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Leaves and bark of *Albizia* shade trees in tea plantation shows both insect attractant and pesticidal properties: a GC-MS based investigation

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Abstract. *Ghosh A, Majumder S, Saha S, Chakraborty S, Bhattacharya M. 2021. Leaves and bark of* Albizia *shade trees in tea plantation show both insect attractant and pesticidal properties: a GC-MS based investigation. Asian J Agric 5: 84-89.* Tea is the major plantation crop of sub-Himalayan region. The bushes are grown under a partial canopy cover of leguminous plants to protect them from scorching sun rays. The shade plants are primarily beneficial but attract several pests. Metabolites present in four *Albizia* trees were detected by GC-MS, and insect attracting and/or repelling phytochemicals were pointed out. A total of thirty-two compounds exhibiting semiochemical properties were detected. 15.84%, 2.52%, 2.61% of semiochemicals are exclusively present in leaves of *Albizia odoratissima*, *Albizia chinensis,* and *Albizia procera.* While in bark 10.73% and 13.35% semiochemicals were exclusively present in *Albizia odoratissima* and *Albizia lebbeck*. A total of nine semiochemicals are exclusively present in AO, out of which seven semiochemicals viz., caryophyllene, epoxide; longiborneol; longifolene; methyl linolenate; methyl linoleate; methyl cis-jasmonate; tonalid are present in leaf and five semiochemicals viz. longiborneol; methyl cis-jasmonate; camphor; isopropyl myristate; tonalid are present in bark. A total of five semiochemicals viz. alpha-santalol; bisabolol oxide A; gamma-Sitosterol; glycidyl oleate (as oleic acid); oleoyl chloride (as oleic acid) are found exclusively in the bark of *Albizia lebbeck.* There were only three semiochemicals that were exclusively found in the leaf of AC, these were 2-decen-1-ol; cyclohexanol, 5-methyl-2-(1-methyl ethyl); acetophenone. In the leaf of AP, a total of five semiochemicals were found to be exclusive, these being dehydro-beta-ionone; oleamide; beta-amyrin; isopropyl linoleate; stigmasterol. GC-MS analysis explored metabolites from shade trees like caryophyllene, epoxide; beta-amyrin; 1, 8-cineol etc. which serve as both attractant and pesticidal components while compounds like longiborneol; longifolene; linalyl acetate, etc. are exclusively pest attractants and compounds like isopropyl myristate are exclusive pest repellants. This cumulative property of shade trees can be utilized to trap insect pests and destroy them with pesticidal activity. Isolation of these metabolites from shade trees, and their utilization as semiochemical/pheromone trap and green pesticides, will control pests by eco-friendly measures along with reducing the production cost.

Keywords: *Albizia*, attractant, GC-MS, insect, pesticide

Abbreviations: AO: *Albizia odoratissima;* AC: *Albizia chinensis;* AP: *Albizia procera*; AL: *Albizia lebbeck*; GC-MS: Gas chromatography-mass spectrometry

INTRODUCTION

Tea, in the sub-Himalayan region, is grown under a canopy of trees which provides partial shade that is quite essential for good tea leaf production by minimalizing burns or damage to the young tea leaves from sun-scorch. A good number of trees are planted mainly to provide shade but, at the same time, can also be beneficial for reducing soil erosion, enriching soil fertility and organic matter content through leaf litter and nitrogen fixation (leguminous plants only) (Kalita et al. 2014; Ghosh et al. 2020). Among the shade trees *Albizia* spp. trees are considered as the best by planters for besides providing shade, as being leguminous they can fix nitrogen and can add huge amount of organic matter by shedding their leaves once a year (Anim-Kwapong 2003). Moreover, because of pinnation in leaves, the amount of sunlight under their canopy gets reduced but not blocked completely which is essential for tea bushes. Besides these benefits, tea plantations suffer some disadvantages from these big nutrition suckers. One of the deadliest effects is occurrence of pest and disease infestations (Beer 1987). Several factors are behind these infestations; like reduced air movement and increased humidity to favor fungal diseases and provide good breeding grounds for different insects, etc.

Positive and negative attributes of shade trees on tea bushes and quality of manufactured tea have been the center of attraction by researchers. But one of the ignored areas is studying insect pests attracting properties by shade trees. A semiochemical is a chemical substance or mixture released by plants and other organisms that affect the behaviors of other individuals. Plants contain such chemicals in the form of pheromones, attractants, allomones, kairomones, and synomones. They occur mainly in the outer part or cuticle in plants and thus are easily released into the air to attract other organisms to facilitate pollination (Majumder et al. 2020). In this research, we intended to study the occurrence of semiochemicals in shade tree leaves and bark to find whether they can play a role in attracting tea pests and pollinators. Considering this objective, a GC-MS based study was conducted to detect semiochemicals of four tea plantation-shade trees belonging to *Albizia* spp and make a comprehensive study on probable semiochemical based interaction between them and pests.

MATERIALS AND METHODS

In this study, leaves and bark of four tea plantation shade trees viz., *Albizia odoratissima* (L.f.) Benth, (AO), *Albizia lebbeck* (L.) Benth (AL), *Albizia chinensis* (Osbeck)Merr. (AC) and *Albizia procera* (Roxb.) Benth (AP) was considered.

Crushed fresh sample (1g) of leaf and bark from each plant was dissolved in acetone to extract components prior to GC-MS analysis. Acetone, being polar and aprotic solvent, was used.

The GC-MS analysis was done following the methodology of Majumder et al. (2020) and Chakraborty et al. (2021). Acetone extract of bark and leaves of four shade trees was used for GC-MS. One microliter of extracts was injected in split mode in the instrument (GCMS-QP2010 Plus). Injection temperature was 260 °C and the interface temperature was set to 270°C. Ion source temperature was adjusted to 230 °C. Helium was used as carrier gas. Total flow rate was 16.3 ml min^{-1} and the column flow rate was 1.21 ml min^{-1}. Mass spectra were recorded at 5 scan s^{-1} with a scanning range of 40-650 m/z. Quantification of compounds was done based on their peak areas. The data obtained from GCMS analysis were further analyzed from available literature. The detected metabolites were screened from available database (El-Sayed 2019) to analyze semiochemical properties.

RESULTS AND DISCUSSION

A total of thirty-two compounds exhibiting semiochemical property viz., longifolene, dehydro-beta-ionone, methyl linolenate, methyl linoleate; cumene; acetophenone; methyl palmitate; phenol; squalene etc. were detected by GC-MS analysis. A greater number of semiochemicals were detected in leaves of shade trees compared to bark. Only thirteen semiochemicals (camphor, alpha-santalol, phenol, gamma-sitosterol, etc.) were detected in the bark samples of which five were identical with compounds of leaf extracts (longiborneol; methyl palmitate; methyl cis-jasmonate; phenol and tonalid). Names of the semiochemicals previously reported as pheromones, kairomones, allomones, attractant for pests like ants, mites, moths, flies, etc. are provided in Table 1.

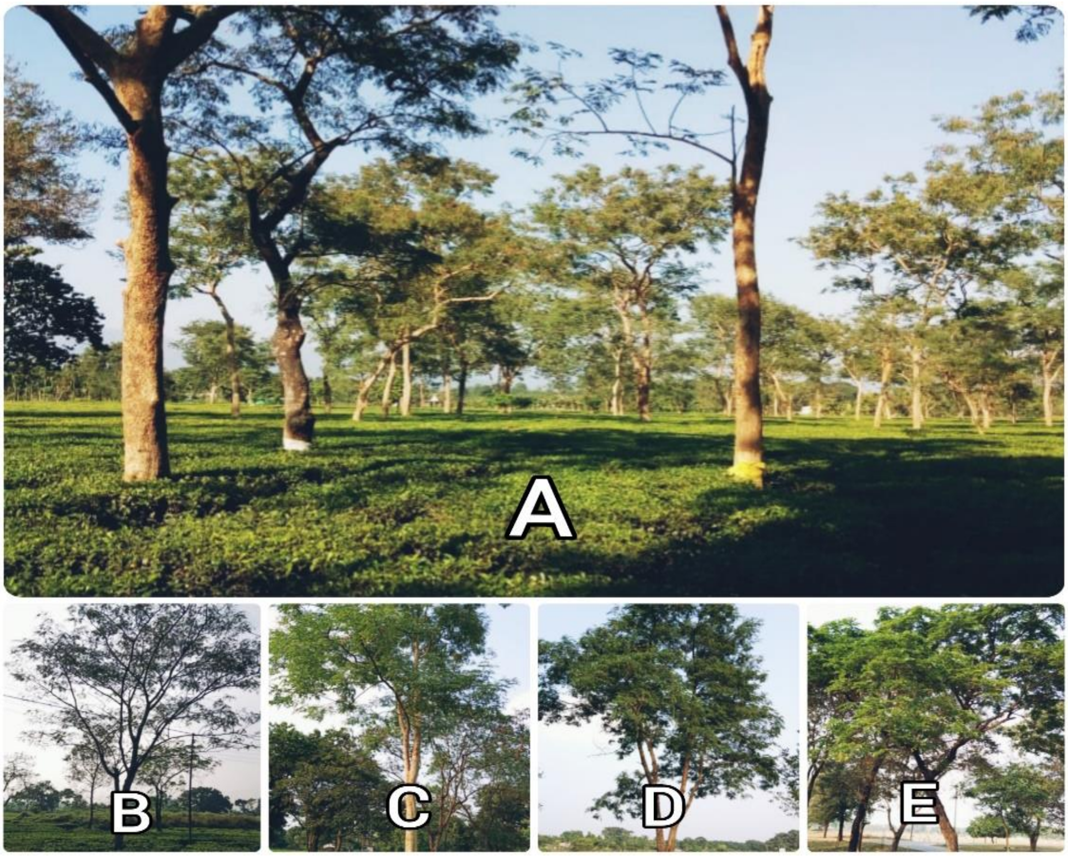

Figure 1. A- Tea Garden of North Bengal University, B- *Albizia chinensis* (AC), C-*Albizia procera* (AP), D-*Albizia odoratissima* (AO), E-*Albizia lebbeck* (AL).

Table 1. Semiochemicals present in four *Albizia* spp. to target wide range of pests.

Metabolites	Type of pests	Attractant and/or insecticidal
Caryophyllene, epoxide	Honeybee, moth, and butterfly (*Papilio* sp.)	Attractant, pheromone, antifeedant, fungi stat, larvicidal, insectifuge pesticidal activity
Longiborneol	*Monochamus alternatus* (Japanese pine sawyer)	Kairomone
Longifolene	Beetle, and pine sawyer	Attractant and pheromone
Linalyl acetate	Butterfly and cockroach	Attractant and pheromone
2-decen-1-ol	Bugs	Nematocidal activity
4,8,12,16-tetramethylheptadecan-4-olide	Crimson-patched longwing	Pheromone
Dehydro-beta-ionone	Beetle, whitefly, and spider mite	Attractant
Oleamide	*Lysmata boggessi* or Peppermint shrimp	Pheromone
Methyl linolenate	Mite, beetle, fly, honeybee, and butterfly	Pheromone, kairomone and allomone
Methyl linoleate	Mite (*Dermatophagoides* sp.), Queen honeybee, bumblebee, leaf-cutting ant, butterfly and leafroller	Attractant and pheromone
Benzene, (1-methylethenyl)	Floral compound or semiochemical	No report
Cumene	Floral compound or semiochemical	Pheromone, kairomone and allomone
Beta-amyrin	*Melanoplus sanguinipes* or Lesser migratory grasshopper	Pheromone and mosquitocidal, larvicidal activity
Cyclohexanol, 5-methyl-2-(1-methylethyl)	*Pogonomyrmex rugosus*-or Rough harvester ant	Pheromone
Acetophenone	Wasp, tick, Dendroctonus beetles and locust	Pheromone, kairomone, attractant and allomone
Methyl palmitate	Spider, mite, thrip, beetle, fruit fly, bumblebee, ant, wasp, butterfly, leafroller, spider, Housefly, Olive fruit fly and leafroller	Pheromone, kairomone, attractant and allomone
Isopropyl linoleate	*Pseudomyrmex ferruginea* or Acacia ant	Pheromone
Methyl cis-jasmonate (Methyl dihydrojasmonate)	Fruit moth	Pheromone and attractant
Neophytadiene	Heliconius sara or Sara longwing	Pheromone
Phenol	Beetle, cockroach, Screwworm fly, tick (*Amblyomma americanum*), moth, beetle, weevil, cockchafer (*Melolontha melolontha*), Blowfly, midge, house mosquito, honeybee Myrmecocystus ants, Locust, and grasshopper	Pheromone, kairomone, attractant and allomone
Phytol	Moth, beetle, and butterfly	Pheromone, attractant and allomone
Squalene	Mites (*Tyrophagus* sp.), seed borer, Tick (*Amblyomma* sp.) weevil, fruit fly, bumblebee, ant, and butterfly	Pheromone, attractant, allomone and pesticidal activity
Stigmasterol 1, 8-cineol	Bee and wood wasp Moth, ant, thrips, weevil, beetle, bug, looper and spider	Pheromone, attractant, allomone, kairomone and acaricidal, nematocidal, insectifuge, pesticidal activity
Camphor	Chafer, weevil, and moth	Attractant and allomone
alpha-Santalol	Fragrance	Mite repellent and acaricidal activity
Bisarbolol oxide A	Floral compound (semiochemical)	Insecticidal activity
gamma-Sitosterol	*Lacerta* sp., *Psammodromus algirus* and *Blanus cinereus*	Pheromone
Glycidyl oleate (as Oleic acid)	Mite, ant, tick (*Amblyomma americanum*), moth, booklouse, honeybee, bumblebee, bee, stem borer and grasshopper	Insectifuge
Oleoyl chloride (as Oleic acid)	Mite, ant, tick (*Amblyomma americanum*), moth, booklouse, honeybee, bumblebee, bee, stemborer and grasshopper	Insectifuge
Isopropyl myristate	Psyllids, beetles, bee, and moths.	Pesticidal activity
Tonalid	Fragrance	No report
Vitamin E	*Blanus cinereus*	Pheromone

Some secondary metabolites exhibiting semiochemical properties are exclusively present in the four *Albizia* spp. (Table 2). Leaf extracts of AO, AC and AP contained 15.84%, 2.52% and 2.61% (nil in AL) area of exclusive semiochemicals while AO and AL contained 10.73% and 13.35% (nil in AC and AP) area of semiochemicals exclusively in bark. A total of nine semiochemicals were exclusively present in AO, out of which seven semiochemicals viz., caryophyllene, epoxide; longiborneol; longifolene; methyl linolenate; methyl linoleate; methyl cis-jasmonate; tonalid were present in leaves and five semiochemicals viz., longiborneol; methyl cis-jasmonate; camphor; isopropyl myristate; tonalid were present in bark.

Table 2. Percentage of exclusive semiochemicals present in *Albizia* shade trees.

Semiochemicals	AO		AL		AC		AP	
	Leaf	**Bark**	**Leaf**	**Bark**	**Leaf**	**Bark**	**Leaf**	**Bark**
Caryophyllene, epoxide	1	0	0	0	0	0	0	0
Longiborneol	3.14	3.45	0	0	0	0	0	0
Longifolene	1.04	0	0	0	0	0	0	0
2-decen-1-ol	0	0	0	0	0.46	0	0	0
Dehydro-beta-ionone	0	0	0	0	0	0	0.1	0
Oleamide	0	0	0	0	0	0	0.25	0
Methyl linolenate	3.96	0	0	0	0	0	0	0
Methyl linoleate	2.13	0	0	0	0	0	0	0
beta-amyrin	0	0	0	0	0	0	0.45	0
Cyclohexanol, 5-methyl-2-(1-methylethyl)	0	0	0	0	0.79	0	0	0
Acetophenone	0	0	0	0	1.27	0	0	0
Isopropyl linoleate	0	0	0	0	0	0	0.75	0
Methyl cis-jasmonate	2.31	3.06	0	0	0	0	0	0
Stigmasterol	0	0	0	0	0	0	1.06	0
Camphor	0	0.53	0	0	0	0	0	0
alpha-Santalol	0	0	0	0.32	0	0	0	0
Bisabolol oxide A	0	0	0	0.88	0	0	0	0
gamma-Sitosterol	0	0	0	9.98	0	0	0	0
Glycidyl oleate	0	0	0	1.37	0	0	0	0
Oleoyl chloride	0	0	0	0.8	0	0	0	0
Isopropyl myristate	0	2.41	0	0	0	0	0	0
Tonalid	2.26	1.28	0	0	0	0	0	0

A total of five semiochemicals viz., alpha-santalol; bisabolol oxide A; gamma-Sitosterol; glycidyl oleate (as oleic acid); oleoyl chloride (as oleic acid) are found exclusively in the bark of AL. Only three semiochemicals have exclusively been found in the leaf of AC, these are 2-decen-1-ol; cyclohexanol, 5-methyl-2-(1-methylethyl); acetophenone. In the leaves of AP, a total of five semiochemicals were exclusive; these were: dehydro-beta-ionone; oleamide; beta-amyrin; isopropyl linoleate; stigmasterol.

Discussion

Semiochemicals like pheromones, allomones, kairomones, attractants, etc. are signaling compounds that can create responses even in minute amounts. But, in our samples, some of the semiochemicals were found to be present in high amounts which made our study more interesting to establish the shade trees as relevant pest attractants. As major components, benzene, (1-methylethenyl)-; cumene; neophytadiene; phytol; squalene; and vitamin E are semiochemicals present in leaf extracts. While phenol and gamma-sitosterol, are two of the prime bark components that are also potent contenders as semiochemicals (Table 1). According to results of this research, leaves of *Albizia* are found to contain more quantity of semiochemicals compared to bark (Figure 2). Summative area percentage of semiochemicals from all the four shade tree leaves amount to 159.59 while that of bark is 34.35.

Comparative analysis among leaf extracts of four shade trees, AO, AC, AL, and AP are depicted in Figure 2. It is observed that the leaves of AO (47.66%), AL (42.29%) and AC (49.34%) contain more amounts of semiochemicals based on area percentage than AP (20.3%). Surprisingly, in bark, the result is quite different. In AC, semiochemical content of leaf shows highest quantity but in bark sample, AL showed highest percentage of semiochemicals (18.27%). In AO, AL, and AC the quantity of semiochemicals in bark is 15.36%, 18.36% and 0.72% respectively.

According to “The Pherobase” (El-Sayed 2019) some of our compounds that have these properties are the following; caryophyllene, epoxide works as pheromone for honeybee and as attractant for moth; longifolene is pheromone for beetle; methyl linolenate is kairomone for mite and pheromone for honeybee; beta-amyrin is a pheromone for grasshopper; methyl palmitate is allomone for flies, thrips, ants, wasp and also attractant as well as kairomone for mites; isopropyl linoleate is a pheromone for ants; methyl cis-jasmonate is pheromone of moth; phytol works as pheromone for moth, and works allomone as well as attractant for beetles; squalene works as attractant and allomone of ticks, etc. Being leguminous, shade trees of *Albizia* sp. generally shed their leaves and other arial parts once in a year (spring/winter) so there is a chance to attract soil pests by their exudates even after shedding of leaves.

In our study, a few compounds were reported to have pest repellent and/or pesticidal properties besides their pest attracting properties (Figure 3). Isopropyl myristate has pesticidal activity and is found in AO (2.41% in bark) (Sharmila et al. 2019); Squalene is found in AO (5.59% in leaf), AL (11.39% in leaf), AC (8.7% in leaf) and AP(1.65% in leaf) having pesticidal activity (Arora and Kumar 2018), (Elakkiya and Murugaiah 2015); Caryophyllene, epoxide has antifeedant, fungistat, pesticidal, larvicidal, insectifugal activity (Duke 1992) and is found 1% in leaf extract of AO . 2-decen-1-ol found in AC (0.46% in leaf), has nematicidal activity. Beta-amyrin has mosquitocidal and larvicidal activity which is found in AP (0.45% in leaf) (Duke 1992); Methyl palmitate, found in AO (2.1% in leaf), has Acaricide and insect (ant) repellant activity (Wang et al. 2009), AL (0.45% in bark); Aphicide compound phytol (Benelli et al. 2020) is found in AO (5.64% in leaf), AL (2.83% in leaf), AC (1.77% in leaf) and AP (3.94% in leaf). Stigmasterol has insecticidal effect and is found in AP (1.06% in leaf) (Nong et al. 2017); 1,8 cineol has acaricidal, nematocidal, insectifugal and pesticidal activities (Duke 1992) that is found in both AL (0.49% in bark) and AC (0.72% in bark); alpha-santalol has acaricidal activity (Misra and Dey 2013) and mite repellant activity against *Tetranychus urticae* and is found in AL (0.32% in bark) (Roh et al. 2012); Bisabolol oxide A has insecticidal activity (de Andrade et al. 2004) and is found in AL (0.88% in bark); glycidyl oleate (1.37%) and oleoyl chloride (0.80%) which are both derivatives of oleic acid is found in the bark extracts of AL, works as insectifuge (Duke 1992).

Besides having both insect attracting and pesticidal properties for pests, some of the metabolites are specifically important for tea plantations as they specifically attract pests of tea bushes. Methyl palmitate and methyl linolenate function as pheromone, kairomone, attractant and allomone for spider, mite, thrip, beetle, ant, wasp, and leaf roller; longifolene functions as pheromone to attract beetles; dehydro-beta-ionone attract beetle, whitefly, spider mite. Spider mites, especially red spider mites cause much damage in tea plantations of sub–Himalayan West Bengal (Roy 2019). Acetophenone act as pheromone, kairomone, attractant and allomone for wasp, tick, beetles, and locust. Phenol is a pheromone, kairomone, attractant and allomone for beetle, cockchafer (*Melolontha melolontha*), locust and grasshopper, while phytol is a pheromone, attractant and allomone for moth and beetle. Camphor is attractant and allomone for moth.

The metabolites of leaf and bark also reduce insect pests. Caryophyllene epoxide is insectifuge with larvicidal and pesticidal activity; 2-decen-1-ol has nematocidal activity specifically on bugs; squalene has pesticidal property on mites, seed borers, ants; beta-amyrin acts on lesser migratory grasshopper. Also, it is mosquitocidal and larvicidal for *Melanoplus sanguinipes* or lesser migratory grasshopper (Duke 1992; El-Sayed 2019). Alpha -santalol has mite repellent and acaricidal activity. Bisabolol oxide A is an insecticide. Glycidyl oleate and oleoyl chloride are insectifuge while isopropyl myristate has pesticidal activity.

Some metabolites have interesting properties; they act as both, attractant and has pesticidal effect on pests. 1,8-cineol is a pheromone, attractant, allomone, kairomone and has acaricidal, nematocidal, insectifugal, pesticidal activity against moth, ant, thrips, weevil, beetle, bug, looper, spider while stigmasterol acts as pheromone and have insecticidal activity. So, considering the beneficial properties of the metabolites, this study suggests that these shade tree organs can be utilized profitably to trap pests in tea plantations and as natural biopesticides.

Shade trees are inseparable partners of tea bushes in tea plantations of sub-Himalayan region. Leguminous trees are the first and foremost choice for providing shade but, non-leguminous trees like neem are planted too for their pesticidal secondary metabolites. Shade tree metabolites serve as insect or pest attractant as revealed by GC-MS analysis, but several metabolites have been reported to exhibit pesticidal activity. So, this dual property established shade trees as insect-attracting plants with insecticidal properties. Moreover, isolation of metabolites from shade trees and their utilization as semi chemical/pheromone trap and green pesticides will not only help in controlling pest in an eco-friendly and profitable aspect, but also will reduce the use of chemical pesticides in tea plantations.

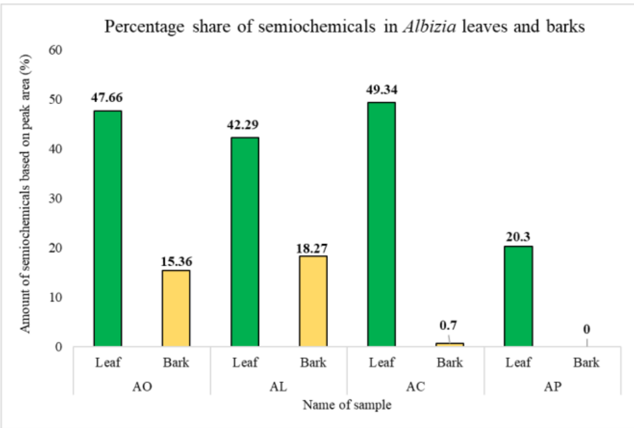

Figure 2. Semiochemicals contents of insect related properties in *Albizia* leaf and bark extracts.

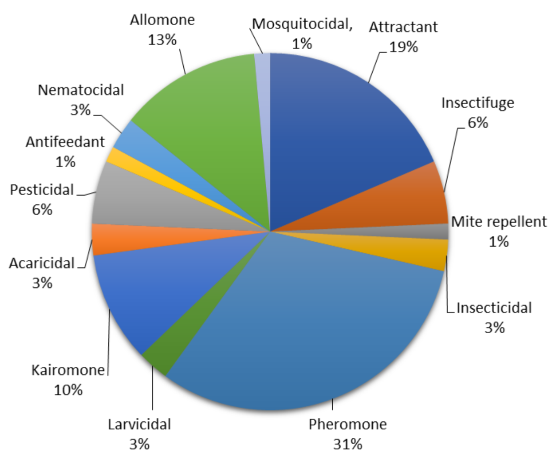

Figure 3. Proportion of insect-related semiochemical activities in *Albizia* extracts revealed by GC-MS.

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