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RESEARCH ARTICLE

TERMITICIDAL EFFECTS OF AFRICAN LOCUST BEAN (*PARKIA BIGLOBOSA*) SEED OIL EXTRACTS

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ABSTRACT

The termiticidal properties of acetone, ethanol and aqueous extract of African locust beans (*P. biglobosa*) seed were investigated on Isoptera: Rhinotermitidae and Termitidae. Mineral compositions of the raw and boiled seeds were evaluated and heavy metals such as cadmium, cobalt, lead, nickel and copper were only detected in raw seeds, which show that heavy metals were leached out of the seeds during boiling process. Extracts from the raw seeds exhibited varying degree of termiticidal activity, while extracts from the boiled seed had no effect on the termites. The aqueous, ethanol and acetone extracts of boiled African locust bean seed had no effect on termite, while extracts of raw seeds affected them. The aqueous extract of raw seeds exhibited concentration dependent termiticidal activity. However the acetone extracts were more active than the aqueous and ethanol extracts. Termites died within 20 min of exposure to paper pad treated with concentration of 5 g mL^{-1} of ethanol and acetone extracts. The heavy metals found in the samples were in safe levels compared to regular or synthetic termiticides. Hence, the extract of African locust bean seeds have termiticidal effects and can be effectively used to control termite infestation instead of the toxic and environmentally unfriendly chemicals. The optimization process shows that the increased application time (0 to 34 s) is safer to detect any health hazard on human (if any) while the survival chances for termites decrease by 400%.

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INTRODUCTION

P. biglobosa (African locust bean) tree is a perennial deciduous tree that grows from 7 to 20 meters high (Teklehaimanot2004; Ojewumi *et al*. 2016a). The pods are flat and have irregular cluster of up to 30 seeds. It is known by different names in different countries - kinda in Sierra Leone and Iru or dawadawa in Nigeria and Ghana (Ojewumi *et al*., 2016a; Azokpota *et al.,* 2005; Odunfa, 1981). Afintin and sonru in Benin Republic (Azokpota *et al*., 2005), while in Japan it is known as natto (Azokpota *et al*., 2005; Beaumont 2002). The tree was listed as one of the plants having real wound healing properties in South-Western Nigeria (Adetutu *et al*., 2011; Ojewumi *et al*., 2016b). Termites are eusocialinsects that are classified at the taxonomic rank of infraorder Isoptera. Termites are among the most successful groups of insects on earth. They colonize most landmasses except for Antarctica. Their colonies range in size from a few hundred individuals to enormous societies with several million individuals.

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Termite queens have the longest lifespan of all insects in the world, with some queens living up to 50 years. Unlike ants which undergo a complete metamorphosis, each individual termite goes through an incomplete metamorphosis that proceeds through egg, nymph, and adult stages. Colonies are described as superorganisms because the termites form part of a self-regulating entity: the colony itself *(Bignel et al., 2010).* Termites are the most destructive insect pests in the world and they cause the most serious damage of all wood-feeding insects. They derive their nutrition from materials containing cellulose such as paper, cotton and other plant products (Femi *et al.* 2008). In addition to timber and wood products, they attack growing trees, leather, papers, rubber, and wool as well as agricultural crops (Teklehaimanot 2004). Significant damage is caused by termites to man-made fabrics, polythene, plastics, metal foils, books, furniture, wooden telephone poles, wooden railway sweepers, and insulators of electric cables (Malaka 1996). Damage caused by termites to wooden structures in the United States of America is estimated to be over 3 billion Dollars annually, with subterranean termites accounting for at least 80% of these damages (Lewis 1997). Costs attributable to a species of termite known as *Coptotermesformosanus* in the Hawaiian Islands alone are greater than 60 million dollars per annum (delate 1995). In Africa and elsewhere in the developing countries, there is hardly data on either the quantum of damage done by termites to agricultural crops, construction timbers, paper, and paper products, or the cost of control or repairing the damage done by these insect pests (Bolarinwa 2012). Tropical rain forests are often associated with low-fertility soils, and termites' recycling of organic matter contributes to the efficient return of nutrients to the vegetation in such forests (Ackerman 2009). Termites are the most populous and most efficient among insects capable of decomposing lignocellulose. In addition, termites harbour nitrogen fixing bacteria and their foraging enhances the nitrogen content of the soil (Hemachandra 2010).Previous biological studies of *P. biglobosa* have reported the antimicrobial activities of the leaf and stem bark (Ajaiyeoba, 2002, Millogo *et al*., 2006, 2007, 2008). The aqueous and acetone extract of *P. biglobosa* raw beans have also demonstrated termicidal properties (Femi *et al*., 2008). All synthetic termicides which contain Chlordane as the active ingredient have side effects on humans, animals or the environment on application. The side effects include: nausea, headaches, shortness of breath and seizures, neurotoxicity, muscle weakness etc. Hence, the need arises for the use of an environmentally friendly and affordable pesticide which can be found in the oil extract of dried African locust beans. Two families of termite which are Rhinotermitidae and Termitidae with subfamilies Rhinotermitinae, Amitermitinae, Macrotermitinae and Termitinae were identified from the termite collections made. Members of the genus Macrotermes were the dominant species. Other species which were also identified were Amitermes spp. Macrotermessubhyalinus, Ancistrotermes spp, Macrotermesnatalensis, Capritermes spp, Coptotermes spp.

MATERIALS AND METHODS

African locust bean seeds were obtained from Itapaji, a village in Ekiti State, Nigeria. All the reagents used were of good analytical grade from SIGMA Manufacturing Industry, USA. African locust bean seeds were processed according to (Ojewumi *et al*., 2016a) before extraction of oil. The seeds were then crushed with binatone electric blender into smaller form to increase the surface area. Figure 1 shows the flowchart for the extraction of oil from African locust bean seeds.

Termites' collection

Termite workers (colonies) were collected from decaying branches of trees at Covenant University farm, Ota, South West Nigeria and brought to the Chemical Engineering Department in Covenant University, Ota. The termites' colony with the decaying wood were kept in a perforated plastic container that was kept moist until needed.

Oil extraction from the dried seed of African locust beans

Oil was extracted from the powdered seed using Soxhlet apparatus and a heating mantle. The Soxhlet apparatus is made up of a condenser, an extractor and a round-bottomed flask which was being heated. African locust bean powder encapsulated in filter paper was placed in the extractor. The 70% acetone and ethanol used for extraction were placed in the flask. The solvent vaporized and rose to the condenser where it was condensed. The condensed organic solvent then dripped on the locust bean powder. The locust bean powder upon contact with hot organic solvent began to secret oil which is soluble in the organic solvent. When the oil and solvent mixture had risen high enough (higher than the siphon tube), the mixture flowed through the siphon tube back into the flask. The oil was then recovered from the organic solvent by heating the mixture with a condenser coupled to the flask. The organic solvent vaporized and was then condensed back into another container. The same process was carried out for boiled African locust beans

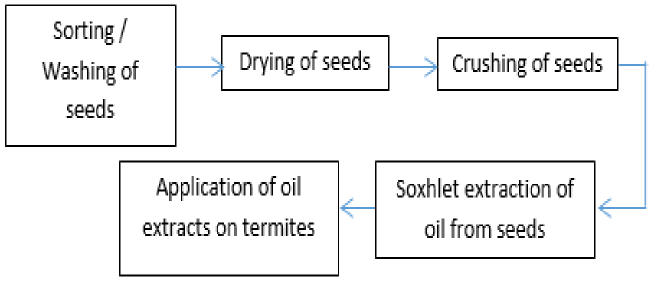

Fig. 1. Flowchart for the extraction of oil from *Parkia biglobosa* seeds

Figure 2 shows the setup for the Soxhlet apparatus where 200 ml of acetone was measured in a measuring cylinder and then poured into a round-bottomed flask and then cocked. The round-bottomed flask was placed on a heating mantle. It was then uncorked and the extractor of the Soxhlet was placed on it. A condenser was placedon the extractor and properly connected to a tap. A piece of filter paper was rolled and used to make a cone. 30g of the locust beanpowder was weighed and poured into the cone. A small rope was attached to the cone to ensure that it could be pulled out without spilling. The cone was lowered into the extractor. The heating mantle was switched on and with the temperature regulated to 50-60^{0}C. The extraction was left to continue for 4 hours. After 4 hours, the heating mantle was switched off and the apparatus was allowed to cool. The condenser was removed and dismantled. The cone was removed from the extractor and any liquid left in the extractor was poured back into the round bottom flask. A condenser was then connected to the round-bottomed flask which still sat on the heating mantle. A conical flask was attached to the other end of the condenser to collect the acetone that vaporised from the mixture in the round bottom flask. This experiment was run for 5 times. Similar procedure as mentioned above was used for ethanol solvent.

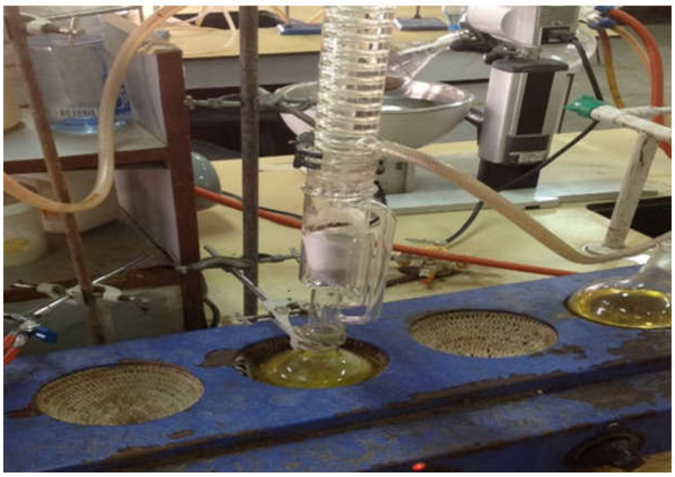

Fig. 2. A setup of Soxhlet apparatus

The oil extract from the boiled seeds of African locust beans clearly did not have an effect on the termites because most of the heavy metals present within would have been leached out.

Paper Pad Treatment

5 g mL $^{-1}$ of the extracted oil sample was injected into a paper pad, allowed to dry and kept in a petri dish. A countable number of termite workers (30 soldiers) were placed on the paper pad and a perforated petri dish was used to cover them to prevent the termites from escaping. All petri dishes were wel tagged and labelled. Observations on the termites' response to the oil samples were noted, monitoring how the termites died per minute with respect to each extract. Figure 3 shows the picture of the exterminated termites after locust bean oil treatment.

Fig. 3. Exterminated termites from Locust bean oil treatment

RESULTS

Table 1. Mineral composition of heavy metals in *Parkia biglobosa* seeds

Minerals	Raw ALB seed (mg . mL $^{-1}$)	Boiled ALB (mg . mL $^{-1}$)
Lead	2.65	ND
Cadmium	0.21	ND
Copper	7.03	ND
Nickel	4.04	ND
Cobalt	4.41	ND

ALB – African locust bean
ND – Not detected

Termite Death Rates Using the Oil Extract

Table 2. Oil extract from acetone

Time(min)	Termites death		Survival chance (%)	
	Run 1	Run 2	Run 1	Run 2
1	3	2	80.0	86.7
2	5	3	66.7	80.0
3	7	4	53.3	73.3
5	9	5	40.0	66.7
6	10	6	33.3	60.0
9	12	8	20.0	46.7
17	14	12	6.67	20.0
20	15	15	0.0	0.0

Table 3. Oil extract from ethanol

Time	No. of dead termites		Survival chance (%)	
	Run 1	Run 2	Run 1	Run 2
1	2	1	86.7	93.3
3	3	3	80.0	80.0
6	6	4	60.0	73.3
8	8	8	46.7	46.7
10	12	12	20.0	20.0
15	15	15	0.0	0.0

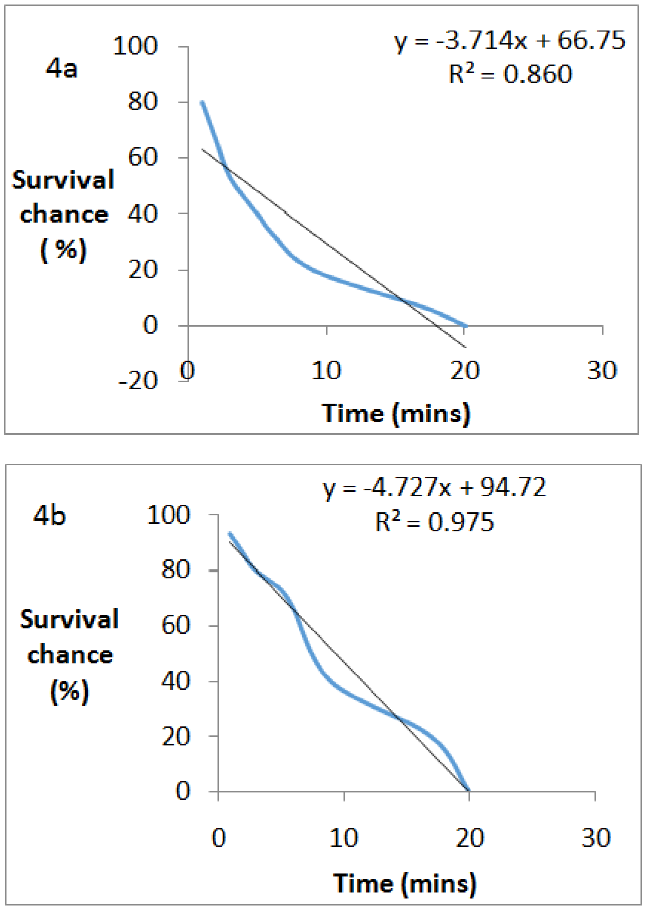

Fig. 4. (a) and (b) survival chance against time for acetone extract

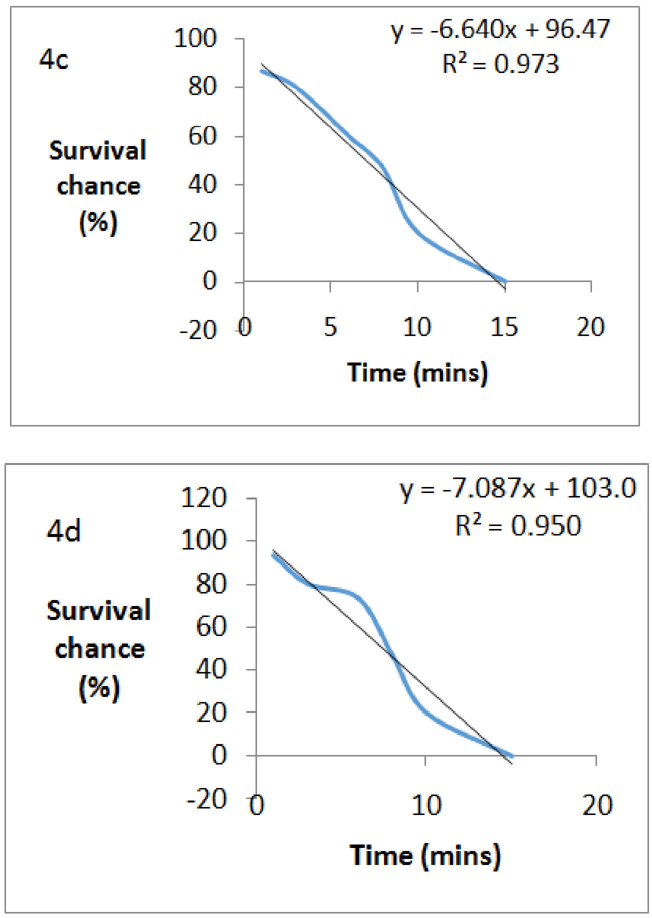

Fig. 4. (c) and (d) survival chance against time for ethanol extract

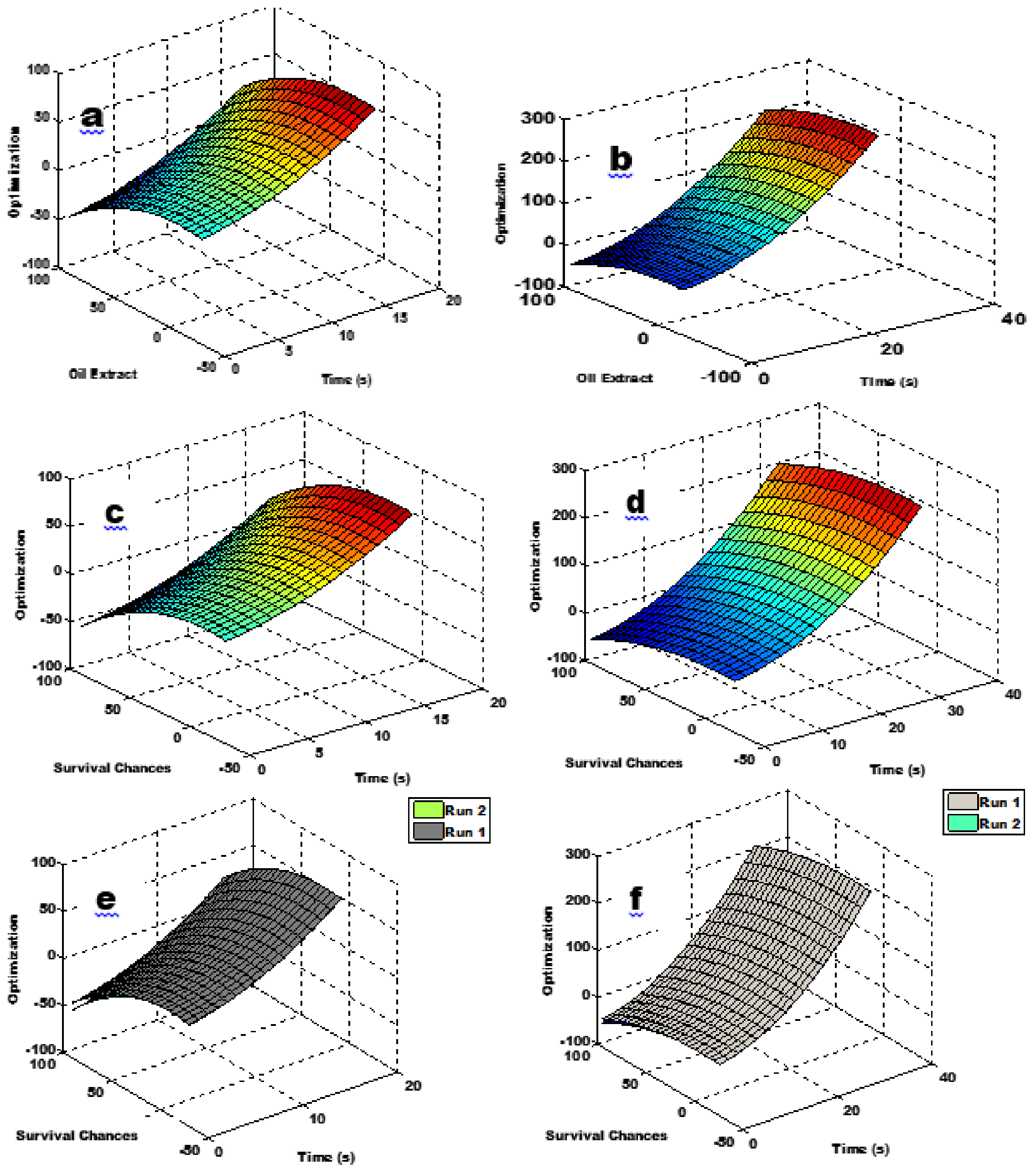

Fig. 6(a). Laboratory optimization (0-16 s) for Run 1; (b) simulated optimization (0-34 s) for Run 1; (c): Laboratory optimization (0-16 s) for Run 2; (d) simulated optimization (0-34 s) for Run 2; (e): Combined laboratory optimization (0-16 s) for both Runs; (f) Combined simulated optimization (0-34 s) for both Runs

An Atomic Absorption Spectrophotometer [AAS] in Covenant University's central laboratory was used to analyze the extracts from acetone and ethanol respectively. There was no difference between the results from both solvents; this shows that the solvents have little or no effect on the mineral composition of the extracts. Heavy metals such as Cd, Co, Cu, Ni, and Pb were found in extracts of the raw seeds but absent in extracts of the boiled seeds [Table 1].

DISCUSSION

Raw *P. biglobosa* seeds have been found to contain some heavy metals such as cadmium, cobalt, nickel, lead, and copper (Cheng 1990), which are basic natural components of the earth crust and are toxic at low concentrations [Table 1]. These elements were lost during the boiling process (Ojewumi 2016c; Alabi *et al.*, 2005).

The presence of these heavy metals in the raw seeds, in addition to the factor of polar organic compounds arising from the interactions between the mineral constituents and the extracting medium may therefore explain the termiticidal action of these extracts on termite. Aqueous, ethanol and acetone extracts of boiled seeds did not have termiticidal effect on termites. This explains why the boiled water obtained during the fermentation stage of preparation is effective in the traditional control of termite infestation in the rural areas (Femi 2008; Malaka 1996). Extracts from several plants have been reported to have destructive effects and feeding deterrents to insects such as termites (Adetutu, 2011; Hemachandra, 2010; Alabi, 2005). It has also been well established that certain extracts found in plant materials acted as natural repellent for termites (Odunfa 1981b; Neya *et al.*, 2004). Figures 4 (a) and (b) show fairly linear graphs which signify a steady state of death of the termites with respect to time. An R^2 value of 1 signifies a perfect linear graph, which is the maximum value attainable. The most suitable oil extract is the one with the value closest to 1. The slope signifies the rate of population decrease with respect to time upon application of the oil extract. From Figures 4 (c) and (d), the graphs both have R^2 values of 0.973 and 0.9503 respectively, giving an average of 0.9617. This slope values are -6.64 and -7.08 respectively. This means that on the average, about 6.64 – 7.08% of the termite population was dead within a minute of application of the locust bean oil extract from ethanol. From the above, the locust bean oil extract from ethanol is the best for termites' extermination. The termites die from heavy metal poisoning as this directly affects their central nervous system. The presence of nickel, cadmium and lead is responsible for this. Extracts from raw seeds of African locust bean seed, which appears to be relatively nontoxic to people, have therefore shown to be an effective termiticide for the control of termite, since there has never been any adverse/toxic effect on local processors of seed as well as consumers of the finished product; the raw extracts may not be harmful to people.

Optimization of Survival Chance

In this section, we optimize the survival chances for trials as shown in Table 3. The objective of this study is to see the likely results if the laboratory concept is applied to a large farm. The polynomial fit for the survival chances Run 1, Run 2 and time in Table 3 is given as:

$$y = -1.6661a^2 - 6.2461a + 96.03 \quad (1)$$
$$y = -2.0268b^2 - 5.0439b + 100.61 \quad (2)$$
$$y = 0.1964c^2 + 1.2821c - 0.3 \quad (3)$$

Run 1 (equation 1) and time (equation 3) were related in the equations below:

$$y = (-0.0051a^2 + 0.198c^2) + (-0.02a + 1.28c) \quad (4)$$
$$y = (-0.006b^2 + 0.198c^2) + (0.0015b + 1.28c) \quad (5)$$

Hence, the governing equations for the yield are equations 5 & 6. Here, y is referred to as the optimization value. The simulation is shown in Figure 6. The oil extracts from the boiled seeds of the *P. biglobosa* powder had no effect on the termites while the oil extracts from raw seed had exterminating effects on the termites. However, the effluent water from boiling the locust bean seeds killed termites. This confirms the earlier study by Ojewumi (2016c) that minerals are leached into boiling, soaking and dehulling water during the processing of *P. biglobosa* seeds to Iru and that a larger percentage of the minerals constituent in the seed resides in the hull of the seed which therefore was leached out during processing (Alabi *et al.*, 2005). These differences accounted for the termiticidal properties in extracts of the raw seeds when compared with extracts from the boiled seeds. From the results displayed in Tables 2 & 3, it was discovered that higher survival chances were obtained in Table 3 with an optimization of 50.3 for Run 1 (Figure 6a) and 50.1 for Run 2. Also, we projected the optimization at longer duration i.e. 0 to 34 seconds; it was observed that the optimization increased about four times as shown in Figures 6c & d. The combined laboratory and simulated optimizations at Runs 1 & 2 are shown in Figures 6e & f respectively. The results show that aside the modification of the laboratory parameters (Tables 2 & 3), if the experimentation time could be doubled, the effectiveness of the process is expected to increase to about 400%. Hence, the increased time is safer to detect its effect on human (if any) while the survival chances for termites decrease by 400%.

Conclusion

This study has shown that oil extracts of the raw seeds of African locust beans have termiticidal properties. The locust bean oil extract from ethanol of the raw seeds was the best for termites' extermination. The presence of some heavy metals such as iron, lead, manganese, cadmium, cobalt, nickel and copper present within the locust bean oil extract and the interacting polar organic compounds in the raw seed extracts could account for this termiticidal property. However further research is required to determine the contributions of each of these chemical constituents to the overall termiticidal action of raw locust bean seed extracts. Farmers, manufacturers of insecticide and pesticide, health experts and medical personnel have for a long time been having problems in creating an environmentally friendly, bio-degradable, non-irritant pesticide for termites' extermination from where they are unwanted. African locust bean oil from a natural source could well be the long lasting solution to the problem. The heavy metals' levels within the oil extract are within the acceptable range by WHO standard for soil application, without disrupting micro organic activity within the soil and aquatic animals. The optimization process shows that the increased application time (0 to 34 s) is safer to detect any health hazard on human (if any) while the survival chances for termites decreases by 400%.

Conflicts of Interest

The authors declare no conflict of interest.

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