MedCrave
Step into the World of Research

International Journal of Avian & Wildlife Biology

Research Article

Open Access

CrossMark

Efficacy of leaf extracts of apple of sodom (*Calotropis procera*) for the control of subterranean termite *Odontotermes Obesus*

Abstract

Despite serious safety concerns, *Calotropis procera* is used for gastrointestinal disorders. The subterranean termites *Odontotermes obesus* is extremely invading wood pests that cause direct economic losses worth billions of dollars throughout the world particularly in humid and subtropic regions. Leaf extract of *Calotropis procera* in two solvent hexane and acetone, is used against workers of subterranean termites, mortality. LC_{50} and tunneling behavior of termites was observed at different concentration of 3%, 7% and 10% respectively and distilled water applied a control. Two solvent hexane and acetone is used for extraction. Data have been analyzed using software Minitab (version 16).

Volume 6 Issue 1 - 2022

Khalid Z Rasib ,Iqra bibi, Palwasha Saif, Warisha Saif, Khushbo Fatima, Zahid Farooq
University of Lahore, Institute of Biology and Biotechnology (IMBB), Pakistan

Correspondence: Khalid Z Rasib, University of Lahore, Institute of Biology and Biotechnology (IMBB), Off Raiwind Campus, Pakistan, Email khalidrasib786@ gmail.com

Received: August 18, 2022 | **Published:** September 2, 2022

Introduction

Calotropis procera commonly called milkweed and a belong to family Apocynaceae. It is low growth weed frequently originate in arid, dry, uncultivated soils. *Calotropis procera* leaves rubber and flowers have been used in Ayurvedic and Unani medicines. Latex of Calotropis. procera has also been shown to have pesticidal property against termites. The latex fillings have been investigated by many scientists and it is a list of mechanisms, include, proteolytic enzymes, gutta-percha rubber, alkaloids, amino acids, carbohydrates, and hydrocarbons resembling with petroleum etc. The latex contains proteolytic enzymes.

Milkweed plant belongs to the family Asclepiadaceae a common harsh environment unwanted plant dispersed in humid and subtropic section of Asia.[1] In native system of Ayurvedic parts of the plant are used as medicine. Similarly, in the recent years milk wild plant gained importance as a potential pesticidal source in contrast to insect pests. It is insecticidal to known have antifeedant,[2] antitermiticidal,[3] antiseptic,[4] nematocidal and antifungal properties.[5] The extract plant of leaves Calotropis procera had shown effectiveness against lepidopterous and sucking pests of some cultivated crops.[6] Extracts of milkweed plant containing insect toxic principles such as cardenolides, cardiac glycosides, flavonoids, giganticness (a novel nonprotein amino acid) and other cytotoxic principles which are effective against an arrangement of insect pests.

Some botanical insecticides, such as *Azadirachta indica* oil, severally effect the immune system of various insect species.[7] This help protects agro-farms and furniture from termite invasion in tropical communities. In addition, the helpfulness of these plant peels found in the area was appreciated. Termites are among the most catastrophic insect pests; they belong to the isopteran order of insects. So named because of the equal shape and venation (An arrangement or system of vein) of the fore and hind wings.[8]

Out of 300 species of termites known so far from Africa, about 35 species. Such as East Africa, in some part of Africa, Macrotermes basis yield of loss 30-60%. Severely damaging agricultural crops and urban organization.[9]

Termites are dominant pests of tree plantation and agriculture crops products of wood in warm temperate, tropical and subtropical areas of the world. Infestation of termites may be controlled or prevented by several ways in different countries.[10] Current methods of termite's management for building include discriminating soil treatments (Interior and exterior perimeter treatment or spot treatment), non-repellent chemical and baiting with slow acting.[11]

The higher termite's species Odontotermes obesus, is fungus growing and a scavenging termite. There are various field and laboratories studies that have been conducted against this specific species which are involving in different insecticides but with different ways of methodologies and purpose.[12]

Protozoa population was directly related to the filter paper area consumption treated with plant extract. And these are found that after two weeks of experience of termites to treated filter paper in no-choice bioassay at the concentration of 20mg/ml of plant leaf extract of Calotropis procera reduced the protozoa population by 62.90% as compared to the untreated negative control.[12] Now a day, compared pyrethroids and organophosphate insecticides, imidacloprid and fipronil as newer chemical gaining acceptance as termicides. for long term safety imidacloprid is popularized in terms of perimeter protocol owing to reduction of active ingredients utilization for this purpose and innately provision of advantage to bring down potentials.[9]

Materials and methods

Collection of plant leaves calotropis procera

The plants leave Calotropis procera were collected the different area from The University of Lahore,[13] the fresh and healthy leaves of plant Calotropis procera were collected for the experiment. Subsequently, plant leaves of Calotropis procera were carried in The University of Lahore laboratory in the Pakistan the leaves were cleaned with distilled water to remove dust and other undesirable soil particle.

Grinding of leaves

The cleaned leaves shade down in the 15 days. The dry leaves were grinded in an electric grinder and kept in the container. Finally, fine powder was collected in the bag to avoid in moisturize.

Collection of termites

The test termite species will be collected from The University of Lahore & brought in the Entomological Research laboratory. Termites will be kept in plastic bag along with trays in the laboratory. Water and tissue paper will be used as the food source/substrate.[14] Termites will be maintained in the laboratory condition under dark environmental using black cloth.

Experimental treatment

The arrangement was complete treatment three replicates in each concentration. Treatment comprised in extract of n-hexane *Calotropis procera* and extract of acetone *Calotropis procera* applied at the different concentration for the control of termites. Untreated the three replicates petri dishes to apply the distilled water for the control of termites. Different concentration of termicides were applied on the toxicity test. The termite's mortality data were recorded every 30-minute interval until 8 h after experience. The mortality was calculated using percentage termite's:

$$Mortality\ (\%) = \frac{Number\ of\ dead\ termites}{Total\ number\ of\ termites} \times 100$$

Filtration process

The solvent mixture shakes in every two hours then I brought Buchner funnel, carbon filter paper and vacuum flask 1000 g in the laboratory of University of Lahore. The filter papers are double coated to wrap in funnel to fit in vacuum flask. The mixture is pass through the filter paper in which liquid fluid are removed but retains the waste solid particle in the Calotropis procera of leave. Either the clarified mixture, and waste particle removed from the mixture may be the clear mixture are formed.

Preparation of plant extracts

The dried leaf (800 g) powder used was extracted with n- hexane (1400 ml) and acetone (1000 ml). And heated at 60–80 C°) for 8 hrs.[8] The extracted crude mixture was stored at refrigerator temp (- 4 to - 60 C°).

Principle of rotary evaporator

Rotary evaporators extract solvent at low temperature with a high degree of repeatability and efficiency. The technique of the action decreases the pressure inside of the round bottom flask and the surface area increase through gentle lowest rotation which improvements process time. The principle is the liquid of the boiling point condense on decreasing their pressure, their allowing solvents to be vaporized at much lower temperatures than they're at normal pressure at boiling point. A condenser at the other end changes the gas back to liquid, which lowered temperatures is required.

Antitermitic bioassays

Antitermitic bioassays using leaf extracts will be performed in Petri dishes at 10 g soil. Every treatment with 3%, 7% and 10% concentration of plant extracts N-hexane and acetone with control will be repeated thrice.[15] Data for mortality will be recorded after an interval of 30 minutes up to 8 hours in a toxicity test.

Effect of plant extracts on galleries formation

Termitidae members of the family make galleries during foraging. This shows the termites in the activity of the soil. The termites in progress making tunnel along the bottom of each Petri dish. The galleries formation termites' response towards for each extract at each concentration after 2 hours was determined.

Statistical analysis

ANOVA test in statistical of Minitab (conversion 16, 19) was carried at significance level $P<0.05$.[2]

Results

Toxicity test

Toxicity test indicated that only one concentration 3% take time more than 8hour to kill the all termites. But other two concentration take less time more than 8hour to kill the all termites. The 7% concentration take less time than 3%concentration to kill the termites. Whereas the 10% concentration take more less time than 8hour in 3% concentrations to kill the all termites. The percentage mortality for subterranean termite in extract of acetone *Calotropis procera* and N-hexane *Calotropis procera* treated with soil, within 8 hours was, 100%, 90%, 85%, 43% in 3%, 7%, 10% and 0% concentration in control group respectively. The lowest percentage mortality was observed in low concentration in0% is 43%. And the highest percentage mortality was noted in highest concentration 10% is 100%. In control treatment, soil is treated only with distilled water and lowest percentage mortality was recorded is 43%. The different in toxicity of each concentration of acetone and N-hexane was significantly different ($p<0.05$).

Toxicity test of n-hexane

Data (%) were transformed before the analysis of variance; means (-standard error) are values three replicates; means with the same lower case alphabet column are not significantly different from each other ($p>0.05$); in different concentration (3%,7% and 10%); p= probability value.

Percentage mortality of termites. *Calotropis procera* using the N-hexane as solvent in the Calotropis procera in toxicity test. Values with percentage mortality rate at every 30-minute shows percentage standard error at each time points (Tables 1-3) (Figures 1 & 2).

Table 1 Time in 8 hours N-hexane

Concentration	1hour	2hour	3hour	4hour	5hour	6hour	7hour	8hour
control	.066±.057	.133±.057	.200±.000	.233±.057	.266±.057	.300±.100	.333±.057	.333±.057
3%	.366±.057	.433±.057	.500±.000	.533±.0577	.600±.000	.633±.570	.666±.057	.700±.000
7 %	.700±.000	.766±.057	.833±.083	.900±.1000	.966±.057	1.00±.000	1.00±.000	1.00±.00
10%	.933±.057	1.00±.000	1.00±.000	1.00±0.00	1.00±.000	1.00±.000	1.00±.000	1.00±.000
P value	0	0	0	0	0	0	0	0

Table 2 Toxicity test of Acetone, time in 8 hours' acetone

Concentration	1hour	2hour	3hour	4hour	5hour	6hour	7hour	8hour
control	.0667±.05774	.1333±.05774	.2000±.000	.266±.057	.333±.577	.366±.057	.433±.115	.466±.152
3%	.3667±.577	.433±.0774	.5000±.0000	.733±.057	1.000±8.00	.833±.057	.9000±.000	.933±.057
7%	.7000±.000	.7667±.057	.833±.057	.866±.0577	.633±.461	.966±.057	1.00±0.00	1.00±0.00
10%	.933±.0577	1.000±.000	.900±.000	1.000±.000	1.00±0.00	1.000±0.00	1.000±0.00	1.000±0.00
P value	0	0	0	0	0	0	0	0

Table 3 Toxicity test to recorded the data of mortality rate 4 h and 8 hours

Time	Doses	X2	df	Mean square	F	Sig
4 hours	Acetone	3.269	15	0.218	24.262	0
8 hours	Acetone	0.38	45	0.008	0.94	0

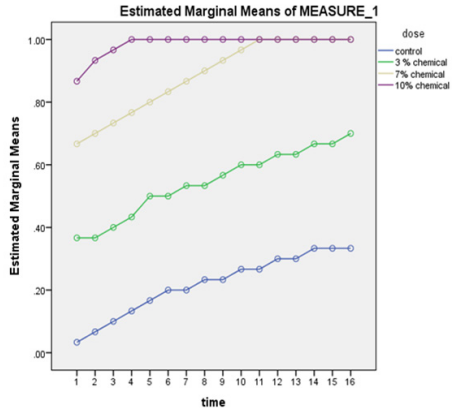

Figure 1 n-hexane *Calotropis procera* of toxicity test for the mortality rate with n-hexane.

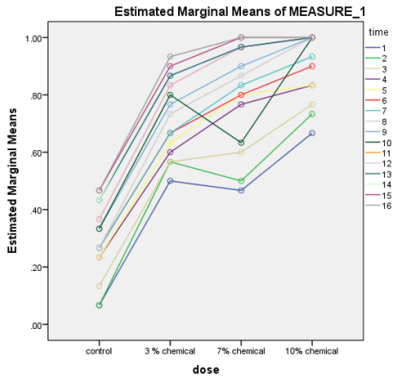

Figure 2 Comparison of LC_{50} values (hrs) of different concentration of leaf extracts of using *Calotropis procera* in different solvent.

In the case of using the leaf extract of N-hexane *Calotropis procera* at high mortality of termites Odontoterms obesus at higher concentration 10% after 8 h of treatment was observed 91.7 % and 81.13% with N-hexane and acetone based on extracts, respectively. At 7% concentration after 8h of treatment mortality was observed 76.0% and 66.1% with N-hexane and acetone based on extracts, respectively. At 3% concentration after 8 h of treatment mortality was observed 54.1% and 43.1% with N-hexane and acetone based on extracts, respectively. Therefore, lowest mortality was observed 43.1% in acetone of extracts of *Calotropis procera* at the lowest dose 3% after 8 h of exposure time.

All concentration (3%, 7% and 10%) of two solvent *Calotropis procera* significant values. N-hexane extracts was comparatively more effective than the extracts of acetone *Calotropis procera*. Therefore, mortality response was influence by exposure time and concentration of the plant extracts of leaves *Calotropis procera*. Probit analysis of N-hexane and acetone extracts of *Calotropis procera* showed LC50 for 8 h (3%, 7% and 10%) was respectively. Table show the significance level of different concentration. All the solvent same the concentration shows significance value in acetone and N-hexane the extracts of *Calotropis procera* (Table 4 & 5) (Figure 3 & 4).

Table 4 LC_{50} values calculated of N-hexane using the extract of *Calotropis procera*

Time	Regression Equation	Significance values	X2	LC50	Lower bound	Upper bound
4 hours	401.617	0	6.726	3.298	2.658	3.98
8hours	673.512	0	8.679	2.55	1.678	2.478

Table 5 LC_{50} values calculated of acetone using the extract of *Calotropis procera*

Time	Regression Equation	Significance values	X2	LC50	Lower bound	Upper bound
4 hours	229.837	0	7.409	5.425	4.349	7.893
8hours	-353	0	8.065	2.243	1.069	2.678

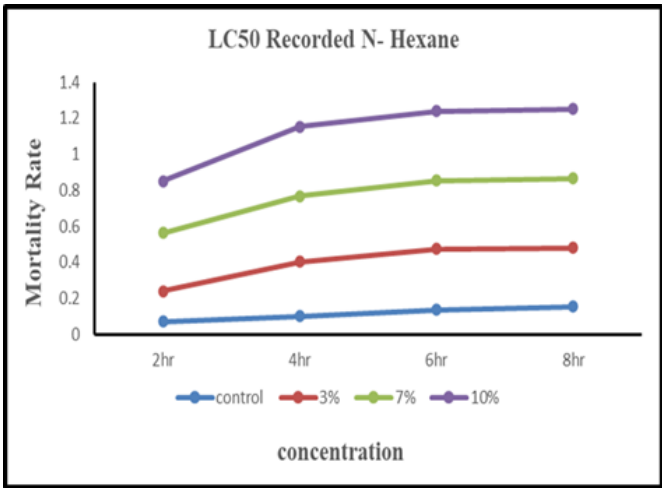

Figure 3 LC_{50} recorded of n-hexane.

Citation: Rasib KZ, bibi I, Saif P, et al. Efficacy of leaf extracts of apple of sodom (*Calotropis procera*) for the control of subterranean termite *Odontotermes Obesus*. *Int J Avian & Wildlife Biol.* 2022;6(1):1–5. DOI: 10.15406/ijawb.2022.06.00176

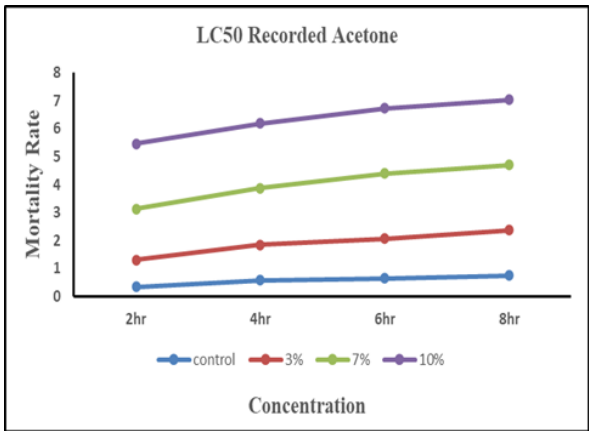

Figure 4 LC_{50} recorded of Acetone.

Discussion

Discussion control of termites was dependent upon on synthetic insecticides and persistent organochloride, organophosphate insecticides.[1,16] The application of pesticides /insecticides triggered pest resistance, environmental degradation and health concerned forced an alternative solution of the problem to more precise and environmentally friendly bio rational program on plant of leaves extracts against pest species. Since biorational application of insecticides is accepted worldwide,[15] and biorational option are much better strategy using plant extracts along with their bioactive compound in managing termites. Since the extracts of leaves plants are investigated in other regions of globe with multi-dimensional parameters comprising of LC50, and other properties.[17,18] In the previous studies, biorational plants insecticides get impeutus in term of claim besides with termite's control. The Antitermitic activity is seem to different plants like *Cinnanomum cassia*, *Cymbopogan, Citratus*, *Vetiveria zizanoides*, *Eucalyptus citrodora*, *Calotropis procera*, *Eucalyptus globules*, *Cedrus atlantica*, *Syzgum aromaticum* and *Coleus amboinicus*.[17,19,20]

The plant extract along with oil of *Calotropis procera* with many steroids. The use of plants extracts has also been reported by several authors to be effectives in the management of termites and serve as an alternative to synthetic insecticides.[21-24] Furthermore, the non-significance chi square values for both j. curcas and A, indica oil shows that toxicity model generated from the probit regression was similar to the theoretical model which described the observed mortality of *Calotropis procera* on treated wood sample as an outcome of the toxic effect of the botanical oil extracts.

The significant negative association between termites' mortality and wood wight loss further shows that the toxicity of the termicides may be responsible for the significance reduction in termites wood consumption mortality, and it was similar to the mortality caused by 10 and 15ml of solignum.

Terpene alcohol may contribute to termite mortality as a result of bioactive compound,[25-28] and termite protection comprising *O. obesus Microterm beesoni, Reticulitermes santonensis and R. virginicus*. Termites' researchers in other portion of globes highlighted and leaves extracts *Calotropis procera* using the solvent acetone and N-hexane used against the control of termites *Odontoterms obesus* to detect tunneling behaviors of termites and mortality rate.[29,30] described those results 10%, 7% and 3% concentration to show the result 93.1%, 83.1% and 71.3% mortality in 8 h application. Similarly, the *larix keptoleplepis* crude extracts in water holding flavonoids in great amount showed effectively termites feeding detereent functions.[31] Extracts of *Lanatana camara*, *Calotropis procera*, *Ocimum sanctum, E.helioscopia* and *S.incanum* and *W.somnifera* was found effective against termites workers.[9,27]

Wood is known as differ in their susceptible to termite's attack; this difference may be attribute to factors such as chemical composition wood density and availability of susceptibility species of termites.[32] However, all the wood tested showed significant susceptibility to *C, sjostedi* attacks. Particularly, the untreated wood samples from all the tested plants species recorded the highest weight loss when compared with treated samples. Based on the results, wood consumption by *C, sajostedti seems* to be higher in *Ceiba pentandra* wood than other wood types tested. These results agree with report of Faruwa who observed a significant impact on bio-based preservatives as control measures against damage caused by fungi and termites in *Triplochiton scleroxylon, Gmelina arborea*, *Ceiba pantandra* wood samples.

The harmful impacts of plants chemical constitutes or crude extracts on insects are established in many methods, involving growth retardation, suppression of calling behaviors, toxicity feeding inhibition, oviposition deterrence, reduction of fecundity and fertility. Searching for anti termitic activity of plants extracts with medicinal attributes could lead to the discovery of new agents for termite's control. So, we can conclude our study that acetone and N-hexane extraction of *Calotropis procera* be used as a potential natural termicides against termites, Isolation of Antitermitic constituents to find out their mode of action against termites. However, the results of our study revealed that leaf extracts of *Calotropis procera* have amazing toxic effect against the termite's species *Odontoterms obesus*.

Conclusion

Increasing MOE was found in all the types of wood tested impregnated with boron, castor bean oil and heat treated. MOR was also indicated as increasing and identical with MOE. The hardness of the radial surface was lower than that of the tangential surface, and increasing radial and tangential hardness were shown but did not occur in the samama wood impregnated with borax. Cell wall thinning happened in samama wood after heat treatment at 160°C but without any fibre damage. The hydrophobic properties of samama wood increased due to the decreasing O element, as indicated by pits covered by boron and oil layers.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgments

This study was not funded by any funding agency.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

1. Mueen Ahmed KK, Rana AC, Dixit VK, et al. PHCOG MAG: Plant Review *Calotropis* species *(Asclepediaceae)* – A comprehensive review. *Pharmacognosy Magazine*. 2005;1(2):48–52.

2. Peri K, Rao PJ, Devakumar C, et al. A novel insect antifeedant non–protein amino acid from Calotropis gigantea. *J Nat Prod*. 1998;61(1):102–104.

3. Badshah H, Farmanullah SA, Salijoqi Shakur M. Toxic effects of AK Calotropis proceraagainst termites (*Heterotermes indicola and Coptermes heime)* Isoptera: Rhinotermitidae. *Pakistan Journal Biology Science*. 2004;7(9):1603–1606.

4. Philip T, Govindaiah AK, Bajpai Datta RK. Effect of certain leaf extracts on hatching and mortality of the root knot nematode, Meloidogyne incognita infesting mulberry. *Indian Journal. Series*.1993;32(1): 37–47.

5. Anil Srivatsava YN, Sukla KN, Sushilkumar D. Recent developments in plant derived antimicrobial constituents – A Review. *J Med Arom Plant Sci.* 2000;22:349–405.

6. Muhammad Faheem Malik, Mohammad Nawaz, Zahid Hafeez. Efficacy of synthetic insecticide and botanical infusions against onion thrips in Baluchistan, Pakistan–I. *Asian Journal Plant Science*. 2003;2(10):779–781.

7. Duarte CM, Agusti S, Barbier E, et al. Rebuilding marine life. *Nature*, 2020;580(7801):39–51.

8. Ito EE, Ighere EJ. Bio–insecticidal Potency of Five Plant Extracts against Cowpea Weevil, Callosobruchus maculatus (F.), On Stored Cowpea, Vigna unguiculata (L). *Jordan Journal of Biological Sciences*. 2017;10(4):317–322.

9. Verma RL, Borongan G, Memon M. Municipal solid waste management in Ho Chi Minh City, Viet Nam, current practices and future recommendation. *Procedia Environmental Sciences*. 2016;35(8):127–139.

10. Howick CD, I Staunton. Colonies in Collision – A Concentrated Chronical of Osipitan Termites and Termites in Australia 1788– 2018. *Termites Pity ltd. Australia*. 2017;43:241–251.

11. Iqbal N, Evans TA. Evaluation of fipronil and imidacloprid as bait active ingredients against fungus–growing termites (Blattodea: Termitidae: Macrotermitinae). *Bull Entomol Res.* 2018;108(1):14–22.

12. Afzal M, Farman M, Rasib KZ, et al. Biocidal action of silver oak (Grevillea robusta) leaf extract on the termite Heterotermes indicola Wasman (Blattodea: Rhinotermitidae). *International Bioterror. Biodegradation*. 2019;11(7):139–177.

13. Hazrat Ali, Jeh Andar Shah, Muhammad Ali, et al. "Medicinal value of Ranunculaceae of Dir valley." *Pakistan Journal of Botany* .2007;39(4):1037–1044.

14. Ahmad Y. The scope and definitions of heritage: from tangible to intangible. *International Journal of Heritage Studies*. 200612(3):292–300.

15. Kang BT, Reynolds L, Atta–Krah AN. Alley farming. *Advances in Agronomy*. 1990;43(1):315–359.

16. Anil Srivatsava YN, Sukla KN, Sushilkumar D. Recent developments in plant derived antimicrobial constituents – A Review. *J Med Arom Plant Sci.* 2000;22:349–405.

17. Zhu YG, Smith SE, Smith FA. Zinc (Zn)–phosphorus (P) interactions in two cultivars of spring wheat (*Triticum aestivum L.*) differing in P uptake efficiency. *Annals of Botany*, 2001;88(5):941–945.

18. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol*. 2006;51:45–66.

19. Singh RP, Chidambara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate *(Punica granatum*) peel and seed extracts using in vitro models. *J Agric Food Chem* 2002;50(1):81–86.

20. Blake R, Turner LM, Smoski MJ. Visual recognition of biological motion is impaired in children with autism. *Psychological Science*. 2003;14(2):151–157.

21. Sen S, Gürhan–Canli Z, Morwitz V. Withholding consumption: A social dilemma perspective on consumer boycotts. *Journal of Consumer research.* 2001;28(3):399–417.

22. Sbeghen AC, Dalfovo V, Serafini LA, et al. Repellence and toxicity of basil, citronella, ho–sho and rosemary oils for the control of the termite *Cryptotermes* brevis (Isoptera: Kalotermitidae). *Sociobiology.* 2002;40(3):585–593.

23. Abdullah R, de Lauretis A, Wickremasinghe M, et al. Rituximab in severe, treatment-refractory interstitial lung disease. *Respirology*. 2014;19(3):353–359.

24. Sohail MS, Bhatnagar R, Sohal AS. A comparative study on the use of third–party logistics services by Singaporean and Malaysian firms. *International Journal of Physical Distribution and Logistics Management*. 2006;36(9):690–701.

25. Ogunsina OO, Oladimeji MO, Faboro EO. Mortality and anti–feedings evaluation of hexane and ethanol extracts of Lantana camara (Verbenaceae), African nutmeg (Monodoro myristica (Gaerth) Dunal) and Enuopiri (Euphorbia Laterifloria, Schum and Thonner) against subterranean termite workers (Macroterme michaelseni). *Toxicological and Environmental Chemistry*. 2009;91(5):971–977.

26. Upadhyay B, Singh KP, Kumar A. Pharmacognostical and antibacterial studies of different extracts of Euphorbia hirta L. *Journal of phytology*. 2010;2(6).

27. Manzoor F, Pervez M, Adeyemi MMH, et al. Effects of three plant extracts on the repellency, toxicity and tunneling of subterranean termite Heterotermes Indicola (Wasmann). *Journal of Applied Environmental and Biological Sciences*. 2011;1(7):107–114.

28. Elango R, Ball RO, Pencharz PB. Recent advances in determining protein and amino acid requirements in humans. *British Journal of Nutrition*. 2012;108.

29. Elsayed NM. Toxicology of blast overpressure. *Toxicology*. 1997;121(1):1–15.

30. Ganapaty S, Thomas PS, Fotso S, et al. Antitermitic quinones from Diospyros sylvatica. *Phytochemistry*, 2004;65(9):1265–1271.

31. Chen Y, Li X, Shen Z. Leaching and uptake of heavy metals by ten different species of plants during an EDTA–assisted phytoextraction process. *Chemosphere.* 2004;57(3):187–196.

32. Gérard P. The links between the gut microbiome and non–alcoholic fatty liver disease (NAFLD). Cellular and *Cell Mol Life Sci*. 2019;76(8):1541–1558.