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Effects of phytoassociates on amount of phytochemical present in Vallabh Priya and Roma varieties of curcuma longa L. (Turmeric)

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Abstract
Curcuma longa L. is atherbaceous spice plant with promising medicinal value. Yellow colour of turmeric is due to presence of bioactive phytochemicals curcumin and other curcuminoids. In present studies we observed cultivation of turmeric is as mostly done in open fields but when it is planted in orchards there these plants find orchard trees as favorable turmeric phytoassociates and the amount of curcumin and curcuminoids vary. Orchard trees and turmeric plants show beneficiary association with respect to soil, nutrition, growth and phytochemical contents. The HPTLC analysis of rhizomes growing under different orchard trees proves that some orchards like Guava, Neem and Jatropha have been a good phytoassociate for turmeric plant and curcumin and curcuminoids content of such rhizome was high compared to rhizomes grown under Mango and litchi plants. Therefore orchards are recommended for turmeric plantation and also the open fields are good area for turmeric cultivation.

Keywords: curcuma longa L., zingiberaceae, phytochemical, phytoassociate, HPTLC

Introduction
Turmeric is an aromatic herbaceous plant and a member of zingiberaceae family with designated to botanical name Curcuma longa L. It has axillary spike inflorescence flowering during winter season [1]. Seven to ten purple pink, vivid violet, purple, red and rarely white petal coloured flowers prims in winter season when plants are green and healthy. At base of each aerial stem is present ellipsoidal rhizome which yield a yellow coloured spice most often used in Asian cuisine for curries, pickle, cheese, breads and cereals etc. Plant rhizomes are used as turmeric for culinary and medicinal uses [2]. Turmeric is grown in Asia, Europe, America and parts of some African countries. India dominates the world trade scenario contributing 80% of production of turmeric followed by China (8%), Maynmar (4%), Nigeria (3%) and Bangladesh (3%). Production of turmeric in India is highest in Tamilnadu, Andhra Pradesh, WestBengal and Telangana. In India 80% of turmeric produced is exported to be consumed in world and annually 20% of produced turmeric is consumed in the country local markets [3]. The common bioactive chemicals of turmeric are curcumin, bismethoxycurcumin and desmethoxycurcumin. It is a phenolic compound with orange crystalline structure. The curcumin is insoluble in water. Curcumin is termed most potent phytochemical of turmeric for its medicinal activity against wounds and infections [4]. Phytochemicals of turmeric are associated with anti-inflammatory, anti-spasmodic, anticarcinogenic and gastrointestinal destocked properties [5-6]. Chandra and Gupta (1972) demonstrated the anti-inflammatory and anti-arthritic effect of volatile oil of curcuma longa L (haldi) [6]. Huang etal (1992) examined the inhibitory effect of curcumin on blood mononuclear cell and smooth vascular muscles. In blood mononuclear cells curcumin was responsive to inactivate mitogens [7]. Antimicrobial action against prevailing infection was reduced considerably with application of curcumin, curcumene and curcuminoid creams and also by oral intake of turmeric tablets as curcumin supplements [8]. Improving bioavilability of curcumin by inhibition of curcuma metabolism and by improving pharmacokinetic profile and the cellular uptake may prove it effective oral supplement for good health [2]. It has been observed E.coli and Staphlyococcus aureus were suspectible to methanol extract of curcumin and food shelf life may also increases by antimicrobial effect of turmeric [9] The Curcuma longa L. plant varieties are found to have ability to grow under various types of orchard trees and these rhizomes may be processed by boiling, sundrying or infrared drying. Some common varieties of turmeric are Roma, Vallabh priya, Suvarna, Roma pahari, Prabha, Vayama, Alleppye etc. Turmeric varieties possess properties for medicinal activity and these may be variable at genetic and physiological biology of its different varieties. Therefore the phytochemical yields with reference to their growth under different plant genus shall be considerably different in percentage amount. Varieties like Roma and Vallabh priya growing under specific orchard trees or phytoassociates like Guava and Neem trees would have high yield of phytochemicals.

Material and Method
The effect of phytoassociates is studied in Roma and Vallabh priya varieties of turmeric for their yield of curcumin and curcuminoids content.

Material samples like turmeric rhizomes were procured from two centers namely
- Sardar Vallabh bhai Patel University, Meerut
- Government Garden, Saharanpur

The Roma and Vallabh priya varieties of turmeric were grown under five different plantations referred as Guava, Litchi, Neem, Mango and Jatropha kurkas.
The rhizomes of Roma and Vallabh priya cultivated in these orchards were dug and samples were processed before study sample were analyzed.
Turmeric samples are usually processed by two methods-
- drying under sunlight
- boiling in water and then sun drying

Processing of Roma and Vallabh priya varieties of these study were also done by both aforesaid methods. Therefore samples used for our study were of following three types-
1. fresh rhizomes
2. sundry rhizomes
3. boiled and sundry rhizomes

These processed samples of Roma and Vallabh priya were prepared as extracts ready for HPTLC analysis of phytochemical concentration in samples.

Chromatographic Conditions
A camag's HPTLC system, consisting of TLC scanner, Linomat 5, and Twin troughdevelopment chamber with Cats 4.05 evaluation software was used.

Preparation of extract for authentic samples
1. Fresh sample
50 mg of fresh sample were taken and extracted with methanol (15x3) on water bath. Extracts were filtered and filterates were collected. The volume of filterate was made to50ml with methanol. These samples were applied to HPTLC analysis.

2. Sundry sample
The sample was extracted with methanol (15x3) and filterate were made to 50ml volume with methanol. These sundry samples were applied to HPTLC analysis.

3. Boiled sundry sample
The sample was extracted with methanol (15x3) and filtered. Filterate were made to volume 50ml and boiled sundry samples were applied to HPTLC analysis.

Formulae of Calculating Percentage Of Curcumin And Curcuminods
To calculate the amount of Curcumin & total curcuminoids linear regression equation was plotted between concentrations of sample and area of different standard concentration using following formulae

$$\%\ of\ curcumin = \frac{conc.of\ std.\ (mg) * Area\ of\ test\ sample}{Area\ of\ std.\ * conc\ of\ sample} * 100$$

$$\%\ of\ total\ curcuminoids = \frac{conc.of\ total\ curcuminoids\ in\ std.* Area\ of\ test\ sample}{Area\ of\ std.\ * conc.\ of\ test\ sample} * 100$$

Result and Discussion
Roma and Vallabh priya harvest grown under different orchards has been processed and analyzed for amount of curcumin and curcuminoids in these samples.
The results are tabulated in table no. - 1 given below. Roma samples from guava orchard have more curcumin (2.02%) when boiled sundried while Roma sundry samples have more curcuminoids (7.34%). Similarly Vallabh priya boiled and sundried rhizome from guava orchard has more cucumin (3.05%) whereas Vallabh priya sundried sample has more curcuminoids (7.96%). Roma samples from Mango orchard showed only traces of curcumin and curcuminoids. The Vallabh priya boiled sundried samples from Mango orchard has curcumin (3.05%) and Vallabh priya sundried sample has more curcuminoids (7.96%). Other samples of Neem and Jatropa as phytoassociate to turmeric plants are also considerably high at phytochemical contents.

Therefore it is concluded that Mango orchard pose some inhibitory impact on phytochemical content of turmeric varieties. This inhibitory effect on phytochemicals of Roma and Vallabh priya varieties may be due to large amount of pesticides applied on Mango trees during the year. The soil and enivornment conditions of mango may not be very favourable for turmeric growth. However the guava orchard as phytoassociate shows enhancing effect on turmeric growth and phytochemical content. Guava orchard soil and environment with almost no use of pesticide makes guava orchards a suitable phytoassociate for turmeric.

Neem plantation with no pesticide spray throughout the year proves it as a suitable place for turmeric growth and study. Neem is also a suitable phytoassociate.

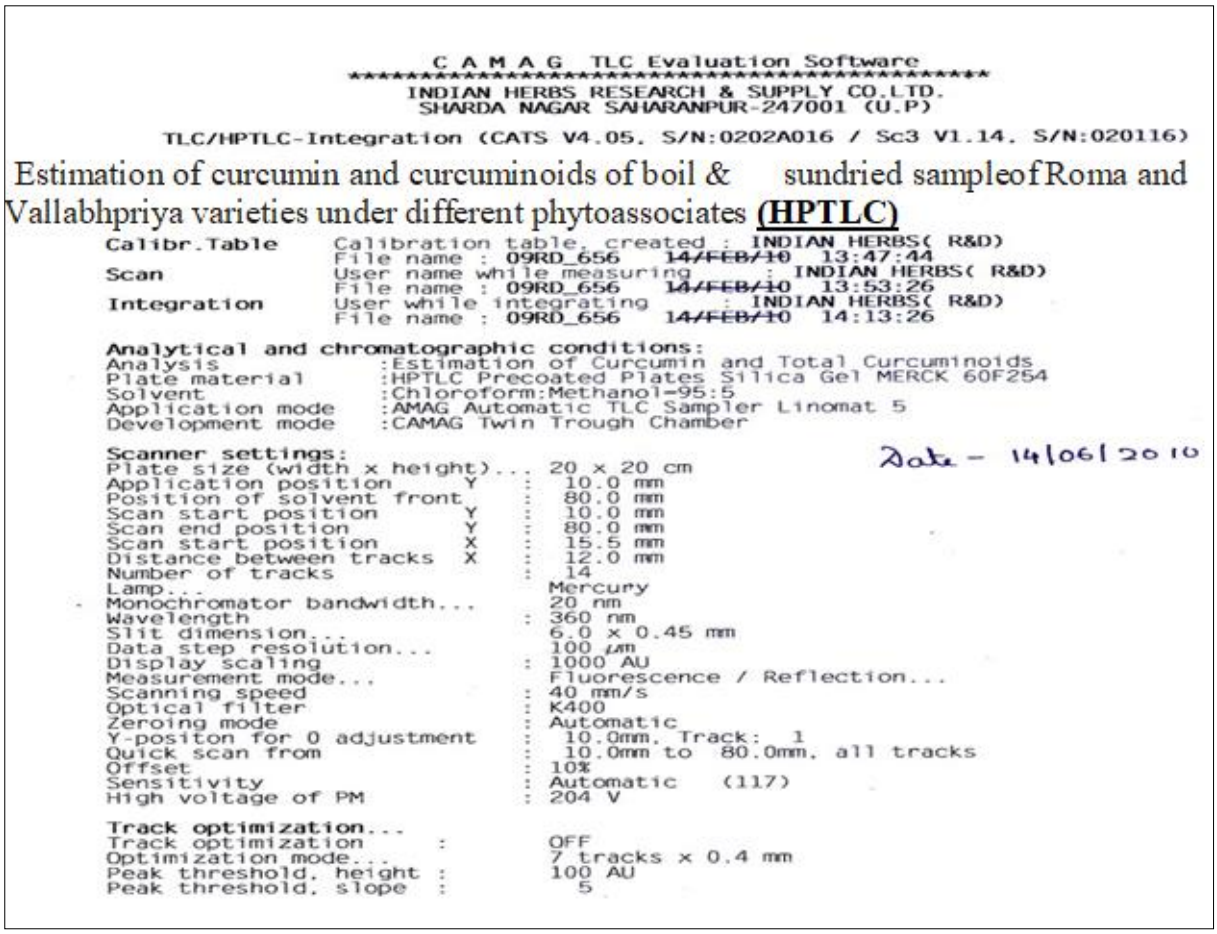
Table 1: Effect of Phytoassociate On Phytochemicals of Turmeric Varieties

Variety	Open Field		Under Mango		Under Neem		Under Litchi		Under Guava		Under Jatropha	
	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD
Roma fresh	0.09	0.37	Traces	Traces	0.55	1.59	0.41	1.41	0.60	1.70	0.38	1.15
Roma sundry	0.50	4.64	Traces	Traces	0.90	3.68	0.19	0.72	1.68	7.34	1.22	5.05
Roma boiled sundried	078	3.59	Traces	Traces	0.88	3.40	0.84	3.07	2.02	5.89	0.59	2.48
Vallabh priya fresh	0.67	2.26	0.41	1.22	0.60	1.92	0.58	1.74	0.92	2.01	0.70	2.01
Vallabh priya sundry	1.47	5.11	1.24	5.36	1.54	6.51	1.20	2.78	2.45	7.96	2.41	6.75
Vallabh priya boiled sundried	1.00	2.01	0.70	3.06	0.98	4.12	0.78	4.78	3.05	6.01	1.08	3.02

CM- curcumin, CMD- curcuminoids

Curcumin and curcuminoids volume are measured in percentage w/w

Estimtion graphs of turmeric varieties by HPTLC


C A M A G TLC Evaluation Software

INDIAN HERBS RESEARCH & SUPPLY CO.LTD.
SHARDA NAGAR SAHARANPUR-247001 (U.P)

TLC/HPTLC-Integration (CATS V4.05, S/N:0202A016 / Sc3 V1.14, S/N:020116)

Estimation of curcumin and curcuminoids of boil & sundried sampleof Roma and Vallabhpriya varieties under different phytoassociates **(HPTLC)**

Calibr.Table — Calibration table, created : INDIAN HERBS(R&D)
File name : 09RD_656 14/FEB/10 13:47:44

Scan — User name while measuring : INDIAN HERBS(R&D)
File name : 09RD_656 14/FEB/10 13:53:26

Integration — User while integrating : INDIAN HERBS(R&D)
File name : 09RD_656 14/FEB/10 14:13:26

Analytical and chromatographic conditions:

Analysis :Estimation of Curcumin and Total Curcuminoids
Plate material :HPTLC Precoated Plates Silica Gel MERCK 60F254
Solvent :Chloroform:Methanol=95:5
Application mode :AMAG Automatic TLC Sampler Linomat 5
Development mode :CAMAG Twin Trough Chamber

Date - 14/06/2010

Scanner settings:

Plate size (width x height)...		20 x 20 cm
Application position	Y	10.0 mm
Position of solvent front		80.0 mm
Scan start position	Y	10.0 mm
Scan end position	Y	80.0 mm
Scan start position	X	15.5 mm
Distance between tracks	X	12.0 mm
Number of tracks		14
Lamp...		Mercury
Monochromator bandwidth...		20 nm
Wavelength		360 nm
Slit dimension...		6.0 x 0.45 mm
Data step resolution...		100 µm
Display scaling		1000 AU
Measurement mode...		Fluorescence / Reflection...
Scanning speed		40 mm/s
Optical filter		K400
Zeroing mode		Automatic
Y-positon for 0 adjustment		10.0mm, Track: 1
Quick scan from		10.0mm to 80.0mm, all tracks
Offset		10%
Sensitivity		Automatic (117)
High voltage of PM		204 V

Track optimization...

Track optimization		OFF
Optimization mode...		7 tracks x 0.4 mm
Peak threshold, height		100 AU
Peak threshold, slope		5

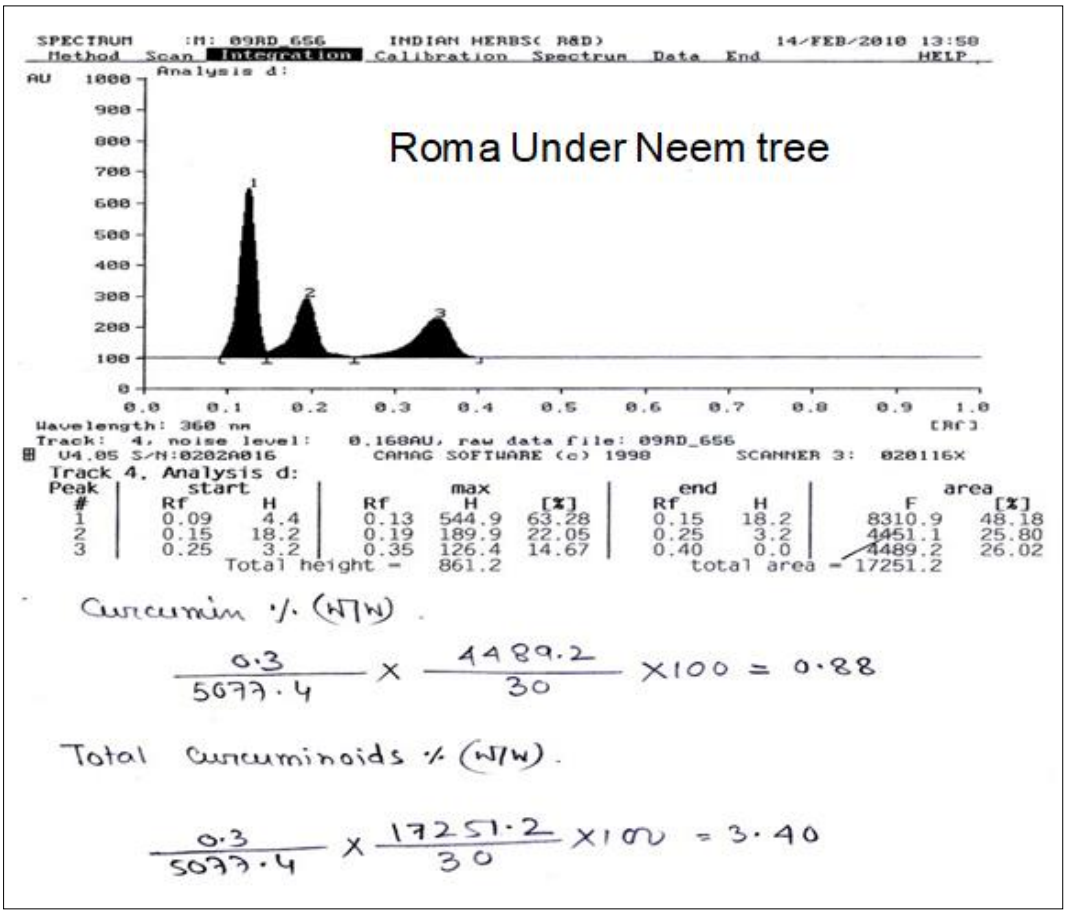

SPECTRUM :M: 09RD_656 INDIAN HERBS(R&D) 14/FEB/2010 13:50
Method Scan Integration Calibration Spectrum Data End HELP
Analysis d:
AU
Roma Under Neem tree
Wavelength: 360 nm
Track: 4, noise level: 0.168AU, raw data file: 09RD_656
U4.05 S/N:0202A016 CAMAG SOFTWARE (c) 1998 SCANNER 3: 020116X
Track 4, Analysis d:
Peak # start Rf H max Rf H [%] end Rf H area F [%]
1 0.09 4.4 0.13 544.9 63.28 0.15 18.2 8310.9 48.18
2 0.15 18.2 0.19 189.9 22.05 0.25 3.2 4451.1 25.80
3 0.25 3.2 0.35 126.4 14.67 0.40 0.0 4489.2 26.02
Total height = 861.2 total area = 17251.2
Curcumin % (W/W)
0.3 / 5077.4 × 4489.2 / 30 ×100 = 0.88
Total Curcuminoids % (W/W).
0.3 / 5077.4 × 17251.2 / 30 ×100 = 3.40

Fig 1

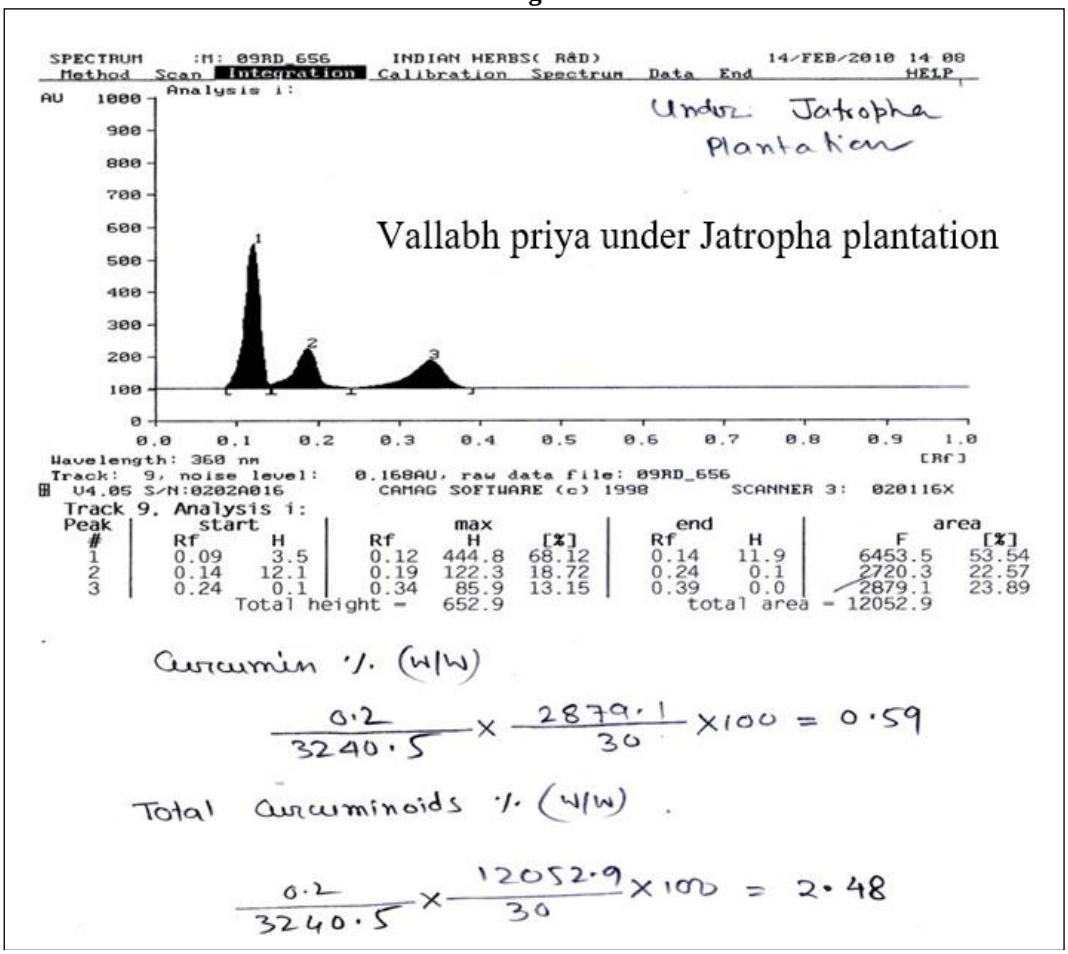

SPECTRUM :M: 09RD_656 INDIAN HERBS(R&D) 14/FEB/2010 14:08
Method Scan Integration Calibration Spectrum Data End HELP
Analysis i:
AU
Under Jatropha Plantation
Vallabh priya under Jatropha plantation
Wavelength: 360 nm
Track: 9, noise level: 0.168AU, raw data file: 09RD_656
U4.05 S/N:0202A016 CAMAG SOFTWARE (c) 1998 SCANNER 3: 020116X
Track 9, Analysis i:
Peak # start Rf H max Rf H [%] end Rf H area F [%]
1 0.09 3.5 0.12 444.8 68.12 0.14 11.9 6453.5 53.54
2 0.14 12.1 0.19 122.3 18.72 0.24 0.1 2720.3 22.57
3 0.24 0.1 0.34 85.9 13.15 0.39 0.0 2879.1 23.89
Total height = 652.9 total area = 12052.9
Curcumin % (W/W)
0.2 / 3240.5 × 2879.1 / 30 ×100 = 0.59
Total Curcuminoids % (W/W)
0.2 / 3240.5 × 12052.9 / 30 ×100 = 2.48

Fig 2

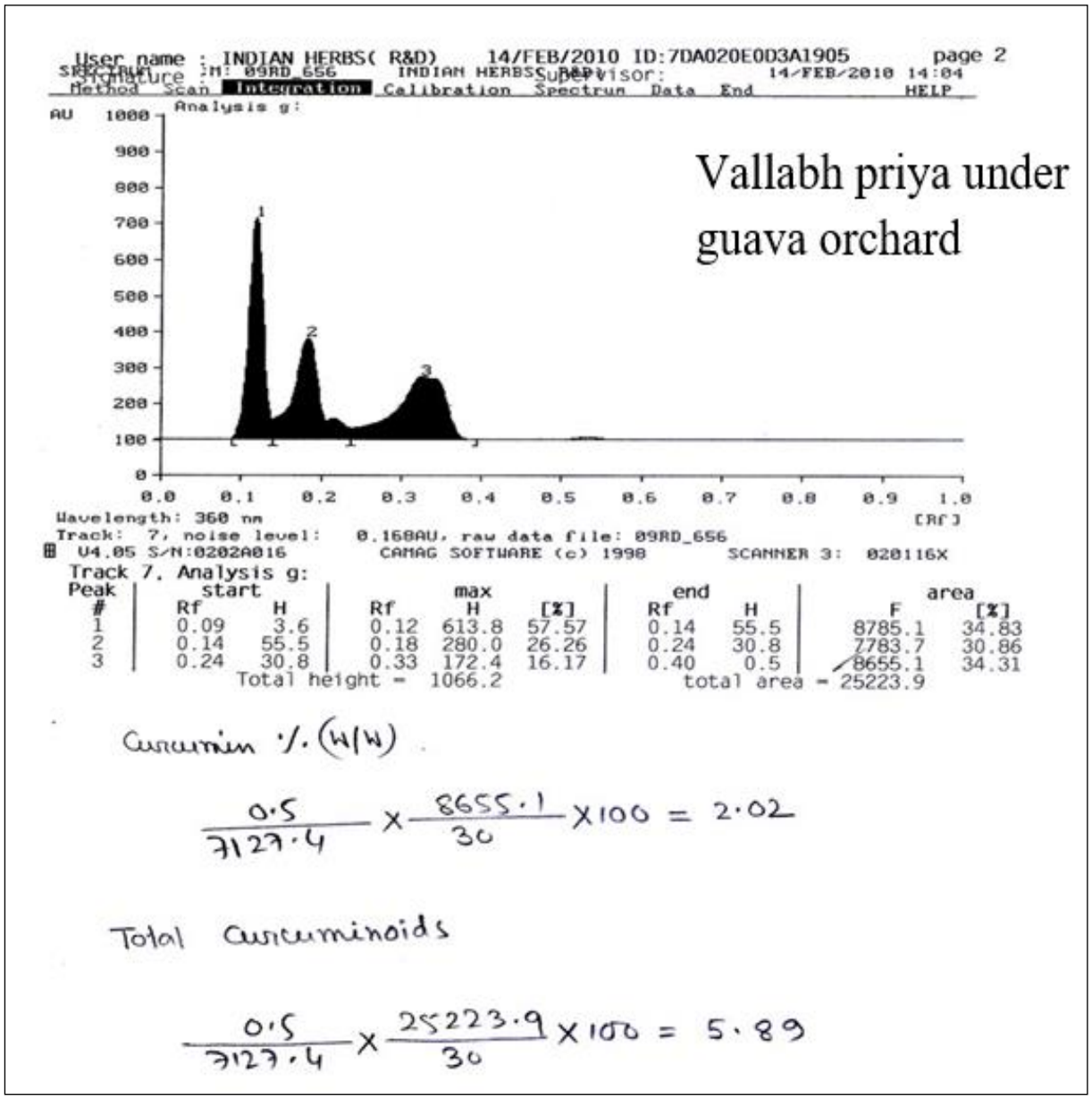


Fig 3

Conclusion

Roma and Vallabh priya varieties were planted under different orchards. Rhizomes under Guava orchard as phytoassciate show high content of turmeric phytochemicals.

Fresh, sundried and boiled sundried samples of Roma and Vallabh priya under guava plantation has curcumin and curcuminoids content as follows

Table 2

A. **Roma**	B. **Vallabh priya**
Curcumin Curcuminoids	Curcumin Curcuminoids
1. Fresh 0.60% 1.70 %	0.92% 2.01%
2. Sundry1.68% 7.34%	2.45% 7.96%
3. Boiled 2.02% 5.89%	3.05% 6.01%

Mango orchard as phytoassociate found to be not amicable for turmeric cultivation. Neem and Jatropha kurkas also found as good phytoassociate to Roma and Vallabh priya varieties, as these samples analysed to HPTLC found yielding appericiable amount of curcumin and curcuminoids.

So, orchard may be a good place for turmeric cultivation yet yield of phytochemicals of such cultivar shall be studied to know the cultivable varieties and appropriate phytoassociates for these turmeric varieties. We may conclude Guava and Neem as favourale phytoassociate for turmeric cultivation and processed rhizomes (sundry and boiled sundry) samples from Guava and Neem orchards shall be rich in phytochemicals like curcumin and curcuminoids.

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