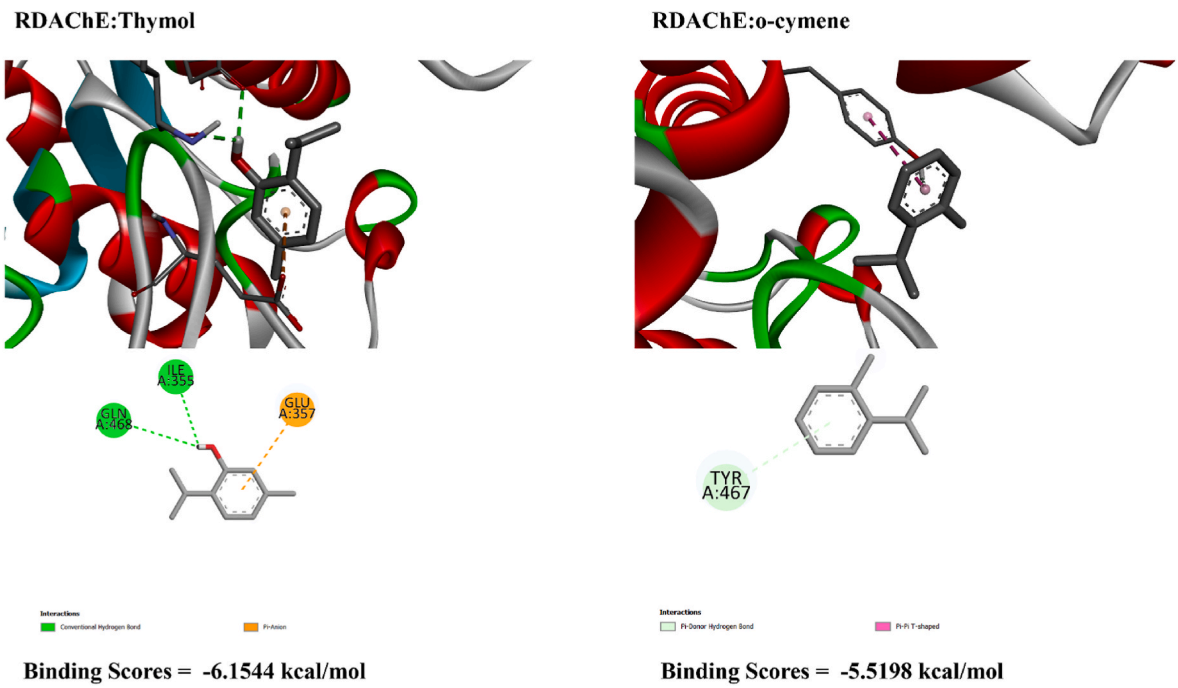

Fig. 7. 2D and 3D visualization of *R. dominica* AchE with docked ligands.

(Carvacrol), *Ocimum kilimandscharicum* (Eucalyptol), and *Syzygium aromaticum* (Eugenol). Almost all of the above components showed feeding deterrence and developmental and reproductive inhibition (Karabörklü and Ayvaz, 2023).

Monarda genus plants contain classes of compounds from monoterpenes, their alcohols, aldehyde forms, and sesquiterpenes (Rashmi et al., 2024). *M. citriodora*, commonly called lemon bee balm, shows tolerance towards cold climatic conditions and hence can be found mainly in colder regions of India like Jammu Kashmir, Himachal Pradesh, and Uttaranchal (Mazza et al., 1987). The literature suggests the presence of thymol as a principal component in the *M. citriodora* EO, but with varying percentages. Thymol content changes according to the climatic conditions and harvesting. The thymol component can share 17.83 %–82.29 % of the total essential oil analysed (Pathania et al., 2013; Grzeszczuk et al., 2020), whereas our reports showed the presence of 49.71 % of thymol in EO which coincides with the results of Lu et al. (2011), other primary compound detected during the study was *o*-cymene with means area of 13.03 %; which can be validated with the study done by Rashmi et al. (2024), where they got 17.92 % cymene content. There was no significant difference between the chemical composition of EO extracted from the different parts of the *M. citriodora* plant, like from leaves and flower the thymol content obtained was 55.10 % and 60.56 % respectively. Secondary compounds like *p*-cymene were 14.90 % and 9.34 % in leaves and flowers, respectively (Rashmi et al., 2024). *M. citriodora* can be considered the thymol-rich EO because thymol is the sole component, taking nearly 50 % share. Significantly fewer plants were observed with this high amount of thymol, except for *Zataria multiflora* Boiss. (47.5 %), *Thymus vulgaris* (48.9 %), *Thymus algeriensis* (56 %), and *Origanum glandulous* Desf. (41.6–81.1 %) (Escobar et al., 2020). Because of their diverse monoterpenes' contents, Monarda species possess diverse biological activities. All species contain mostly thymol and *o*-cymene as their active components. *M. didyma* and *M. fistulosa* were studied for anti-microbial activity, whereas insect repellent property was explored in *M. fistulosa* and *M. bradburiana* (Tabanca et al., 2013). The studies reported anti-microbial, antibacterial, antifungal, and *in vitro* anti-cancer activities of *M. citriodora* (Lu et al., 2011; Di Vito et al., 2019; Deepika et al., 2020). While considering the Monarda genus, leaves of *M. didyma* were given to the growing larvae of fall armyworm (*Spodoptera frugiperda*), it showed feeding deterrence and no choice towards the leaves, whereas an inhibitory effect was seen in the overall larval growth (Rabinowitz et al., 2015). Furthermore, hydrolate of the same showed potent activity toward the field pest *Trialeurodes vaporariorum* (Mariani et al., 2020). The EO and ethanolic extract from *M. fistulosa* were explored for their insecticidal activity towards the Colorado potato beetle and showed significant ovicidal, antifeedant, and overall insecticidal activity (Elisoveţ;caia et al., 2018). Silver nitrate nanoparticles synthesized from *M. citriodora* essential oil were tested for their efficacy on different microbes, such as *Bacillus cereus* and *Micrococcus luteus*, which showed promising results (Yadav et al., 2024). *M. citriodora* EO inhibited the storage grain molds, including *Aspergillus flavus* and 15 other molds (Deepika et al., 2020). However, there is no report of testing *M. citriodora* EO against the storage pest insect, so we evaluated its activity, and we got significant fumigant and contact toxicity results against the test *R. dominica* within 12 and 24h, requiring less volume to have good insecticidal activity when no food source was considered.

Thymol in the *M. citriodora* EO accounts for its bioactivity, as thymol-rich EO showed good results against the stored product insects. *Origanum vulgare* and *Mosla chinensis* share thymol as a major compound and showed fumigant toxicity against *Ephestia kuehniella* and *S. zeamais* (Erler, 2005; Kim et al., 2010). EOs from *O. vulgare* and *T. ammi* exhibited substantial contact toxicity towards *R. dominica*, and the thymol in oils played an important role in the activity (Kanda et al., 2017). The fumigant toxicity of *M. citriodora* with sorghum and pearl millet showed differential mortality rates. In pearl millet, we can see more effectiveness, at the end of 168 h, a minimum of 10 μL of oil treatment showed nearly 75 % mortality of adult *R. dominica*, whereas, in sorghum, we required higher volumes. When the contact toxicity studies were done on *R. dominica* and *T. castaneum*, thymol showed effectiveness and was more toxic than other aroma compounds tested (Kanda et al., 2017). The same results can also be seen in our results, as *M. citriodora* is highly rich in thymol content; higher mortality can be seen in sorghum with a minimum of 10 μL dose after 24 h. At the end of the 168 h, a 5 μL dose gave more than 75 % mortality, and all others showed complete mortality. In pearl millet, the same trend can be seen. The above results showed the effectiveness of *M. citriodora* against the

R. dominica infesting sorghum, and pearl millet and can be a good candidate for fumigant and contact insecticide. Binary combinations of EOs with other EOs or aroma compounds can be a broad-spectrum strategy for combating pests. A study by Kanda et al. (2017) showed that thymol is least effective towards *S. oryzae*. In contrast, linalool was toxic towards *S. orzyae* but not others, so using a binary combination served as toxic to all the insect species tested. Our study tested the binary combination of *M. citriodora* EO with *O. gratissimum*, lemongrass, citronella oil, geraniol, and estragole to check their interaction in insect mortality. The results showed that combining *M. citriodora* oil with other oils and aroma compounds showed an antagonistic effect, causing lesser mortality of *R. dominica* than expected. Compared to this, the estragole combination showed an additive effect but no synergistic effect, so using *M. citriodora* alone can serve the best. The post-treated grains were tested by GC-MS for the persistence of EO residues on the sorghum and pearl millet grains and showed no traces of the compounds, making it a good candidate as a grain protectant and can usually be used for the grains for export purposes. Moreover, the treatment did not hamper germination, as demonstrated with the *Rosmarinus officinalis* EO treatment (Kiran and Prakash, 2015).

The enzyme system of the insect body plays an important role in detoxifying foreign material or toxins. As per the literature, EO specifically targets the nervous system of insects by targeting acetylcholinesterase. Other modes include inhibition of P450 cytochrome, different ATPases, cholinergic system, modulation of GABA receptors, and octopaminergic system (Karaborklü and Ayvaz, 2023). We have assessed the effect of *M. citriodora* EO on the AChE as well as a detoxifying enzyme called Glutathione-S-Transferase. Acetylcholine esterase is a primary site for synthetic insecticides where the acting component binds to the Acetylcholine esterase enzyme and inhibits its activity, resulting in the accumulation of messenger signal acetylcholine at the synapse, leading to continuous signal transduction, making the insect hyperactive and finally collapsing of the nervous system (Nascimento et al., 2024). One of the actions of EO is the inhibition of AChE, as already reported in the literature. Major compounds associated with the inhibition can be listed as anethole, carvacrol, carvone, cinnamaldehyde, citral, citronellol, eucalyptol, α-pinene, etc. (Karaborklü and Ayvaz, 2023). In *R. dominica*, linalool, fenchone, and γ-terpine cause AChE inhibition (López et al., 2008). When we checked the effect of *M. citriodora* EO with different concentrations on the enzyme extracted from *R. dominica*, a dose-associated response was observed *in vitro*. The treatment of 10 % ***M. citriodora*** EO showed maximum AChE inhibition of 75 %, comparable with the standard drug donepezil, which is suggested as a medication for inhibiting AChE in Alzheimer's (Dhage et al., 2021). Thymol shows AChE inhibitory properties when checked in neurodegenerative disease rat models (Hamdan et al., 2022). The extract of *M. fistulosa* showed inhibitory activity when tested against AChE (Georgiev et al., 2022). Molecular docking with *Tetronarce californica* AChE enzyme showed binding affinities of nearly identical scores. Thymol with −7.2 kcal/ml, *p*-cymene −7.1 kcal/mol, whereas standard drugs such as galantamine showed the highest score with −9.2 kcal/mol (Aanniz et al., 2025). At the same time, our study showed similar scores of −6.1 kcal/mol (Thymol) and −5.5 kcal/mol (*o*-cymene) with Rd AChE.

Typically, insecticides cause an increase in the activity of detoxifying enzymes, and insects that are tolerant and resistant to pesticides show an elevation in detoxifying enzymes. GST is the main target of insecticide action (Kinareikina and Silivanova, 2023). Our study focused on the GST activity when treated with the same dose. We discovered that the GST activity was reduced drastically with the *in vitro* treatment of 1 % *M. citriodora* EO and decreased significantly with increased concentration. The results can be correlated with the work done by (Waliwitiya et al., 2012), which suggests there is inhibition in GST activity when exposed to EO or synthetic insecticide for a longer duration with higher concentration. *Citrus sinensis* peel EO can prominently inhibit the GST activity in *Callosobruchus maculatus* and *S. zeamais* (Oyedeji et al., 2020).

5. Conclusion

In the above investigation, we evaluated the insecticidal property of *M. citriodora* essential oil against the stored product insect pest, especially *R. dominica*. Moreover, to complement the biorational traits of *M. citriodora* EO, it showed significant fumigant and contact toxicity in both with and without sorghum and pearl millet grains. Enzyme systems like AChE and GST were inhibited significantly *in vitro* at lower concentrations, which was advocated by molecular docking studies. In addition, the seed viability of grains was unaffected due to the lower persistence of the EO. Hence, the EO from *M. citriodora* can be a good candidate for developing novel biorational strategies against stored insect pests.

CRediT authorship contribution statement

Ambre Vicky Vilas: Writing – original draft, Formal analysis. **D.G. Mokshith:** Writing – original draft, Formal analysis. **C.S. Vivek Babu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declared that they do not have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2025.102841.

Data availability

Data will be made available on request.

References

Aanniz, T., Elouafy, Y., Bouyahya, A., Aguerd, O., Chamkhi, I., Ullah, R., Ali, E.A., Akhazzane, M., Gallo, M., Benali, T., 2025. Characterization of volatile compounds and biological effects capacities of Moroccan *Thymus capitatus* L. Chem. Biodivers. https://doi.org/10.1002/cbdv.202403501 essential oils: *in vitro* and *in silico* approaches.

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18 (2), 265. https://doi.org/10.1093/jee/18.2.265a, 26.

Anagha, K.K., 2023. Millets: nutritional importance, health benefits, and bioavailability: a review. Pharm. Innov. 12.

Antony Ceasar, S., Maharajan, T., 2022. The role of millets in attaining United Nation's sustainable developmental goals. Plants People Planet 4. https://doi.org/10.1002/ppp3.10254.

Bhatt, P., Chauhan, N., Urvashi, Sendri, N., Bhandari, P., Reddy, S.G.E., 2024. *Thalictrum foliolosum* DC against aphid, *Aphis craccivora* Koch and mealybug, *Planococcus lilacinus* Cockerell: a potential bioinsecticide. Biocatal. Agric. Biotechnol. 60, 103326. https://doi.org/10.1016/J.BCAB.2024.103326.

Bincy, K., Remesh, A.V., Reshma Prabhakar, P., Vivek Babu, C.S., 2023. Differential fumigant and contact biotoxicities of biorational essential oil of Indian sweet basil and its active constituent against pulse beetle, *Callosobruchus chinensis*. Food Biosci. 51. https://doi.org/10.1016/j.fbio.2022.102283.

Chandrashekar, A., Satyanarayana, K.V., 2006. Disease and pest resistance in grains of sorghum and millets. J. Cereal. Sci. 44, 287–304. https://doi.org/10.1016/J.JCS.2006.08.010.

Daglish, G.J., Singarayan, V.T., Jagadeesan, R., Reid, R., Ebert, P.R., Nayak, M.K., 2024. Seasonal flight activity and associated frequency of rph2 phosphine resistance allele in *Rhyzopertha dominica* at bulk storage facilities in southern Queensland, Australia. J. Stored Prod. Res. 109, 102447. https://doi.org/10.1016/J.JSPR.2024.102447.

Deepika, Singh, A., Chaudhari, A.K., Das, S., Dubey, N.K., 2020. Nanoencapsulated *Monarda citriodora* Cerv. ex lag. essential oil as potential antifungal and antiaflatoxigenic agent against deterioration of stored functional foods. J. Food Sci. Technol. 57. https://doi.org/10.1007/s13197-020-04318-4.

Dhage, P.A., Sharbidre, A.A., Dakua, S.P., Balakrishnan, S., 2021. Leveraging hallmark Alzheimer's molecular targets using phytoconstituents: current perspective and emerging trends. Biomed. Pharmacother. https://doi.org/10.1016/j.biopha.2021.111634.

Di Vito, M., Bellardi, M.G., Mondello, F., Modesto, M., Michelozzi, M., Bugli, F., Sanguinetti, M., Sclocchi, M.C., Sebastiani, M.L., Biffi, S., Barbanti, L., Mattarelli, P., 2019. *Monarda citriodora* hydrolate vs essential oil comparison in several anti-microbial applications. Ind. Crops Prod. 128, 206–212. https://doi.org/10.1016/j.indcrop.2018.11.007.

Edde, P.A., 2012. Corrigendum to " A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. J. Stored Prod. Res. 48, 1–18. https://doi.org/10.1016/j.jspr.2011.10.004, 2012.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7. https://doi.org/10.1016/0006-2952(61)90145-9.

Erler, F., 2005. Fumigant activity of six monoterpenoids from aromatic plants in Turkey against the two stored-product pests confused flour beetle, *Tribolium confusum*, and Mediterranean flour moth, *Ephestia kuehniella*. Z. Pflanzenkr. Pflanzenschutz 112. https://doi.org/10.1007/bf03356158.

Escobar, A., Pérez, M., Romanelli, G., Blustein, G., 2020. Thymol bioactivity: a review focusing on practical applications. Arab. J. Chem. https://doi.org/10.1016/j.arabjc.2020.11.009.

Georgiev, B., Nikolova, M., Aneva, I., Dzhurmanski, A., Sidjimova, B., Berkov, S., 2022. Plant products with acetylcholinesterase inhibitory activity for insect control. BioRisk 309–315. https://doi.org/10.3897/biorisk.17.77052, 2022.

Ghosh, M., Schepetkin, I.A., Özek, G., Özek, T., Khlebnikov, A.I., Damron, D.S., Quinn, M.T., 2020. Essential oils from *Monarda fistulosa*: Chemical composition and activation of Transient Receptor Potential A1 (TRPA1) channels. Molecules 25. https://doi.org/10.3390/molecules25214873.

Grzeszczuk, M., Wesołowska, A., Stefaniak, A., 2020. Biological value and essential oil composition of two monarda species flowers. Acta Scientiarum Polonorum, Hortorum Cultus 19, 105–119. https://doi.org/10.24326/asphc.2020.4.10.

Hamdan, A.M.E., Alharthi, F.H.J., Alanazi, A.H., El-Emam, S.Z., Zaghlool, S.S., Metwally, K., Albalawi, S.A., Abdu, Y.S., Mansour, R.E.S., Salem, H.A., Abd Elmageed, Z.Y., Abu-Elfotuh, K., 2022. Neuroprotective effects of phytochemicals against aluminum chloride-induced alzheimer's disease through ApoE4/LRP1, Wnt3/β-Catenin/GSK3β, and TLR4/NLRP3 pathways with physical and mental activities in a rat model. Pharmaceuticals 15. https://doi.org/10.3390/ph15081008.

Hegde, K.R., Bhavana, M.R., Buvaneswaran, M., Sinija, V.R., Rawson, A., Hema, V., 2025. Exploring millet proteins: extraction techniques, post-processing, and modification using novel approaches. Food Human. 4, 100504. https://doi.org/10.1016/J.FOOHUM.2025.100504.

Hou, L., Wu, Y., Wang, S., 2019. Thermal death kinetics of *Cryptolestes pusillus* (Schonherr), *Rhyzopertha dominica* (fabricius), and *Tribolium confusum* (jacquelin du val) using a heating block system. Insects 10. https://doi.org/10.3390/insects10050119.

Joshi, J., Kumar, S.S., Rout, R.K., Rao, P.S., 2025. Millet processing: prospects for climate-smart agriculture and transition from food security to nutritional security. J. Future Foods 5, 470–479. https://doi.org/10.1016/J.JFUTFO.2024.08.004.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S.A.A., Ballard, A.J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A.W., Kavukcuoglu, K., Kohli, P., Hassabis, D., 2021. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589. https://doi.org/10.1038/s41586-021-03819-2.

Kanda, D., Kaur, S., Koul, O., 2017. A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents. J. Pest. Sci. 90. https://doi.org/10.1007/s10340-016-0800-5, 2004.

Karabörklü, S., Ayvaz, A., 2023. A comprehensive review of effective essential oil components in stored-product pest management. J. Plant Dis. Prot. 130. https://doi.org/10.1007/s41348-023-00712-0.

Ketoh, G.K., Koumaglo, H.K., Glitho, I.A., Auger, J., Huignard, J., 2005. Essential oils residual effects on *Callosobruchus maculatus* (Coleoptera: bruchidae) survival and female reproduction and cowpea seed germination. Int. J. Trop. Insect Sci. 25. https://doi.org/10.1079/IJT200565.

Kim, S. Il, Yoon, J.S., Jung, J.W., Hong, K.B., Ahn, Y.J., Kwon, H.W., 2010. Toxicity and repellency of origanum essential oil and its components against *Tribolium castaneum* (Coleoptera: tenebrionidae) adults. J. Asia Pac. Entomol. 13. https://doi.org/10.1016/j.aspen.2010.06.011.

Kinareikina, A., Silivanova, E., 2023. Impact of insecticides at sublethal concentrations on the enzyme activities in adult *Musca domestica* L. Toxics 11. https://doi.org/10.3390/toxics11010047.

Kiran, S., Prakash, B., 2015. Toxicity and biochemical efficacy of chemically characterized Rosmarinus officinalis essential oil against *Sitophilus oryzae* and *Oryzaephilus surinamensis*. Ind. Crops Prod. 74, 817–823. https://doi.org/10.1016/J.INDCROP.2015.05.073.

Kozhissery Sreekrishnakumar, A., Lokesh, M., Sahu, U., Vendan, S.E., 2025. Can volatile sulphides act as fumigant or contact toxicant in cowpea weevil, *Callosobruchus maculatus* F.?: a comparative analysis. Nat. Prod. Res. https://doi.org/10.1080/14786419.2025.2451220.

Lokesh, M., Sreekrishnakumar, A.K., Sahu, U., Vendan, S.E., 2024. Influence of molecular descriptors of plant volatilomics on fumigant action against the three major stored product beetle pests. Environ. Sci. Pollut. Control Ser. 31, 35455–35469. https://doi.org/10.1007/s11356-024-33483-8.

López, M.D., Jordán, M.J., Pascual-Villalobos, M.J., 2008. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. J. Stored Prod. Res. 44. https://doi.org/10.1016/j.jspr.2008.02.005.

Lu, Z.G., Li, X.H., Li, W., 2011. Chemical composition of antibacterial activity of essential oil from *Monarda citriodora* flowers. In: Advanced Materials Research, pp. 920–923. https://dx.doi.org/10.4028/www.scientific.net/AMR.183-185.920.

Madhusudhana, R., Vinutha, M., Dheeraj, M., Rajappa, P.V., 2025. Millets for diversification and risk mitigation in rainfed agriculture. Ind. Farm. 75 (1), 32–35.

Manivannan, S., Subramanyam, B., 2023. Insecticidal efficacy of two candidate diatomaceous earth powders against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: bostrichidae). J. Stored Prod. Res. 104. https://doi.org/10.1016/j.jspr.2023.102212.

Mariadoss, A., Kumar, M.A., Srihari, P., Sankarganesh, E., Alice, C., Sujeetha, R.P., 2024. Feeding preference and development of lesser grain borer, *Rhyzopertha dominica* (Fabricius) in different millet grains. J. Entomol. Res. 48, 326–329. https://doi.org/10.5958/0974-4576.2024.00064.3.

Mariani, V., Turci, S., Pollini, A., Biffi, S., Cencetti, G., Bellardi, M.G., 2020. Prove Preliminari Di Efficacia Insetticida Dell'Idrolato Di Monarda Didyma Contro Trialeurodes Vaporariorum.

Mazza, G., Chubey, B.B., Kiehn, F., 1987. Essential oil of *Monarda fistulosa* L. var. Menthaefolia, a potential source of geraniol. Flavour Fragrance J. 2. https://doi.org/10.1002/ffj.2730020310.

Meena, S.R., Gochar, R., 2018. Domestication and nutrient management of *Monarda citriodora* cer.ex lag. In: Sub Tropical Region of Jammu (India).

Meng, E.C., Goddard, T.D., Pettersen, E.F., Couch, G.S., Pearson, Z.J., Morris, J.H., Ferrin, T.E., 2023. UCSF ChimeraX: tools for structure building and analysis. Protein Sci. 32, e4792. https://doi.org/10.1002/pro.4792.

Mirdita, M., Schütze, K., Moriwaki, Y., Heo, L., Ovchinnikov, S., Steinegger, M., 2022. ColabFold: making protein folding accessible to all. Nat. Methods 19, 679–682. https://doi.org/10.1038/s41592-022-01488-1.

Nascimento, G.J., Oliveira, L.H.G., Rique, H.L., Leite, R.T., Nunes, F.C., 2024. Repellence and insecticidal activity mediated by necrosis in *Aedes aegypti* mosquitoes exposed to thymol. Arq. Bras. Med. Vet. Zootec. 76. https://doi.org/10.1590/1678-4162-13027.

Oppert, B., Morgan, T.D., 2013. Improved high-throughput bioassay for *Rhyzopertha dominica* (F.) (Coleoptera: bostrichidae). J. Stored Prod. Res. 52. https://doi.org/10.1016/j.jspr.2012.11.001.

Ou, G., Zhu, S., Huang, Q., Wei, Y., Zhang, Z., Cheng, D., 2025. Mechanism and synergy evaluation of a mixture of croton essential oil and spirodiclofen on *Phenacoccus solenopsis* (Hemiptera: pseudococcidae). Crop Prot. 190, 107104. https://doi.org/10.1016/J.CROPRO.2024.107104.

Oyedeji, A.O., Okunowo, W.O., Osuntoki, A.A., Olabode, T.B., Ayo-folorunso, F., 2020. Insecticidal and biochemical activity of essential oil from *Citrus sinensis* peel and constituents on *Callosobruchus maculatus* and *Sitophilus zeamais*. Pestic. Biochem. Physiol. 168, 104643. https://doi.org/10.1016/J.PESTBP.2020.104643.

Pathania, A.S., Guru, S.K., Verma, M.K., Sharma, C., Abdullah, S.T., Malik, F., Chandra, S., Katoch, M., Bhushan, S., 2013. Disruption of the PI3K/AKT/mTOR signaling cascade and induction of apoptosis in HL-60 cells by an essential oil from *Monarda citriodora*. Food Chem. Toxicol. 62. https://doi.org/10.1016/j.fct.2013.08.037.

Rabinowitz, R., Rowley, L., Brown, J.T., Meagher, R.L., Rathinasabapathi, B., 2015. Vegetable section feeding deterrence and inhibitory effects of Bee balm (*Monarda didyma*) leaves on fall armyworm. Proc. Fla. State Hort. Soc. 128, 162–165.

Rajendran, S., Chayakumari, 2003. Insect infestation and control in stored grain sorghum and millets. J. Food Sci. Technol. 40 (5), 451–457.

Rajendran, S., Sriranjini, V., 2024. Insect Pest management in stored millets. Indian J. Entomol. 1–14. https://doi.org/10.55446/IJE.2024.2403.

Ramírez-Cabrera, C., López-Díaz, G., Canal, N.A., Bacca, T., 2024. Control failure of deltamethrin in Colombian populations of *Rhyzopertha dominica* (F.) (Coleoptera: bostrichidae). Revista de Ciencias Agrícolas 41, e1229. https://doi.org/10.22267/rcia.244101.229.

Rashmi, Tandon, R., Kalia, A., Raju, B., Silakari, O., 2024. Bioactive molecules isolated from Cymbopogon flexuosus and *Monarda citriodora* essential oils: chemical profiling, antimycotic potential, and molecular docking studies against green and blue molds of Kinnow. J. Essential Oil Beari Plant 27, 1130–1147. https://doi.org/10.1080/0972060X.2024.2392708.

Remesh, A.V., Raveendran, A., Bincy, K., Wagh, V.S., Dastager, S.G., Babu, C.S.V., 2022. Insights on biorational potential of *Ocimum gratissimum* essential oil and its binary combination with monoterpene phenol for control of rice weevil (*Sitophilus oryzae*) and aflatoxigenic fungi. Food Biosci. 50. https://doi.org/10.1016/j.fbio.2022.102019.

Ren, Y., Wang, T., Wang, C., D'Isita, I., Hu, Q., Germinara, G.S., Cao, Y., 2023. Population development of *Rhyzopertha dominica* (F.) (Coleoptera: bostrichidae) on different stored products. Entomol. Res. 53. https://doi.org/10.1111/1748-5967.12670.

Singh, K.D., Koijam, A.S., Bharali, R., Rajashekar, Y., 2023. Insecticidal and biochemical effects of *Dillenia indica* L. leaves against three major stored grain insect pests. Front. Plant Sci. 14. https://doi.org/10.3389/fpls.2023.1135946.

Suman, S., Chandra, S., 2024. Introduction to millet and challenges of millet production due to extreme environmental conditions in India: a review. Cereal Res. Commun. https://doi.org/10.1007/s42976-024-00551-1.

Swamy, S.V.S.G., Raja, D.S., Rao, V.V., 2023. Preference and progeny development of stored product insects in response to grain characteristics of millets. Cereal Res. Commun. https://doi.org/10.1007/s42976-023-00471-6.

Swamy, S.V.S.G., Wesley, B.J., 2021. Incidence and diversity of stored product insects in processed millets. Indian J. Entomol. 83. https://doi.org/10.5958/0974-8172.2020.00224.2.

Tabanca, N., Bernier, U.R., Ali, A., Wang, M., Demirci, B., Blythe, E.K., Khan, S.I., Baser, K.H.C., Khan, I.A., 2013. Bioassay-guided investigation of two Monarda essential oils as repellents of yellow fever mosquito *Aedes aegypti*. J. Agric. Food Chem. 61. https://doi.org/10.1021/jf402182h.

Trott, A.J. Olson, 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461. https://doi.org/10.1002/jcc.21334.

Vendan, S.E., Manivannan, S., Sunny, A.M., Murugesan, R., 2017. Phytochemical residue profiles in rice grains fumigated with essential oils for the control of rice weevil. PLoS One 12. https://doi.org/10.1371/journal.pone.0186020.

Verma, M.K., Chandra, S., 2013. *Monarda citriodora* cerv. Ex lag.: an alternative rich source of thymol. Indian Perfum. 57, 43–48.

Waliwitiya, R., Nicholson, R.A., Kennedy, C.J., Lowenberger, C.A., 2012. The synergistic effects of insecticidal essential oils and piperonyl butoxide on biotransformational enzyme activities in *Aedes aegypti* (Diptera: culicidae). J. Med. Entomol. 49. https://doi.org/10.1603/ME10272.

Yadav, G.K., Ul Baligah, S.H., Jeet, S., Kumar, V., Bhanwaria, R., 2024. Therapeutic potential and chemo-morphological characterization of silver nanoparticles synthesized using aqueous extracts of *Monarda citriodora* Cerv. ex Lag. Biocatal. Agric. Biotechnol. 57. https://doi.org/10.1016/j.bcab.2024.103076.