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Identification and Evaluation of Semiochemicals of Tea Stems and Live Wood Termite G*lyptotermes dilatatus* Bugnion & Popoff (Isoptera: Kalotermitidae)

H.M.A Niluka [1], R.D.P.D Senanayake [2], A.D.N.T Kumara [3*] and P.M.G.A Yasara[4]

[1,3,4] *Department of Biosystems Technology, Faculty of Technology, South Eastern University, Sri Lanka*
[2] *Entomology and Nematology Division, Tea Research Institute, Talawakelle,, Sri Lanka*

*Corresponding Author: adntkumara@seu.ac.lk || ORCID: 0000-0002-4670-2919

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Abstract- The *Glyptoterms dilatatus*, commonly referred to as the low country live wood termite, primarily resides in decayed wood caused by fungal infections in the pruned stems of the tea plant, Camellia sinensis. This study explores the response of *G. dilatatus* to both decomposed and healthy tea stems of the TRI 4042 cultivar, along with the initial colonies of termite alates. The total chemical composition of initial colonies of C. sinensis and *Gliricidia sepium* is compared with that of rotted and healthy tea stems (TRI4042). Utilizing a choice chamber bioassay, the impact of different parts of the tea plant on termite behavior is assessed. Results demonstrate that rotted stem pieces of TRI 4042 are more attractive to alates, with a mean percentage response of 10.83±1.32, compared to 4.16±1.70 for healthy stem pieces. The analysis of volatile extracts from rotted stems using GC-MS identifies 20 compounds, with four being common in all replicates. Furthermore, termite initial colonies exhibit 42 compounds, with 20 additional compounds compared to healthy and rotted stem pieces. Specific compounds, such as Phenol, 3,5-bis(1,1-dimethylethyl)- C_{14} H_{22} O, are common in rotted stem pieces and termite initial colonies. In the extraction of body volatiles from termite alates, compounds such as n-Hexane, 1-Hexene, 5-methyl, and Eugenol have been identified. Further studies are necessary to ascertain the compounds that elicit behavioural responses and their use for developing management practices.

Keywords: Choice chamber bioassay, Gliptoterms dilatatus, termite behavior, tea stems volatiles, termite initial colonies, TRI 4042.

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1. Introduction

Tea is a one of the major plantation crop grown in Sri Lanka (Rajakaruna et al., 2016). Ceylon tea, *Camellia sinensis* (L.) O.Kuntze which is widely recognized as the tea from Sri Lanka, has gained recognition on a global scale. Sri Lanka's tea sector, which is the fourth-largest producer of tea globally and the top producer of value-added tea, it is essential to the national economy. This sector is the greatest producer and exporter of orthodox tea in the world, accounting for approximately 90% of its production for the exporter (Gamage & Wickramaratne, 2019). Climate has a major effect on the development and productivity of tea plants (Carr, 1972). Based on elevation, Sri Lanka's tea growing regions (*Camellia sinensis*) are divided into three agro-ecological zones: Low-grown tea is cultivated at altitudes below 610 meters above mean sea level , medium-grown tea is produced at altitudes ranging from 610 to 1220 meters amsl,and high-grown tea is sourced from elevations exceeding 1220 meters amsl. Estimates indicate that 42.2% of the country's total tea-growing area is comprised of land dedicated to low-grown tea. About 60% of the entire amount of tea produced in the country is derived from low-grown tea (Anonymous, 2011).

Tea plants flourish in deep, well-drained acidic soils with a pH range of 4.5 to 5.0. They require an annual rainfall of 1500 to 2000 mm, along with mild temperatures between 18°C and 20°C, and ample sunlight. These optimal conditions are generally found in tropical regions at elevated altitudes, where temperatures are consistently maintained between 13°C and 30°C.The optimal sunlight intensity for tea growth is between 50% to 80% of full radiation (Eden 1976; Purseglove 1968), (Heinrich Lehmann-Danzinger, 2000). The central highlands and low-elevation regions of Sri Lanka are ideal for cultivating fine tea because of their humidity, cool temperatures, and rainfall. However, climate change is impacting tea production by causing soil erosion, increasing pests and diseases, reducing biodiversity, and disrupting ecosystem function. These factors are leading to a decrease in crop yield and affecting the country's extensive tea production (Jayasinghe et al., 2020). Tea plants are susceptible to a variety of pests, including the Up Country and Low Country Live Wood Termites, Nettle Grub, Red Mite, Pink Mite, Spider Mite, Scarlet Mite, Purple Mite, Lobster Caterpillar, Looper Caterpillar, Tea Tortrix, Red Slug, Lygus Bug, and Shot Hole Borer. Furthermore, different parts of the tea bush are affected by specific diseases. The leaves may develop Grey Blight, Brown Blight, Blister Blight, Black Blight, Red Rust, Oil Spot, and Phloem Necrosis Disease. The stems can be impacted by Wood Rot, Collar Branch Canker, Stem and Branch Canker and Hypoxylon. Additionally, the roots are susceptible to Black, Brown, Violet, White, Red and Charcoal Root diseases. (*Sri Lanka Tea under Threat from Pests, Fungal Outbreaks, Weeds due to Agro-Chemical Ban*, 2021).

Low-grown tea plantations in Sri Lanka are heavily affected by the low-country live wood termite (LCLWT), a significant economic pest (Pradeesh & Senaratne, 1977). Infections can cause yield losses of up to 3000 kg/ha in clonal tea, affecting over 50% of the stand in ten years (Sands, 1977). Therefore, a new control method is needed to prevent such losses. Prior studies have examined the behaviour of the low country live wood termite (LCLWT) and the efficacy of semiochemicals in controlling them in tea cultivars. Wood rot fungi, including *F. solani, Gliocladium roseum,* and *Myrothecium roridum*, *Fusarium oxysporum, Lasiodiplodea theobromae* have been found to contribute to die-back and decomposition in pruned stems (Balasooriya A., 1998). Tea cultivars with soft-wooded frames that yield high amounts are particularly vulnerable to termite infestations (Pradeesh Sivapalan et al., 1977). Research has shown that extracts from decayed tea stems can attract LCLWT (Senanayake et al., 2015), highlighting the potential of semiochemicals in managing insect pests.

Semiochemicals are signalling molecules that communicate information between organisms to change their behaviour (Dicke & Sabelis., 1988).Plants release semiochemicals known as Herbivore-Induced Plant Volatiles (HIPVs), which repel pests and attract their natural enemies (Khan etal., 2008) In agricultural settings, semiochemicals like pheromones and allerochemicals can successfully manage insect pests by interfering with their ability to survive and reproduce (Rizvi et al., 2021). The study aimed to evaluate the effects of chemical elements in tea plant stems against *G. dilatatus*, investigate effective compounds of termite initial colony and decayed tea stems, extracts of rotted and healthy wood of termite susceptible cultivars, and extracts of LCLWT initial colony wood of tea and *G. sepium*. The ultimate goal was to build a device for capturing LCLWT alates to implement the current LCLWT Integrated Pest Management program among Sri Lankan tea growers. The main steps included identifying attractive parts of the tea plant, isolating chemical constituents in volatile tea stems, and identifying body extracts released by *G.dilatatus*.

2. Methodology

Colonies of *Glyptotermes dilatatus* were gathered from tea bushes affected by termite infestation at the St. Joachim Estate in Rathnapura, situated at a longitude of 80°23'57" E, latitude of 6°40'58" N, and an elevation of 128 meters. The termite-infested tea bushes were reared in plastic boxes in the entomology laboratory at the Tea Research Institute, Rathnapura, where the temperature (28±2℃) and humidity (80%±2) were maintained until alates emerged from the tea stems. Test insects were obtained from these laboratory-reared colonies, with alates (winged reproductive termites) used for the choice chamber bioassay. Healthy and infected stems of the termite-susceptible tea cultivar TRI 4042 were collected from an experimental site, while termite-infested wood stems from initial colonies were gathered separately from *Gliricidia sepium* and *Camellia sinensis*. The plant materials were air-dried to lower the moisture content to 14-15% and subsequently preserved at -5℃ in the Entomology Research Laboratory at TRI, Rathnapura. To evaluate the behavioral responses of *G. dilatatus* to different plant parts of *C. sinensis*, a choice chamber bioassay was conducted using the termite-susceptible TRI 4042 cultivar. The bioassay employed a four-armed choice chamber consisting of four transparent 250 ml plastic containers connected to a central container by plastic tubing (1.2 cm diameter, 4 cm length). The setup was placed in a plastic box and covered with black paper. In two containers, 30 grams of 1 cm-long pieces of rotted and healthy TRI 4042 stems were introduced, while the remaining two containers served as controls without plant materials. Twenty undifferentiated alates, freshly emerged from the tea stems, were introduced into the central container. After 14 hours, the number of alates that moved into the baited and unbaited containers was recorded. The experiment was replicated six times using six (four-arm) choice chambers (Senanayake et al., 2015).

A. Samples preparation for GC

Randomly selected three healthy tea samples and three rotted tea samples from bioassay were used to identify behavioral response of alates of *G. dilatatus*. That test samples had been used for volatile identification by using GC-MS technology. Dynamic headspace approach was used to extract volatile materials from stem pieces.30 (g) gram of stem pieces had been kept in the sample container and sterilized (80℃ for 2 hours oven dried) porapak tube in line with the purge gas flow. HEPA-CAP Disposable in line filter devise and suction pump connected to sample collector using NS 29/32 connector. Absolutely sealed the exposes using thread seal and tapes. Setup became connected to power. After 24 hours that porapak tube take away and acquire volatile extract from the porapak tube using Dichloromethane (DCM- CH2CI2) setups and pipette.The extracts were amassed in to the GC-MS vial.

B. Samples preparation for MS and chemical profile identification

Volatilized extraction was injected by GC inlet in GC-MS system and the Machine was ran 1 hour for each sample to detect volatile materials. Before introduced the samples in to sample head space system sample collector should be cleaned with De- ionized water and then cleaned with hexane (95%).Two micro liters of each derivatized sample were automatically injected onto the column in split mode (10:1 split ratio). The analysis was carried out with an oven temperature program, using injection in sandwich mode with a fast plunger speed and no viscosity delay or dwell time. The oven ramped from 60 °C (with a 1-minute hold) to 325 °C at a rate of 10 °C per minute, followed by a 10-minute hold before cooling, resulting in a total run time of 15-60 minutes. The injection temperature, MS transfer line temperature, and ion source temperature were set at 250 °C, 250-300 °C, and 230 °C, respectively. Helium was utilized as the carrier gas at a consistent flow rate of 1-2 ml/min. The detector was operated in electron ionization (EI) mode at 70 Ev.

1) Initial colony samples arrange for GC-MS

Stem fragments sourced from termite initial colonies derived from *G.sepium* and *Camellia sinensis* were utilized to identify the volatile compounds responsible for the attraction of *G. dilatatus*. That colony carried stems were grind into small pieces (1cm length) and introduce into sample collector. Vaporized volatile materials were collected using above mentioned steps until inject by GC-MS system.

2) Alates sample prepare for GC-MS

Alates of *G. dilatatus* were prepare for body extract identification using GC-MS 30 matured alates of *G. dilatatus*(male and female) were introduced into sample collector and collect body extracts of alates after 24 hours. Volatile collection was done in the room temperature (25±2 ℃ and 77% RH).

3) Analysis of results

The bioassay data was analyzed using Chi- Squared test (significance level at 0.05). Gathered GC-MS data was analyzed with MetaboAnalyst data analyzing tool.

3. Results and discussion

According to the analysis results in the table 1 there has significant difference between the termites attracted to different kinds of *C. sinensis* parts (x^2 = 83.66,df=3,P= 5.0157) at significance level in 0.05. A mean comparison between rotted stems and healthy stems indicated a significant response of alates to the rotted stems. The mean value of alates attracted to healthy stems is 4.16, and the mean value of alates attracted to rotted stems is 10.83(10.83>4.16).

Table 1

The response of alates of G. dilatatus to different parts of the tea cultivar (TRI4042) of C. sinensis with a choice chamber bioassay

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		Response of Alates (Mean ± SE)		
Tea cultivar	Rotted stem	Healthy stem	Not respond	Untreated
TRI404	12±1.52	4.33±1.05[b]	1.5±0.6	3±O.85[b]

Twenty volatile compounds were identified in the rotted tea stems. Out of these compounds, four were common to all six replicates

Table 2
Chemical constituents of volatiles of rotted stem of TRI4042

Volatile compound	Abbreviation	Retention time	Peak area
cyclobutanol	$C_4 H_8 O$	1.1	191702.4
D-Limonene	$C_{10} H_{16}$	10.433	81379.3
Cyclopentane, 1,1,3-trimethyl	$C_8 H_{16}$	19.658	38050.61
1-Undecene, 9-methy	$C_{12} H_{24}$	23.743	64999.79
Pentane, 2,2,3,4- tetra methyl	$C_9 H_{20}$	9.6	40156.31
1-Hexanol, 2-ethyl-	$C_8 H_{18} O$	10.567	176638.9
Nonanal	$C_9 H_{18} O$	12.704	74806.49
Cyclopropane, pentyl-	$C_8 H_{16}$	14.976	86545.49
Cyclopropane, nonyl-	$C_{12} H_{24}$	19.652	176453.1
3,4-Hexanedione, 2,2,5-trimethyl	$C_9 H_{16} O_2$	19.817	29355.45
Phenol, 3,5-bis (1,1-dimethylethyl)-	$C_{14} H_{22} O$	22.197	164950.2
Phthalic acid, 4- cyanophenyl nonyl ester	$C_{24} H_{27} N O_4$	30.525	287635.9
1,2-Benzenedicarboxylic acid, bis(2- methylpropyl) ester	$C_{16} H_{22} O_4$	32.382	490687.6
Phthalic acid, cyclobutyl isobutyl ester	$C_{16} H_{20} O_4$	34.615	234300.9
1-Hexanol, 2-ethyl-	$C_8 H_{18} O$	10.573	166691.7
1-Hexanol, 4-methyl-	$C_7 H_{16} O$	12.704	75834.56
Cyclopropane, octyl	$C_{11} H_{22}$	19.652	158848.2
1,3,6,10- Dodecatetraene, 3,7,11- trimethyl-, (Z,E)-	$C_{15} H_{24}$	21.166	92296.79
Butane, 2,2-dimethyl-	$C_6 H_{14}$	23.87	77453.56
Ethylene glycol monoisobutyl ether	$C_6 H_{14} O_{20}$	9.593	44259.94

Table 3
Chemical constituents of volatile of healthy stem of TRI 4042

Volatile compound	Abbreviation	Retention time	Peak area
3,3-Dimethylbutane-2-ol	$C_6H_{14}O$	6.699	248700
Pentane, 2,2,3,4- tetramethyl	C_9H_{20}	9.606	37801.56
2-Oxo-4-phenyl-6-(4- chlorophenyl)-1,2-dihydropyrimidine	$C_{16}H_{11}C_lN_2$	9.746	57092.86
Limonene	$C_{10}H_{16}$	10.439	380550.2
1-Hexanol, 2-ethyl-	$C_8H_{18}O$	10.573	297558.1
1-Hexanol, 4-methyl-	$C_7H_{16}O$	12.711	108286.2
Benzene, 1,2- dimethoxy	$C_8H_{10}O_2$	13.881	96873.47
Citronellyl butyrate	$C_{14}H_{26}O_2$	14.524	159968.8
(S)-3-Ethyl-4- methylpentano	$C_8H_{18}O$	14.988	91899.63
Oxalic acid, butyl propyl ester	$C_{10}H_{16}O_4$	15.37	23473.63
Cyclopropane, octyl-	$C_{11}H_{22}$	19.658	159040.9
3,4-Hexanedione, 2,2,5- trimethyl	$C_9H_{16}O_2$	19.823	30812.44
Cyclopropane, nonyl-	$C_{12}H_{24}$	23.736	245758.9
Butane, 2,2-dimethyl-	C_6H_{14}	23.876	106078
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	30.531	299847
Dibutyl phthalate	$C_{16}H_{22}O_4$	32.389	494912.9
Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	34.622	343816.9
D-Limonene	$C_{10}H_{16}$	10.439	181474
1-Hexene, 5-methyl-	C_7H_{14}	10.579	76521.65
Cyclopropane, pentyl-	C_8H_{16}	19.652	84546.37
Pentanoic acid, 5- hydroxy-, 2,4-di-tbutylphenyl esters	$C_{19}H_{30}O_3$	22.197	69908.85
1-Decene, 8-methyl-	$C_{11}H_{22}$	28.043	105188.2
Phthalic acid, 4- cyanophenyl nonyl ester	$C_{24}H_{27}NO_4$	32.376	146964.5
1-Butanol, 2-methyl-	$C_5H_{12}O$	10.541	52471.42
1-Butanol, 3-methyl-, propanoate	$C_8H_{16}O_2$	8.747	148006.7
1-Undecanol	$C_{11}H_{24}O$	28.037	333820.5
Ethylene glycol monoisobutyl ether	$C_6H_{14}O_2$	9.593	44259.94

***Note**: The table shows the chemical compounds detected in healthy stem pieces. A total of 27 volatiles were detected, with each compound presenting with a different peak area and* retention time. One retention time and one peak area were selected to be included in the table.

Table 4

Chemical constituents of volatile of stem pieces of termite initial colonies

Ethylene glycol monoisobutyl ether	$C_6H_{14}O_2$	6.686	113623.2
Ether, hexyl pentyl	$C_{11}H_{24}O$	17.724	93831.2
n-Butyl ether	$C_8H_{18}O$	6.673	245229.4
2-Propenal	C_3H_4O	9.746	23405.47
1-Hexanol, 2-ethyl-	$C_8H_{18}O$	10.567	260113.9
dl-Menthol	$C_{10}H_{20}O$	14.517	107483.4
1,3,6,10- Dodecatetraene, 3,7,11- trimethyl-, (Z, E)-	$C_{15}H_{24}$	21.166	77356.04

Table 5

Chemical compounds present in the extractions from the body of G. dilatatus

Chemical compound	Abbreviation	Retention time	Peak area
n-Hexane	C_6H_{14}	1.692	878354.8
1-Hexene, 5-methyl	C_7H_{14}	10.586	61911.98
Decane, 2,4-dimethyl	$C_{12}H_{26}$	17.158	35691.93
Pentane, 2,3,3- trimethyl	C_8H_{18}	18.214	67186.32
Eugenol	$C_{10}H_{12}O_2$	18.729	526663.6
Cyclopentane, 1,1,3- trimethyl	C_8H_{16}	19.671	37699.69
Cyclohexene, 3-methyl6-(1-methylethylidene)	$C_{10}H_{16}$	20.67	64131.99
1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9aoctahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)	$C_{15}H_{24}$	20.892	219620.2
1-Undecene, 9-methyl	$C_{12}H_{24}$	23.743	65768.97

***Note**: Table was displayed the volatiles from termite body extractions. The extractions were obtained from the matured alates of G. dilatatus (winged reproductive). A total of 28 alates*

were used to collect the extractions using GC-MS technology. All of the compounds were collected using the head space method.

The two axes, PC1 and PC2, represent the two most important principal components, which account for the most variance in the data. The text labels, "HW", "IC", and "RW", likely represent different kind of samples (HW=Healthy wood, RW=Rotted wood, IC=Initial colony). Each data point represent the sample in the clusters. Data points that are closer together on the plot have more similar chemical compositions. Conversely, data points that are further apart have more dissimilar chemical compositions. The text at the bottom of the plot, "PC 1 (47.5%)" and "PC 2 (26.8%)", indicates the percentage of variance explained by each principal component. In this case, PC1 explains 47.5% of the variance in the data, and PC2 explains 26.8%.

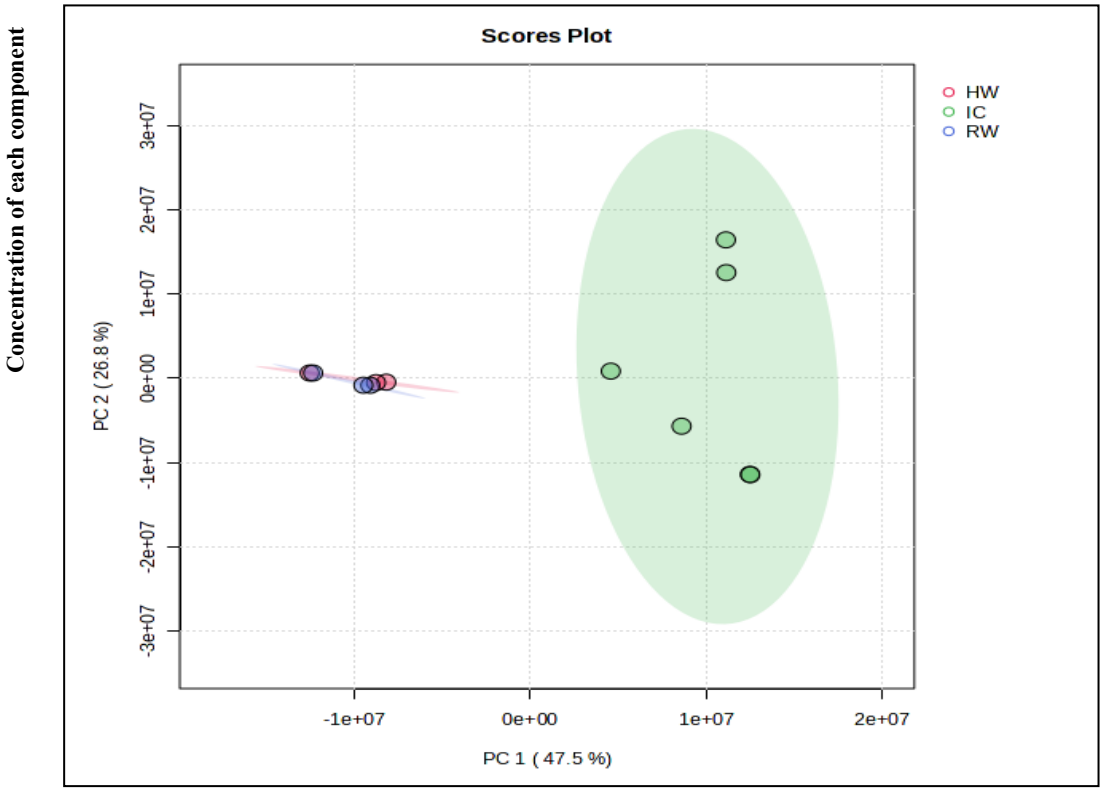

Figure 1. *PCA Scores plots of headspace volatiles of different plant stem materials*

Together, these two components explain 74.3% of the variance in the data. The data points are spread out more along PC1 than PC2. "HW" data points appear to be clustered together in the upper right quadrant of the plot. This suggests that the chemical composition of Healthy wood is relatively similar across the samples. The "IC" data points are more spread out than the c data points. This suggests that there is more variation in the chemical composition of initial colonies across the samples. There is some overlap between the "IC" and "RW" data points. This suggests that the chemical composition of Initial colonies samples is similar to the chemical composition of some Rotted wood samples.

According to the chemical composition of the sample replicates, there are two main separate clusters. The first cluster contains termite initial colonies, and the second cluster is further divided into two subclusters. These subclusters consist of healthy and rotted tea stem samples, indicating that some volatile compounds are present in both rotted and healthy stems.

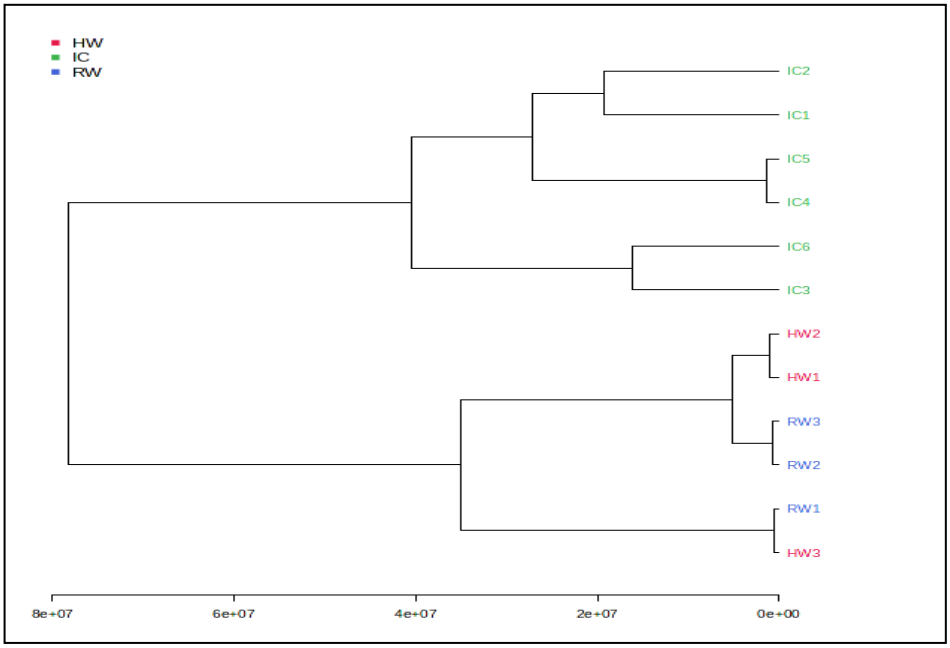

Figure 2. *Hierarchical Clustering Dendrogram different tea stem volatile*

4. Conclusion

Integrated pest management (IPM) is an effective method for controlling the pest *G. dilatattus*, which is an economically important pest of low-grown tea plantations in Sri Lanka. Controlling this pest is extremely challenging due to its concealed habit within the living plant. In this study, semiochemicals found in decaying stems of TRI4042 and rotted stems that inhabit the initial colonies were assessed for the development of an environmentally friendly pest control agent. The average response of alates to the decaying stems was higher than that of healthy stems. When the chemical characteristics of the rotten and healthy stems were assessed in comparison to rotten stems inhabited with initial termite colonies, it was established that the rotten stems with the termite colonies contained more volatile compounds in greater proportions. The reason for this is that rotted pruning cuts have a specific stage of rotting that allows them to produce semiochemicals in the right ratio and concentration to attract LCLWT and termites are dependent on a significant quantity of water for their survival. Consequently, wood that is in a state of decay due to prolonged exposure to moisture is especially appealing to these insects. The reason for this is that the cellulose content in wood, along with water consumption, are the only essential requirements for the sustenance of termites. Therefore, wood that has been saturated with water provides a convenient source of nutrition for termites, making wood rot an enticing option for their habitation. This study also provided guidance for advancing two key areas: a better version of the Integrated Pest Management (IPM) package for the management of *G. dilatatus* and enhancement of the pheromone trap.

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