ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Formulation of Neem Leaf and Croton Seed Essential Oils as a Natural Insecticide Tested on Mosquitoes and Cockroaches

Yassin A. Endris and Kedir D. Mekonnen*

Cite This: *ACS Omega* 2023, 8, 15052−15061

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Essential oils are highly aromatic plant oils utilized as a new insect control alternative to synthetic insecticides because of environmental concerns. As a result, the purpose of this study was to evaluate the effects of essential oil extraction conditions on neem leaf and cotton seed, as well as to identify their chemical composition using gas chromatography−mass spectrometry. Moreover, both extracted oils were subjected to bioinsecticide formulation in individual as well as mixed forms, which were tested on mosquitoes and cockroaches using the conventional roach killer and a blank control as a comparison. The results revealed that the maximum yields of essential oils from neem leaf and croton seed were respectively 30.54 and 14.75%. The extraction process was greatly affected by the particle sizes because of the mass transfer limitation between solute−solvent interactions. The insecticidal evaluation showed that the blended form of essential oils at the 20% concentration has better efficiency than the individual oils, which accounted for 80% mosquito and 71.8% cockroach death rates within 4 h, whereas the synthetic roach killer completely killed all of the insects within the same time frame. Terpineol, α-terpinyl acetate, eucalyptol, and δ-cadinene are the active insecticidal compounds in neem leaf oil, whereas the active compounds of croton seed oil are epiglobulol, copaene, δ-cadinene, α-cubebene, and β-guaiene.

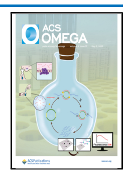
INTRODUCTION

In the past two decades, the use of pesticides has dramatically increased worldwide owing to the increase of human population and crop production.[1] The remarkable primary aids of using diverse types of pesticides are used in daily life to kill pests in areas of agricultural activities and public health. Consequently, pesticides are critically important to reduce or eliminate enormous diseases such as malaria and improve agricultural crop yields. Historically, hexachlorocyclohexane (BHC), dichlorodiphenyltrichloroethane (DDT), endrin, chlordane, parathion, β-aldrin, dieldrin, captan, and 2,4-D have been used as synthetic pesticides since 1945. The drawbacks of many of these products include high rates of application, high toxicity, and lack of selectivity. The pesticide group that can be used to kill insects liable for diseases is commonly called insecticides.[2]

Insecticides are one of the main factors responsible for global environmental poisoning and human health problems. The indiscriminate use of synthetic pesticides has resulted in ecological and health hazards along with the development of resistance in insect pests. This has led to the popularity of pest control agents derived from plants as they are biodegradable, environmentally compatible, and less toxic to nontarget organisms. In developing countries, malaria control programs based on the application of insecticides (e.g., DDT) and antiplasmodia agents are facing difficulties because of the development of resistance to DDT by some species of mosquito. The other problem associated with the use of DDT is its deleterious effect on the environment. To suppress the environmental as well as human health problems, the development of biopesticides needs a great emphasis.[3−6]

Botanically originated insecticides are selective and biodegradable, can delay insect resistance mechanisms, and are an eco-friendly alternative to synthetic insecticides. Essential oils derived from various plant species show an extensive range of insecticidal, repellent, antifeedant, growth regulatory, oviposition deterrent, and antivector activities.[1] Essential oils are highly aromatic/odorous plant oils commonly isolated by some physical processes mainly from leaves, fruits, peels, seeds, flowers, buds, bark, twigs, rhizomes, wood, bulbs, roots, and resin of plants, and a few of them are produced by microorganisms or obtained from animal sources; in plants, essential oils impart their unique and commonly diagnostic odor.[7−9] As evidence, plants have been used since ancient

Received: December 17, 2022
Accepted: April 6, 2023
Published: April 24, 2023



https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

Table 1. Factors and Their Levels Used in Factorial Design

sample type	variables	unit	code	coded variable level level 1	level 2	level 3
for neem leaf	particle size	mm	A	0.5−1	1−1.18	1.18−1.4
	extraction time	h	B	2	3	4
	solvent type and ratio	%	C	50% hexane−50% ethanol	40% hexane−60% ethanol	60% hexane−40% ethanol
for croton seeds	particle size	mm	A	0.5−1	1−1.18	1.18−1.4
	extraction time	h	B	2	3	4
	solvent type and ratio	%	C	50% ethanol−50% hexane	hexane	ethanol

times to repel/kill blood-sucking insects like mosquitoes in human history. Mosquitoes in the larval stage are attractive targets for pesticides because of breeding in water, and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in water sources, however, introduces many risks to people and/or the environment. Plant-originated natural pesticides are the promising alternative because of their eco-friendly nature. *Croton macrostachyus* is one of the eight *Croton* species found in Ethiopia and is known as medicinal and insecticidal plants used by rural communities;[10] this plant belongs to the species of the genus *Croton L.*, Euphorbiaceae family, commonly known as the spurge family. Numerous species of *Croton* are producers of essential oils, which have significant medicinal activities.[10,11] It is also regarded as a multipurpose tree by subsistence farmers for the provision of several ecosystem goods and services such as increasing soil productivity in semiarid areas and pharmacological activities in Ethiopia, Kenya, and Tanzania.[12,13] In addition to *Croton macrostachyus*, the neem (*Azadirachta indica* A. Juss) plant, which belongs to the family of Meliaceae, contains several biologically active compounds responsible for diverse activities such as insect antifeedant, growth disrupting, insecticidal, nematicidal, fungicidal, and bactericidal. Neem is a life-giving drought-resistant tree, especially for the dry coastal, southern districts. In the past two decades, scientists have focused their attention toward neem because of its environmental and human being benefits.[3,14,15] However, the plant architecture, fruiting, flowering, phytochemical compositions (i.e., bioactive compounds), and in situ competition with other species are dependent on various agricultural factors, such as the environment, climatic conditions, soil type, seasonal variation, altitude and humidity, salinity, light, harvesting time, and postharvest management prior to isolation.[7,16−18] As a result, different essential oils can be sometimes produced by different parts of the same plant.[9]

Therefore, the purpose of this research was to extract essential oils from neem leaf and *Croton macrostachyus* seed, with an impact analysis of the extraction conditions on the yield, identification of chemical compositions, formulation, and evaluation of their insecticidal properties in individual as well as mixed forms.

■ MATERIALS AND METHODS

Materials. The main raw materials, neem leaf and croton seeds, were respectively collected from Bahirdar and Mersa, North Wollo, Ethiopia. Normal hexane (99.9%) and ethanol (97%) were used during the extraction process as oil extractive solvent chemicals. In addition, distilled water, sodium hydroxide, ethanol, oxalic acid, phenolphthalein, chloroform, iodine bromide solution, sodium thiosulfate, starch solution, and an emulsifier (liquid soap) were also used in the characterization phase. All chemicals and reagents used were of analytical grade. The equipment list used throughout the experimental work is presented in the Supporting Information.

Sample Preparation. Nondefective fresh neem leaf and mature croton seeds were collected, and both samples were dried at a temperature of 110 °C for 2−6 h to facilitate size reduction and sieved to 0.5−1.4 mm. The advantage of size reduction is that it allows a higher surface area to volume ratio required for high oil extraction efficiency due to the diffusion of the solvent toward the accessible area of the sample.[19]

Experimental Design for Oil Extraction. A full factorial design of three factors on three levels of treatment was applied to investigate the effects of constraints on the response variable, oil yield, by keeping the extraction temperature at 70 °C.[20] Upon the combination of factor levels (Table 1), 27 randomized experimental runs were generated for each raw material subjected for oil extraction. Oil extraction was conducted via a Soxhlet apparatus using *n*-hexane and ethanol solvents (mixed at different proportions); about 200 mL of the solvent (either in a mixed or pure form) for every 30 g of the solid sample was applied to leach the oil contained in it. Each independent experimental run was replicated two times to assure that the actual result is not a default, and the average results were reported. Once the extraction was completed, complete removal of the solvent was performed in the most efficient sytem with a rotary vaccum evaporator at a temperature of 50 °C. Finally, the response variable (amount of oil) extracted from each solid sample was calculated using eq 1.[21] The detailed experimental run sheets created as per the factor level arrangement are described in the Supporting Information (Tables S1 and S2).

$$\%\text{oil yield} = \frac{\text{mass of oil extracted}}{\text{initial mass of the sample used}} \times 100 \quad (1)$$

Physicochemical Characterization of Neem Leaf and Croton Seed Oil. The characterization of solvent-free extracted oil properties was performed by following ASTM and AOAC standard techniques; acid value (ASTM D664), saponification value (AOAC, 2000b), specific gravity (ASTM D1298), iodine value (AOAC, 2000), and kinematic viscosity (ASTM D445). Furthermore, the chemical compositions of both extracted oils were examined via gas chromatography with mass spectrometry (GC−MS) analysis to evaluate the presence of bioactive constituents in medicinal plants by following scientific procedures.[17,22]

Formulation and Evaluation of Insecticidal Effects. The formulation of the insecticide was prepared by mixing water with the neem leaf and croton essential oils. Because of the immiscible nature of oil with water, liquid soap was incorporated as an emulsifier, which acted as an intermediate solvent to obtain a complete oil−water mixture that can be sprayed as an insecticide. Distilled water (1000 mL) was first measured followed by the addition of 2% liquid soap and oils

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

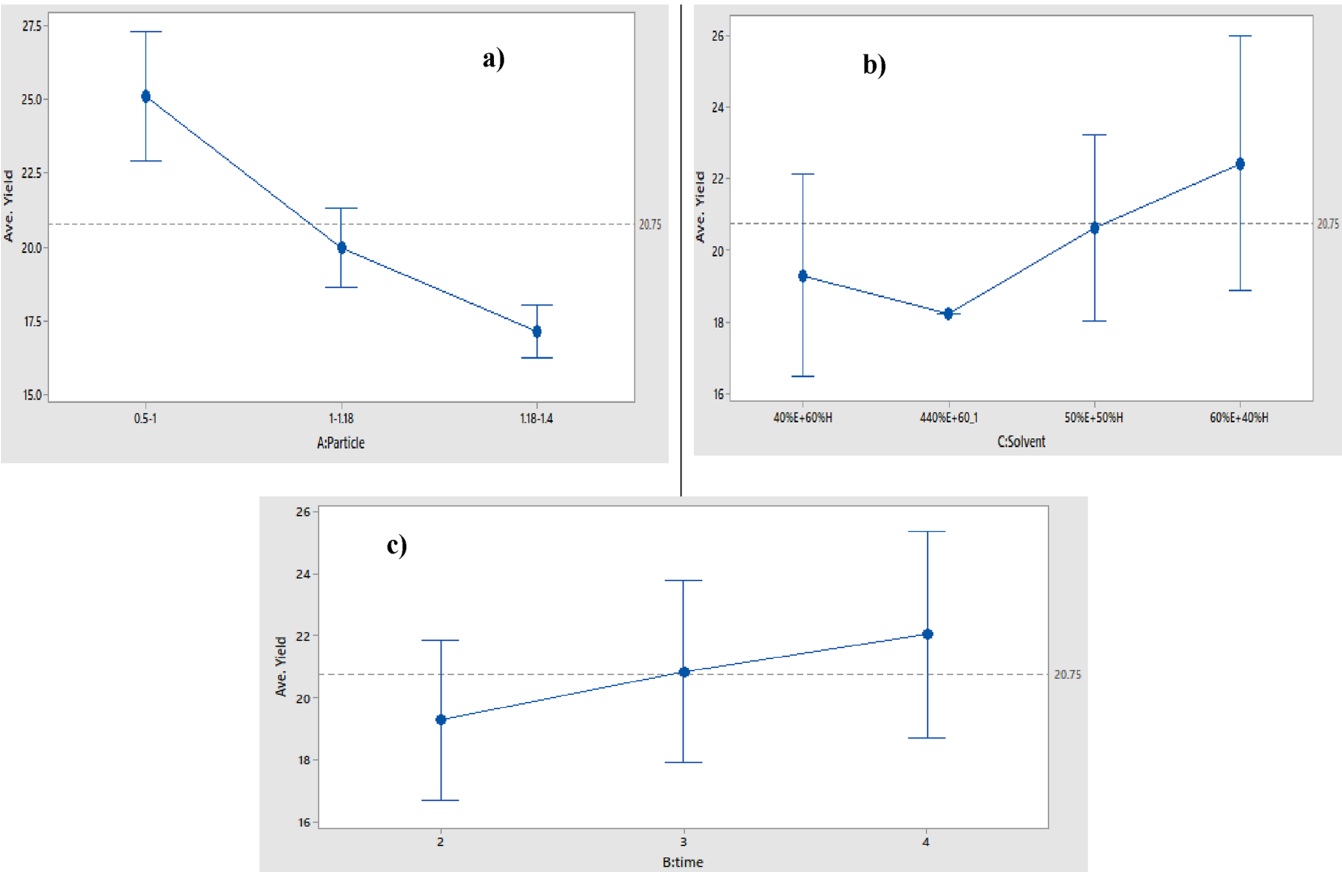


Figure 1. Main effect plots of neem leaf oil yield by particle size (a), solvent type ratio (b), and extraction time (c).

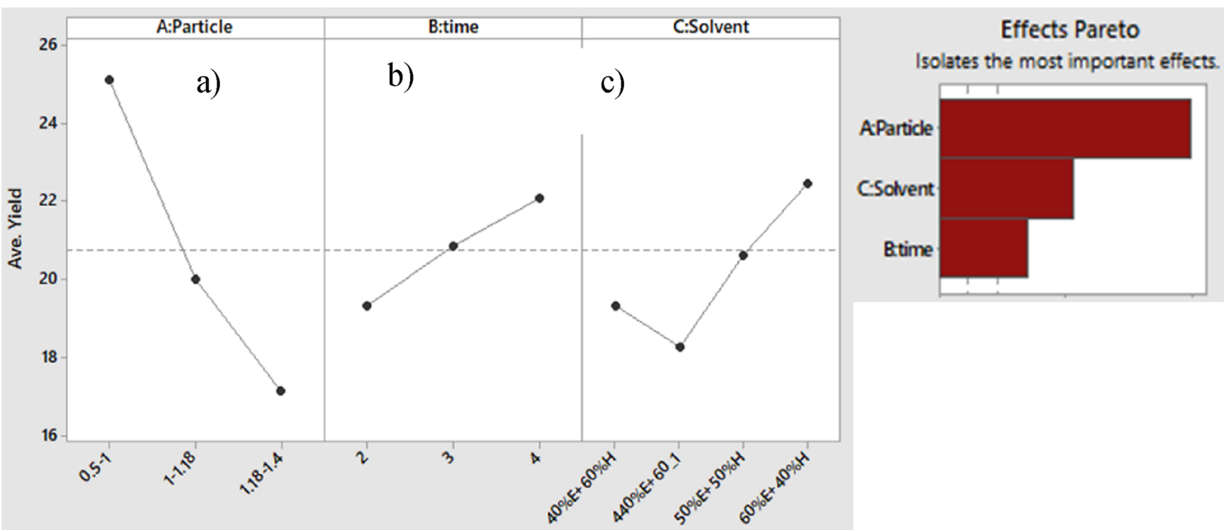


Figure 2. Main effect screener plots for neem leaf oil yield: (a) particle size effect, (b) extraction time effect, and (c) effect of the solvent type ratio.

at 1, 5, 10, and 20%. The three components were properly shaken for complete mixing. To test the insecticidal effects of the formulated mixture, mosquitoes and cockroaches were collected in polyethylene containers. To begin, a few pieces of bread, banana peels, and water were added to the container as a source of food, into which enough insects were drawn, before closing the container with a mesh lid and transferring it to the test room (laboratory). The container was also punctured with needles to allow for air exchange. Then, a modified bioassay method was employed to evaluate the insecticidal activity of the oil. After the insects had recovered, test and knockdown counts were recorded for every 2 h time interval.

RESULTS AND DISCUSSION

Oil Yield Determination. All the percentage yields of essential oils from neem leaf and croton seeds based on the experimental parameter combinations of factorial design with their physicochemical characteristics are shown in the Supporting Information (Tables S1−S3). Thus, the maximum oil yield of 30.54% was obtained from neem leaf at the

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

Table 2. Analysis of Variance for Neem Oil Extraction[a]

source	sum of squares	Df	mean squares	F-value	p-value prob > F	significance
model	757.04	6	126.17	152.89	<0.0001	significant
A-extraction time	71.66	2	35.83	43.42	<0.0001	significant
B-particle size	588.16	2	294.08	356.34	<0.0001	significant
C-ratio of solvent	97.22	2	48.61	58.90	<0.0001	significant
residual	38.79	47	0.83			significant
lack of fit	31.27	20	1.56	5.62	<0.0001	insignificant
pure error	7.51	27	0.28			
cor total	795.83	53				

[a]Std. dev. = 0.91; mean = 20.74; C.V. = 4.38%. R^2 = 0.9513; adjusted R^2 = 0.9450; predicted R^2 = 0.9357; adeq. precis. = 42.999. Significant $P \leq 0.05$; insignificant when $P > 0.05$.

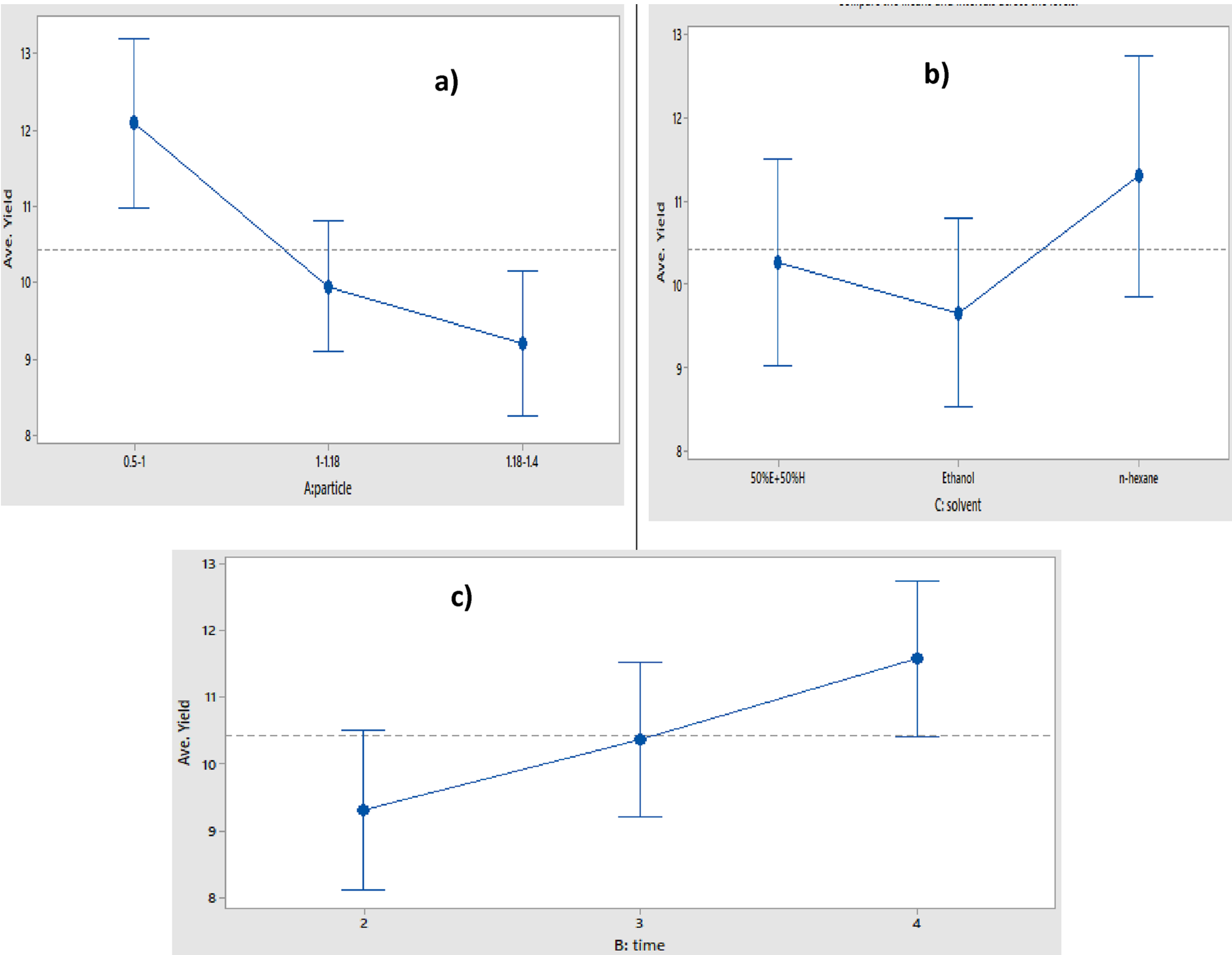


Figure 3. Main effect plots of croton seed oil yield by particle size (a), solvent type (b), and extraction time (c).

combination of a particle size range of 0.5−1 mm, a 4 h extraction time, and a solvent ratio of 60% ethanol and 40% *n*-hexane. Meanwhile, the maximum oil yield of croton seed was 14.75% at a minimum particle size range of 0.5−1 mm, a maximum extraction time of 4 h, and an *n*-hexane solvent. Such results are almost similar to previously reported values in the literature except for smaller deviation, a neem leaf oil yield of 37.73%[23] and 19% oil from croton seeds.[24] The smaller deviations may arise from the variety of geographical locations where the plants were grown, seasonal variation, and the degree of plant stalk maturation, or other extraction conditions may be the case.

Effects of Extraction Parameters on the Yield of Neem Leaf Essential Oil. *Main Effect Plots and Screener.* Figure 1 is the main effect plots of operating conditions, which can indicate the potential differences in the means or variability across the levels of variables, and generated with Minitab-17 software. Accordingly, the effect of particle size on neem leaf oil yield is shown Figure 1a. It can be noted that a smaller particle size gives a higher oil yield than the larger one because smaller-sized particles are desired for attacking the accessible

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

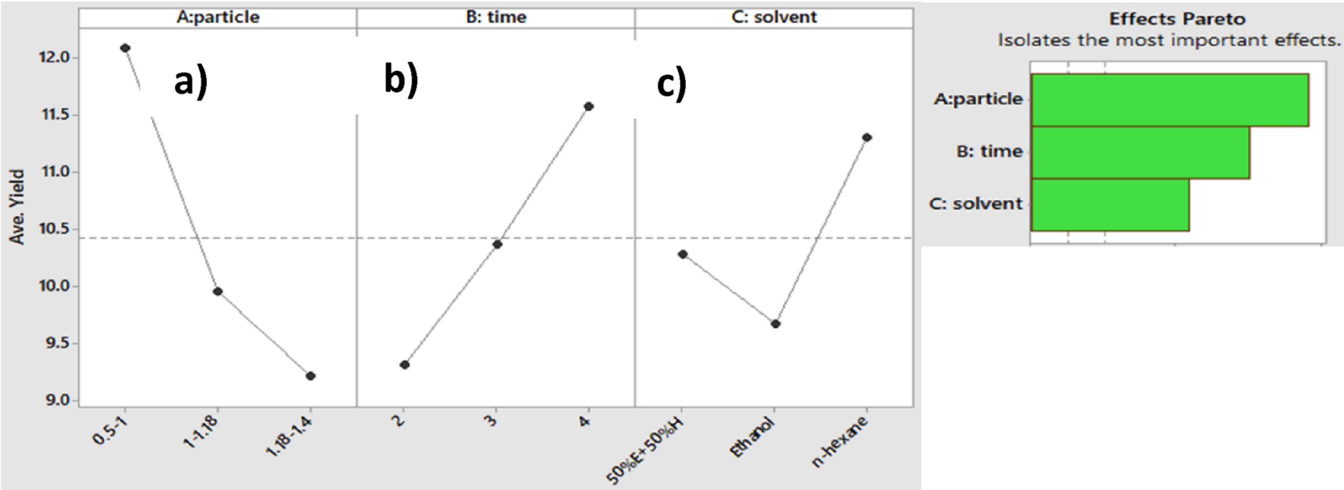


Figure 4. Main effect screener plots for croton seed oil yield: (a) particle size effect, (b) extraction time effect, and (c) effect of the solvent type ratio.

Table 3. Analysis of Variance for Croton Seed Oil Extraction[a]

source	sum of squares	Df	mean squares	*F*-value	*p*-value prob > *F*	significance
model	150.89	6	25.15	115.15	<0.0001	significant
A-extraction time	46.01	2	23	105.34	<0.0001	significant
B-particle size	80.17	2	40.09	183.55	<0.0001	significant
C-type of solvent	24.71	2	12.36	56.58	<0.0001	significant
residual	10.26	47	0.22			significant
lack of fit	6.01	20	0.30	1.94	0.0547	insignificant
pure error	4.21	27	0.16			
cor total	161.16	53				

[a]Std. dev. = 0.47; mean = 10.42; C.V. = 4.49%. R^2 = 0.9363; adjusted R^2 = 0.9282; predicted R^2 = 0.9159; adeq. precis. = 40.250. Significant $P \leq 0.05$; insignificant when $P > 0.05$.

area of the particles easily.[19] As a result, it is quite clear that the oil yield of the 0.5−1 mm-sized sample is superior to that of the 1.18−1.4 mm-sized one. The solvent ratio plays a great role in the percentage yield of neem leaf oil. Figure 1b shows that a 60% ethanol and 40% *n*-hexane mixture gave the maximum amount of oil extracted from neem leaf. Baboo[21] reported that the extraction efficiency of the ethanol−hexane mixture is better than ethanol or hexane because the mixture reduces the flammability usually associated with the pure hexane solvent. Extraction time is also another important parameter that affects the percentage yield of oil. A better oil yield was achieved at the maximum time of extraction as depicted in Figure 1c because a longer time is essential for contacting the solid sample with the liquid solvent to leach out most of the oil parts contained in it.

Meanwhile, the main effect screener plots of neem leaf oil extraction are shown in Figure 2. The graphical interpretation easily infers that the variation of particle size has a great effect on the yield of oil relative to the rest of variable influences even though they have an impact on the extraction. This is due to the fact that a solid sample having a smaller particle size is very important to allow a better surface area to volume ratio needed for oil extraction because of the solvent diffusion toward the surface of the solid.[19,25,26]

Statistical Analysis of Neem Leaf Oil Extraction. The statistical analysis of neem leaf oil extraction was performed by applying the principles of analysis of variance (ANOVA) with the *p*-value (probability error value) as a significance analysis tool (Table 2). Thus, the model *F*-value of 152.89 implies that the model is significant; there is only a 0.01% chance that a "model *F*-value" this large could occur due to noise. A *P*-value less than 0.05 indicates the significance of terms involved in the process. In this case, A, B, and C are all significant terms. Values greater than 0.05 indicate that the terms are not significant. The *F*-value of "lack of fit" of 5.62 implies that the lack of fit is not significant relative to the pure error. There is only a 0.01% chance that a "lack of fit *F*-value" this large could occur due to noise. In any case, an insignificant lack of fit is desired to show good agreement between experimental data and the model. A significant lack of fit is bad. Furthermore, the model adequacy was examined with a good treated value of adjusted R^2 (0.9450) and predicted R^2 (0.9357) and a large value of adequate precision (42.999), which implies the precision and reliability of the model.

Effect of Process Parameters on *Croton macrostachyus* Seed Oil Extraction. The effects of extraction parameters on the croton seed oil yield are almost of similar inference with those of the neem leaf oil extraction scenarios as illustrated in the main effect plots (Figure 3), screener plots (Figure 4), and ANOVA results (Table 3).

Chemical Composition of Neem Leaf and Croton Seed Essential Oil. Usually, the insecticidal active components of essential oils are generally lipophilic, which can be diffused through the cell membranes of insects to affect digestive and neurological functions. Active compounds accountable for insecticidal activity, for example, terpenoids, alkaloids, and steroids, might be made by plants.[22] Researchers reported that chemical compounds δ-cadinene,[27] α-terpinyl

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

Table 4. Chemical Composition of Neem Leaf Oil

	lab no.: J-0032/1207/19	customer ID: NM			
S/N	name	formula	area	% area	RT
1	hexanoic acid, methyl ester	$C_7H_{14}O_2$	290191	0.15	5.624
2	eucalyptol	$C_{10}H_{18}O$	1405258	0.74	6.195
3	3-hexenoic acid, methyl ester, (*E*)-	$C_7H_{12}O_2$	261731	0.14	7.151
4	2-hexenoic acid, methyl ester, (*E*)-	$C_7H_{12}O_2$	870341	0.46	7.866
5	octanoic acid, methyl ester	$C_9H_{18}O_2$	152119	0.08	9.819
6	α-gurjunene	$C_{15}H_{24}$	1590182	0.83	11.177
7	β-gurjunene	$C_{15}H_{24}$	687006	0.36	13.562
8	aromadendrene	$C_{15}H_{24}$	8370823	4.39	14.75
9	(+)-9-epi-β-caryophyllene	$C_{15}H_{24}$	2658998	1.39	17.266
10	decanoic acid, methyl ester	$C_{11}H_{22}O_2$	636722	0.33	18.2
11	alloaromadendrene	$C_{15}H_{24}$	539026	0.28	18.805
12	(+)-δ-cadinene	$C_{15}H_{24}$	295951	0.16	21.516
13	α-terpinyl acetate	$C_{12}H_{20}O_2$	3068714	1.61	22.7
14	terpineol	$C_{10}H_{18}O$	298227	0.16	23.398
15	dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	2650919	1.39	26.687
16	neophytadiene	$C_{20}H_{38}$	1503257	0.79	30.104
17	methyl tetradecanoate	$C_{15}H_{30}O_2$	1461177	0.77	34.324
18	epiglobulol	$C_{15}H_{26}O$	1456496	0.76	34.57
19	ledol	$C_{15}H_{26}O$	1383545	0.72	38.456
20	globulol	$C_{15}H_{26}O$	5143973	2.70	39.34
21	rosifoliol	$C_{15}H_{26}O$	220505	0.12	40.092
22	β-eudesmol	$C_{15}H_{26}O$	817387	0.43	40.486
23	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	37281588	19.53	42.888
24	hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	1043810	0.55	44.19
25	7-hexadecanoic acid, methyl ester, (*Z*)-	$C_{17}H_{32}O_2$	939604	0.49	44.39
26	nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	357081	0.19	45.624
27	methyl stearate	$C_{19}H_{38}O_2$	3948722	2.07	48.88
28	9-octadecenoic acid (*Z*)-, methyl ester	$C_{19}H_{36}O_2$	4179183	2.19	49.797
29	9,12-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	11273491	5.91	51.594
30	9,12,15-octadecatrienoic acid, methyl ester, (*Z,Z,Z*)-	$C_{19}H_{32}O_2$	92603790	48.52	53.781
31	9,12,15-octadecatrienoic acid, ethyl ester, (*Z,Z,Z*)-	$C_{20}H_{34}O_2$	3465567	1.82	54.368

acetate, α-terpineol, 4-terpineol acetate and linalool,[28] cadinene, α-copaene, germacrene D and B, α-cubebene, β-elemene, α-guaiene, α-humulene, aromadendrene, β-selinene, α-selinene, caryophyllene oxide, nerolidol, spathulenol, and δ-cadinene[29] are all the major components found to have insecticidal activity to kill and repel insects. However, some active components of the essential oil may not be present, for instance, azadirachtin in neem leaf essential oil, because of several reasons linked to the plant growth environment that can cause changes in chemical composition.[7,9,16−18] Thus, the chemical compositions of both neem leaf and croton seed essential oils were also analyzed with the help of GC−MS analysis, which is the most useful and popular tool for identifying bioactive phytoconstituents in medicinal plants.[17] Consequently, more than 31 types of chemical compounds were identified by means of their integral values of the peaks linked with retention times for each oil, as presented in Tables 4 and 5; the mass spectral peaks of the total ion chromatogram (TIC) for neem leaf and croton seed oil extracts are also shown in Figures 5 and 6, respectively. Terpineol, α-terpinyl acetate, eucalyptol, and δ-cadinene are the active components found in neem leaf essential oil. The main active insecticidal compounds in croton seed essential oil are epiglobulol, copaene, δ-cadinene, α-cubebene, and β-guaiene. The MS spectra of the chromatograms for all active compounds with their structures are also presented in Supporting Information, Tables S7 and S8. Thus, the result implies that both essential oils contained chemical compounds that are toxic to insects.

Insecticide Formulation and Performance Evaluation. The bioinsecticides used in this study for testing on mosquitoes and cockroaches were created by combining each individual oil as well as their blends in a 1:1 ratio and then mixing them with additives to determine their contribution to insect killing. In each batch of tests, a double-hour mortality rate was performed; throughout the experiment, tests with a blank control and a commercial roach killer (i.e., insecticide aerosol, manufactured by Kafr El Zayat International Pesticides and Chemicals, Egypt) were implemented as a comparison to know the potential effects of the natural bioinsecticide product. All the detailed formulations of both oils are tabulated in the Supporting Information (Tables S4−S6). Thus, after 4 h, the roach killer could kill 100% of mosquitoes, whereas 20% concentrations of neem leaf oil and croton oil-based products killed 73.33 and 76.66% of mosquitoes, respectively, as revealed in Table S4 and Figure 7. This rate of mortality indicates that the bioinsecticide activity is comparable to synthetic insecticides and superior to blank controls at all time−concentrations tested. For complete assassination of insects at the 20% concentration, both bio-oil-derived insecticides need about 8 h of treatment. At lower concentrations of oil, it takes several hours to kill the insects on which it is sprayed.

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

Table 5. Chemical Composition of Croton Seed Oil

	lab no.: J-0033/1207/19	customer ID: CR			
S/N	name	formula	area	% area	RT
1	octanoic acid, methyl ester	$C_9H_{18}O_2$	121413	0.07	9.819
2	α-gurjunene	$C_{15}H_{24}$	199890	0.11	11.206
3	cyperene	$C_{15}H_{24}$	314040	0.17	12.132
4	α-muurolene	$C_{15}H_{24}$	1218536	0.68	18.75
5	(-)-β-cadinene	$C_{15}H_{24}$	6763451	3.75	19.427
6	benzoic acid, methyl ester	$C_8H_8O_2$	7734591	4.28	20.366
7	(+)-δ-cadinene	$C_{15}H_{24}$	1752206	0.97	21.528
8	copaene	$C_{15}H_{24}$	523751	0.29	22.416
9	α-cubebene	$C_{15}H_{24}$	380131	0.21	23.165
10	α-curcumene	$C_{15}H_{22}$	637259	0.35	23.973
11	cis-3-decen-1-ol	$C_{10}H_{20}O$	178769	0.1	25.952
12	τ-cadinol acetate	$C_{17}H_{28}O_2$	3385222	1.88	26.763
13	*cis*-calamenene	$C_{15}H_{22}$	2342258	1.3	27.081
14	α-cadinene	$C_{15}H_{24}$	365269	0.2	27.69
15	epicubebol	$C_{15}H_{24}$	576691	0.32	27.888
16	β-maaliene	$C_{15}H_{24}$	2706424	1.5	29.039
17	epiglobulol	$C_{15}H_{26}O$	4398768	2.44	29.208
18	β-guaiene	$C_{15}H_{24}$	726635	0.4	30.375
19	guaia-1(10),11-diene	$C_{15}H_{24}$	1802028	1	30.996
20	α-ylangene	$C_{15}H_{24}$	821809	0.46	33.052
21	methyl tetradecanoate	$C_{15}H_{30}O_2$	3650930	2.02	34.32
22	(*R*)-cuparene	$C_{15}H_{22}$	3430441	1.9	35.196
23	γ-eudesmol	$C_{15}H_{26}O$	782042	0.43	42.274
24	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	29496287	16.34	42.862
25	(±)-*trans*-nuciferol	$C_{15}H_{22}$	452019	0.25	46.33
26	methyl stearate	$C_{19}H_{38}O_2$	5432176	3.01	48.85
27	6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol	$C_{15}H_{24}O$	720518	0.4	49.252
28	9-octadecenoic acid (*Z*), methyl ester	$C_{19}H_{36}O_2$	39612562	21.94	49.839
29	ethyl oleate	$C_{20}H_{38}O$	1251329	0.69	50.698
30	9,12-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	16247619	9	51.607
31	9,12,15-octadecatrienoic acid, methyl ester, (*Z*,*Z*,*Z*)-	$C_{19}H_{32}O_2$	13201999	7.31	53.679
32	9,12-octadecadien-1-ol, (*Z*,*Z*)-	$C_{18}H_{34}O$	1751225	0.97	55.548
33	benzyl benzoate	$C_{14}H_{12}O_2$	22766154	12.61	58.534
34	2-methoxybenzyl acetate	$C_{10}H_{12}O_3$	4086064	2.26	68.289

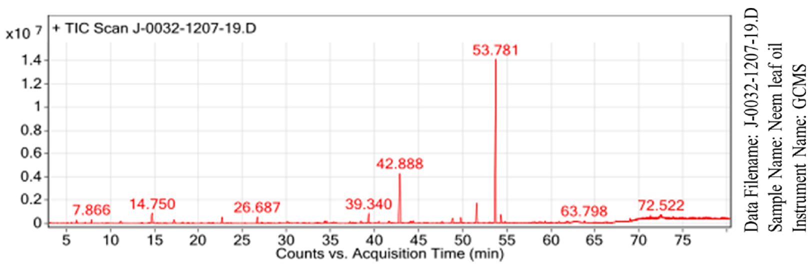


Figure 5. Typical GC−MS chromatogram of neem leaf essential oil showing the separation of chemical components.

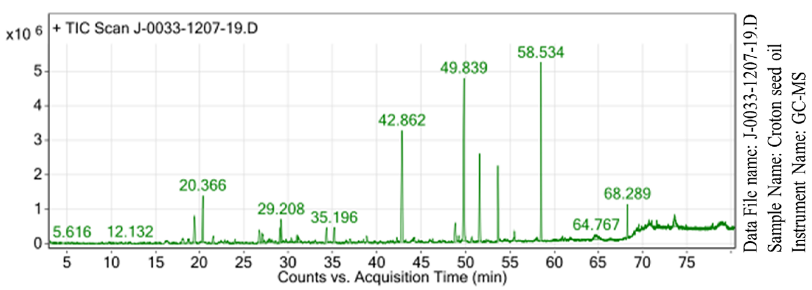


Figure 6. Typical GC−MS chromatogram of croton seed essential oil showing the separation of chemical components.

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

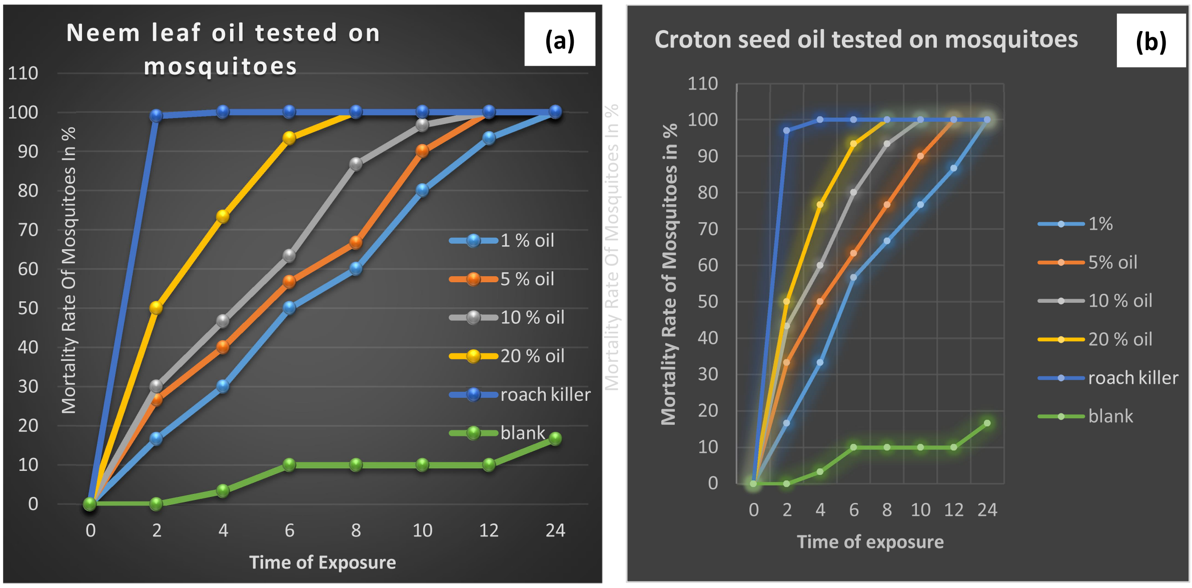


Figure 7. Insecticide tested on mosquitoes: neem leaf oil (a) and croton seed oil (b).

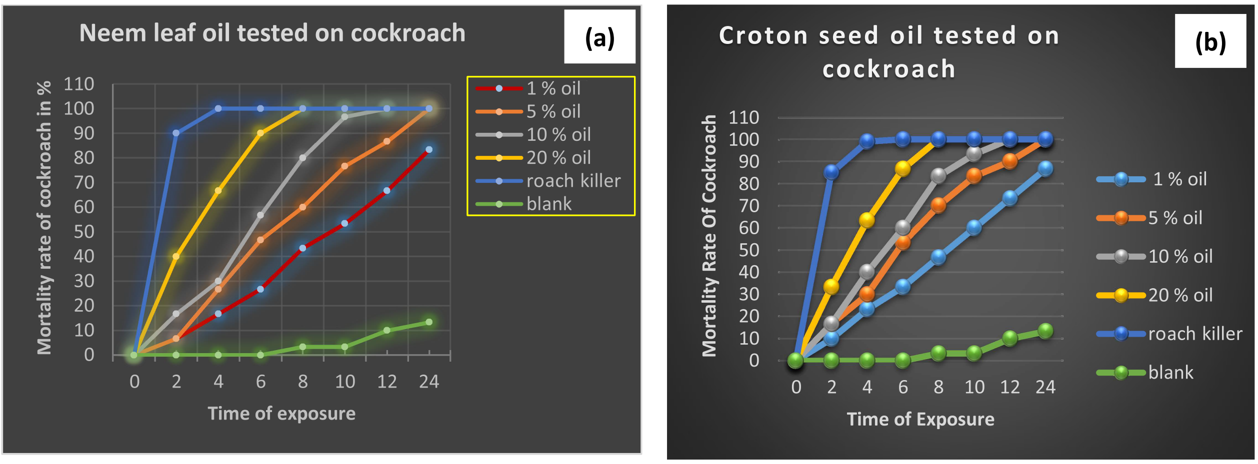


Figure 8. Insecticide tested on cockroach: neem leaf oil (a) and croton seed oil (b).

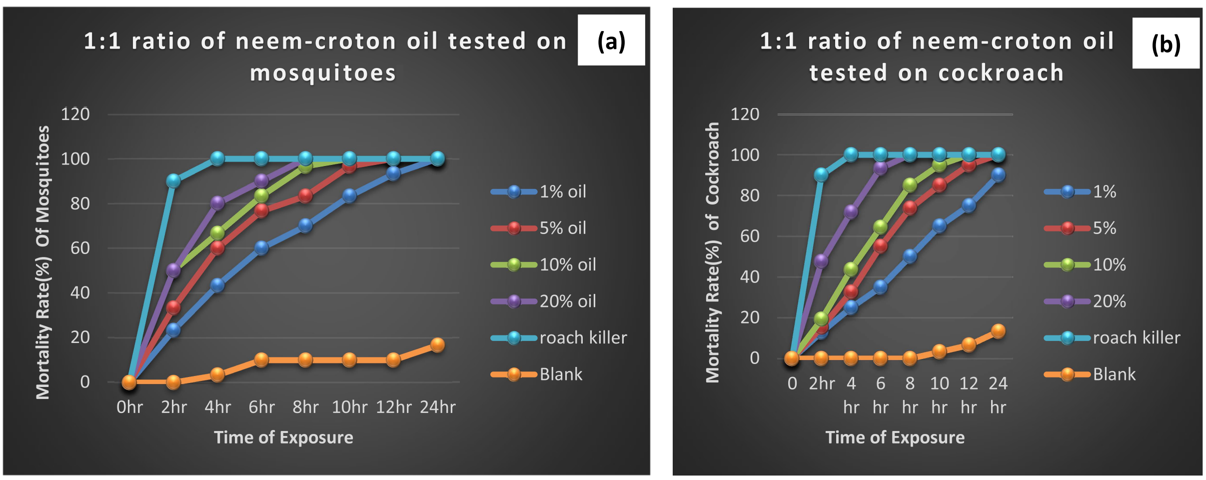


Figure 9. Mixed oil insecticide (1:1) tested on mosquitoes (a) and cockroach (b).

Similarly, Table S5 and Figure 8 also suggest almost the same inferences as those about mosquito effects, except for slight percentage deviations. As the concentration of neem leaf oil and/or croton oil is increased, the mortality rate of cockroaches increases within a shorter time. As a comparison using a synthetic insecticide, the roach killer effectively killed

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061

cockroaches within a treatment time of 4 h, whereas a 20% bioinsecticide executes about 66.66 (neem oil) and 63.33% (croton oil) at this time of contact; however, all of the insects were deceased after 8 h.

Furthermore, the effect of a one-to-one blend of neem leaf and croton seed essential oils was tested for its efficiency, as shown in Supporting Information, Table S6 and Figure 9. The blending of essential oils can reduce, increase, or even hinder a specific biological activity via the molecular interactions of their composing substances. When mixing essential oils, new compounds can be formed because of the reaction between some chemical components, which may change the phytochemical profile of the mixture and influence its insecticidal activity. Mixtures of essential oils significantly delay the selection of resistant insects as a result of the different bioactive substances' complex arrangements with various mechanisms of action. Relative to the pure essential oils (unblended), the insecticidal activity of the mixture is increased, which is advantageous such that the active component of the blend could effectively penetrate more into the pests, enhancing its toxicity.[22] The results showed that the effects of the mixed oil-based insecticide were more effective than the individually formulated ones at killing the insects used in the experiments. While the roach killer entirely killed all of the exposed mosquitoes and cockroaches within 4 h, the 20% concentration mixed oil formula only moderately killed around 80% of mosquitoes and 71.8% of cockroaches within the same time frame. This implies that the biopesticide potential of the mixed oil makes a very significant contribution to pest control.

■ CONCLUSIONS

From this study, it can be concluded that neem leaf and croton seed essential oils could be utilized for insecticide formulation instead of the conventional one. In this research, the effects of particle size, extraction time, and solvent type, which cause variation in oil yield, were assessed. Of these parametric effects, the particle size greatly influences the response variable, oil yield, since it determines the diffusion of the solvent toward the active surface of the sample for the leaching of the oils contained in it; the maximum oil yield was achieved at smaller particle sizes in the range of 0.5−1. Also, the active insecticidal compounds of both oils were evaluated with the help of GC−MS; accordingly, terpineol, α-terpinyl acetate, eucalyptol, and δ-cadinene were identified as the main active components of neem leaf essential oil, whereas *Croton macrostachyus* oil comprises epiglobulol, copaene, (+)-δ-cadinene, α-cubebene, and β-guaiene. For insecticidal efficiency evaluation, various bioinsecticide formulations were made and tested on mosquitoes and cockroaches in comparison with the synthetic roach killer as well as a blank control. The test results showed that the 20% concentration of the mixed oil-based one has better efficiency than the individual oil, which accounts for 80% of mosquito deaths and 71.8% of cockroach deaths within 4 h, whereas the synthetic roach killer completely kills all of the insects within the same time frame. This implies that the biopesticide potential of the mixed oil makes a very significant contribution to insect control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c08026.

Details of equipment lists, experimental run sheet for oil extraction and physicochemical properties, insecticidal formulation, and MS spectra of the chromatograms for both oils' active insecticidal compounds with their structures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Kedir D. Mekonnen − *School of Mechanical and Chemical Engineering, Kombolcha Institute of Technology-Wollo University, Wollo 208, Ethiopia;* orcid.org/0000-0001-9612-9324; Phone: +251 925216653; Email: kedirched@gmail.com, kedirched@kiot.edu.et

Author

Yassin A. Endris − *School of Mechanical and Chemical Engineering, Kombolcha Institute of Technology-Wollo University, Wollo 208, Ethiopia*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.2c08026

Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript and contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge financial support from the Kombolcha Institute of Technology, Wollo University, Ethiopia (https://kiot.wu.edu.et/).

■ REFERENCES

(1) Warra, A. A.; Narasimha, M.; Prasad, V. *African Perspective of Chemical Usage in Agriculture and Horticulture — Their Impact on Human Health and Environment*; LTD, 2020, DOI: 10.1016/B978-0-08-103017-2.00016-7.

(2) Tudi, M.; Ruan, H. D.; Wang, L.; Lyu, J.; Sadler, R.; Connell, D.; Chu, C. Agriculture Development , Pesticide Application and Its Impact on the Environment. *Environ. Res. Public Heal.* **2021**, *18*, 1−23.

(3) Jadeja, G. C.; Maheshwari, R. C.; Naik, S. N. Extraction of Natural Insecticide Azadirachtin from Neem (Azadirachta Indica A. Juss) Seed Kernels Using Pressurized Hot Solvent. *J. Supercrit. Fluids* **2011**, *56*, 253−258.

(4) Chowański, S.; Kudlewska, M.; Marciniak, P.; Rosiński, G. Synthetic Insecticides - Is There an Alternative? *Polish J. Environ. Stud.* **2014**, *23*, 291−302.

(5) Berhanu, A.; Asfaw, Z.; Kelbessa, E. Ethnobotany of Plants Used as Insecticides, Repellents and Antimalarial Agents in Jabitehnan District, West Gojjam. *Ethiop. J. Sci.* **2006**, *29*, 87−92.

(6) Padmaperuma, G.; Butler, T. O.; Shuhaili, F. A. B. A.; Almalki, W. J.; Vaidyanathan, S. *Microbial Consortia : Concept and Application in Fruit Crop Management*; Elsevier Inc., 2020, DOI: 10.1016/B978-0-12-818732-6.00025-3.

(7) Narayanan, B. S. and C. S. ESSENTIAL OILS/Isolation and Production. In *Encyclopaedia of Food Science, FoodTechnology andNutrition*; 2003; pp. 2185−2189, DOI: 10.1016/B0-12-227055-X/00426-0.

(8) Lawal, O. A.; Ogunwande, I. A. Essential Oils from the Medicinal Plants of Africa. In *Medicinal Plant Research in Africa*; Elsevier Inc., 2013; pp. 203−224, DOI: 10.1016/B978-0-12-405927-6.00005-9.

(9) Buckle, J. Basic Plant Taxonomy, Basic Essential Oil Chemistry, Extraction, Biosynthesis, and Analysis. In *Clinical Aromatherapy*; 2015; pp. 37−72, DOI: 10.1016/B978-0-7020-5440-2.00003-6.
(10) Macrostachyus, C. Chemical Analysis and Medicinal Activities of Volatile Components from the Seeds of Croton Macrostachyus Plant. *Int. J. Sci. Basic Appl. Res.* **2018**, *37*, 316−330.
(11) De Oliveira, L. F.; Damasceno, C. S.; Campos, R.; De Souza, M.; Joslin, G.; Ferreira, D. A.; De Fátima, J.; Dias, G.; Miguel, O. G.; Miguel, M. D. Chemical Composition of the Volatile Oil of Croton Glandulosus Linnaeus and Its Allelopathic Activity Chemical Composition of the Volatile Oil of Croton. *Nat. Prod. Res.* **2020**, *0*, 1−4.
(12) Karunamoorthi, K.; Ilango, K.; Euphor, C. D. Stapf . and Croton Macrostachyus Del . against Anopheles Arabiensis Patton , A. *Eur. Rev. Med. Pharmacol. Sci.* **2010**, 57−62.
(13) Maroyi, A. Ethnomedicinal Uses and Pharmacological Activities of Croton Megalobotrys Müll Arg: A Systematic Review. *Trop. J. Pharm. Res.* **2017**, *16*, 2535−2543.
(14) Tinghui, X.; Wegener, M.; O'Shea, M.; Deling, M. World Distribution and Trade in Neem Products with Reference to Their Potential in China. *Agric. Resour. Econ. Soc.* **2001**, 1−15.
(15) Boadu, K. O. Production of Natural Insecticide from Neem Leaves (Azadirachta Indica). *Asian J. Plant Sci. Res.* **2011**, *1*, 33−38.
(16) Ramasar, R.; Naidoo, Y.; Dewir, Y. H. Seasonal Change in Phytochemical Composition and Biological Activities of Carissa Macrocarpa (Eckl .) A . DC . Leaf Extract. *Hortic. Artic.* **2022**, *8*, 1−13.
(17) Kumar, S.; Yadav, M.; Yadav, A.; Yadav, J. P. South African Journal of Botany Impact of Spatial and Climatic Conditions on Phytochemical Diversity and in Vitro Antioxidant Activity of Indian Aloe Vera (L .) Burm . F. *South African J. Bot.* **2017**, *111*, 50−59.
(18) Burm, L. Effect of Climate Change on Phytochemical Diversity , Total Phenolic Content and in Vitro Antioxidant Activity of Aloe. *BMC Res. Notes* **2017**, 1−12.
(19) Mekonnen, D.; Sendekie, Z. B. NaOH-Catalyzed Methanolysis Optimization of Biodiesel Synthesis from Desert Date Seed Kernel Oil. *ACS Omega* **2021**, *6*, 24082−24091.
(20) Bayisa, Y. M.; Bullo, T. A. Optimization and Characterization of Oil Extracted from Croton Macrostachyus Seed for Antimicrobial Activity Using Experimental Analysis of Variance. *Heliyon* **2021**, *7*, No. e08095.
(21) Baboo, P. *Advancement in Neem Oil Extraction Process ADVANCEMENT IN Author*. 2016, No. January.
(22) Carvalho, S.; Justino, R.; Costa, O.; Andrade, S.; Alexandre, P.; Sandra, L.; Antonia, I.; Souza, D.; Buarque, N.; Gusm, D. Phytochemical and Toxicological Evaluation of a Blend of Essential Oils of Croton Species on Aedes Aegypti and Mus Musculus. *South African J. Bot.* **2020**, *132*, 188−195.
(23) Evbuomwan, B.; Felix-Achor, I.; Opute, C. Extraction And Characterization Of Oil From Neem Seeds , Leaves And Barks. *Eur. Int. J. Sci. Technol.* **2015**, *4*, 1−7.
(24) Alemayehu, G. Ethnobotanical Profile of Croton Macrostachyus (Euphorbiaceae) in Ethiopia : Review of the Literature. *Int. J. Res. Pharm. Pharm. Sci.* **2018**, *3*, 209−222.
(25) Nguyen, T.; Do, L.; Sabatini, D. A. Biodiesel Production via Peanut Oil Extraction Using Diesel-Based Reverse-Micellar Microemulsions. *Fuel* **2010**, *89*, 2285−2291.
(26) Rodríguez-miranda, J.; Hernández-santos, B.; Herman-lara, E.; Gómez-aldapa, C. A.; Garcia, H. S.; Martínez-sánchez, C. E. Effect of Some Variables on Oil Extraction Yield from Mexican Pumpkin Seeds. *CyTA − J. Food* **2014**, *12*, 9−15.
(27) García, M.; Gonzalez-Coloma, A.; Donadel, O. J.; Ardanaz, C. E.; Tonn, C. E.; Sosa, M. E. Insecticidal Effects of Flourensia Oolepis Blake (Asteraceae) Essential Oil. *Biochem. Syst. Ecol.* **2017**, *35*, 181−187.
(28) Liu, X. C.; Li, Y. P.; Li, H. Q.; Deng, Z. W.; Zhou, L.; Liu, Z. L.; Du, S. S. Identification of Repellent and Insecticidal Constituents of the Essential Oil of Artemisia Rupestris L. Aerial Parts against Liposcelis Bostrychophila Badonnel. *Molecules* **2013**, *18*, 10733−10746.
(29) Murugesan, S.; Rajeshkannan, C.; Babu, D. S.; Sumathi, R.; Manivachakam, P.; Breeding, T.; Puram, R. S. Identification of Insecticidal Properties in Common Weed - Lantana Camara Linn by Gas Chromatography and Mass Spectrum (GC-MS-MS). 2012, 3 (5), 2754−2759.

https://doi.org/10.1021/acsomega.2c08026
ACS Omega 2023, 8, 15052−15061