

To Examine the oxidative and toxic effect of *oleo rheumea* (ayurvedic herbal oil) on *Periplaneta americana* (American cockroach)

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Abstract

The American cockroach, a common household pest known as *Periplaneta americana*, hails from tropical regions and can be found in places like India. Not only is it a nuisance in homes, but it also poses a public health risk as a carrier of certain human pathogens. In response to these challenges, there has been interest in exploring alternative solutions, such as plant extracts and herbal oils. Our study focused on the effectiveness of "*Oleo rheumea*" herbal oil against the American cockroach. The research utilized bioassays to assess both the repellent activity and the impact on biomolecules like lipids and proteins. During the study, cockroaches were exposed to sub-lethal concentrations of *Oleo rheumea* for varying durations (24, 48, 72, and 96 hours) to evaluate repellence. Notably, a 50% mortality rate was observed at a 0.05% concentration of the oil solution. Analysis of mortality data was conducted using Grafpad software. The research uncovered significant changes in lipid peroxide levels, correlating with the concentration of *Oleo rheumea*. Additionally, a dose-dependent depletion in protein sulphhydryl levels and the formation of protein carbonyls were observed. Behavioral changes in cockroaches, including a knockdown effect and the development of black residues, were also noted. Herbal oils, with their complex mixtures of biogenetically related phenols, monoterpenes, and sesquiterpenes, could serve as effective and safer alternatives. *Oleum rheumea* emerges as a promising repellent against American cockroaches and could find applications in surface treatments or fumigation in cockroach control programs.

Keywords: Protein damage, mortality, *Oleum Rheumea*, *Periplaneta americana*, topical bioassay

Introduction

According to the Insect Pest Management and Control (IPC), an insect becomes a pest when it disturbs the quality or quantity of food or fiber in production and has a negative impact on public health and the economy, resulting in serious disease to human health. Insect and pest management is the regulation or management of species, a member of the insect kingdom that affects human activities. Human response depends on the severity of the damage caused and will vary from tolerance, deterrence and management, to attempts to eradicate the pest completely. Pest control may be carried out as part of a comprehensive pest management plan (Amjad *et al.* 2022) [2]. According to Kim *et al.* (2021) [14], a wide range of chemicals and environmental factors can disturb the integrated processes at the various levels which determine animal behaviour. The adjustment in integrated physiological status that behavioural alterations represent is why they are regarded as a key sign of chemical toxicity.

The ability of cockroaches to mechanically transmit human intestinal parasites, animal diseases, and human allergies has long been recognised. Synthetic organic pesticides in the form of baits, aerosols, foggers, and crack treatments are the main control methods for cockroaches. The American cockroach is one of the most prevalent species in sewer systems and is especially prevalent in commercial structures. These cockroaches are thought to transmit diseases including typhus and diarrhea to people. Additionally, they lead to phobia, discomfort, and financial disadvantage. To reduce their negative effects on public health and cleanliness, these cockroaches must be managed

(Fazeli-Dinan *et al.* 2022) [10]. Currently, residual insecticides (such as dichlorvos, chlorpyrifos, propoxur, and pyrethroids), stomach toxins (such as hydramethylnon, boric acid, and sulfluramid), and insect growth regulators (such as noviflumuron and lufenuron) are the most common chemical-based methods for controlling cockroaches (Kafle *et al.* 2022) [13]. As a result, a lot of work has been put into discovering substitute repellents that are ecologically sound and friendly to the environment (Nour *et al.* 2022) [19].

Numerous natural substances that have been extracted from plants have shown a wide range of biological functions. As synthetic insecticides contain chemicals that are hazardous to both human health and the environment, naturally occurring insecticides found in plants can be employed as repellent to many insects (Mitoriana *et al.* 2020) [17]. Presently, bioinsecticides, especially those derived from plant origin, have been increasingly evaluated in controlling insects of medical importance (Nour *et al.* 2017) [20]. Many essential oils are intricate blends of volatile chemical molecules that are typically produced in plants as secondary metabolites. They are made up of oxygenated chemicals (alcohols, esters, ethers, aldehydes, ketones, lactones, and phenols) and hydrocarbons (terpenes and sesquiterpenes). Different groups of essential oils have demonstrated strong arthropod species repellency (Servi, 2021) [23].

Oleo Rheumea, herbal liniment oil is known for its anti-inflammatory property which reduces pain from muscles and joints, thereby reducing pain (Buckle *et al.* 2015) [4]. Dohnul khardal and Dohnul darchini make up the majority of this oil and are present in amounts of 28% and 15%, respectively. In varied doses, these two chemicals serve as

insect repellent in various essential oils. The present study was undertaken to evaluate the cytotoxic effects and biomolecular damage of “*Oleo Rheumea*” herbal oil against on american cockroaches, *Periplaneta americana*.

Material and methods

1. Test insect: Experiments were performed on adult American cockroaches, *Periplaneta americana* (L.), both sexes. Insects were reared at $26\pm 2^\circ\text{C}$ under a 12 hr:12 hr light-dark photoperiod regime in plastic containers. They

were fed oat flakes and apples and provided with water ad libitum. For the repellency test, we used 10–20d-old adult cockroaches since they showed the highest activity to chemicals. To avoid the effects of anesthesia or physical stress that may be caused by handling the cockroaches with tweezers, the insects and their shelters were transferred to repellent test containers by The number of cockroaches was then adjusted by removing extra ones from the test cage with tweezers.



Fig 1: Experimental setup

Substance tested: Commercial *Oleo Rheumea* (OR), herbal oil was purchased from “Khojati herbals” from MIDC, Nashik factory, Pune. To prepare the different concentrations *Olea rheumea*, acetone (0.001%) was used as a medium.

2. Acute toxicity test (LD50): To perform the acute toxicity assay five plastic jars with holes for the aerations were used. Four different concentrations of *Olea rheumea* (0.01%, 0.01%, 0.1%, 1%, and 10%) were prepared in acetone (0.01%) along with control (acetone-0.01%). The cockroaches were not fed during the period of exposure. LD50 activity of the oil was evaluated at five concentrations to produce a range of mortality from 10 to 95 percent along with control. Ten insects were collected for each group and gently transferred into plastic jars. Pads of cotton soaked with different *Olea rheumea* solutions were placed in the respective jars. Mortality of cockroaches was determined at the end of the 24-96 hr recovery period. Percent mortality was corrected by using Abbott’s formula. LC50 with their 95 percent confidence limits of the oil were determined using Log probit analysis test (Campbell *et al.*, 2017) [5].

$$\text{Corrected Mortality} = \frac{\% \text{ Test Mortality} - \% \text{ Control Mortality}}{100 - \% \text{ control mortality}} \times 100$$

3. Quantitating oxidative damage to biomolecules

3.1. Lipid Damage: Oxidative damage to lipids in cockroaches induced by *Olea rheumea* at different concentrations was measured in terms of nmoles of malondialdehyde equivalents formed (Devasagayam, 1986) [9] and expressed as nmoles of TBARS/100 mg tissue.

3.2. Damage to Proteins: Oxidative damage to protein carbonyls is based on the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine (DNPH) to form a 2,4-dinitrophenylhydrazone, which can be measured at 366 nm.

The amount of carbonyls formed was expressed as nmoles of protein carbonyls formed/100 mg tissue (Song *et al.* 2020) [25] and protein sulphhydryls were quantitated using Ellman’s reagent (5,5-dithiobis-2-nitrobenzoic acid) and expressed as nmoles of protein sulphhydryls/100 mg tissue (Ellmann, 1959) [11].

Estimation of protein

Protein was estimated following the method of Lowry *et al.* 1951 [15]. All the assays were replicated five times and the values given are the average of these replicates.

4. Statistical analysis

The mortality (%) data obtained were used to calculate the 24, 48, 72, and 96 hr LC50 values by Probit analysis method, using a statistical package (Grafpad software). ANOVA was used to compare the LC50 values of *Oleum rheumea* oil to test organisms after 96 hrs. One-way analysis of variance (ANOVA) test associated with Tukey’s test was used to determine the statistical significance of the differences among experimental groups. All the statistical analyses were done using SPSS 17.0 software. The data was presented as mean \pm S.D. of samples at least five times. Differences were considered significant at $p < 0.001$ and $p < 0.05$.

Results

1. Behavioural changes in cockroaches under treatment

Experimental cockroaches were introduced into the concentrations of the oil sample, started showing discomfort within a few minutes, and started feeling suffocated hence, began to move rapidly and tried to move away from the cotton balls injected with the oil sample. Experimental cockroaches tried to come out from the plastic jars and were getting anesthetic due to the strong odor of an oil sample compared to the control. During the period of treatment with toxicant cockroaches showed black and white residue after death.

2. Assessment of the lethal dose (LD50)

The concentrations of the toxicant at which 50% of the test animals die are referred to as the median lethal dose (LD50) or medial tolerance limit (Mitoriana *et al.*, 2020) [17]. The result of the lethal toxicity of *Oleo Rheumea* oil was exposed to different concentrations from 0.01%, 0.1%, 1%,

and 10%. LD50 of oil sample for the experimental cockroaches have been summarized in given Table 1. The number of dead cockroaches was recorded every 24 hours and they were removed immediately to avoid the contamination of the exposure solution.

Table 1: Lethal toxicity (LD50) values for *Periplaneta americana* exposed to different concentration of *Oleo Rheumea* (OR)

Different Conditions Used	Total no. of cockroach	After 96 hours No. of dead cockroaches	Percent mortality (%)	Corrected mortality (%)
Control	10	2 ± 0.5	20	0
0.01% OR	10	4 ± 1.5	40	25
0.1% OR	10	7 ± 1.8*	70	62.5
1% OR	10	10 ± 2.3**	100	100
10% OR	10	10 ± 3.6**	100	100

The present investigation showed the rate of mortality of *Periplaneta americana* at different concentrations of *Olea rheumea*. In OR-treated cockroaches, the rate of percent mortality was significantly (p<0.05) increased compared to control ones. Percent mortality of cockroaches treated with

different concentrations of *Olea rheumea* were 0.01% (40%), 0.1% (70%), 1%, and 10% (100%) respectively. In control, 20% mortality was observed. Corrected mortality was calculated to ensure the correct LD50 value (figure 2).

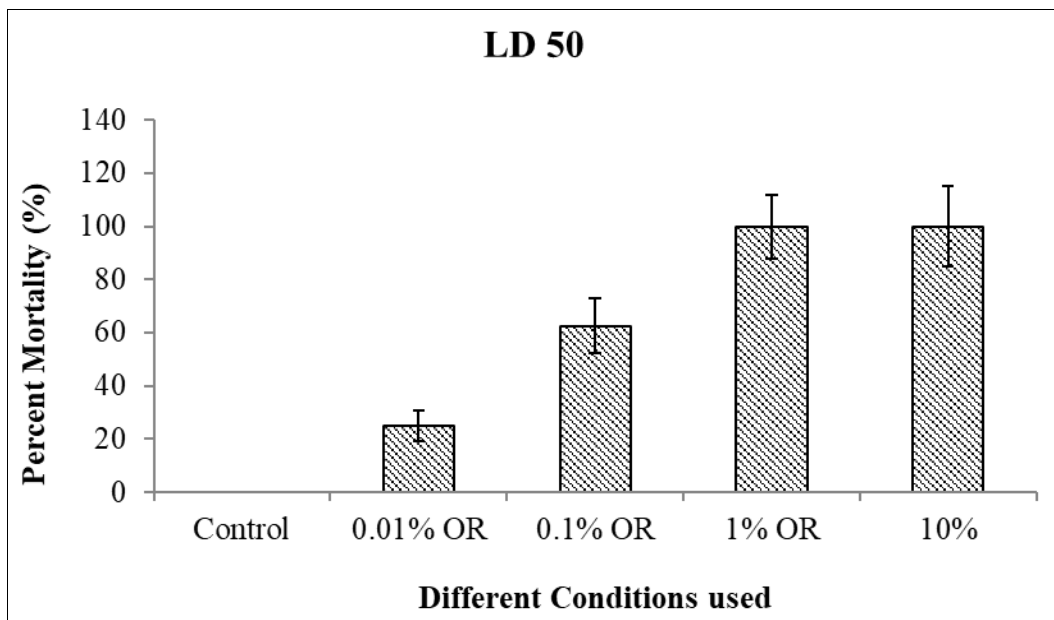


Fig 2: Percent Mortality of cockroaches treated with different concentrations of *Oleo Rheumea* (OR)

3. Damage to biomolecules

The dead cockroaches at each concentration of *Olea rheumea* were dissected. Head and abdominal sections of cockroaches were assessed for damage to biomolecules (lipids and proteins).

3.1. Determination of Lipid Peroxidation

Oxidative damage caused due to *Olea rheumea* was estimated in terms of thiobarbituric acid reactive substances and is expressed in %. The lipid peroxidation showed a

significant (p<0.001) increase in the amount of TBARS formation with an increase in the concentration of the oil sample as compared to the control (Table 2). There was a significant (p<0.05) increase in the amount of TBARS % in the abdominal region as compared to the head region (Figure 3). Maximum lipid damage was observed at 10% concentration of OR (Head- 40 % ± 2.3, Abdomen- 80% ± 2.3) compared to control (Head- 5 % ± 1.2, Abdomen- 10% ± 2.1).

Table 2: Percent of lipid damage in cockroaches treated with Different concentration of *Oleo Rheumea* (OR)

Different Conditions Used	Lipid Peroxidation (%)	
	Head	Abdomen
Control	5 ± 1.2	10 ± 2.1
0.01% OR	10 ± 2.3	26 ± 1.6
0.1% OR	21 ± 3.9	38 ± 2.6
1% OR	35 ± 1.2	63 ± 4.5
10% OR	40 ± 2.3	80 ± 2.3

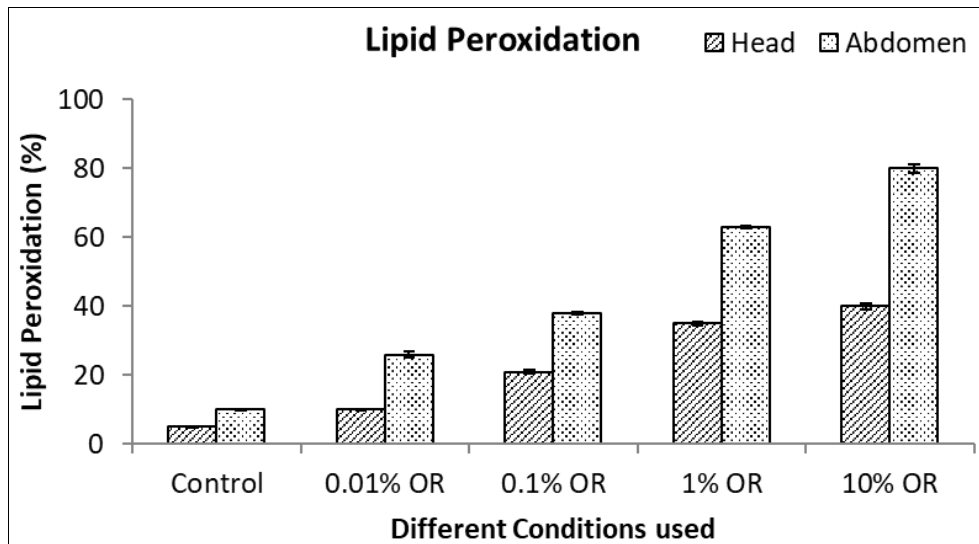


Fig 3: Percent of lipid damage in cockroaches treated treated with different concentrations of *Oleo Rheumea* (OR)

a) Determination of protein carbonyl

Protein oxidation results in protein carbonyl formation. With an increase in the concentration of *Olea rheumea* significant ($p < 0.05$) increase in protein carbonyl formation was observed (Table 3). Maximum carbonyl formation was observed the abdominal region compared to head region at all the concentrations of *Olea rheumea*. In Figure 4 a substantial significant ($p < 0.001$) increase was observed

(Head- 6.3 ± 1.4 μ moles protein carbonyl/mg protein, Abdomen- 9.6 ± 1.8 μ moles protein carbonyl/mg protein) respectively in protein carbonyl formation at 10 % concentration of *Olea rheumea*, compared to control, acetone treated cockroaches (Head- 0.2 ± 0.2 μ moles protein carbonyl/mg protein, Abdomen- 1.0 ± 0.1 μ moles protein carbonyl/mg protein).

Table 3: Amount of Protein Carbonyl formed in cockroaches treated with Different concentration of *Oleo Rheumea* (OR)

Different Conditions Used	μ M of Protein Carbonyl formed/ mg protein	
	Head	Abdomen
Control	0.2 ± 0.2	1.0 ± 0.1
0.01% OR	2.6 ± 0.3	4.4 ± 1.1
0.1% OR	4.2 ± 1.2	6.9 ± 0.8
1% OR	5.6 ± 1.1	8.3 ± 1.1
10% OR	6.3 ± 1.4	9.6 ± 1.8

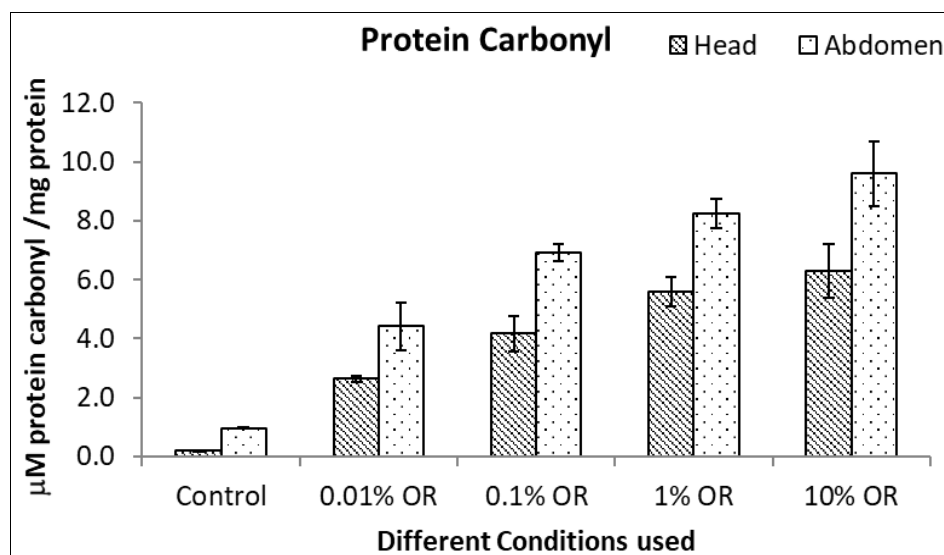


Fig 4: Amount of Protein Carbonyl formed in cockroaches treated with different concentrations of *Oleo Rheumea* (OR)

b) Determination of protein sulphhydryls

Figure 5 shows the depletion of protein sulphhydryl in *Olea rheumea* treated cockroaches at all the concentrations compared to the control. At 10% concentration of *Olea rheumea* significant ($p < 0.05$) depletion protein sulphhydryls was observed (Table 4) in head (0.3 ± 0.1 μ moles protein

sulphhydryl/mg protein and abdominal region(1.6 ± 0.1 μ moles protein sulphhydryl/mg protein) respectively compared to control (Head - 4.3 ± 0.3 μ moles protein sulphhydryl/mg protein; Abdomen- 7.0 ± 0.1 μ moles protein sulphhydryl/mg protein).

Table 4: Amount of Protein Sulphydryl depleted in cockroaches treated with Different concentration of *Oleo Rheumea* (OR)

Different Conditions Used	µM of Protein Sulphydryl Depleted/ mg protein	
	Head	Abdomen
Control	4.3 ± 0.3	7.0 ± 0.8
0.01% OR	3.6 ± 1.1	6.4 ± 0.2
0.1% OR	2.2 ± 0.6	4.9 ± 0.1
1% OR	1.2 ± 0.4	2.3 ± 0.4
10% OR	0.3 ± 0.1	1.6 ± 0.1

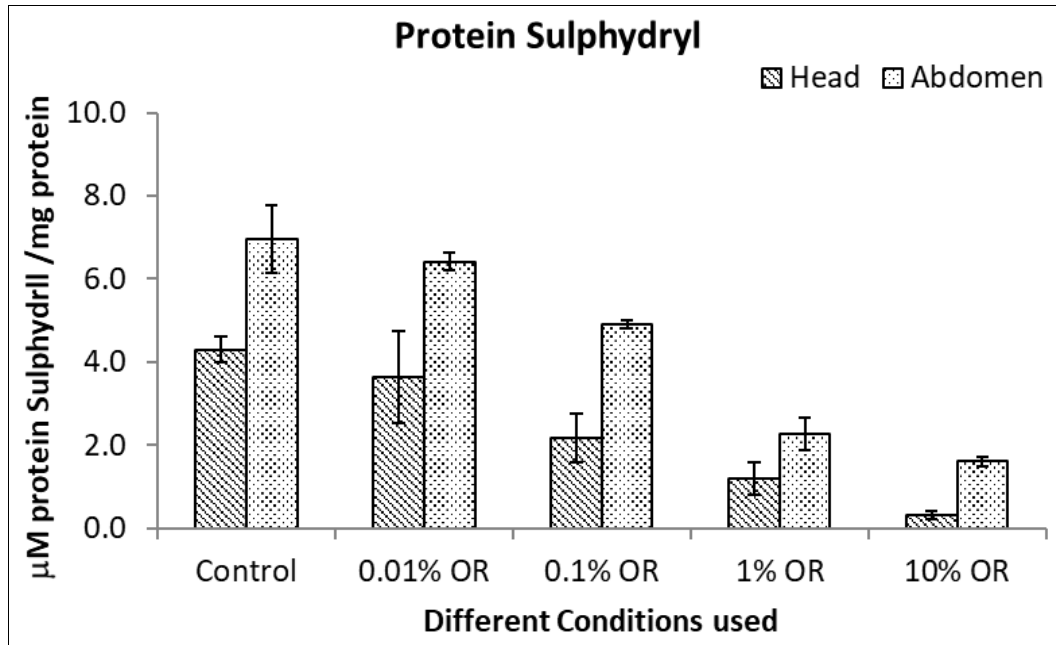


Fig 5: Amount of Protein Sulphydryl depleted in cockroaches treated with different concentrations of *Oleo Rheumea* (OR)

Discussion

Chemical insecticides are a common method of controlling cockroaches, but this has been limited by many problems, including cockroaches developing natural resistance and harming human health (Mackingsley *et al.* 2021) [6]. Therefore, there is a strong emphasis on alternative control agents, such as essential oils, which are environmentally safe and environmentally friendly. (Thavara *et al.* 2007; Cansian *et al.* 2015) [6, 26].

It has been reported that the two main components of the oil “*Oleo Rheumea*” i.e. “*Dohnul Darchini*”, scientifically called *Cinnamomum verum* also called Ceylon cinnamon, and “*Dohnul Khardal*”, scientifically called *Brassica nigra*, commonly known as black mustard act as a strong repellent for insects specifically for cockroaches (Buckle *et al.* 2015) [4]. This oil also contains components like laung and camphor having very strong odour in specific percentages.

During the present work, there was significant repellency observed in treated groups as compared to the control group. Oils were considered toxic if some or all cockroaches behaved sluggishly and were moribund or dead (Sharififard *et al.* 2015). It was evident from the observation table 1 that *Olea rheumea* oil which are present in maximum percentage acts as highly repellent at 1% and 10% after 24 hours of treatment. We also observed a certain number of deaths in control treatment may be due to the direct interaction of cockroaches with the acetone due to the high percentage leading to damage to the respiratory system of an organism.

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products

(Yang *et al.* 2020) [27]. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell including proteins, lipids, and DNA. Oxidative stress can cause disruptions in the normal mechanism of cellular signaling (Akagawa, 2021) [1].

The LD50 value was estimated for the sample “*Oleo Rheumea*” against *Periplaneta americana* using EPA probit analysis program. With the increase in the concentration of the oil sample at 0.01%, 0.1%, 1%, and 10% there was an increase in the mortality of cockroaches. The LD50 value proved that at 10% concentration, oil sample is highly toxic in nature and may be used as repellent in the future to control the growth of insects causing severe health and hygiene problems (Ubulom *et al.* 2021) [22]. Fumigant toxicity of essential oils and their constituents has been reported for various insects including cockroaches, house flies, etc (Mitoriana *et al.* 2020; Coetzee *et al.* 2022) [7, 17].

According to the observation tables, the highest knockdown value was recorded in 1% and 10% concentration as it shows 100% effect on the experimental cockroaches while the least deaths were observed in 0.01% having 20% mortality rate.

The study of molecular damage of lipids and proteins is performed by using three bioassays i.e. lipid peroxidation, protein carbonyl, and protein sulphydryl using different reagents in each bioassay. Damage to the biomolecules is mainly observed in the head and abdomen region of the experimental cockroaches by using reagents like 2-thiobarbituric acid for lipid peroxidation, DNPH for protein carbonyl, and Ellmans reagent for protein sulphonation. Lipid peroxidation is the oxidative degradation of lipids,

Oxidative degradation of lipids occurs due to the loss of electrons (Thiobarbituric acid assay). TBARS detects the level of malondaldehyde (MDA) which is a major lipid peroxidation product. Lipid peroxidation indicates the oxidative stress in an organism. During the present work, we can observe that it shows the increasing amount of TBARS % with increasing concentration of oil sample as compared to control. We can also observe the TBARS % is higher in the abdomen region as compared to the head which shows that production of MDA is higher in the abdomen region and hence the oxidative stress can be seen (Olmedo *et al.* 2015; Maliszewska *et al.* 2018) ^[16, 21].

Protein carbonylation is the appearance of carbonyl groups such as aldehyde or ketone groups in proteins as the result of several oxidative modification reactions. Protein carbonylation is a standard marker for oxidative stress. Protein carbonyl formation was increased with the increasing conc. of *Olea rheumea* concentration as compared to control. In Table 3 with the increase in the concentration of *Olea rheumea* protein damage was observed in the head and abdomen region of cockroaches. As there is increase in oxidation, the formation of carbonyl is also increased during the process (Akagawa, 2021, Hauck *et al.* 2019) ^[1, 12].

The presence of intact disulfide linkages appears necessary to the physiological behaviour of the active principles such as insulin and the appearance of free-SH groups coinciding with loss in activity (Mark *et al.* 2015). Protein sulfhydryl showed decreasing concentration with increasing *Olea rheumea* concentration. We can see there is a depletion in the concentration of Protein sulfhydryl in the head region which shows that the abdomen is more affected with the *Olea rheumea* sample (Żebrowska *et al.* 2019) ^[28].

The toxicity and repellency of *Oleo rheumea* is probably due to the presence of two main components of oil which are mustard oil and cinnamon which can be used as a repellent against cockroaches (Isman *et al.* 2021). The presence of Allyl isothiocyanate (C₄H₅NS) in mustard oil and Cinnamaldehyde (C₆H₅CH=CHCHO) in cinnamon oil has a strong-smelling odor that burns the nostrils of most of the pests hence acts as an effective insect repellent (Buckle *et al.* 2015) ^[4]. Pest management methods ought to be effective because urban pests frequently occupy areas close to human habitations. Environmentally safe, human-friendly, and non-targeted animal-friendly pest management techniques are constantly in demand in place of synthetic, poisonous pesticides for the control of urban pests.

In the current investigation, the possibility of using *Oleo rheumea* as a natural insecticide against *Periplaneta American* (American cockroaches) checked. This is the first report on *Oleo rheumea* as an insecticide for American cockroaches. These findings suggested that American cockroaches could be directly sprayed with *Oleo rheumea* oil that has been converted into an aerosol. *Oleo rheumea* emerges as a promising repellent against American cockroaches and could find applications in surface treatments or fumigation in cockroach control programs. More research is needed to make a controlled formulation from this insecticide that could be used in different places.

Declaration of competing interest

We have read and approved the final version of the manuscript and it has not been published previously nor it is being considered by any other peer-reviewed journals. The

authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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