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NEUTRAL VOLATILE COMPOUNDS IN FCV TOBACCO

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It is reported that about 41 compounds are found in substantial quantities to enable the smoker to perceive their impact on smoke flavour. With the objective of analysing neutral volatile compounds responsible for smoke flavour in FCV tobacco grown in Northern Light Soils (NLS), Karnataka Light Soils (KLS) & Southern Light Soils (SLS), steam volatile compounds were extracted and gas chromatography-mass spectrometry (GC-MS) analysis was carried out. As authentic standards of the compounds are not available for quantification, the area normalization method was adopted and the proportion of a particular compound in the total neutral volatile fraction was calculated. Composition of the neutral volatile fraction varied in tobacco samples from different agro-climatic zones. Higher proportion of neophytadiene (40.8%), megastigmatrienone isomers (10.1%), thunbergol (7.2%), 3-hydroxysolavetivone (6.7%), solavetivone (4.6%) and duvatriendiol (4.1%) was observed in NLS samples. Neophytadiene (21.0%), dibutyl phthalate (21.5%), viridiflorol (13.9%), 3-hydroxysolavetivone (12.3%), 1-nonadecene (12.0%), thunbergol (11.3%), solavetivone (8.1%), cembrene (6.3%) and megastigmatrienone isomers (5.9%) were the important compounds in the fraction in KLS samples. These differences could be attributed to the climatic conditions and agronomic practices, the variety Kanchan being common. In the samples from SLS region, the proportion of neophytadiene (28.4%), thunbergol (23.5%), viridiflorol (17.2%), caryophyllene oxide (9.6%), duvatrienediol (9.2%) and megastigmatrienone isomers (8.9%) was high, thus implying the varietal effect, as Hema and Siri are predominantly cultivated in the region. Identification of compounds *viz.*, rishitin, solavetivone, nerolidol, farnesol and linalool necessitates further investigations on their role in insect-pest management.

INTRODUCTION

Tobacco smoke contains more than 4000 compounds of which about 1200 are directly derived from tobacco and they have a positive or negative influence on the smoke flavour. Some of the constituents are transferred from tobacco to smoke by volatilization without any change in the structure. Flavour from any compound can only be identified if the compound exists in sizeable quantities because only 1/3rd of any volatile compound transfers directly from tobacco to the smoke (Wakeham, 1972). Demole and Berthet (1972) characterized volatiles and semi-volatiles contributing to burley tobacco flavour notes by GC-MS. Lloyd *et al.* (1976) identified 276 acidic, basic and neutral volatile compounds for the first time from flue-cured tobacco and confirmed that many of the identified compounds contributed to the flavour of blended cigarettes. As per the literature, about 41 compounds are found in substantial quantities to enable the smoker to perceive their impact on smoke flavour (Roberts, 1988). The compounds are broadly classified as acids, alcohols, aldehydes, amides, anhydrides, esters, ethers, hydrocarbons, ketones and lactones. Extensive studies conducted over the past 20 years have established the relationship between volatile compounds particularly the neutral fraction, and smoke flavour. Sakaki *et al.* (1986) observed that smoking quality of flue-cured tobacco can be evaluated by the relative abundance of the volatiles that are related to tobacco variety and stalk position. Wahlberg and Enzell (1987) reported that two major classes of diterpenoids viz., monocyclic cembronoids and the bicyclic labdanoids are found in tobacco. Virginia and Burley tobaccos contain only the cembronoids, while Oriental and Cigar tobaccos contain both the labdanoids & cembronoids. Weeks *et al.* (1989) employed chromatography for evaluation of volatiles from flue-cured tobacco varieties. Thus, analysis of neutral volatile compounds will help in establishing qualitative and quantitative profiles of cultivars/germplasm accessions. It will also help in evaluating the influence of production practices on the levels of the flavour constituents.

Comprehensive studies on aroma bearing and aroma precursor compounds in FCV tobacco conducted at CTRI established that carbonyl compounds and volatile acids are directly correlated to tobacco aroma. It is observed that higher fatty acids and amino acids are the precursors of carbonyl compounds (Prabhu, 1981). Results of the preliminary study conducted at CTRI indicated that higher levels of neutral volatile compounds like solanone, neophytadiene and isomers of megastigmatrien-3-one are present in FCV tobacco samples from NLS compared to other regions.

MATERIALS AND METHODS

With the objective of analysing neutral volatile compounds responsible for smoke flavour in 23 FCV tobacco samples (KLS:12, NLS: 7, SLS: 5) were subjected to steam distillation, the distillate was extracted with dichloromethane and concentrated to 1ml (Wu *et al.*, 1992). The GC-MS analysis was carried out using a QP 2010 Plus GC-MS system equipped with AOC - 20i auto sampler (Single quadrupole, Shimadzu Corporation, Kyoto, Japan). A ZB-5 MS (5% Phenyl, 95% Dimethyl polysiloxane) (Zebron™ – Phenomenex, USA) capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The column oven temperature was programmed to rise from an initial temperature of 60 °C (hold for 1 min) to 140 °C (hold for 5 min) @ 6 °C/min, from 140 °C to 180 °C (hold for 5 min) @ 6 °C/min and to a final temperature of 210 °C @ 6 °C/min, the final temperature was held for 14 min with a total run time of 50 min. Helium was used as the carrier gas with a flow rate of 1 ml/min. The inlet and interface temperatures were kept at 250 °C. The Electron Ionisation (EI) source was operated at 200 °C and the quadrupole temperature was 150 °C. All samples were analysed in scan mode with a mass range of 50 to 500 units. One micro liter (µl) of the sample was injected in split less mode by the auto sampler. The obtained peaks were identified using US National Institute of Standards and Technology (NIST) standard mass spectral library database. As authentic standards of the compounds are not available for quantification, the area normalization method was adopted and the mean per cent of a particular compound in the total neutral volatile fraction of all the samples from a zone was calculated.

RESULTS AND DISCUSSION

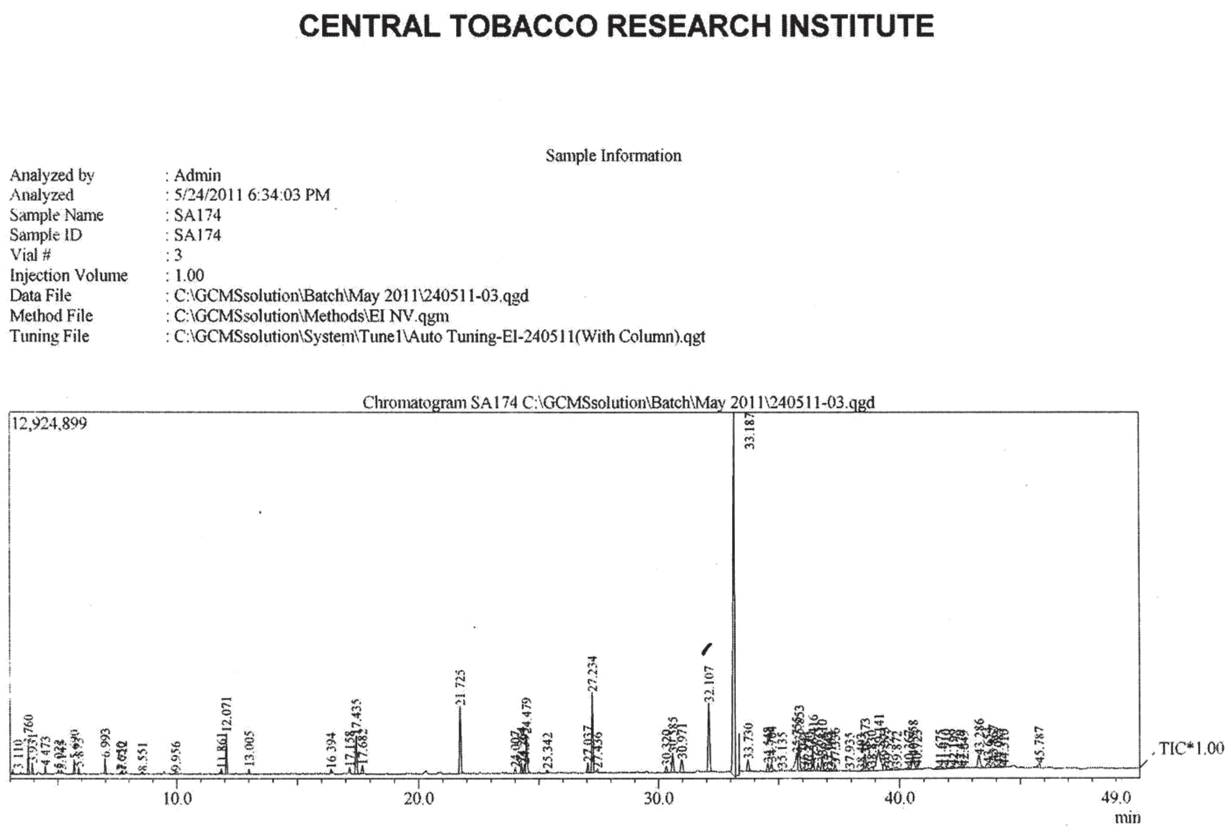
It is inferred from the mean values of samples analysed that with their higher proportion in the neutral volatile fraction, the following compounds (Table 1 & Fig. 1) significantly contribute to smoke flavour: neophytadiene, thunbergol, viridiflorol, dibutyl phthalate, megastigmatrienone isomers, 1-nonadecene, 3-hydroxysolavetivone, duvatrienediol, caryophyllene oxide, solavetivone, retinol acetate, globulol and solanone. The other important compounds identified are: rishitin, diethyl phtalate, cycloisolongifolene, valencene, lineolone, 3-oxo-á-ionol, nootkatone, trimethyl-2,4,4 hexene-1, benzyl alcohol, phenylacetaldehyde, furfuryl alcohol and protoanemonine. Wahlberg *et al.* (1977) reported that neutral volatile compounds like isomers of megastigmatrien-3-one, damascenone, dihydroactinidiolide, neophytadiene, solanone and furfuryl alcohol increase on curing of tobacco and reach maximum values after 6 months ageing. GC-MS analysis of volatile neutral fraction has focused attention on 20 compounds, which had the greatest chance of being transferred from tobacco to smoke and have a positive relation with smoke flavour and also correlate well with the sensory evaluation (Wu *et al.*, 1992). In the comparative analysis of cut tobacco from China, Huang *et al.* (2006) estimated the relative content of volatile compounds viz., megastigmatrienones (11.36%), thunbergol (1.43%), 3-oxo-á-ionol (1.26%), retinol acetate (0.58%), 2-acetylpyrrole (0.44%), nootkatone (0.34%), solanone (0.27%), phenylethyl alcohol (0.26%), farnesyl acetone (0.24%) and phytol (0.14%). Even though, differences existed in the content of some compounds, which could be attributed to the climate, variety and cultural practices, a similar trend is observed in the case of FCV tobacco grown in different regions in India.

It is observed from the data that all the reported compounds in the neutral volatile fraction were detected in all the samples, though the

Table 1: Important neutral volatile compounds in FCV tobacco

Compound	Per cent of neutral volatile fraction	Compound	Per cent of neutral volatile fraction
Neophytadiene	30.1	Rishitin	2.3
Thunbergol	14.0	Globulol	2.1
Viridiflorol	12.3	Solanone	0.7
Dibutyl phthalate	8.7	Diethyl Phtalate	1.6
Megastigmatrienones	8.3	Cycloisolongifolene	1.4
1-Nonadecene	8.0	Valencene	1.3
3-Hydroxysolavetivone	6.8	Lineolone	1.3
Duvatrienediol	6.5	3-oxo-á-ionol	1.1
Caryophyllene oxide	5.1	Nootkatone	0.9
Solavetivone	4.4	Benzyl Alcohol	0.5
Retinol aetate	4.1	Phenylacetaldehyde	0.3

Fig. 1: A typical GC-MS chromatogram



composition of the fraction varied in different agro-climatic zones (Table 2 & Fig. 2). The identified compounds accounted for 83.4, 93.2 and 89.4% of the total fraction in samples from NLS, KLS and SLS, respectively. Zhu *et al.* (2009) have identified and quantified 39 volatile components of the tobacco flavour samples accounting for 86.54% of the total content. They reported that the 12 samples had 28 peaks in common. The proportion of neophytadiene, and megastigmatrienone isomers was higher in NLS samples (Table 2). Fujimori *et al.* (1978) reported that in air-cured and aged burley tobacco, the largest peak was neophytadiene but it contributes little to tobacco flavour. It is concluded that 15-30% of neophytadiene generated during flue-curing might have been produced by dehydration of phytol, a metabolite from chlorophyll hydrolysis (Amin,1979). Neophytadiene has been suggested to be a tobacco flavour enhancer and is considered as a flavour carrier by entrapping volatiles in the tobacco smoke aerosol (Leffingwell and Leffingwell, 1988). In KLS samples, higher levels of 1-nonadecene, 3-hydroxysolavetivone and dibutyl phthalate were recorded. These differences could be attributed to the climatic conditions and agronomic practices, the variety Kanchan being common. In NLS, production practices viz., fertilization (N: 100 kg/ha; K_2O: 120 kg/ha), irrigation and topping contribute to higher levels of leaf nicotine and total nitrogen. Whereas in KLS, the crop is a rainfed, it is topped only in certain

Table 2: Composition (%) of neutral volatile compounds in FCV tobacco samples from different zones

Compound	NLS	KLS	SLS
Neophytadiene	**40.82**	21.01	28.37
Thunbergol	7.24	11.30	**23.46**
Viridiflorol	5.87	13.89	**17.17**
Dibutyl phthalate	1.85	**21.45**	2.90
Megastigmatrienones	**10.10**	5.90	8.87
1-Nonadecene	4.04	**11.99**	-
3-Hydroxysolavetivone	6.66	**12.34**	1.46
Duvatrienediol	4.06	6.23	**9.23**
Caryophyllene oxide	4.25	1.43	**9.62**
Solavetivone	4.61	**8.13**	0.34
Retinol acetate	3.21	**5.22**	3.98
Cembrene	ND	**6.33**	1.41
Globulol	1.34	**3.10**	1.93
Rishitin	1.65	**2.94**	ND
Valencene	1.14	**2.12**	0.62
3-oxo-á-ionol	0.52	**2.12**	0.68
Nootkatone	0.32	**1.53**	ND
Solanone	0.69	0.40	1.09
Phytol	**1.20**	0.08	ND
Phenylethyl alcohol	0.55	0.31	0.77
Benzyl Alcohol	0.48	0.55	0.50
Phenylacetaldehyde	0.17	0.07	**0.51**
2-Acetylpyrrole	0.16	0.17	0.26
Â-Damascenone	0.08	0.06	ND
Neryl acetone	0.26	0.14	0.15
Clovene	1.55	2.21	ND
Farnesyl acetone	ND	6.52	1.52

ND: Not detected

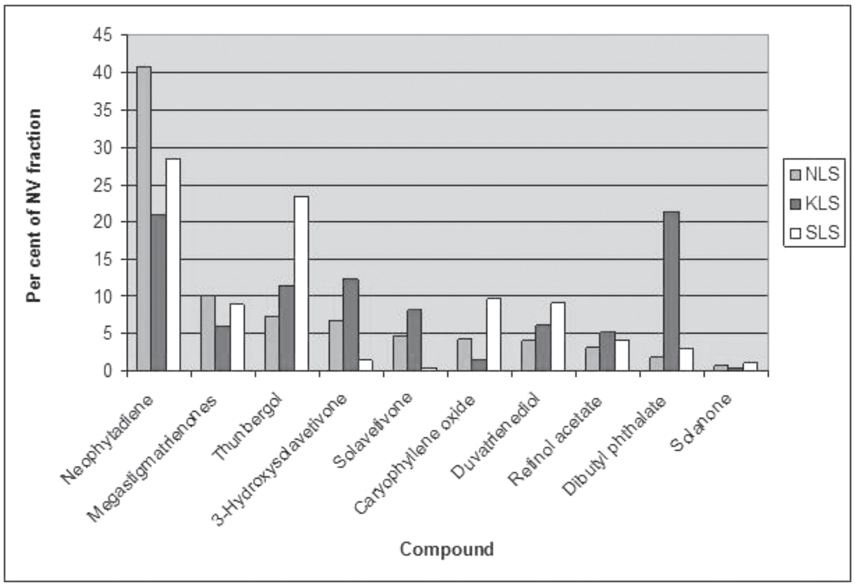


Fig. 2: Composition of neutral volatile compounds in FCV tobacco samples from LS, KLS and SLS

pockets and 60 kg N/ha is applied resulting in leaf with medium nicotine and nitrogen contents. According to Court *et al*. (1984) nitrogen fertilization increased neophytadiene in flue-cured tobacco. Shi *et al*. (2009) observed that flavour quality of tobacco is highly correlated with nicotine and total nitrogen contents. Megastigmatrienones (derived from carotenoids) and solanone (derived from thunberganoids) are the representative burley compounds. In the samples from SLS region, the levels of thunberganoids (thunbergol, viridiflorol, duvatrienediol) and caryophyllene oxide were high, thus implying the varietal effect, as Hema and Siri are predominantly cultivated in the region.

In the background of reports on fungitoxic terpenoids from tobacco (Fuchs *et al*., 1983), accumulation of six sesquiterpenoid phytoalexins in tobacco leaves infiltrated with *Pseudomonas lacrymans* (Guedes *et al*., 1980) and antifungal activity of *Phytophthora infestans* of sesquiterpenoids from infected potato tubers (Engstrom *et al*., 1999), identification of the compounds viz., rishitin, solavetivone, nerolidol, farnesol and linalool in FCV tobacco, in the present study, necessitates further investigations on their role in insect-pest management. It is concluded that important neutral volatile compounds responsible for tobacco flavour viz., neophytadiene, thunbergol, viridiflorol, megastigmatrienone isomers, 3-hydroxysolavetivone, duvatrienediol, solavetivone, thunbergol, viridiflorol, duvatriendiol and solanone were identified in FCV tobacco samples and composition of the fraction varied in samples from different agro-climatic zones. The differences could be attributed to the climatic conditions, agronomic practices and the variety grown. Identification of compounds viz., rishitin, solavetivone, nerolidol, farnesol and linalool necessitates further investigations on their role in insect-pest management.

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