



Unmasking the Lethal Potential:

Investigating the Impact of essential oils of Eucalyptus (*Eucalyptus saligna*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Turpentine oil and Petrol (Gasoline) on Mortality rate of Subterranean Termite (*Coptotermes heimi*)

Falak Ashraf Siddiqui^a and Barish E. James^{b*}

^{a, b} Department of Zoology, Isabella Thoburn College, University of Lucknow, Lucknow 226001, Uttar Pradesh, India.

Abstract: -

This experiment presents the findings focused on assessing the effectiveness of various oils, including petrol, as wood protectants against termite infestation. The collected data revealed that petrol exhibited the highest mortality rate among termites causing 96.5% mortality in 5 hours of the experiment, compared to other oils, while neem oil exhibited the lowest mortality rate with 46%. Additionally, the study indicates that turpentine oil, eucalyptus oil, and garlic oil demonstrated a high level of resistance against termite infestation and have the potential to be utilized as alternative wood preservatives, offering an environmentally friendly option to synthetic counterparts. The mortality pattern revealed by the findings is as follows: Petrol>Turpentine oil>Eucalyptus oil>Garlic oil>Neem oil. Additionally, the effectiveness of these oils in preventing damage to wood was investigated. Analysis of Variance (ANOVA), and the Test Significant Difference ($p < 0.05$) were employed for data analysis. The results revealed a significant increase in the mortality rate of termites exposed to petrol, turpentine oil, eucalyptus oil, garlic oil and neem seed oil than in the control treatment. These findings highlight the potential of these oils in effectively controlling termite infestation and minimizing wood damage.

Keywords: - Subterranean termites, *Coptotermes heimi*, Mortality rate, Petrol, Garlic oil, Neem oil, Eucalyptus oil, Turpentine oil, Natural alternatives.

Introduction: -

After experiencing earthquakes, several developing countries concentrated on adopting wood-framed constructions to lessen casualties (Haseeb, et al., 2011). Termites, social insects belong to the Isoptera order (Horwood & Eldridge, 2005). Termites, a dangerous pest, cause enormous damage to buildings, furniture, and all cellulose-containing materials (Mohanny & Ghada, 2023). Termites are prevalent wood pests, and the utilization of synthetic insecticides for their control poses a significant risk to the environmental well-being (Alamu and Ewete, 2023). The colony of Subterranean termite consists of three interdependent groups, or 'castes' with particular forms and functions: workers, soldiers, and reproductive forms. Workers form the colony's majority. They are wingless, blind, and infertile with round, pale heads and delicate bodies. They work tirelessly to feed, construct, and care for the colony, and are responsible for most of the damage when infesting timber. Soldiers, like workers, lack wings, are sterile, and blind. They may have large mandibles and a noticeable snout, with possible variations within a colony. Their primary duty is defending the colony against their main adversaries – ants (Horwood and Eldridge, 2005). Subterranean termites, specifically, establish their colonies in soil or trees, with a preference for areas characterized by high moisture levels. In certain cases, their nests can extend beyond ground level, resulting in the formation of mounds (Horwood & Eldridge, 2005). Termites in the genus *Coptotermes* are widespread pests (Gentz, et al., 2008).

Coptotermes spp. is recognized as the most aggressive species of subterranean termite (Kuswanto, et al., 2015). Due to their vulnerability to desiccation, subterranean termites are widely acknowledged to rely heavily on the relative humidity levels in their inhabited environments for their survival. In tropical regions, where the diversity of termites is high, attacks on buildings typically originate from a ground nest. Termites construct galleries on trees or building walls as pathways to access the cellulose, such as wood, within the structure. Even after infiltrating the building, termites maintain connections with the ground for moisture and the nest center for communication.

As a result, they pose a significant threat and cause substantial damage in tropical areas with their extensive presence (Ghaly & Edwards, 2011). Subterranean termite attacks contribute to approximately 90% of the overall economic loss and account for around 70% of the construction damage incurred (Kuswanto, et al., 2015). Termite damage has resulted in significant economic losses for various countries, with Malaysia, India, Australia, China, Japan, and the United States experiencing losses of 10, 35, 100, 375, 800, and 1,000 million US dollars, respectively. (Ghaly & Edwards, 2011)

*Corresponding author: Termites, classified as insects of the order Isoptera, encompass a diverse group of approximately 2,500 species, with around 300 of them recognized as pests (Ahmed, et al., 2016) and out of the 38 species of subterranean termites, the genus *Coptotermes* stands out with the highest number of species, followed by *Macrotermes*, *Reticulitermes*, and *Odontotermes* (Rust & Su, 2012). Termites are abundant in India. Around 220 different termite species, each with its own distinctive traits and habitats, are found in India. Several termite species are commonly found in various regions of North India. *Odontotermes obesus* and *Coptotermes heimi* are found in UP, Punjab, Haryana, and Rajasthan. *Coptotermes gestroi* is common in Delhi, UP, and Bihar; *Microcerotermes beelsoni* is found in UP and Uttarakhand; *Heterotermes indicola* is widely distributed in UP, Punjab, and Haryana; and *Macrotermes* spp. is commonly found in UP, Punjab, and Haryana.

Of the 2,700 termite species known in the world, 80 termite species were considered serious

The use of chemical insecticides has negative impacts on organisms that are not the intended targets, as well as the environment and water sources (Nisar, et al., 2022) and also has several drawbacks, including strong odors, handling issues, challenges in painting applications, potential skin irritation for workers, toxicity to non-target organisms, and the risk of flammability (Edlich, et al., 2005). Considering the limitations associated with traditional preservatives, an alternative method for wood protection involves utilizing hydrophobic plant, vegetable oils or essential oils, which offer advantages such as reduced toxicity to non-target organisms and the environment (Ahmed, et al., 2020). A notable advantage of using essential oils as an alternative wood preservative is their environmental friendliness, causing no harm to the ecosystem and offering a naturally sustainable solution (Meisyara, et al., 2021). Many research investigations have found that essential oils repel termites and other insects and are hazardous to them (Verma, et al., 2009). In addition to their toxicity, some oils have the capacity to transmit toxicant deeply into the wood (Ahmed, et al., 2013), (Ahmed, et al., 2014), (Fatima & Morrell, 2015).

India has enormous plant diversity, which is the source of essential oils. Yet, there are countless essential oils that have never been evaluated for their termiticidal properties. Thus, this study aimed to evaluate the anti-termite activity of few essential oils, such as Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus saligna*), Garlic (*Allium sativum*), Turpentine oil (Pine oil) and Petrol against subterranean termites (*Coptotermes heimi*). Various oils, including those from *Jatropha*, *Jajoba*, *Neem*, and *Eucalyptus*, have reportedly been discovered to be toxic, possess antifeedant qualities, and efficiently ward off insects, including termites (Batish, et al., 2008), (Singh & Sushilkumar, 2008), (Manzoor, et al., 2012), (Himmi, et al., 2013), (Alavijeh, et al., 2014), (Adebawo, et al., 2015).

More than 55 nations currently employ *Neem tree (Azadirachta indica)* extracts for pest management. *Neem* products have been utilized for over 2,500 years in several parts of Asia, including India and Burma (Ahmed, et al., 2016). According to a trailblazing researcher (Gahukar, 2010), *Neem (Azadirachta indica A. Juss.)* is one of the promising plant species that can produce adequate quantities of active compounds and has been extensively used for large-scale pest management. A promising method of wood preservation uses the oil from the seeds of some tree species to create biodegradable chemicals that can increase the useful life of non-durable wood. One species that has this capability is *Neem (Azadirachta indica)* (Okanlawon, et al., 2020). The leaves and seeds of *Neem (Azadirachta indica)*, the bulbs of *Garlic (Allium sativum)*, and the seeds of *Physic nut (Jatropha spp.)* have all been used to successfully manage *Macrotermes* spp. Because of the harm it does to household and agricultural goods, *Macrotermes* spp. is the most important termite pest (Addisu, et al., 2014) hence control measures should be taken.

Clove bud and Garlic oils demonstrated the strongest antitermitic activity among the various plant essential oils tested after testing 59 oils on the Japanese termite, *Reticulitermes speratus* Kolbe (Park & Shin, 2005). Garlic essential oil has been shown in many studies to have insecticidal action against *Blattella germanica* Linnaeus (Blattodea: Blattellidae) (Tunaz, et al., 2009), *Lycoriella ingenua* Dufour (Diptera: Sciaridae) (Park, et al., 2006), *Reticulitermes speratus* Kolbe (Isoptera: Rhinotermitidae) (Park & Shin, 2005), and several grain storage insects as *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), *Sitophilus oryzae* Linnaeus, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and *Tribolium castaneum* Herbst (Coleoptera: Curculionidae) (Huang, Chen, & Ho, 2000), (Mikhael, 2011). *Eucalyptus*, which has over 700 species worldwide, supplies a range of components extracted from its essential oil, including an acaricide agent, which is an insecticide (Samani, et al., 2015). *Eucalyptus* spp. essential oils are thought to have repellent and insecticide properties (Nerio et al., 2010). Among the several components of *Eucalyptus* essential oil, 1,8-cineol is the most prominent and plays an important role in its insecticidal effect (Duke, 2004). Petroleum oils have repellent or toxic effects on ants, termites, and other soil insects when

applied as pesticides (Rahman, 2007). Turpentine is also a natural insecticide that has been proven to be effective at killing termites. Turpentine, derived from pine resin and pine oil, is used to repel boring larvae in tree preparations as well as flea, tick, and lice treatments for pets (Buchanan, 2011).

Considering the suitable effect of plant essential oils on pests, this study is devoted to analysing the toxicity of four essential oils and petrol on an important pest, the subterranean termite (*Coptotermes heimi*), and whether they can be used as natural substitutes for synthetic wood protectants. Existing methods of subterranean termite control and their success are discussed.

Materials and Method: -

The study was conducted during March 2023 at $24.17 \pm 5^\circ\text{C}$ and $70\% \pm 5\%$ humidity.

Collection of Insects and Soil: Eastern Subterranean Termites (*Coptotermes heimi*) were collected from field colonies in the study area. The soil used came from the near vicinity of a field termite colony (termite's natural habitat). Distilled water was sprayed onto the soil before to the experiment, and this moistened soil was employed in experimental units.

Preparation of Containers: Plastic containers were selected for the experiment. 60 plastic containers (7.5 cm L x 3 cm H x 6 cm W) used for housing the termites. Each container was filled with moistened soil, creating a 0.5 cm thick layer at the bottom. 5 grams of moistened soil were layered in each container.

Ventilation: To provide ventilation, the top lids of the plastic containers were pricked with sterilized needle to make several holes, allowing for airflow while maintaining a controlled environment.

Placement of Wooden Blocks: 60 wooden blocks weighing 5 grams each were used for serving as feeding substrates for the termites. They were placed inside the plastic containers. Each container received one wooden block, ensuring that it was in contact with the soil layer. After that, the wooden blocks were treated with the respective oils (0.5 ml).

Selection and application of Oils: This study used various types of oil extracts and petrol. Turpentine oil, Eucalyptus oil, Neem oil, Garlic oil, and Petrol (Gasoline) were among the oils used. A syringe was used to measure them at 0.5 ml. The oils were applied dropwise to the respective wooden blocks, which served as food substrates for the termites, using the same syringe for easy application. On each wooden block, 0.5 ml of oil was applied uniformly.

Placement of Insects: Each box contained 20 termites that had been meticulously put.

Replication: The above steps were repeated with five different oils consisting of 10 replicas each. The design of the experiment was completely randomized with 5 treatments and 10 replications of each. Control without any treatment was also included.

Control set-up: Plastic boxes were utilized with a 0.5 cm thick layer of moistened soil (5 grams) at the bottom. The lids of the boxes were equipped with holes to allow for ventilation. Wooden blocks of 5 grams each, without any oil treatment, were placed inside the boxes as the feeding substrates for termites. 20 termites were carefully placed in each container. A total of 10 replicas were created for this group, ensuring a sufficient sample size for comparison with the experimental groups. This control setup will provide a baseline against which the effects of the different oils can be evaluated, allowing for a more accurate assessment of their impact on termite behaviour and mortality.



Figure 1: Sample Model (Plastic Container containing soil and a wooden block)

The percentage of death was determined by counting the number of insects that died every hour until 100% mortality was reached, which took 5 hours. The mortality index was computed as the ratio of the mean number of dead insects to the total number of insects treated. The following formula was employed: -

$$\text{Mortality index} = \frac{\text{Mean no. of insects dead}}{\text{No. of insects treated}} \times 100 \%$$

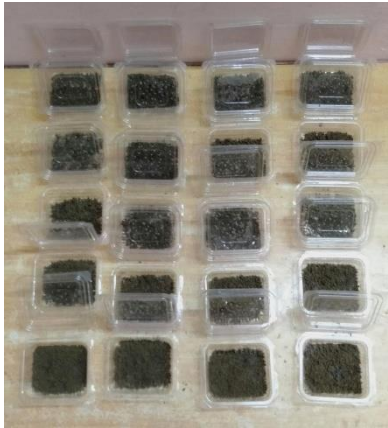


Figure 2: Plastic containers filled with moistened soil.



Figure 3: Experimental set-up



Figure 4: Termites (*Coptotermes heimi*) (S- Soldier, W- Worker)



Figure 6: *Coptotermes heimi* Soldier



Figure 5: *Coptotermes heimi* Worker

Statistical Analysis: - The data was projected for statistical analysis in order to detect significant differences between distinct essential oils of Neem, Eucalyptus, Turpentine, Garlic, and Petrol treatments. The dependent variable is Mortality; Oil types are factors. The analysis of Variance (ANOVA) was used. The test's significance level was $P < 0.05$. The computations were done with IBM SPSS statistical software, and the graphs were made with GraphPad Prism 9 software for Microsoft.

Observation: -

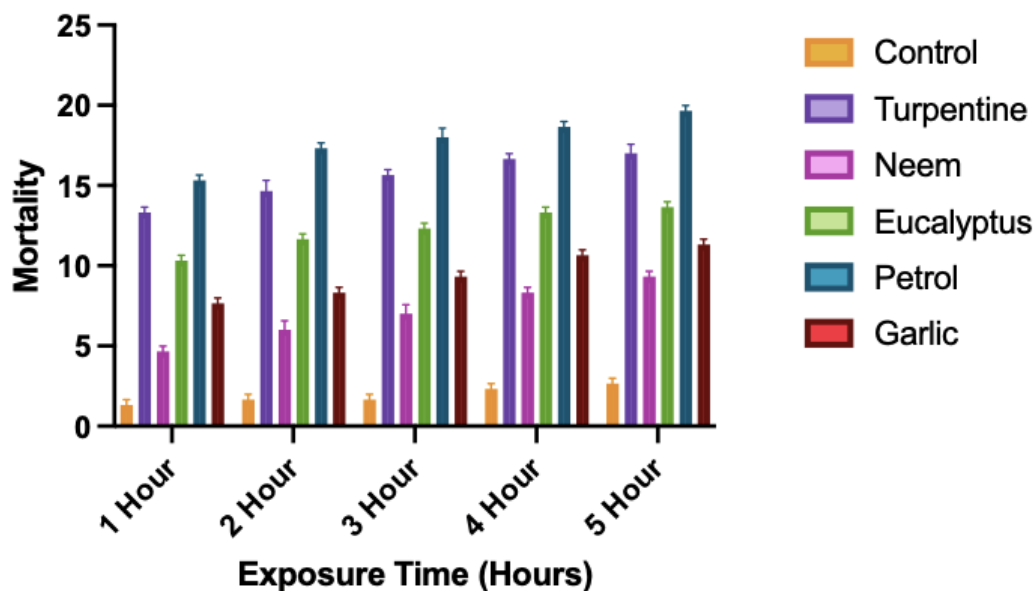
Table- 1

Mortality Rate of different Essential oils and Petrol against Subterranean Termite (*Coptotermes heimi*)

S. No	Treatment	N	Mortality (Mean \pm SE)				
			1hr	2hr	3hr	4hr	5hr
1	Control	20	1.33 \pm 0.33	1.66 \pm 0.33	1.66 \pm 0.33	2.33 \pm 0.33	2.66 \pm 0.33
2	Turpentine oil	20	13.33 \pm 0.33	14.66 \pm 0.66	15.66 \pm 0.33	16.66 \pm 0.33	17.00 \pm 0.57
3	Neem oil	20	4.66 \pm 0.33	6.00 \pm 0.57	7.00 \pm 0.57	8.33 \pm 0.33	9.33 \pm 0.33
4	Eucalyptus oil	20	10.33 \pm 0.33	11.66 \pm 0.33	12.33 \pm 0.33	13.33 \pm 0.33	13.66 \pm 0.33
5	Petrol	20	15.33 \pm 0.33	17.33 \pm 0.33	18.00 \pm 0.57	18.66 \pm 0.33	19.66 \pm 0.33
6	Garlic oil	20	7.66 \pm 0.33	8.33 \pm 0.33	9.33 \pm 0.33	10.66 \pm 0.33	11.33 \pm 0.33

- Mean of three replications are considered.
- N = No. of Insects (Termites)
- SE = Standard Error
- Test Significance level $P < 0.05$.

Graph- 1



Mean Mortality of different Essential oils and Petrol against Subterranean Termite (*Coptotermes heimi*)

Result: -

As table 1 reveals, the mean of the Control is 1.33, 1.66, 1.66, 2.33, and 2.66 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The mean of Turpentine oil is 13.33, 14.66, 15.66, 16.66, and 17.00 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The mean of Neem oil is 4.66, 6.00, 7.00, 8.33, and 9.33 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The mean of Eucalyptus oil is 10.33, 11.66, 12.33, 13.33, and 13.66 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The mean of Petrol is 15.33, 17.33, 18.00, 18.66, and 19.66 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The mean of Garlic oil is 7.66, 8.33, 9.33, 10.66, and 11.33 for 1 hour, 2 hour, 3 hour, 4 hour, and 5 hour respectively. The application of essential oils used in this study and petrol had a substantial effect on termite control. According to the findings, maximum mortality was observed when termites were fed on Petrol treated wood, i.e., 96.5%. However, significantly lower mortality of termites when fed on wood treated with Turpentine oil with 84% mortality followed by Eucalyptus oil with 67% was observed compared to other oil treatments. Garlic and neem oils showed significantly lower mortality rates compared to other oil treatments for this species, with 57% mortality in garlic oil and 46% mortality in neem oil. The study revealed the following trend in mortality: Petrol>Turpentine oil>Eucalyptus oil>Garlic oil>Neem oil. The F value for different treatments varied significantly for each hour of observation, with the maximum being $F = 316.800$ for Hour 4 and the lowest being $F = 163.509$ for Hour 2, according to the results of the ANOVA.

Discussion: -

Present study shows the potential of several oils as natural replacements for synthetic wood protectants, as well as their ability to control pests. After 5 hours of examination, each essential oil was found to have a mortality effect on the subterranean termite *Coptotermes heimi*. Petrol (Gasoline) had the greatest mortality rate, i.e., 96.5%, after 5 hours of the treatment. This phenomenon is consistent with the findings of a study conducted by (Rahman, 2007), who claimed that when used as insecticides, petroleum oils have repellent or toxic effects on ants, termites, and other soil insects, confirming that petrol can be used as an alternative to synthetic pesticides to kill termites in a very short period of time.

The rapid onset of toxicity from essential oils in insects, including termites, may indicate a neurological mode of action. Using the American cockroach as a model, Enan demonstrated that essential oil contents poison insects by inhibiting octopamine receptors (Enan (a), 2005). In arthropods, octopamine acts as a neuromodulator and neurotransmitter. Enan's findings in *Drosophila* and the American cockroach (Enan (a), 2005), (Enan (b), 2005) suggest that eugenol, cinnamic alcohol, and trans-anethole bind to and activate G-protein-

coupled neurological and olfactory receptors, causing a metabolic disruption that may kill the insect. In insects, cell membrane disruption or tracheal obstruction may be alternative routes of rapid-onset toxicity.

Other treatments had a lower mortality rate and were shown to be less potent when compared to petrol. After petrol, the mortality was highest in Turpentine oil. Taking into account the 84% mortality rate of turpentine oil and the 67% mortality rate of eucalyptus oil on termites. The outcome of eucalyptus oil in this study is also supported by the study conducted by (Manzoor et al., 2012) where 100% mortality was observed after nine days of exposure for *M. obesi* at all doses of eucalyptus oil, but does not correlate with the study of (Ahmed, Fatima, & Hassan, 2020) where eucalyptus was least effective. The insecticidal characteristics of Eucalyptus oil were discovered in a study by (Alavijeh et al., 2014), as a high feeding level in a toxicity trail might be ascribed to the effect of eucalyptus oil on the nervous system of termites. Furthermore, eucalyptus oil has a broad range of biological activity, including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal, and nematocidal properties (Batish et al., 2008). It should be noted that eucalyptus oil is a highly effective product that may be used as a biopesticide to control termites.

The garlic oil results in this study are consistent with the findings of (Huang & Lam, 2000), who discovered that garlic extract and its principal components, diallyl disulphide, diallyl trisulphide, and diallyl sulphide, are toxic to *Sitophilus zeamais* and *T. castaneum*. Garlic extract has been shown to be harmful in the workplace, other than to insect pests (Park & Shin, 2005). (Mobki et al., 2013) reported a similar outcome, achieving 83.3% death of *Tribolium castaneum* larvae within 48 hours.

In this study, termites fed on wood treated with neem oil had the lowest mortality rate of the five treatments, and the findings corroborate with those of (Fatima & Morrell, 2015), where neem had less mortality rate of the oils tested. However, this does not correlate with the study by (Ahmed, et al., 2020), where neem showed high mortality among termites. Bioactive compounds derived from the plant neem (*Azadirachta indica* A. Juss) have been demonstrated to have biological effects on insects such as feeding deterrent, oviposition and growth suppression, and contact poisoning. The effectiveness of the Defatted Neem Oil (DNO) formulation against subterranean termites, *Coptotermes*, has been extensively evaluated in wood protection and soil barrier studies. The formulation product contains DNO as an active ingredient as well as other inert chemicals that aid in the delivery of the active ingredients to control the target pest and boost the product's use (Himmi, et al., 2013). Both protozoa genera were absent from the stomachs of workers exposed to Neem, Eucalyptus, or Jatropha oils after a one-week sampling, suggesting that these oils had a profound and practically immediate adverse effect on termite health (Fatima & Morrell, 2015).

Essential oils may also operate synergistically with existing preservatives, allowing low preservative concentrations to be equally effective while also being more environmentally friendly. Newly developed wood protection systems with insecticidal qualities that limit decay and mould fungi would have a greater applicability in the field of forest products. Additives to coatings and sealants, fumigants for warehouse storage, additives to engineered composites, and surface treatment for framing timber during construction are all possible applications (Clausen & Yang, 2008). It can be inferred that these essential oils also contain some bioactive compounds that induce toxicity in termites, causing their mortality.

Conclusion: -

This study evaluated the effects of Petrol and four different oils on the mortality of subterranean termite (*Coptotermes heimi*). Additionally, it looked at these oils' effectiveness as termite mortality for wood, including petrol. According to the findings, within 5 hours of treatment, all essential oils and petrol produced termite mortality. However, petrol was the most successful in producing a rapid 96.3% mortality rate within five hours of the experiment. Turpentine oil, eucalyptus oil, and garlic oil came in second, third, and fourth place, respectively, while neem oil showed the lowest mortality rate, i.e., 46%. The use of petrol, turpentine oil, eucalyptus oil, and garlic oil as termite repellents for wood was very encouraging and could be used as natural substitutes for synthetic wood protectants. The results illustrate that oils and petrol have the ability to control this species with natural products, while the long-term implications of these volatile compounds remain unknown. However, repeated use of these natural oils and petrol will be necessary for long-term sustainability.

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