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Comparative analysis of volatile compounds in normal leaves and mite induced leaf galls of *Litchi chinensis* Sonn

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Abstract
Litchi chinensis Sonn. is an important component of the Indian fruit basket. Its production is severely affected by the *Aceria litchii* mite, which induces formation of blister like galls on the leaves. GC-MS analysis of the methanolic extracts of the normal and galled leaves was carried out to study the alterations in the volatile chemical compounds in the leaf galls as a result of *Aceria* mite infestation. It was found that the leaves were rich in sugars, sesquiterpenes, terpenes, linoleic acid esters, vitamins, antioxidants, glycerol and steroids. Amount of Alpha methyl mannofuranoside reduced in the galled leaves to 60.82% from 72.49% in the normal leaves. Amount of Vitamin E reduced from 1.80% in normal leaves to 0.40% in galled leaves. Caryophellene increased in galled leaves (2.04%) when compared to normal leaves (0.38%). Betulinaldehyde and.gamma.-Sitosterol were absent in galled leaves but present in normal leaves (4.95% and 2.12% respectively). Lupeol and beta-Longipinene were present only in galled leaf (1.98% and 4.54% respectively) and absent in the normal leaf. The results obtained from this study show that the metabolic processes of the leaf cells have been altered by the *Aceria* mite, leading to changes in the chemical composition of the leaves. This study will help to elucidate the mechanism of gall formation and plant defence in *Litchi*, further.

Keywords: *Aceria litchii*, gall, GC-MS, phytochemicals

Introduction

Litchi chinensis Sonn, a member of family Sapindaceae, is native to Southeast Asia. India ranks second in Litchi production, China being the first. The Asian countries leading in Litchi production are China, India, Thailand and Vietnam [1]. In US maximum production comes from Florida followed by Hawaii and California [2]. In India it is mainly cultivated in Bihar, West Bengal, Uttar Pradesh, Uttarakhand, Jharkhand, Assam, Tripura & Punjab. The litchi tree is dense, round topped, evergreen tree having elliptical oblong, pointed leaves [3]. Litchi plant is cultivated for its juicy and fleshy fruits. These fruits are eaten directly or used in various industries for preparation of juices, jellies and squashes [4]. Among the pests affecting litchi production across the globe, the mite *Aceria litchii* (Keifer) (Acari: Eriophyidae) has been reported to be a serious one. The nymphs as well as adults of *Aceria* damage the leaves, inflorescence and developing fruits while puncturing them with their rostrum to suck the cell sap. The mite infestation leads to formation of blister like galls on the upper surface of leaves. The undersurface of the leaves shows growth of hair like structures which are initially white in colour but gradually turn brown, forming a dark brown, velvety 'erineum'. The leaves attacked by the mite become thick and curled, shrivel and eventually fall off. When infected at young stage, the flower and fruit set too gets impeded [5]. Though present throughout the year, Litchi mite is most active from March to September and hibernates during harsh winters [6, 7]. This mite severely affects the growth and production of Litchi [8]. The current experiment was conducted to find out the alterations in volatile compounds upon infection by *Aceria litchii* in the leaves of *Litchi chinensis*.

Material and Method

Plant Extract Preparation

The mite infected leaves and healthy leaves of Litchi were collected from litchi garden in Dehradun. The leaves were shade dried separately at room temperature and powdered using a grinder. 10gms powder, each of normal and galled leaves was weighed and extracted in 80% methanol using Soxhlet apparatus for almost 24 hours. Among the several chemicals used for extraction of volatile compounds present in leaves, methanol is an effective one [9]. The extract thus obtained was syringe filtered and further used for GC-MS analysis.

GC-MS analysis

GC-MS is an analytical technique used to separate components present in a given sample mixture. In this experiment, GC-MS analysis of both, the galled and normal litchi leaves extract was carried out on a shimazdu GCMSQP2010 Ultra system. The temperature of injector was kept at 280℃. The samples were injected in the split mode with split ratio being 60 to 1. The volume of the sample injected was 1µl. A capillary column composed of Rtx-5MS (5% Diphenyl-95% Dimethyl Polysiloxane) was used. Its dimensions were 30m × 0.25mm × 0.25µm. Helium was used as the carrier gas with a constant flow of 1.00 ml/min. At the beginning of the process, the temperature was kept at 60℃ for 2 min which was increased to 10℃/min up to 260℃ that was held for 10 min. The ionization potential of mass spectrophotometer was 70eV. The interface temperature was 260℃ and that of the ion source was 280℃. The mass scan range was 40-550.

Phytochemical Identification

The phytochemicals present in the extracts were identified with the help of the data available in National Institute of Standards and Technology (NIST) library.

The mass spectra of the unknown compounds were matched with the spectra of the known compounds available in NIST. 2.3. The molecular weight, structure and molecular formulae of the phytochemicals were thus confirmed with the help of NIST.

Result and Discussion

GC-MS analysis of methanolic extracts of normal and galled leaves confirmed the presence of 43 phytochemicals in normal leaves (Figure 1) and 42 in the galled leaves (Figure 2). These chemical compounds mostly comprised of sesquiterpenes, terpenes, esters, antioxidants, steroids, vitamins, fatty acids etc. Table 1 and Table 2 show the retention time and area percentages of the various phytochemicals detected and identified in the GC-MS analysis of normal and galled leaves respectively.

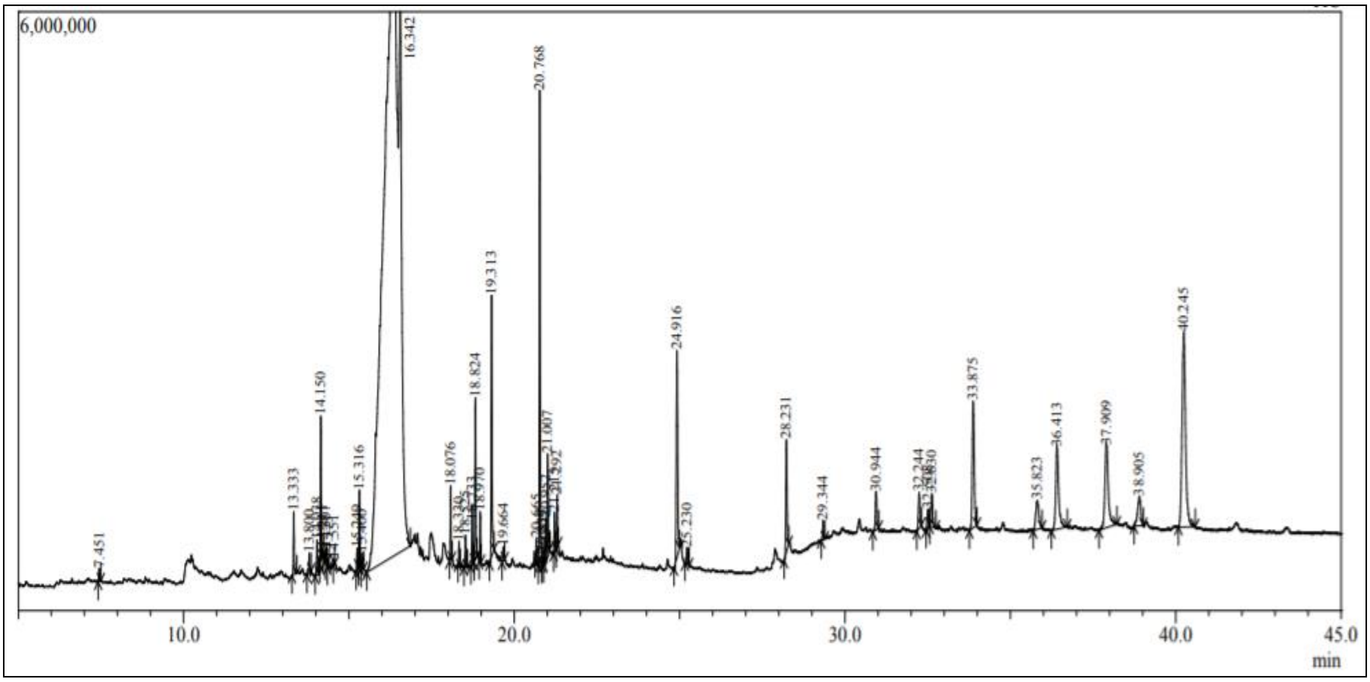

Fig 1: GC-MS chromatogram of methanolic extract of normal *Litchi* leaves

Table 1: Components detected in GC-MS analysis of methanolic extract of normal leaves of *Litchi*.

Peak Report TIC						
Peak#	R.Time	F.Time	Area	Area%	A/H	Name
1	7.451	7.495	246596	0.08	1.92	Benzeneacetaldehyde
2	13.333	13.430	1101878	0.38	1.75	Caryophyllene
3	13.800	13.860	301535	0.10	1.57	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-
4	14.038	14.085	690051	0.24	1.98	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl
5	14.150	14.185	2265748	0.78	1.57	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyc
6	14.211	14.235	86283	0.03	1.19	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2
7	14.307	14.330	214663	0.07	1.55	.alpha.-Muurolene
8	14.373	14.400	123054	0.04	1.55	.alpha.-ylangene
9	14.551	14.570	75664	0.03	1.24	isoledene
10	15.249	15.280	349304	0.12	1.64	Megastigmatrienone
11	15.316	15.350	1226194	0.42	1.60	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7
12	15.400	15.450	323829	0.11	2.03	Caryophyllene oxide
13	16.342	16.860	210533777	72.49	35.09	.alpha.-Methyl mannofuranoside
14	18.076	18.115	1100535	0.38	1.48	Neophytadiene
15	18.330	18.370	358797	0.12	1.49	Phthalic acid, butyl undecyl ester
16	18.525	18.590	524313	0.18	1.62	Neophytadiene
17	18.733	18.780	1022491	0.35	2.87	3,7,11,Trimethyl-8,10- dodecedienylacetate
18	18.824	18.880	2810457	0.97	1.73	3,7,11,Trimethyl-8,10- dodecedienylacetate
19	18.970	19.000	730671	0.25	1.46	Hexadecanoic acid, methyl ester
20	19.313	19.390	4817428	1.66	1.84	l-(+)-Ascorbic acid 2,6-dihexadecanoate
21	19.664	19.710	301224	0.10	2.13	trans-Sinapyl alcohol

Peak#	R.Time	F.Time	Area	Area%	A/H	Name
22	20.665	20.700	323467	0.11	1.29	8,11,14-Docosatrienoic acid, methyl ester
23	20.768	20.830	8351655	2.88	1.76	Phytol
24	20.835	20.890	202600	0.07	1.84	Benzoic acid, 3-acetylamino-5-chloro-4-methy
25	20.905	20.905	75347	0.03	0.82	2-[1-(2-Hydroxy-ethyl)-1H-benzoimidazol-2-y
26	20.952	20.975	389548	0.13	1.34	Linoelaidic acid
27	21.007	21.075	1762217	0.61	1.91	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
28	21.215	21.250	666027	0.23	1.68	Ethyl iso-allocholate
29	21.292	21.325	839435	0.29	1.60	Thunbergol
30	24.916	25.005	5860589	2.02	2.82	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
31	25.230	25.295	490715	0.17	2.89	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthal
32	28.231	28.300	2897070	1.00	2.58	Octadecanoic acid, 2,3-dihydroxypropyl ester
33	29.344	29.390	440845	0.15	2.25	Squalene
34	30.944	31.025	1212147	0.42	3.14	.delta.-Tocopherol
35	32.244	32.310	1196831	0.41	3.33	.beta.-Tocopherol
36	32.508	32.560	571145	0.20	3.17	.gamma.-Tocopherol
37	32.630	32.755	1134140	0.39	3.47	(R)-2,8-Dimethyl-2-((3E,7E)-4,8,12-trimethyl
38	33.875	33.990	5225223	1.80	4.11	Vitamin E
39	35.823	35.965	1859642	0.64	6.41	Ergost-5-en-3-ol, (3.beta.)-
40	36.413	36.720	5174293	1.78	6.38	Stigmasterol
41	37.909	38.220	6169045	2.12	7.41	.gamma.-Sitosterol
42	38.905	39.025	2005115	0.69	6.77	24-Noroleana-3,12-diene
43	40.245	40.590	14386365	4.95	7.55	Betulinaldehyde
			290437953	100.00		

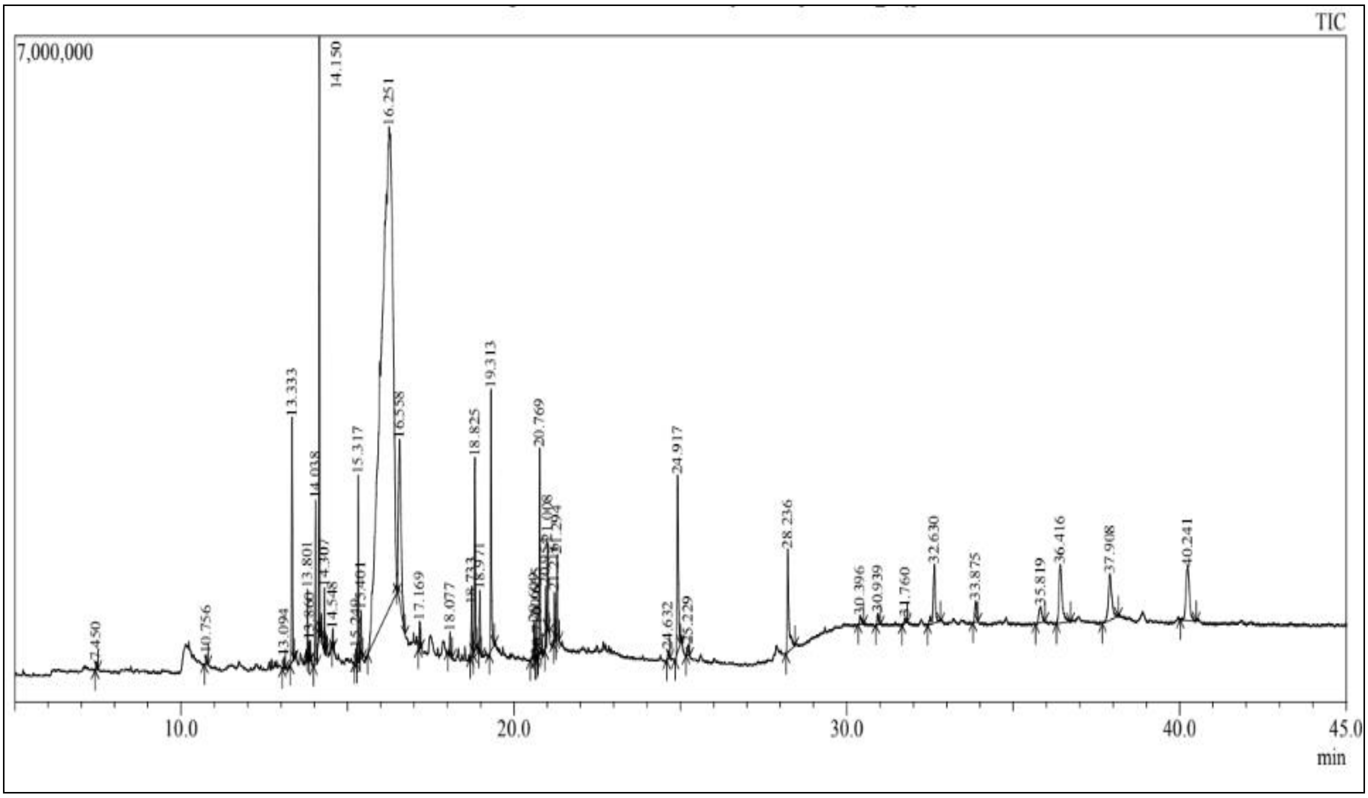

Fig 2: GC-MS chromatogram of methanolic extract of galled *Litchi* leaves

Table 2: Components detected in GC-MS analysis of methanolic extract of galled leaves of *Litchi.*

Peak#	R.Time	F.Time	Area	Area%	A/H	Name
						Peak Report TIC
1	7.450	7.500	168679	0.08	1.96	Benzeneacetaldehyde
2	10.756	10.805	177609	0.09	1.80	Propanal, 2-methyl-3-phenyl-
3	13.094	13.125	198302	0.10	1.65	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-
4	13.333	13.395	4204352	2.04	1.63	Caryophyllene
5	13.801	13.835	962031	0.47	1.38	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-
6	13.860	13.890	217648	0.11	1.34	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methy
7	14.038	14.085	3236037	1.57	1.87	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl
8	14.150	14.185	9374836	4.54	1.50	.beta.-Longipinene
9	14.307	14.335	723183	0.35	1.30	.alpha.-Muurolene
10	14.548	14.570	239813	0.12	1.32	isoledene
11	15.249	15.280	290489	0.14	1.93	syn-Tricyclo[5.1.0.0(2,4)]oct-5-ene, 3,3,5,6,8,
12	15.317	15.360	3413809	1.65	1.73	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-
13	15.401	15.460	605721	0.29	1.41	Caryophyllene oxide
14	16.251	16.490	125539981	60.82	24.78	.alpha.-Methyl mannofuranoside
15	16.558	16.730	9017441	4.37	5.35	.alpha.-Methyl mannofuranoside
16	17.169	17.200	453154	0.22	1.60	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-,
17	18.077	18.145	303514	0.15	1.29	Neophytadiene
18	18.733	18.780	1203568	0.58	2.82	3,7,11,Trimethyl-8,10- dodecedienylacetate
19	18.825	18.880	3420895	1.66	1.68	3,7,11,Trimethyl-8,10- dodecedienylacetate
20	18.971	19.020	848518	0.41	1.36	Hexadecanoic acid, methyl ester
21	19.313	19.420	4923646	2.39	1.79	l-(+)-Ascorbic acid 2,6-dihexadecanoate
Peak#	R.Time	F.Time	Area	Area%	A/H	Name
22	20.609	20.630	751432	0.36	1.95	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
23	20.665	20.690	753642	0.37	1.71	8,11,14-Docosatrienoic acid, methyl ester
24	20.769	20.845	3770667	1.83	1.73	Phytol
25	20.954	20.985	890087	0.43	1.57	Linoelaidic acid
26	21.008	21.060	1492166	0.72	1.64	Dichloroacetic acid, tridec-2-ynyl ester
27	21.216	21.250	867049	0.42	1.69	Ethyl iso-allocholate
28	20.710	20.710	19459	0.01	0.81	Acetamide, N-methyl-2-[(5-methyl-1,3,4-thiad
29	21.294	21.360	1654219	0.80	1.85	Thunbergol
30	24.632	24.685	240877	0.12	2.67	p-Heptylbenzonitrile
31	24.917	25.005	5247988	2.54	2.81	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
32	25.229	25.280	296453	0.14	2.70	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthale
33	28.236	28.440	3795384	1.84	5.00	Octadecanoic acid, 2,3-dihydroxypropyl ester
34	30.396	30.515	307501	0.15	3.47	Hexatriacontane
35	30.939	31.015	329518	0.16	2.90	.delta.-Tocopherol
36	31.760	31.820	185515	0.09	3.00	Senkyunone
37	32.630	32.820	2707335	1.31	4.77	(R)-2,8-Dimethyl-2-((3E,7E)-4,8,12-trimethyl
38	33.875	33.945	818465	0.40	3.79	Vitamin E
39	35.819	35.950	1043442	0.51	6.32	Ergost-5-en-3-ol, (3.beta.)-
40	36.416	36.720	4050740	1.96	7.05	Stigmasterol
41	37.908	38.160	3581684	1.74	7.51	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-
42	40.241	40.500	4094466	1.98	7.56	Lupeol
			206421315	100.00		

Table 3: showing peak area percentages of notable phytochemicals in normal and galled leaves.

S.No.	Name of the compound	Nature of the compound	Area %	
			Normal leaf	Galled leaf
1	Alpha methyl mannofuranoside	Glycoside	72.49	60.82
2	Vitamin E	Antioxidant	1.80	0.40
3	Phytol	Diterpene	2.88	1.83
4	Caryophellene	Sesquiterpene	0.38	2.04
5	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Sugar acid	1.66	2.39
6	Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl)ethyl ester	Palmitic acid ethyl ester	2.02	2.54
7	Octadecanoic acid,2,3-dihydroxypropyl ester	Linoleic acid ester	1.00	1.84
8	Betulinaldehyde	Triterpenoid	4.95	-
9	.gamma.-Sitosterol	Steroid	2.12	-
10	beta.–Longipinene	Sesquiterpene	-	4.54
11	Lupeol	Triterpene	-	1.98

Table 3 shows comparison of area percentage of compounds present in significant amounts in normal and galled leaves. The area percentage of alpha.-Methyl mannofuranoside was 72.49% in normal leaves which reduced to 60.82% in galled leaves. Phytol reduced from 2.88% in normal leaves to 1.83% in galled leaves and Vitamin E reduced from 1.80%

in normal leaves to 0.40% in galled leaves. Some chemical compounds increased in galled leaves in comparison to normal leaves. Caryophyllene increased from 0.38% in normal leaves to 2.04% in galled leaves, l-(+)-Ascorbic acid 2,6-dihexadecanoate increased from 1.66% in normal leaves to 2.39% in galled leaves, Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester increased from 2.02% in normal leaves to 2.54% in galled leaves and, Octadecanoic acid,2,3-dihydroxypropyl ester increased from 1.00% in normal leaves to 1.84% in galled leaves. Some compounds were present only in galled leaves like beta-Longipinene (4.54%) and Lupeol (1.98%). Some phytochemicals were absent in galled leaves like.gamma.-Sitosterol (2.12%) and Betulinaldehyde (4.95%). The above mentioned chemical compounds are of great importance because of their medicinal properties and their role in plant defence.

The compound alpha methyl mannofuranoside acts as a plant growth regulator by modulating glycoregulation in plants [10]. Its reduced quantity in the galled leaves shows that the metabolic processes involved in the normal growth of the plant have been adversely affected due to mite infestation, thus leading to gall formation. Another significant compound, Vitamin E, which reduced substantially in the galled leaves, is an antioxidant which protects cell membranes from damage caused due to light [11]. Its reduced quantity in the galled leaves suggests that the mite has altered the pathway involved in the plant defence. Quantity of Phytol, a diterpene alcohol, also reduced in galled leaves. Phytol shows antibacterial activities against *Staphylococcus aureus.* It has antioxidant and anticancer activities as well [12]. It has potential to be used for the treatment of rheumatoid arthritis and other chronic inflammatory diseases [13]. Among the chemical compounds whose quantity increased in the galled leaves, Caryophellene is an important one. It shows antimicrobial activities and plays a major role in plant defence mechanism [14]. It has been observed to be released from non-floral parts of the plant to act against herbivores [15]. For example, in Cotton and *Hymenaea*, Caryophellene reduced the growth of the insects feeding on them [16]. Its higher amount in galled leaves suggests that the mite infestation triggered the plant defence system to release more of Caryophellene. l-(+)-Ascorbic acid 2, 6-dihexadecanoate also increased in the galled leaf. It has many medicinal properties. It is antiallergic, antianemic, antibacterial, antibronchitic, antidiabetic, anti-inflammatory etc. [17].

Some compounds were completely absent in the galled leaves but present in normal leaves. A significant compound Betulinaldehyde, absent in galled tissue, is a pentacyclic triterpenoid which possesses antitumour activity [18]. Its presence in the normal leaves suggests that the mite infection has affected the synthesis of betulinaldehyde, leading to formation of gall. Betulinaldehyde also shows antimicrobial activities against bacteria and fungi. It has been found to be effective against the bacterium *Staphylococcus aureus* [19]. Another important compound found absent in galled leaves but present in normal leaves is.gamma.-Sitosterol (2.12%). It is a naturally occurring plant steroid which acts as an effective antidiabetic drug [20]. Its complete loss in the galled tissue suggests that either it was completely used during gall formation or the pathway involved in its synthesis was altered by the mite. Unlike above mentioned phytochemicals, some compounds were found only in galled leaves. Lupeol, a pentacyclic triterpenoid, is one such compound. It is anti-inflammatory and is used for therapeutic purposes [21]. It acts as a precursor for other triterpenoid metabolites which play a crucial role in the plant defence [22]. The presence of lupeol in only galled leaves shows that mite infection stimulated the production of lupeol in galled tissue as a mechanism of plant defence. Beta longipinene is another significant compound which was present only in galled leaves. Alpha-longipinene, an isomer of beta-longipinene shows anti-biofilm activity against certain fungal strains [23]. This shows that it plays a major role in plant defence.

Conclusion

The results obtained in this study suggest that compounds like alpha methyl mannofuranoside, Phytol and Vitamin E reduced in the *Litchi* leaves due to mite infestation. Amount of terpenoids and steroids increased under the stress conditions induced by the mite. Quantity of phytochemicals like Caryophyllene increased in the galled leaves. Betulinaldehyde and.gamma.-sitosterol were completely absent in the galled leaves whereas beta.-Longipinene and Lupeol were present only in galled leaves. Role of these significant compounds in plant defence during gall formation in *Litchi* still remains unexplored. A detailed study of phytochemicals specific to these galls can help in understanding the host pathogen interaction in *Litchi* leaf galls induced by mite. The medicinal value of the compounds too can be explored further for the human welfare.

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