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RESEARCH ARTICLE

Biotoxicity and repellency of *Vitex negundo* (L.) oil nanoemulsion towards *Rhyzopertha dominica* (F.) on stored rice

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Abstract

Rhyzopertha dominica F. (lesser grain borer) is an important primary pest of stored rice that causes substantial economic loss. This pest developed resistance to a wide range of hazardous chemical pesticides due to its great degree of adaptability. This study proposed to prepare *Vitex negundo* oil nanoemulsion (VNO NE) to improve its efficacy against this target pest. *Vitex negundo* oil (VNO) contains key compounds such as Aromandendrene, β-caryophyllene, Squalene, 3-octen-5-yne,2,7-dimethyl-,(E)-,5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate, Farnesyl bromide, 4-terpineol and Elemol. VNO NEs were prepared using a high-speed homogenizer from which nanoemulsion having 5% VNO mixed at a 1:2 (w/w) ratio with tween80 was found to be optimum considering different characterization parameters. The mean zeta potential, polydispersity index and hydrodynamic diameter of the nanoemulsion were -3.4 mV, 0.263 and 166.62 nm respectively. For contact toxicity lethal dose 50 (LD_{50}) value of VNO NE was 0.517 µL cm^{-2} against *R. dominica* which was 46.03% less, compared to bulk VNO. LD_{50} value of VNO NE for fumigant toxicity against *R. dominica* was 245.38 µL L^{-1} which was 32.05% less than that of crude oil. The highest repellency increased by 30.14% than VNO, when treated with VNO NE in *R. dominica*. Significant inhibition of glutathione transferase enzyme was also detected in insects treated with VNO NE than VNO and control. These results indicated that VNO NE is an effective novel pesticide that can be recommended for the management of *R. dominica* in stored rice.

Keywords

Vitex negundo oil; GC-MS; nanoemulsion; contact toxicity; fumigant toxicity; repellency

Introduction

Rice is consumed by more than half of the world's population. Grain storage after harvesting is required for future usage and to ensure food security. Approximately, 70% of farm output is stored by farmers for different purposes (1). In postharvest activities, about 1/3rd of food is lost or wasted, which is still a major global problem (2).

Insect infestations resulted in up to 3.9-6% storage loss in cereals and in India storage loss in rice is 3.51% due to pests (3, 4). Apart from direct quantitative losses, qualitative losses spawned due to changes in chemical constituents like proteins, carbohydrates and amino acids that resulted in

the nutritional depletion of grains. Additional losses such as food contamination by allergens (like uric acid, dead bodies, cast skins and excreta) pose significant obstacles to the food industries and the export of food commodities (5). There are around a thousand species of stored grain pests infesting various stored commodities worldwide from which 2 orders, Coleoptera and Lepidoptera make up the majority of stored grain pests (6).

Rhyzopertha dominica (Fabricius) (Coleoptera: Bostrichidae), often known as lesser grain borer is an important primary pest of cereal crops in many parts of the world (7, 8). This pest can cause substantial economic losses in starch-containing substrates like rice, maize and wheat (7, 9). Adult insects consume the grain externally and hide themselves in cracks (7, 10). In addition to grain weight loss, it also causes grain infestation by copious amounts of frass containing larval exuviae, feces, pieces of immature insects and other wastes (7). Affected grains produce unpleasant smell and lack nutrients that are not fit for human consumption (9).

Synthetic pesticides are frequently used for controlling storage pests including *R. dominica* (11). The widespread usage of these chemicals led to adverse consequences on environmental contamination as well as human health (11-13). *R. dominica* is resistant to a wide range of chemical groups, including pyrethroids, organophosphates and insect growth regulators due to its great degree of adaptability (9, 14-16). Resistance to phosphine fumigation is a matter of concern since the world's stored commodities mostly depend on this fumigant as a pest protectant. These drawbacks led to replacing many chemical pesticides with more sustainable and eco-friendly alternatives from natural sources which can be referred to as green chemistry.

Botanicals with a special emphasis on essential oils fit perfectly into the theme of organic food production. Essential oils have strong aromatic bioactive compounds that are volatile in nature and show toxic, repellent and antifeedant properties against stored grain pests (17). Potential essential oils from plants like *Eucalyptus floribunda*, *Acorus calamus*, *Mentha pulegium*, *Lavandula stoechas*, *Ruta chalepensis* and *Ocimum gratissimum* show insecticidal properties against storage pests.

Vitex negundo L. (family: Lamiaceae), is a large-sized medicinal shrub with aromatic nature found across India. Numerous phytochemicals, including lignans, terpenes, iridoids, flavonoids and steroids are present within it (18). This shrub possesses pesticidal properties against various stored grain pests like *Sitophilus zeamais, Tribolium castaneum, Oryzaephilus surinamensis*, *Callosobruchus chinensis* and *S. oryzae* (19-21).

Despite their promising insecticidal properties, the greatest barriers to the practical application of essential oils are their low water solubility, lack of physical stability and degradation (22). Nanoemulsion formulation of essential oil is a superior way to get rid of all the above-mentioned limitations since it has improved properties owing to its controlled release (23). The smaller particle size of nanoemulsion has a significant impact on pesticide activity by accelerating insecticide penetration through the insect cuticle (24). In the current study, we developed VNO NE using a high-speed homogenizer and evaluated its efficacy against *R. dominica* in comparison to bulk oil.

Materials and Methods

Plant material

Vitex negundo (L.) leaves were gathered from a village near ICAR-National Rice Research Institute, Cuttack, Odisha, India (20°45' N latitude, 85°93′ E longitude and 36 m altitude). Cuttack district is situated in the East and South coastal plains zone of Odisha.

Chemicals

Emulsifier Tween 80 i.e polyoxyethylene sorbitan monooleate required for nanoemulsion was procured from Merck, India and Glutathione-S-transferase (GSTs) assay kit was procured from Sigma-Aldrich, Merck, India.

Oil extraction

Oil extracted from *V. negundo* leaves following the hydrodistillation method. Clevenger apparatus was used for this method where leaves were boiled at 70 °C for 4 h. Oil is released from oil glands in vapor form along with water due to boiling. This vapor mixture passed through a cooled condenser to separate and collect oil. The oil collected was measured and quantified in g at the end of this method. The oil obtained was converted to a % (25).

$$\text{Recovery of oil (\%)} = \frac{\text{Wt.of the oil (g)}}{\text{Wt.of the sample (g)}} \times 100$$

Chemical characterization of Vitex negundo oil

The extracted oil was chemically characterized using GC-MS/MS equipment (Shimadzu TQ8040, Shimadzu Corporation, Kyoto, Japan). The Rxi-5-Sil MS capillary column (30 m×0.25 mm, 0.25 µm) was employed in the gas chromatography system. Pure helium (99.99%) was utilized as the carrier gas. Each chemical compound was identified by comparing its retention index to the C8-C40 alkane standards and its MS spectra to the reference spectra in the National Institute of Standards and Technology (NIST) collection (26).

Insect culture

R. dominica (lesser grain borer) was obtained from the Grain Entomology Laboratory of Crop Protection Division, National Rice Research Institute. Over the course of the study period, test insects were cultured and maintained in a laboratory setting at 65 ± 5% RH and 28 ± 2 °C and rice as feeding material. Same-age adults of 7-10 days old were used in our research.

Preparation of nanoemulsion

To create different VNO NEs, a variety of VNO concentrations (2.5, 5 and 7.5%) were used. Tween 80 was used as a surfactant to formulate VNO NE, based on the hydrophilic-

lipophilic balance of the VNO. VNO and emulsifiers were mixed at different ratios on a weight basis starting from 1:1 to 1:3 (w/w). To prepare an emulsion, the required amount of water was added to the mixture and the mixture was vortexed (Maxi Mix II, Thermolyne, USA) for 2-3 min. Using a high-speed homogenizer (IKA T25 digital ULTRA TURRAX, T 25 D S22, Germany), the bulk emulsion was homogenized at 3 different rotation speeds (10000, 15000 and 20000 rpm) and 3 different durations (10, 15 and 20 min).

Characterization of nanoemulsions

Stability study

Heating cooling freezing and thawing stress tests were conducted to check the stability of VNO NEs (27, 28). Formulated nanoemulsions were centrifuged at 3000 rpm for 30 min to see any phase separation. In the alternate heating and cooling cycle, VNO NEs first heated at 40 °C followed by cooling at 4 °C and each temperature was altered after 48 h. Similarly, for alternate freezing and thawing cycles, 2 temperatures i.e. -21 °C and 25 °C were taken and each temperature was altered after 48 h. Both cycles were repeated twice and the experiment was performed in triplicate. After the investigation, phase separation, froth production etc. were recorded. Stable VNO NEs that passed the stress tests were examined further.

Dynamic light scattering (DLS)

The hydrodynamic diameters, polydispersity indices (PDI) and zeta potential values (ζ-potential) of VNO NEs were measured using a particle size analyzer (Malvern Instruments Pvt Ltd, UK).

Biotoxicity of V. negundo oil

Contact toxicity

Contact toxicity of VNO against *R. dominica* was carried out (29). The contact toxicity assay was performed on cemented petri plates with a 64 cm^2 surface area. A completely randomized design (CRD) was followed to conduct this experiment. Doses of VNO for different treatments against *R. dominica* were 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 µL cm^{-2} and control. These doses were diluted in soybean oil to a predetermined amount of 500 µL each for smooth application in cemented petri plates. Ten adult insects were released into each petri plate with 1 g of rice. To prevent insects from escaping, petri plates were sealed with paraffin film and pin holes were made for air movement. Each treatment was replicated thrice and mortality was recorded at 24 and 48 h after treatment (HAT). Lethal doses (LD) were calculated using 1.5EPA probit analysis software. Doses of treatments, the total number of insects (30 per treatment) and the number of dead insects per treatment were inputted into this software to obtain lethal doses.

Fumigant toxicity

Fumigation toxicity tests were performed using 1150 mL fumigation chambers (30). Ten adults of *R. dominica* were kept in a perforated pouch with 1 g rice, tied with rubber bands and placed inside the fumigation chamber. Filter paper (Whatman No. 1) was cut into 64 cm^2 pieces and was put at the top of the air-tight fumigation chamber close to the lid after being treated with VNO. Different doses of VNO used for treatment were 300, 400, 500 and 600 µL per liter air (µL L^{-1}) and control. Each treatment was replicated thrice and adult mortality was recorded 5 days after treatment (DAT). Lethal doses (LD) were calculated.

Repellency study

A 64 cm^2 glass petri plate with filter paper (Whatman No. 1, cut into 64 cm^2 pieces) was utilized for the repellency investigation (31). The filter paper was divided into 2 halves in which one was treated with VNO treatment and the other half was controlled i.e. treated with only soybean oil. A repellency test was conducted with low oil concentrations (i.e. 1, 3 and 5% oil) to avoid mortality. According to these concentrations, different doses of VNO (1.5, 4.5 and 7.5 µL) were taken and mixed with soybean oil to prepare VNO treatments and volume in each treatment was made up to 150 µL. Petri plates were subjected to fan drying and insects were released in the middle area of the filter paper. Each treatment was replicated 7 times and the number of insects present in the 2 parts was recorded at 2, 6 and 12 HAT. For every considered time interval, the % repellency of different VNO treatments was measured using the formula (32):

$$PR(\%) = \frac{(Nc - Nt)}{(Nc + Nt)} \times 100$$

Here, Nc is the number of insects in the untreated half paper and Nt is the number of insects in the treated half.

Biotoxicity of VNO NE

The same methods were followed for studying the contact, fumigant and repellent toxicity of VNO NE as described in the VNO biotoxicity test. Doses of VNO NE were taken based on LD_{50} values of contact and fumigant toxicity of VNO. For contact toxicity against *R. dominica* doses were 0.7, 0.95, 1.2, 1.45 µL cm^{-2} and control. In the case of fumigant toxicity treatment doses of VNO NE were 250, 300, 350, 400 µL L^{-1} and control. Lethal doses were calculated in all cases. For the repellency study, the treatment of one-half filter papers was VNO NEs (150 µL volume of VNO NEs with different oil concentrations (1.5, 4.5 and 7.5 µL) other half was controlled i.e. treated with only soybean oil. Each treatment was replicated 7 times and the number of insects present in the 2 parts was recorded at 2, 6 and 12 HAT. For every considered time interval, the % repellency of different VNO NE treatments was calculated.

Glutathione-S-transferase (GSTs) assay

Enzyme extraction

Adults of *R. dominica* were treated with different contact toxicity treatments (LD_{50} dosage of crude oil, LD_{50} of nanoformulated oil and control) at varying exposure times (1, 6 and 24 HAT). Before exposing the adults to bioassay, all the beetles were pre-weighed and the weight of *R. dominica* was 3.26 mg (per 3 insects) After the exposure time, both the treated and untreated samples were kept at -80 °C and then crushed with 1 mL Dulbecco's phosphate-buffered saline (DPBS) in Eppendorf tube. The crushed samples were centrifuged at 10000 rpm and the superna-

tants were collected in the fresh tube and kept for further analysis (36).

Enzyme assay

The glutathione S-transferase (GSTs) enzyme activities were measured by using the commercially available GST assay kit (CS0410, Sigma-Aldrich, Merck, India). The reaction solution had 4 µL of enzyme solution, 196 µL of substrate solution (192.08 µL of DPBS, pH 7.2, 196 µL of 200 mM Glutathione reduced and 1.96 µL of 100 mM CDNB (1-chloro-2,4-dinitrobenzene). The GSTs' activities were documented in the microplate reader (Agilent, USA; EpochTM2 microplate reader) at 340 mm and 1 min intervals. At 5 min intervals, the absorbance change was noted. The CDNB (1-chloro, 2,4-dinitrobenzene) extinction coefficient (0.0096) was used to determine the total GST activity (33).

Statistical analysis

The Probit regression analysis was done using 1.5 EPA Probit Analysis Program software (24). Lethal doses (LD_{10}, LD_{50} and LD_{90}) were estimated. GST data were obtained using GEN5 absorbance microplate software and analyzed using one-way ANOVA at $P<0.05$ in Microsoft Excel version 2016 (34).

Results and Discussion

Chemical composition of VNO

The amount of oil recovered from the dried leaves of *V. negundo* (L.) was 0.65% having a pale yellow coloration (Fig. 1). This is roughly comparable to the 0.5% oil extracted in previous studies (25). As per several other sources, varying quantities of oil were extracted (35, 36). These differences might be due to variations in environmental, soil, climate conditions, extraction methods and genetic makeup of the *V. negundo* (18).

Fig. 1. *V. negundo* oil.

Gas chromatography-mass spectrometry (GC-MS) technique identified a total of 40 chemicals from VNO, in which major compounds were Aromandendrene, β-caryophyllene, Squalene, 3-octen-5-yne,2,7-dimethyl-, (E)-,5-(1-isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3methyl-2-pentanol acetate, Farnesyl bromide, 4-terpineol and Elemol (Fig. 2). Some compounds found in our results, such as α-Pinene, β-Pinene, 3-octanone, p-cymene, 1,8-cineole, γ-terpinene, Linalool, 4-terpineol, α-terpineol, β-elemene, β-caryophyllene, Elemol, Nerolidol, Caryophyllene oxide, epi-α-cadinol and β-eudesmol are corroborated with previous studies (18, 37, 38). Earlier reports suggested the primary constituents of VNO as caryophyllene oxide, T–eudesmol, β-caryophyllen, trans-β-farnesene, βeudesmol, α-terpinol and α-terpinene (39).

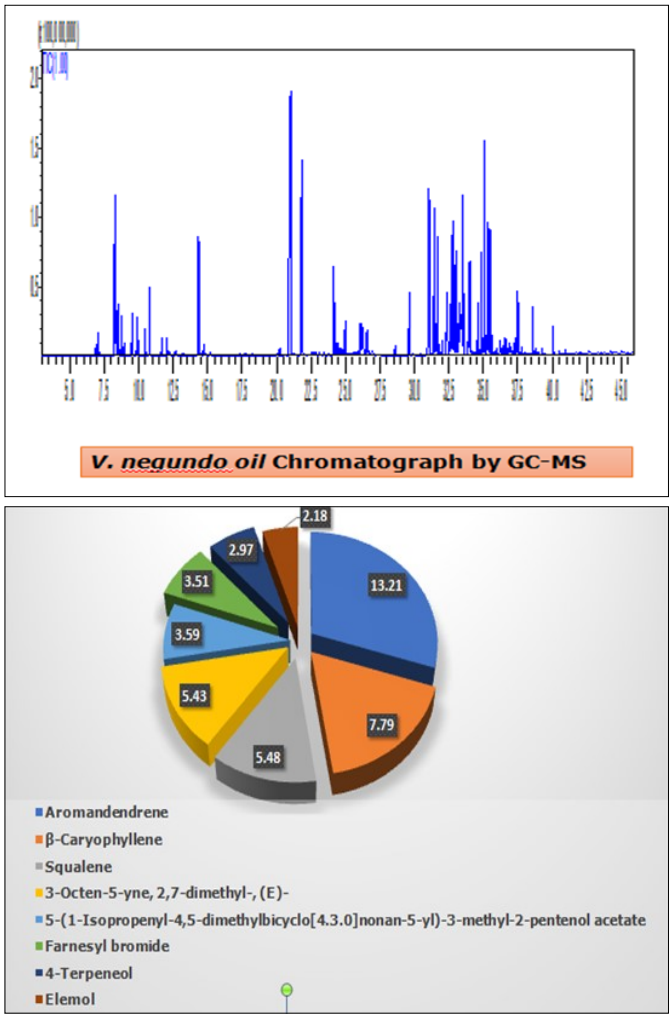

Fig. 2. Chemical constituents of *V. negundo* oil using GC-MS.

Preparation and characterization VNO NE

Several nanoemulsions were formulated with various oil concentrations and VNO, emulsifier ratios while maintaining a consistent time (15 min) and rotation speed (20000 rpm) (Fig. 3). VNO and surfactant at 1:2 and 1:1 ratios of 5% VNO concentration were found stable nanoemulsions as they survived the stress tests. Increased homogenization

time led to the reduction in droplet size in *Momordica cochinchinensis* oil emulsion (40). Transparent white, super white and milky-coloured emulsions resulted in our experiment which might be due to the lower average oil droplet size of nanoemulsions as compared to the visible spectrum wavelengths even at fractions of large droplet

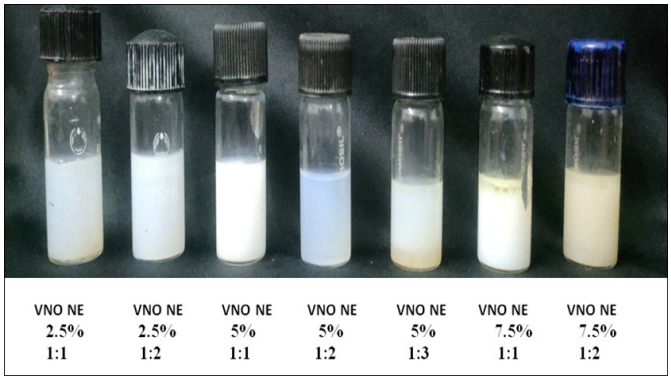

Fig. 3. Different *V. negundo* nanoemulsions (VNO NEs) (rotation speed : 20000 rpm, duration : 15 min).

volume (41). Phase separation was seen when the VNO's loading capacity was raised to 7.5%. These unstable nanoemulsions might have resulted from oil coalescence and bigger droplets created by Ostwald ripening (42).

VNO NEs (VNO and tween80 at 1:1 and 1:2 ratios of 5% oil concentration) that passed the stress tests were further characterized for hydrodynamic diameter, PDI and ζ-potential (Table 1). Both the stability and biological activity of nano-formulations depend on variables such as droplet size, polydispersity index and zeta potential (43).

The droplet sizes of VNO: surfactant at 1:1 and 1:2 of 5% oil concentration were recorded at 185.41 and 166.62 nm respectively (Table 1). The results conform with previous reports that the particle size of VNO NE was within 200 nm (25). The excessive mechanical force created during higher rotation speed might be responsible for the lesser droplet size of nanoemulsion (44). The rise in surfactant concentration led to a drop in interfacial free energy, which in turn induced a decrease in particle size and smaller droplet sizes led to weaker attractive forces and more stable nanoemulsions (45). Reduced droplet size led to improved stability against droplet flocculation, coalescence and sedimentation due to weakened attraction forces operating between droplets (46). Nano-sized oil droplets in nanoemulsions contribute to their stability (47).

VNO: tween80 at 1:1 and 1:2 of 5% oil concentration have PDI values of 0.281 and 0.263 respectively (Table 1). The formulation's non-uniform size distribution was assessed using the Polydispersity index (48). A PDI score of less than 0.6 indicated greater homogeneity and stability in the emulsion (43).

The zeta potential of VNO: tween 80 at 1:1 and 1:2 ratio were -4.3 and -3.4 mV respectively (Table 1). This is in line with previous reports, that the hydroxyl ion adsorbed at the O/W interface and the ethylene oxide group contained in tween 80 enable the droplets to have higher negative charges (49). Negative zeta potential resulted from more repulsion between droplets thus making the formulation more stable (50).

Table. 1. Hydrodynamic diameter, polydispersity index and zeta potential of *Vitex negundo* oil nano-emulsions (VNO NE)

	Hydrodynamic diameter (nm)	Polydispersity index	Mean zeta potential (mV)
*Nanoemulsion 1	185.41	0.281	-4.3
*Nanoemulsion 2	166.62	0.263	-3.4

*Nanoemulsion 1: Contain 5% VNO and VNO: emulsifier at 1:1 ratio, Nanoemulsion 2: Contain 5% VNO and VNO: emulsifier at 1:2 ratio

VNO NE (VNO: tween 80 at 1:2 ratio with 5% oil concentration) was found to be stable in most nanoemulsions after considering all the characters.

Biotoxicity study

A comparison between the biotoxicity of VNO NE (VNO: tween 80 at 1:2) and bulk oil was done. At 24 HAT, the LD_{50} values for contact toxicity of VNO NE (a.i.) against *R. dominica* was 0.517 µL cm^{-2}, which was 46.03% less compared to VNO. However, the LD_{50} value of VNO NE (a.i.) at 48 HAT was 0.401 µL cm^{-2} which was 41.88% less than the crude oil (Table 2). While considering the fumigant toxicity, the LD_{50} values of VNO NE (a.i.) for *R. dominica* (i.e 245.38 µL L-1) was 32.05% less than that of crude oil (Table 3). The insecticidal properties of the oil might be due to the presence of secondary metabolites (51). Terpenes specifically monoterpenes were thought to be the main compounds responsible for the essential oils' efficacy against

Table. 2. Contact toxicity of *Vitex negundo* oil and its nanoemulsion against *Rhyzopertha dominica* F. (Lesser grain borer).

Time (HAT)	No. of insects tested	Lethal dose 50 (LD_{50}) (a.i.) (µL/Cm^2)	95% fiducial limits		Lethal dose 10 (LD_{10}) (a.i.) (µL/Cm^2)	Lethal dose 90 (LD_{90}) (a.i.) (µL/Cm^2)	Slope	Standard error	X^2 calculated	df	P value
			Lower	Upper							
***Bulk VNO**											
24	180	0.958	0.596	1.541	0.154	5.952	1.616	0.105	1.000	4	0.909796
48	180	0.690	0.407	1.167	0.087	5.459	1.429	0.117	0.998	4	0.910099
***VNO NE**											
24	180	0.517	0.368	0.728	0.198	1.349	3.120	0.076	0.995	2	0.608049
48	180	0.401	0.270	0.597	0.147	1.090	3.002	0.088	0.990	2	0.609571

*Bulk VNO: *Vitex negundo* oil, VNO NE: *Vitex negundo* oil nanoemulsion (5% VNO; VNO and emulsifier was mixed at 1:2 ratio), HAT: Hours after treatment.

Table.3. Fumigant toxicity of *Vitex negundo* oil and its nanoemulsion against *Rhyzopertha dominica* F. (Lesser grain borer).

Time (DAT)	No. of insects tested	Lethal dose 50 (LD_{50}) (µL/L)	95% fiducial limits		Lethal dose 10 (LD_{10}) ((µL/L)	Lethal dose 90 (LD_{90}) (µL/L)	Slope	Standard error	X^2 calculated	df	P value
			Lower	Upper							
*Bulk VNO											
5	120	361.16	267.89	486.90	137.65	947.59	3.094	0.066	0.928	2	0.628764
*VNO NE											
5	120	245.38	209.12	290.47	153.60	391.85	6.371	0.035	0.955	2	0.620332

*Bulk VNO: *Vitex negundo* oil, VNO NE: *Vitex negundo* oil nanoemulsion (5% VNO; VNO and emulsifier was mixed at 1:2 ratio), DAT: Days after treatment.

insect pests (52). Previously reported that *R. dominica* species were more susceptible to essential oils or their constituents (53).

Our results revealed that the lethal dosages (LD_{50}) of VNO NEs were lower than those of crude oil, indicating the enhanced effectiveness of nanoemulsion. Our results are in line with previous reports where VNO NE showed superior larvicidal efficacy than that of VNO against *A. aegypti*. The nanoemulsion of essential oils offered added advantages like regulated release, avoidance of quick vaporization, promotion in consistency and lowered effective dose requirement (54). The active components of nanoemulsion spread and penetrate well in the target site due to their small size as compared to bulk oil (55). The smaller particle size of nanoemulsion has a significant impact on pesticide activity by accelerating insecticide penetration through the insect cuticle (24).

Repellency study

The highest repellency of VNO NE for *R. dominica* was 30.14% more than VNO (Table 4.). Our findings are consistent with past research showing that nanoemulsion of *Citrus sinensis* (sweet orange) oil demonstrated more repellency than ordinary oil against certain storage pests (31). It has also been noted that the mint and eucalyptus oil nanoemulsion repels better than bulk oil (56).

Table 4. Percentage repellency of *Vitex negundo* oil and its nanoemulsion against *Rhyzopertha dominica* F. (Lesser grain borer).

Concentration (µL/64 Cm^2)	2 HAT	6 HAT	12 HAT
*Bulk VNO			
1.5 µL	25.71	22.86	14.28
4.5 µL	38.57	31.43	20.00
7.5 µL	51.43	42.86	31.42
S.E(m) ±	3.93	4.29	3.56
CD (0.05)	11.67	12.73	10.59
*VNO NE			
1.5 µL	40.00	30.00	25.71
4.5 µL	57.14	44.27	37.14
7.5 µL	72.86	60.00	45.71
S.E(m) ±	4.44	3.75	5.26
CD (0.05)	13.19	11.14	15.63

*Bulk VNO: *Vitex negundo* oil, VNO NE: *Vitex negundo* oil nanoemulsion (5% VNO; VNO and emulsifier was mixed at 1:2 ratio), HAT: Hours after treatment.

Biochemical assay

A significant decrease in GST activity of both VNO and VNO NE was noticed as compared to the control and VNO NE showed significantly lower GSTs activity in beetles than bulk VNO (Fig. 4). This is in line with earlier reports that certain detoxification enzymes in the tissues and organs of target insects were either removed or hindered following exposure to plant extracts (57). Essential oil nanoemulsions suppress GST activity in storage pests (58).

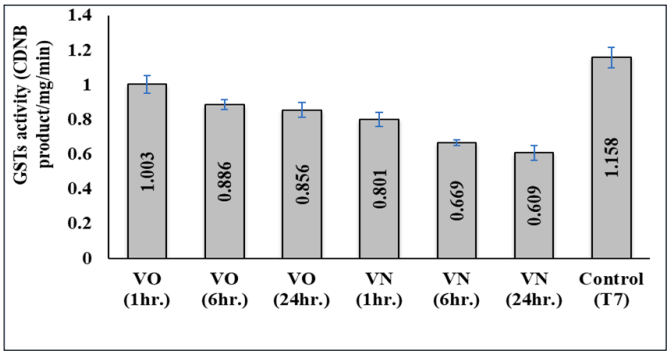

Fig. 4. GSTs inhibition activities of *V. negundo* oil and its nanoemulsion (CDNB product/mg/min) in *R. dominica* adults.

Conclusion

Secondary metabolites in *V. negundo* oil might be the reason of their insecticidal properties. VNO NE outperformed VNO against *R. dominica* in terms of biotoxicity and repellency. GSTs enzyme inhibition was significantly more in VNO NE-treated insects than in bulk oil-treated insects. Nanoemulsion formulation of essential oil emerged as a novel substitute for synthetic pesticides, offering lower dosages and more effective pest control against storage pests.

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Authors' contributions

NB, PR and TA conceptualised and supervised the research design and experimental planning. PP carried out the experiment and analysis. GP participated in the GSTs enzyme assay. BG, PG, SD and PC participated in the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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