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Chemistry of semiochemicals used as trail pheromones in tropical fire ant (*Solenopsis geminata*)

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Abstract

Trail pheromones were extracted with petroleum ether from the cuticular abdominal glands of tropical fire ant (*Solenopsis geminata)* and sixteen pheromonal compounds were characterised using Gas Chromatography-Mass Spectrometry (GC/MS) Technique in combination with Fourier Transform-Infrared Spectroscopy (FT-IR). Undecane (31.71 %) constituted the bulk of the pheromone compounds. Other ones analysed include 1,4-dimethylbenzene (6.06 %), nonane (5.66 %), decane (3.63 %), dodecane (1.09 %), tridecane (1.11 %), hexadecanoic acid (10.47 %), (z)-9-octadecenoic acid (10.51 %), octadecanoic acid (3.71 %), 2-methyleicosane (2.26 %), octacosane (3.79 %), 1,2-benzenedicarboxylic acid dioctyl ester (3.02 %), 2,6,10,14-tetramethylheptadecane (5.37 %), dotriacontane (4.78 %), hexatriacontane (4.22 %) and 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol [(E,E)- farnesol] (2.61 %). FT-IR analysis of the extract showed peaks at 1541.18, 1645.33, 2094.76, 2942.51 and 3430.51 cm^{-1} indicating the presence of aromatic compounds, alkene, alkane, and alcohol as well as carboxylic acid compounds. These compounds constituted 63.62 % hydrocarbons, 24.69 % fatty acids, 9.08 % aromatic compounds and 2.61 % alcohol. This investigation has revealed that the trail pheromone in tropical fire ant (*S. geminata*) is not just a single compound but a mixture of compounds. These pheromones could artificially be used to lure and mass trap the ants in integrated pest management thereby reducing their negative effects on agricultural produce and around residential areas.

Key words: Fire ant (*Solenopsis geminata*), Pheromones, GC/MS Analysis, FT-IR, Pest management.

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1. Introduction

The use of toxic chemicals as pesticides to control insects and other pestiferous organisms has drawn public concern due to environmental and health implications hence the need for alternative techniques of integrated pest managements that are environmentally friendly. One of such techniques is the use of pheromones to control the behaviour of insect pests. Pheromones convey information that produces specific responses in individuals of the same species [1]. Pheromones have been applied in monitoring a population of insects to determine if they are present or absent in an area or to determine if enough insects are present to warrant a costly treatment [2]. Pheromones are also applied in mass trapping insects to remove large numbers of insects from the breeding and feeding population as well as in the disruption of mating in populations of insects [2]. The tropical fire ant, *Solenopsis geminate* belongs to the family *Formicidae* and subfamily *Myrmicinae* [3]. Foragers will mass recruit to a food source via trail pheromones and generally forage within 15 m of the nest. They primarily feed on the ground. Foragers mark areas they explore chemically, and marks last for 6 hours [4]. Unmarked areas are actively invaded and areas marked with a colony's pheromone are actively defended. Foragers are slow to find food but are effective at defending resources once found [3,5]. *S. geminate* possesses a painful sting causing injury to humans and domestic animals, and cases of anaphylactic shock have been reported [6]. They can be common around urban areas, are attracted to electric fields [7], and can cause chewing damage to PVC coatings of electrical wiring [8]. They also build ugly mounds in lawns, steal seeds from seedbeds, bite holes in fabrics and feed on a range of household foods [3]. Workers tend honeydew-producing homoptera, especially mealybugs. This increases pest populations and can reduce seed set and yields [3]. They can also girdle citrus trunks, which may introduce disease [9]. *S. geminata* has the potential to invade native communities and affect both animals and plants in that community. This is especially the case in disturbed ecosystems, which it reinvades rapidly after disturbance and probably acts as an important organiser of the arthropod community [3].

Foragers also prey on vertebrates. They have been reported to feed on hatching quail, entering piped eggs and consuming the entire chick, decreasing nesting success, and

causing adult quail to abandon the nest; attack and consume young birds in their nest or that have fallen from their nest. They have also been observed killing young rats and may kill young mongooses in their burrows [3]. Foragers have also been recorded feeding on the seeds and seedlings of sorghum, tomato, citrus, avocados, coffee, cocoa, corn, and tobacco [3,5,10]. Losses can be significant (e.g., 11% of potato and tomato crops had gnawed tubers and girdling of stems [10], 90% of tomato seeds destroyed in a paddock [5]. *S. geminata* is an opportunistic omnivore and hence is considered both a pest and a beneficial predator [11]. Its presence in argroecosystems can alter the invertebrate community significantly. Many studies have demonstrated that *S. geminata* reduces densities of invertebrate pests e.g., a citrus pest *Diaprepes abreviatus* in the Caribbean, various rice pests in the Philippines [11], *Sitophilus* spp. on maize, various pests in Florida soybean crops, larval and adult flies in Puerto Rico, and fall armyworm in maize [3].

S. geminata in South East Nigeria can invade residential areas attacking both human and livestock. Wild animals held in local traps are destroyed and rendered useless by *S. geminata* causing agony to hunters. This insect causes discomfort to farmers in Nigeria as they destroy agricultural produce including stored products. Therefore the insect is purely considered a pest in Nigeria and needs to be controlled. This investigation focuses on harnessing and studying trail pheromones from *S. geminata* and their possible application in controlling the pest.

2. Materials and Methods

2.1. Insect Collection

Colonies of *S. geminata* were collected from Umuariaga village near Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and housed in circular glass nests. The organism was identified and authenticated in the Zoology Department of the aforementioned university. About 200 adults of *S. geminata* were used for the investigation. Hereafter, the words 'carpenter ants' refer only to the adults of *S. geminata* unless otherwise stated.

2.2. Extraction of Pheromonal compounds

Cuticular abdominal glands of *S. geminata* were excised with fine brand new razor blade after anaesthetising the organisms by cleaning with chloroform which also removes cuticular surface contaminants. The tissue was extracted in petroleum ether for 20 min. at room temperature. Extract was placed in screw cap vials and stored at −15 ℃ until analysis.

2.3. GC/MS Analysis

GC analysis was carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 80-280 ℃ held at 80 ℃ for 1 min, and at 200 ℃ for 4 min (rate 10 ℃/min), and finally at 280 ℃ for 5 min (rate 10 ℃/min). The injection temperature was 250 ℃. GC/MS analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with column oven temperature of 80 ℃. The carrier gas was Helium with a pressure of 108.0 Kpa and linear velocity of 46.3 cm/s. Total flow was 6.2 mL/min, column flow was 1.58 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 1.0. Also, ion source temperature was 230 ℃, interface temperature was 250 ℃, solvent cut time was 2.5 min., detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spec., start time was 3.0 min., end time was 28.0 min, event time was 0.5 s, scan speed was 1250, and start m/z was 40 while end m/z was 600.The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge, Germany, was used. All solvents used were of analytical grade and were procured from Merck, Germany.

2.4. Components Identification

The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature.

2.5. FT-IR Analysis

FT-IR measurement of the extract was performed using FTIR-8400S Fourier Transform Infrared Spectrophotometer, SHIMADZU, Japan, in a diffused reflectance mode at a resolution of 4 cm^{-1} in sodium chloride (NaCl) pellets in the range 4500-400 cm^{-1}.

3. Results and Discussion

The chemistry of pheromones in fire ants was examined using GC/MS in combination with FT-IR techniques. Sixteen peaks were analysed in the GC chromatogram shown in Fig. 1 indicating the presence of sixteen pheromonal compounds. The compounds analysed included 1,4-dimethylbenzene (6.06 %), nonane (5.66 %), decane (3.63 %), undecane (31.71 %) , dodecane (1.09 %), tridecane (1.11 %), hexadecanoic acid (10.47 %), (z)-9-octadecenoic acid (10.51 %), octadecanoic acid (3.71 %), 2-methyleicosane (2.26 %), octacosane (3.79 %), 1,2-benzenedicarboxylic acid dioctyl ester (3.02 %), 2,6,10,14-tetramethylheptadecane (5.37 %), dotriacontane (4.78 %), hexatriacontane (4.22 %) and 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol [(E,E)- farnesol] (2.61 %). Undecane constituted the bulk of the pheromone compounds. Fig. 2 shows the FT-IR spectra of the extract. The FT-IR analysis of the extract showed a peak at 1541.18 cm^{-1} indicating the presence of C=C functional group from an aromatic compound while another peak at 1645.33 cm^{-1} was also indicative of C=C functional group but from an alkene molecule. The spectra showed yet another peak at 2942.51 cm^{-1} due to C−H functional group from an alkane. A broad peak at 3430.51 cm^{-1} was as a result of the presence of O−H functional group from an alcohol or carboxylic acid. The pheromonal extract showed the presence of aromatic compounds, alkenes, alkanes and alcohols as well as carboxylic acid compounds. The composition of the compounds was 63.62 % hydrocarbons, 24.69 % fatty acids, 9.08 % aromatic compounds and 2.61 % alcohol. The highest component was 11-octadecenoic acid methyl ester followed by 1,12-tridecadiene. Table 2 shows the nomenclatures, molecular formulae, molecular weights, retention times, peak areas and the nature of these compounds. The mass spectra of the two most abundant compounds in the pheromonal extract are shown in Figs. 3

and 4 while the structures of the sixteen compounds analysed are shown in Fig. 5.

The pheromonal compounds isolated from cuticular abdominal glands of *S. geminata* consist of several compounds, the bulk of which are hydrocarbons (63.62 %). It has been reported with substantial evidence that synthesizing pure chemicals by insects in pheromone secreting glands is very difficult, if not impossible [12]. Therefore, it could be that during the biochemical synthesis of the active pheromonal compounds, other chemicals are synthesized which could either promote the pheromone functionality of the compounds or be used for other communication purposes by the insect. Octacosane, dotriacontane and hexatriacontane i.e. compounds 11, 14 and 15 have high carbon contents meaning that they will have low volatility compared to other compounds. These compounds are required to be persistent when used as trail pheromones and so need to be less volatile. However, the volatility gradient will be appropriate for the fulfilment of different communication functions such as orientation and attraction. It has been reported that a very good food source will trigger trail laying with plenty of an attractive chemical, whereas food sources of low quality will be signalled with low amounts of this chemical laid on the trail [4]. The tropical fire ant requires chemical trails to perform the function of attraction and orientation of nest mates. The ant has to produce a range of chemicals for these two purposes. Out of the sixteen pheromonal compounds analysed, ten were hydrocarbon compounds. In housefly, it was reported that saturated and unsaturated female cuticular hydrocarbons tested separately elicited low responses but when combined at a ratio of 65 %: 35 % saturated: unsaturated, induced male activity [13]. When methylheptacosanes and methylnonacosenes were combined with (Z)-9-tricosene male activity strongly increased compared to (Z)-9-tricosene alone and that the two compounds producing the highest activity in combination with (Z)-9-tricosene were 4,8-dimethylheptacosene and 13-methylnonacosene [13]. This then means that the hydrocarbon compounds observed in the extract of the tropical fire ants are used in synergism by the insects to achieve maximum interactive results. Although no unsaturated hydrocarbon was found in the extract of the fire ant, mixtures of the available saturated hydrocarbons might be responsible for effective communication amongst the ants.

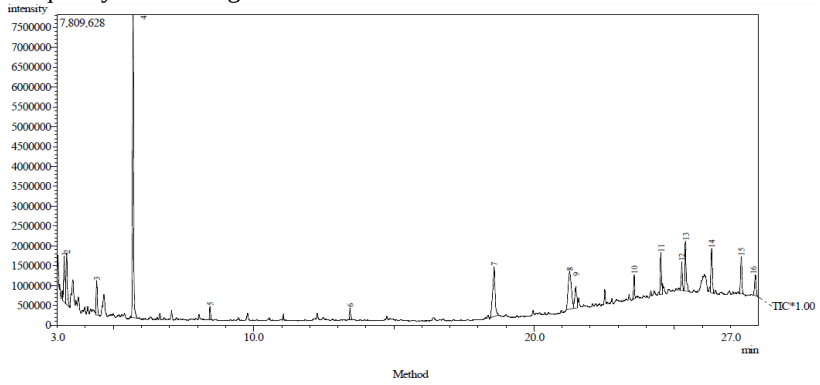

Fig. 1: GC-MS chromatogram of tropical fire ant pheromone extract

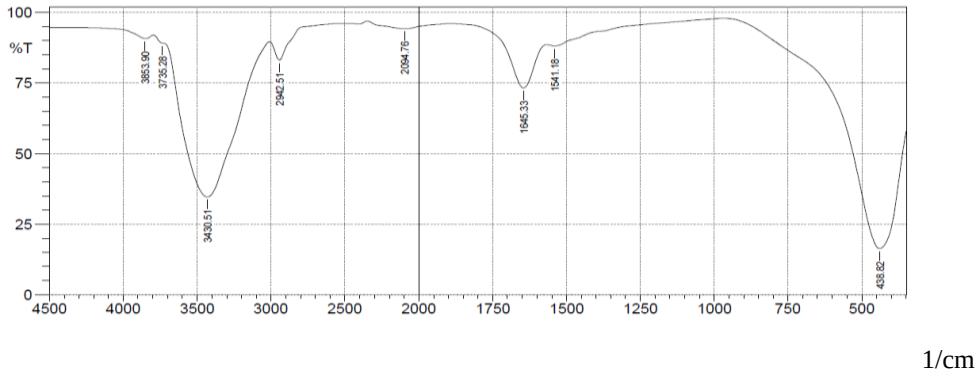

Fig. 2: FT-IR spectra of tropical fire ant pheromone extract

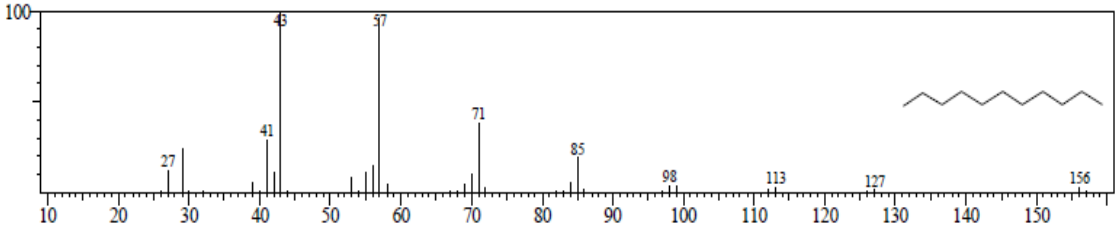

Fig. 3: Mass spectra of undecane

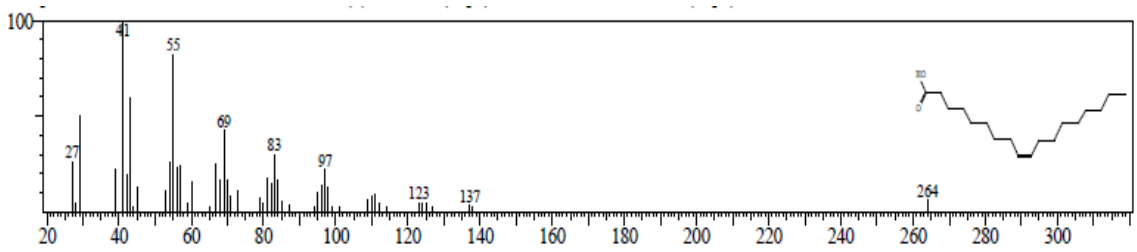

Fig. 4: Mass spectra of (z)-9-octadecenoic acid (Oleic acid)

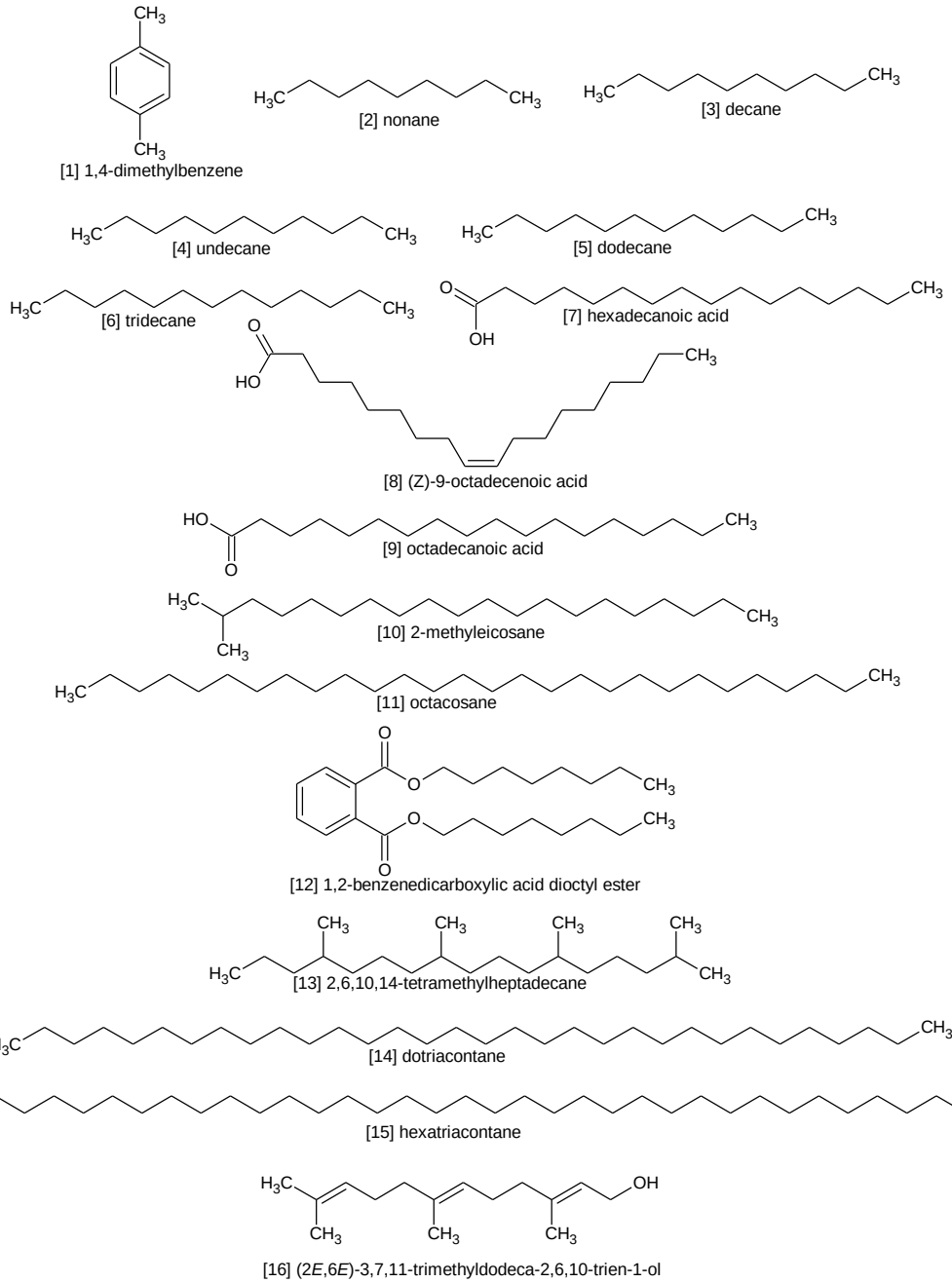

Fig. 5: Structures of pheromonal compounds from Tropical Fire Ant (*Solenopsis geminata*)

Table 1: FT-IR absorption of the extract from tropical fire ant (*Solenopsis geminata*)

S/N	FT-IR absorption (cm^{-1})	Functional group	Nature of compound
1	1541.18	C=C	Aromatic
2	1645.33	C=C	Alkene
3	2942.51	C−H	Alkane
4	3430.51	O−H	Alcohol or carboxylic acid

Table 2: Pheromonal compounds identified from the GC-MS analysis of extract from tropical fire ant (*Solenopsis geminata*)

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak area (%)	Nature of compound
1	1,4-Dimethylbenzene	C_8H_{10}	106	3.245	6.06	Aromatic
2	Nonane	C_9H_{20}	128	3.337	5.66	Hydrocarbon
3	Decane	$C_{10}H_{22}$	142	4.404	3.63	Hydrocarbon
4	Undecane	$C_{11}H_{24}$	156	5.705	31.71	Hydrocarbon
5	Dodecane	$C_{12}H_{26}$	170	8.450	1.09	Hydrocarbon
6	Tridecane	$C_{13}H_{28}$	184	13.433	1.11	Hydrocarbon
7	Hexadecanoic acid (Palmitic acid)	$C_{14}H_{28}$	256	18.573	10.47	Fatty acid
8	(z)-9-Octadecenoic acid (Oleic acid)	$C_{16}H_{32}O_2$	282	21.263	10.51	Fatty acid
9	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284	21.489	3.71	Fatty acid
10	2-Methyleicosane	$C_{21}H_{44}$	296	23.568	2.26	Hydrocarbon
11	Octacosane	$C_{28}H_{58}$	394	24.518	3.79	Hydrocarbon
12	1,2-Benzenedicarboxylic acid dioctyl ester	$C_{24}H_{38}O_4$	390	25.263	3.02	Aromatic ester
13	2,6,10,14-tetramethylheptadecane	$C_{21}H_{44}$	296	25.398	5.37	Hydrocarbon
14	Dotriacontane	$C_{32}H_{66}$	450	26.328	4.78	Hydrocarbon
15	Hexatriacontane	$C_{36}H_{74}$	506	27.383	4.22	Hydrocarbon
16	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol [(E,E)- farnesol]	$C_{15}H_{25}O$	222	27.883	2.61	Alcohol

Also, Z,E-alpha-farnesene, E,E-alpha-farnesene, Z,E-alpha-homofarnesene, Z,Z-alpha-homofarnesene and Z,Z,Z-allofarnesene have been reported as the trail pheromones of *Solenopsis invicta* (also known as *Solenopsis geminata*) [14]. In this article, E,E-alpha-farnesol otherwise known as 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol is reported as trail pheromone component of the tropical fire ants which is also used as a trail pheromone in many other insects. Other compounds detected from the GC/MS analysis of the tropical fire ant extract were fatty acids and aromatic esters. The fatty acids include palmitic, oleic and stearic acids. The hydrocarbon pheromones of many insects are synthesized from fatty acids in a series of steps [15]. These involve chain shortening or elongation, desaturation and also through modification of the functional group by reduction, acetylation or sometimes, oxidation [15]. It is possible that the ten hydrocarbon pheromones observed were synthesized by the fatty acids through a variety of processes some of which are aforementioned. The longer hydrocarbon chains might have been broken down to the shorter ones while oxidation produced other related compounds. Oleic acid is emitted by the decaying corpses of a number of insects, including bees and *Pogonomyrmex* ants, and triggers the instincts of living workers to remove the dead bodies from the hive. If a live bee [16] or ant [17] is daubed with oleic acid, it is dragged off for disposal as if it were dead. The oleic acid smell also may indicate danger to living insects, prompting them to avoid others who have succumbed to disease or places where predators lurk [18]. Besides acting as precursors for pheromone biosynthesis, fatty acids can also be used as pheromones by the insects. It is noteworthy that these compounds that have been isolated from tropical fire ants could not have only been used as trail pheromones but also as sex, aggregating, alarm and defence pheromones by the ants. More promising, but little considered until recently, is the use of pheromones to cause low-density populations of solitary form individuals of the insects followed by their destruction. The pheromones could also be used to cause the insect to undergo phase transformation and migrate prematurely. Pheromones, during unfavourable conditions, could be used for migration. It has been hypothesized that pheromone application on insects at low

population levels would cause inopportune migration, and dispersion of individuals so wide that mate finding would be nearly impossible [1].

4. Conclusion

The tropical fire ant (*S. geminata*) causes discomfort to farmers in Nigeria as they destroy agricultural produce including stored products. Therefore the insect is purely considered a pest in Nigeria and needs to be controlled. The cuticular abdominal extract of the ant was examined with GC/MS and FT-IR spectroscopy and sixteen pheromonal compounds were analysed. These compounds constituted 63.62 % hydrocarbons, 24.69 % fatty acids, 9.08 % aromatic compounds and 2.61 % alcohol. This investigation reveals that the trail pheromone in tropical fire ants is not just a single compound but a mixture of compounds. However, behavioural bio-assays are necessary to authenticate the pheromonal functionality of these compounds and also to know which combinations of compounds would give the best response as well as to verify if the compounds could artificially be used to lure and mass trap the ants in integrated pest management thereby reducing their negative effects on agricultural produce and around residential areas. More research in this area is therefore required.

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