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ORIGINAL RESEARCH ARTICLE

Volatile constituents of different smoking materials in bee honey harvesting and detection of mean differences in calming time using completely randomized design

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ABSTRACT

Beekeeping and honey production provide a wide range of economic contributions through income generation from marketing of honey and related products, and creation of non-gender-biased employment opportunities. Traditionally, honey harvesting has been accomplished by making use of naked flames to rid of or even destroy honey bees or employing smoke to suppress their aggression. However, there is limited data on the effectiveness of different smoking materials used to calm honey bees during harvesting, necessitating research into establishing effective options that maximize honey yield while preserving the bee colony. This study evaluated the adaptability of variation in time taken to calm the honey bees, *Apis mellifera scutellata*, during honey harvesting using two fungi species *Calvatia cyathiformis* and *Calvatia gigantean*, air-dried hay from *Chloris gayana* and *Nasiwa setaria*, and the bark of *Juniperus procera* (African pencil cedar) as smoking agent sources. Gas chromatography-mass spectrometry (GC-MS) analysis of the volatile compounds released by the smoldering smoking materials identified naphthalene, 2-(2 octenyl)-cyclopentanone, 1,3-diethylbenzene, 2-methoxyphenol, 1,5,5-trimethyl-6-(3-methyl-buta-1dienyl)-cyclohexene, creosol, 4-ethyl-2-methoxyphenol, 2,4-bis(1,1-dimethylethyl)-phenol and cedrol as the major components of the calming smokes. Completely randomized design was used to compare the mean calming time among the smoking materials. Significant differences in mean calming time among the groups was reported. Post -ANOVA analysis using Turkey's HSD test revealed significant differences among the smoking material types, with *C. cyathiformis* and *C. gigantean* emerging as the most effective calming agents.

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Introduction

The development of skills and small enterprises, along with the creation of employment opportunities, is crucial for alleviating poverty in rural areas of Kenya. Many of the communities living in these poverty nodes possess prime beekeeping resources, yet they remain largely unaware of the potential benefits and are not fully capitalizing on them (Benedict, 2015). With a global shortage of honey bee products, especially those that meet organic and fair-trade standards, there is a significant opportunity to establish beekeeping businesses in these unpolluted rural areas. Such enterprises could meet the rising demand for organic, fair-trade honey bee products, thereby contributing to both local economies and global markets (Conrad, 2013).

However, the potential impact of these opportunities on social development is hindered by the use of expensive and inefficient honey harvesting methods. These methods often disregard the importance of timely harvesting and the safety of worker bees, thus compromising the viability of beekeeping businesses. In Kenya, honey is harvested annually by beekeepers from both artificial hives and natural hives found in the wild. Understanding the strategies beekeepers use to sustain their harvests is an area of significant interest (Gichora, 2003).

The documentation of traditional or indigenous beekeeping practices is essential for preserving this knowledge and creating a database for potential replication in other regions. Despite the value of traditional methods, modern technology has led scientists to discourage some of these practices, particularly the use of naked flames to suppress bees. Instead, modern methods favor the use of smoke, which is considered more effective and safer for both bees and beekeepers (Natalie, 2014). The choice of smoking material used to produce smoke in beekeeping is critical, as it must meet several conditions: it should be easy to ignite, burn slowly, produce a dense stream of smoke quickly, be non-toxic to bees and beekeepers, have a non-objectionable smell, and produce cool, even smoke (Carroll, 2006).

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Beekeepers often choose smoking materials based on local availability, with common options including dried cattle dung, soft rotten wood, wood shavings, dry bark, coconut husks, rice husks, dry pine needles, maize cobs, tightly packed hay, dried lucerne, dry papaya trunk fibers, Giant puffballs slices (dried), dried dung of local ruminants, dried camel dung, old sacking burlap from jute natural fibres (not plastics), thick sisal rope and cotton or rags (Koisianga, 1996; Tewodros et al., 2017). While these materials are widely used, the process of honey harvesting remains challenging. Bees are highly defensive when their hives are opened, leading to painful stings for beekeepers. To mitigate this, beekeepers often use protective gear such as bee veils, suits, and gloves (Tew, 2004). Smoke from the chosen smoking material is then used to calm the bees, allowing beekeepers to inspect the hive with reduced risk of being stung. The smoke disrupts the bees' communication by masking the alarm pheromone emitted by guard bees, causing the colony to retreat and allowing the beekeeper to harvest honey (Jones et al., 2024).

Kenya's Agricultural Shows have played a significant role in educating beekeepers about preferred practices and keeping them informed of the latest developments in beekeeping. Although scientists have not identified a single preferred smoking material, they recommend using any natural material that meets the necessary conditions. Despite the wide range of available smoking materials, Kenyan beekeepers and scientists have yet to determine which is the most effective. This research therefore, aimed to investigate which materials best calm bees during honey harvesting while fulfilling the required conditions, thereby enabling beekeepers to manage their bees without causing distress during the harvesting process.

Figure 1. *Calvatia cyathirfomis*.

Figure 2. *Calvatia gigantea*.

Materials and methods

Collection of smoking agents

All the smoking agents; *Calvatia cyathiformis*, *Calvatia gigantea* (Figures 1 and 2, the two types of fungi), dry hay from *Chloris gayana* Kunth and *Nasiwa setaria*, and bark of *Juniperus procera* (Cedar tree) were all collected in the month of December, 2015. *C. cyathiformis* and bark of cedar tree were collected from Koibatek forest in Baringo county, Kenya. Mature wild fungi *C. gigantea* were carefully harvested from grazing fields and bushy areas in the Bik-Kwen region. The dry hay, a mixture of *C. gayana* and *N. setaria* grass, was collected from animal feeding areas.

Pre-treatment of smoking agents

The collected smoking materials were air-dried under shade for five days and then stored in brown sample envelopes at room temperature. This drying process ensured that the materials were sufficiently dehydrated to withstand the storage conditions during the entire study duration. The dried materials were then divided into two batches: one batch was used for field experiments to determine the calming time of bees exposed to each smoke type, and the other batch was used in the laboratory to analyze the chemical composition of the smoke produced by each smoking material.

Trapping and exposure of the bees to smoke

A non-probability sampling technique was used since there was no need of estimating the probability of each bee being included in the sample. Sampling was conducted at strategic locations where bees were observed foraging as they exited the hives. Bees were attracted to sampling bowls using honey and then trapped with homemade nets. A swam of sixty bees trapped in the bowls were subsequently exposed to smoke from the four different smoke materials: *C. cyathiformis*, *C. gigantea*, dry hay, and cedar bark. Smoke was introduced into the bowl's enclosure for 15 s using a standard bee smoker. Immediately after exposure, the smoke source was removed, and the bees were closely observed for signs of pacification. This exposure was repeated four times with different samples of bees, and the recovery time for each smoke type was recorded so as to determine calming time. After each experiment, the trapped bees were released. In this context, the calming time is the duration for which the bee colony remains sufficiently subdued (docile or passive) for safe hive inspection or honey extraction.

Trapping of volatile constituents of the smoking agents

The smoking agents were individually smoldered in a crucible, and the resulting volatile constituents were trapped using C_8 adsorbent packed in 4×4 cm wire mesh sachets made from wire mesh 150. The sachets were cleaned using a Soxhlet apparatus with dichloromethane for 8 h, loaded with 50 mg of adsorbent, and cleaned again for 4 h. The prepared sachets were hooked to an inverted glass funnel positioned above the smoldering smoking material in the crucible using a tripod stand (Figure 3). The setup was run for 8 h to ensure adequate trapping of the volatile constituents. To desorb the trapped components, the adsorbent was placed in a Pasteur pipette plugged with cotton wool and eluted with GC-grade dichloromethane (DCM) into vials for subsequent GC-MS analysis.

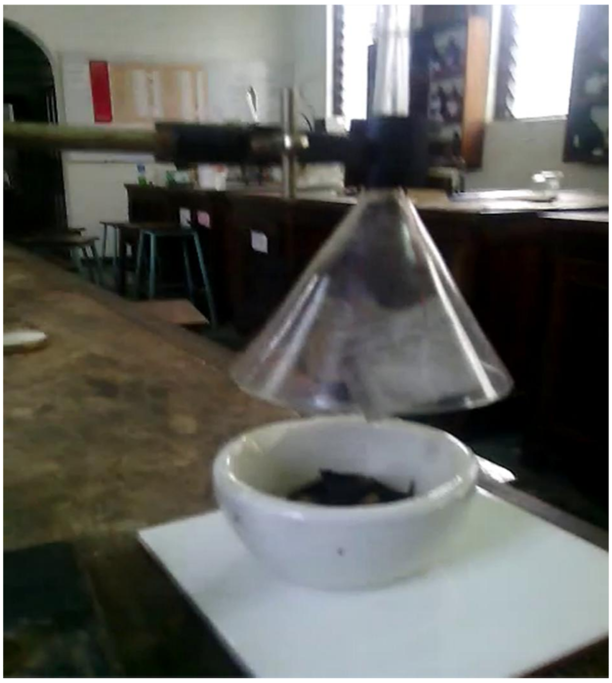

Figure 3. Trapping of the smoke volatile components.

Analysis of the smoke volatile constituents

The volatile constituents obtained from the natural smoking materails were analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis was performed on a GC-17A gas chromatograph coupled with a GC-MS QP 5050 A mass spectrometer, equipped with a fused silica capillary column. The column temperature was initially set at 50 °C for 2 min, then increased to 250 °C at a rate of 3 °C/min. Helium was used as the carrier gas at a pressure of 90 kPa. The identification of the compounds was done by comparing the recorded mass spectra with those in the literature, supplemented by the Wiley 7th Mass Spectral Library and the NIST MS Search 2.0 GC-MS libraries. Kovats retention indices (RI) were derived using Kovats formula (Kovàts, 1958). Quantitative data were obtained from the electronic integration of peak areas and retention times.

Detection of differences in the mean calming times of various smoking materials

The experiment was conducted using a completely randomized design (CRD). All experimental units were grouped homogeneously into four groups namely *Calvatia cyathiformis, Calvatia gigantean, Chloris gayana* Kunth & *Nasiwa setaria*, and *Juniperus procera*.

The statistical model for CRD is defined by equation 1 as:

$$Y_j = \mu + t_i + e_{ij} \tag{1}$$

Where:

μ = overall mean effect

t_i = true effect of the ith treatment

e_{ij} = error term of the jth unit receiving ith treatment

In CRD the null hypothesis is that there is no significant mean differences among the groups. This hypothesis is rejected wherever the p value is less than the specified level of significance (0.05). When the null hypothesis is rejected, a post ANOVA test is done to determine which specific groups have mean differences. In this research, Tukey's HSD test was used to carryout post ANOVA test.

Results

Mean differences in smoking material calming times

Mean calming times for the individual smoking materials were computed as given in Table 1.

Table 1 shows that *Calvatia cyathiformis* smoke had the highest calming time of 35.06 min, while that of Juniperus procera displayed the minimal effect with a mean calming time of 7.99 min.

A completely randomized design (CRD) was employed to statistically determine if there exist significant mean differences in calming time among the different smoking materials. The main objective was to determine the best natural smoking material for calming bees by assessing the variability in mean calming times among the different treatments. Using CRD, Table 2 was generated.

Table 2 shows that the p value was 0.000 which implies that the null hypothesis was rejected. This means that there is at least significant mean difference between any pair. Therefore, post ANOVA test was done using Tukey̓s HSD and results given in Table 3.

The Tukey's HSD results indicate significant differences between most treatments. Treatments 1 and 2 were significantly different from all other treatments. On the other hand, treatments 3 and 4 did not significantly differ from each other. The findings necessitated analysis of the volatile chemical constituents of the smoking materials so as to determine the cause of variation in the mean calming times.

Table 1. Smoking materials mean calming times.

Group	Mean (Minutes)	Standard deviation
Calvatia cyathiformis	35.06	3.516
Calvatia gigantea	14.31	1.139
Chloris gayana Kunth & *Nasiwa setaria*	6.60	1.390
Juniperus procera	7.99	0.470

Table 2. CRD results on mean time differences amongst smoking materials.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2074.952	3	691.651	174.984	.000
Within Groups	47.432	12	3.953		
Total	2122.384	15			

Volatile constituents of the smoking agents

The chemical constituents of Calvatia cyathiformis smoke

The analysis of *C. cyathiformis* smoke revealed that the majority of the identified constituents were aromatic compounds, accounting for 85.6% of the total composition (Figures S1 and S2; Table 4). The predominant compound was naphthalene, which constituted 24.27% of the smoke's chemical profile. Other notable aromatic components included 4-ethyl-benzaldehyde (7.29%), y-chlorobutyrophenone (6.98%), 1,3-diethylbenzene (6.11%), benzyl nitrile (4.87%), and 2,4-bis(1,1-dimethylethyl) phenol (4.48%). The non-aromatic components were 1,5,5-trimethyl-6-acetylmethylcyclohexane (7.66%) and cis-p-mentha-2,8-dien-1-ol (6.78%). Unidentified components with a composition ≥2.98% accounted for 22.01% of the total smoke constituents. Table 4 provides the volatile compounds and their retention indices.

The chemical constituents of calvatia gigantea smoke

The chemical profile of *C. gigantea* smoke was predominantly composed of non-aromatic hydrocarbons, which accounted for 66.97% of the identified constituents, while aromatic compounds made up 27.39% (Figures S1 and S3; Table 5). The major non-aromatic hydrocarbons included 1,2,3,3,4-pentamethylcyclopentene (25.71%), 2,6,10-trimethyltetradecane (15.28%), and 2,6,10,15-tetramethylheptadecane (10.05%). Among the aromatic constituents, naphthalene (11.26%), 2,4-bis(1,1-dimethylethyl) phenol (10.31%), and 4-chloro-2,6-bis(1,1-dimethyl) phenol (5.82%) and (3,3-dimethylcyclohexylidene)-(Z)-Acetaldehyde (3.23%) were the most prevalent. Earlier investigations revealed that *C. gigantea* in its natural environment releases naphthalene, 2,6,10-trimethyltetradecane and 2,4-bis(1,1-dimethylethyl)-phenol in trace amounts (Leffigwell & Alford, 2011).

Table 3. Paired mean differences between smoking materials.

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	20.74750*	1.40582	.000	16.5738	24.9212
	3	28.46250*	1.40582	.000	24.2888	32.6362
	4	27.07250*	1.40582	.000	22.8988	31.2462
2	1	−20.74750*	1.40582	.000	−24.9212	−16.5738
	3	7.71500*	1.40582	.001	3.5413	11.8887
	4	6.32500*	1.40582	.004	2.1513	10.4987
3	1	−28.46250*	1.40582	.000	−32.6362	−24.2888
	2	−7.71500*	1.40582	.001	−11.8887	−3.5413
	4	−1.39000	1.40582	.758	−5.5637	2.7837
4	1	−27.07250*	1.40582	.000	−31.2462	−22.8988
	2	−6.32500*	1.40582	.004	−10.4987	−2.1513
	3	1.39000	1.40582	.758	−2.7837	5.5637

*The mean difference is significant at the 0.05 level.
1 = *Calvatia cyathiformis*, 2 = *Calvatia gigantean*, 3 = *Chloris gayana* Kunth & *Nasiwa setaria*, 4 = *Juniperus procera*.

Table 4. Volatile constituents of *Calvatia cyathiformis* smoke.

S/No.	Retention time (Minutes)	Kovats RI	Compound	% Composition
1.	5.61	950	1,5,5-trimetyl-6-acetylmethylcyclohexane	7.66
2.	7.71	1031	1,3-diethylbenzene	6.11
3.	7.82	1043	cis-p-Mentha-2,8-dien-1-ol	6.78
4.	7.91	1056	Unidentified	3.32
5.	7.99	1064	y-Chlorobutyrophenone	6.98
6.	8.07	1070	Unidentified	5.18
7.	8.27	1095	1-Phenyl-1-butene	2.43
8.	9.11	1097	Benzyl nitrile	4.87
9.	9.48	1162	Naphthaline	24.27
10.	9.82	1181	4-Ethylbenzaldehyde	7.29
11.	10.61	1200	1,5-Bis(benzylidene)-3-phenylpentane	4.48
12.	10.94	1210	Unidentified	5.93
13.	13.98	1450	Unidentified	2.98
14.	14.16	1519	2,4-Di-tert-butylphenol	4.48
15.	19.46	1600	Unidentified	4.60

Table 5. Volatile constituents of *Calvatia gigantean* smoke.

S/No.	Retention time (Minutes)	Kovats RI	Compound	% Composition
1.	5.26	970	6-Acetyl-1,5,5-trimethylcyclohex-1-ene	6.54
2.	5.58	990	Unidentified	5.62
3.	5.61	995	1,2,3,3,4-pentamethylcyclopentene	25.71
4.	6.40	1030	(3,3-dimethylcyclohexylidene)-(Z)- Acetaldehyde	3.23
5.	7.80	1041	2,6,10-trimethyltetradecane	15.28
6.	8.50	1125	2,6,10-trimethyltetradecane (Repeated)	6.16
7.	9.82	1162	Naphthalene	11.26
8.	11.11	1220	2,6,10,15-tetramethylheptadecane	10.05
9.	14.16	1519	2,4-bis(1,1-dimethylethyl)phenol or 2,4-Di-tert-butylphenol	10.31
10.	14.54	1530	4-chloro-2,6-bis(1,1-dimethyl)phenol	5.82

Table 6. Volatile constituents of *Juniperus procera* bark smoke.

S/No.	Retention time (Minutes)	Kovats RI	Compound	% Composition
1.	4.88	831	Furfural	3.45
2.	5.47	920	Unidentified	1.40
3.	5.60	940	2-(2-octenyl)-Cyclopentanone	10.81
4.	6.36	955	5-Methyl- 2-furancarboxaldehyde	4.70
5.	6.42	960	2-Isopropylidene-5-methylhex-4-enal	3.64
6.	7.72	1032	1,3-diethylbenzene	2.87
7.	7.80	1040	•Pivalatelimonen-6-ol	4.39
8.	8.08	1050	Unidentified	1.98
9.	8.34	1075	2-methoxyphenol	9.18
10.	9.48	1135	1,5,5-Trimethyl-6-(3-methyl-buta-1,3-dienyl)-cyclohexene	3.35
11.	9.82	1162	Naphthalene	10.79
12.	9.91	1165	Creosol	13.08
13.	11.15	1247	4-ethyl-2-methoxyphenol	6.92
14.	12.36	1320	Unidentified	1.66
15.	13.06	1414	2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethylmethanoazulene	3.26
16.	14.16	1519	2,4-Di-tert-butylphenol	2.07
17.	15.18	1550	3-(2-Methyl-propenyl)-1H-indine	2.54
18.	15.42	1589	Cedrol	4.84
19.	21.49	1680	Unidentified	2.75

Table 7. Volatile constituents of smoke of hay from *Chloris gayana* Kunth and *Nasiwa setaria*.

S/No.	Retention time (Minutes)	Kovats RI	Compound	% Composition
1.	5.58	990	1,2,3,3,4-pentamethylcyclopentene	23.79
2.	8.34	1075	2-methoxyphenol	9.65
3.	9.48	1110	2-ethylphenol	13.98
4.	9.82	1162	Naphthalene	18.72
5.	10.23	1205	1,2-Benzenedimethanol	17.11
6.	11.15	1247	4-ethyl-2-methoxyphenol	7.01
7.	11.91	1275	Unidentified	4.12
8.	14.23	1380	Unidentified	5.81

The chemical constituents of Juniperus procera bark smoke

The smoke from *J. procera* bark was predominantly composed of oxygenated hydrocarbons (Figure S4; Table 6). The key constituents included creosol (13.08%), 2-(2-octenyl)-cyclopentanone (10.81%), naphthalene (10.79%), 2-methoxyphenol (9.18%), 4-ethyl-2-methoxyphenol (6.92%), cedrol (4.84%), 5-methyl-2-furancarboxaldehyde (4.70%), and pivalatelimonen-6-ol, (4.39%) (Figure S1).

The chemical constituents of smoke of hay from Chloris gayana Kunth and Nasiwa setaria

1,2,3,3,4-pentamethylcyclopentene constituted the major proportion of the smoke volatiles at 23.9% with the other constituents being naphthalene

(18.72%), 1,2-Benzenedimethanol (17.11%) 2-ethylphenol (13.98%), 2-methoxyphenol (9.65%) and 4-ethyl-2-methoxyphenol (7.01%) (Figures S1 and S5; Table 7).

Discussion

Paired mean differences between smoking materials, as provided by the Tukey's HSD results, indicate significant differences between most treatments. Treatments 1 and 2 were significantly different from all other treatments, indicating superior calming time, as supported by the established smoking material mean calming times. On the other hand, treatments 3 and 4 did not significantly differ from each other, suggesting they have comparable calming effects. These findings align with existing literature, which shows that the effectiveness of various smoking materials in honey bee smokers can vary significantly, influencing how quickly and effectively bees are calmed during hive inspections (Carroll, 2006; Jones et al., 2024).

The results from the GC-MS analysis of the smoke volatiles from the smoking materials reveal both qualitative and quantitative differences in the various chemical constituents. *C. cyathiformis*, *C. gigantea* and hay from *C. gayana* Kunth and *N. setaria* smoke are rich in aromatic compounds, while *Juniperus procera* bark smoke is characterized by a high content of oxygenated hydrocarbons. Interestingly, the terpene and terpenoid constituents previously reported for *C.gigantea* were not detected in its smoke volatiles (Leffingwell & Alford, 2011). This diversity in chemical composition suggests that the choice of smoking material can significantly influence the type and concentration of smoke volatiles, potentially affecting the effectiveness of the smoke in calming bees during honey harvesting.

The calming effect of the most effective smokes on the bees could be associated with the presence of aromatic hydrocarbons. The smoke of the most active smoking agent, *C. cyathiformis* had naphthalene in highest proportion relative to other constituents. Naphthalene, though present in a lower proportion, was also detected in the smoke of *C. gigantean*, which had the second-best calming effect among the four evaluated smoking agents. The oxygenated aromatic hydrocarbons as well as other oxygenated compounds, may have a minimal calming effect, as demonstrated by the relatively short calming duration of the smoke from *C. gigantean*, *J. procera,* and hay from *C. gayana* Kunth and *N. setaria*.

The recovery of all the test bees post-calming duration indicates that the evaluated smoking materials are safe, meet the required conditions, and can be deployed in modern honey bee harvesting technology (Carroll, 2006; Natalie, 2014).

Conclusions

The results demonstrated that each smoking material has a unique chemical profile, which influences its efficacy in calming honey bees. The findings provide valuable insights for beekeepers in selecting the most suitable smoking material to optimize honey harvesting while ensuring the well-being of both the bees and the beekeeper.

Therefore, we conclude that; best natural smoking agent is *C. cyathiformis*. The use of *C. cyathiformis* should be encouraged and be developed into a commercial smoking agent for the bee farmers by Kenya Agricultural Research Institute in collaboration with the Kenya Forestry Research Institute.

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Disclosure statement

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

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