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SHORT COMMUNICATIONS

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EFFECT OF NITROGEN AND LEAF POSITION ON QUALITY CONSTITUENTS OF *LANKA* TOBACCO (*NICOTIANA TABACUM*, L.) GROWN IN ANDHRA PRADESH

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***Lanka* tobacco, an indigenous air-cured tobacco used for cheroot making, is famous for its characteristic pungent taste and strong aroma and is cultivated on alluvial flood plains of Godavari River in East and West Godavari, and Khammam districts of Andhra Pradesh. A field experiment was conducted during 2010-11 in Rekhapalli, Khammam district, Andhra Pradesh to study the effect of nitrogen levels (Recommended practice; N1=300 kg/ha and farmers practice; N2=1000 kg/ha) and leaf position (bottom, middle and top) on biochemical quality constituents in *Lanka* tobacco. Nicotine, reducing sugars (RS), starch, proline, petroleum ether extractives (PEE) and acid value (positively related to quality) decreased whereas chlorophyll a, chlorogenic acid, rutin and nitrate nitrogen (negatively related to quality) increased with an increase in nitrogen level from 300 to 1000 kg/ha. Nicotine, RS, chlorogenic acid, PEE, starch, nitrate nitrogen and proline contents increased whereas rutin content decreased with increase in leaf position from bottom to top. Nicotine content decreased by 10.9% in the top leaves with increase in nitrogen levels from 300 to 1000 kg/ha. Reducing sugar content varied from 0.46 to 1.41% among the leaf position and nitrogen levels. Reducing sugars and proline contents decreased significantly by 14.39 and 79% respectively, whereas chlorogenic acid content increased by 17.68% with increased levels of nitrogen from 300 to 1000 kg/ha. The chlorogenic acid content in the top leaves was 36.79 and 40% higher than the middle and bottom leaves, respectively. The maximum content of nitrate nitrogen (4.06 mg/g) was in the top position leaves under 1000 kg N/ha. Nitrate nitrogen content increased by 82.53, 48.16 and 129.45% in top, middle and bottom position leaves, respectively, with increase in nitrogen from 300 to 1000 kg/ha. Results revealed that in *Lanka* tobacco accumulation of quality constituents was maximum in the top position followed by middle position at 300 kg N/ha. The Recommendation of 300 kg N/ha for *Lanka* tobacco was found to be optimum for higher yields with balanced quality constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).**

Key words: *Lanka* tobacco, Leaf position, Nitrogen, Quality characters

INTRODUCTION

Lanka tobacco (*Nicotiana tabacum* L.) is an indigenous air-cured tobacco type used for cheroot making, is cultivated on alluvial flood plains (Entisols) of Godavari and Krishna rivers in East and West Godavari districts, Krishna and Khammam districts of Andhra Pradesh. Country cheroot made of *Lanka* tobacco is liked very much by the people as it has a characteristic pungent taste and strong aroma. Chemical quality parameters *viz.*, nicotine, reducing sugars (RS) and chlorides and biochemical constituents *viz.*, starch, petroleum ether extractives (PEE), polyphenols, free amino acids and free fatty acids are some of the leaf constituents responsible for aroma and quality of tobacco and these parameters are influenced by quantity of manures, position of leaf on stalk, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew, 1981). A change in any of these factors can markedly alter the leaf composition and thus effect the smoke quality. Nitrogen is the key nutrient in tobacco fertilization and tobacco is sensitive to nitrogen nutrition. In tobacco, leaf being the economic product, inadequate or excess nitrogen show adverse effect on growth and chemistry of flue-cured tobacco. From the seedling stage to final harvest, the soil nitrogen regimes affect the process of plant development and chemistry of cured tobacco more than any element (McCarts

and Woltz, 1967). From the time of harvest to that of consumption, the tobacco leaf undergoes several processing steps. Some of these steps involve biochemical and thermal transformation which change the chemical composition and flavour properties. Nitrate concentration increases in the leaf as the nitrogen fertilization increases. The concentration of nitrate nitrogen in tobacco leaf influences the health related smoke constituents.

Tobacco is multilevel harvesting crop and leaves are harvested from the bottom whenever the leaves are matured. Hence, the leaves present in the middle (L position) and top (T position) will remain more days on the plant and chemistry of these leaves will be different from lower leaves. *Lanka* tobacco derives its name from the islands or 'Lankas' of Godavari and Krishna rivers during heavy floods. Even though CTRI recommended 300 kg N/ha, farmers used to apply 1000 kg N/ ha in the form of urea expecting good quality and quantity. Urea being applied to the soil surface by broadcasting, considerable loss of nitrogen as ammonia into the atmosphere results in wastage and pollution of environment. The objective of the present work is to study the effect of nitrogen and leaf position on biochemical quality constituents in *Lanka* tobacco.

MATERIALS AND METHODS

A field experiment was conducted during *rabi* season in 2011-12 in Rekhapalli, Khammam district, Andhra Pradesh. Lanka soils are moderately alkaline (pH 7.8 to 8.4) with low CE (10 to 12 m.e./100 g soil). The tobacco variety *Lanka* was grown with recommended fertilizer package of practice by CTRI (300 kg N/ha) and the practice followed by the farmers (1000 kg N/ ha). The cured leaf samples were collected replication wise from nitrogen treatments and different leaf positions on the stalk (bottom, middle and top). Midribs were separated from the leaf and lamina was dried in the hot air oven at 60°C for 6 hours, powdered, passed through 40 micron mesh and used for chemical analysis.

The powdered samples were analyzed for biochemical constituents *viz.,* chlorophyll pigments, carotenoids (Hiscox and Iscrelstons, 1979), petroleum ether extractives (Andersen *et al.*, 1977), free fatty acids (Chu *et al.,* 1972), starch (Gaines and Meudt, 1968), polyphenols (Siva Raju *et al.*, 2005), nitrate nitrogen by using salicylic acid-sulphuric acid reagent (Padmavathy, 2008), proline (Bates *et al.*, 1973) and nicotine, reducing sugars and chlorides (Harvey *et al.*, 1969). The data were statistically analyzed (Panse and Sukhatme, 1957).

RESULTS AND DISCUSSION

Chlorophyll a (Chl a) content varied from 0.602 to 1.032 mg/g among the leaf positions and nitrogen treatments (Table 1). Chl a content increased significantly (42.37%) with increase in nitrogen levels from 300 to 1000 kg/ha and with increase in leaf position from bottom to top. Chl a content in top leaves was 7.6 and 20.6% higher than the middle and bottom leaves respectively. Chl a content increased with increase in leaf position from bottom to top in each nitrogen level and was maximum in the top leaves. There was non-significant variation in Chl b content in either leaf positions or nitrogen levels (Table 1). Carotenoid content varied from 0.676 to 0.767 mg/ g among the leaf positions and nitrogen levels (Table 1). There was non-significant increase in carotenoid content with increase in nitrogen from 300 to 1000 kg/ha. Top position leaves contained significantly higher levels of carotenoids over the bottom leaves. Break down of the chlorophyll pigments was one of the important biochemical transformations during curing to get desired colour of the tobacco. Chandrasekhararao *et al.* (2013) reported an increase in Chlorophyll a, Chl b and total chlorophyll content with increase in leaf position from bottom to top on stalk and a decrease with increase in nitrogen levels. Court *et al.* (1982) reported that Chlorophyll a and b concentrations in the cured leaf were about 1% of the amount measured at harvest. Carotenoids are precursors to many of the volatile aroma components of tobacco in addition to being the major colour pigments (red-orange to yellow). The chemical breakdown products of pigments during the curing have been reported to give rise to numerous flavor components (Shi *et al.,* 2012).

The starch content varied between 2.14 and 8.12 mg/g among the leaf positions and nitrogen levels (Table 1). Starch content increased

Table 1: Effect of nitrogen levels and leaf position on pigments and starch in *Lanka* tobacco

Leaf position	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Carotenoids (mg/g)			Starch (mg/g)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	0.765	1.032	0.899	0.23	0.24	0.24	0.766	0.767	0.767	8.12	5.32	6.72
Middle	0.678	0.992	0.835	0.21	0.21	0.21	0.755	0.750	0.753	5.68	4.62	5.15
Bottom	0.602	0.888	0.745	0.20	0.22	0.21	0.676	0.753	0.715	4.83	2.14	3.48
Mean	0.682	0.971		0.213	0.23		0.732	0.757		6.21	4.03	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm(±)**	**CD (P=0.05)**	
Nitrogen	**0.009**	**0.027**		**0.012**	**0.038**		**0 .06**	**0.17**		**0.36**	**1.08**	
Leaf position	**0.034**	**0.108**		**0.016**	**0.065**		**0.01**	**0.04**		**0.55**	**1.25**	
Nitrogen x Leaf position	**0.048**	**0.134**		**0.015**	**0.049**		**0.04**	**0.13**		**0.08**	**0.26**	

significantly with increase in the leaf position from bottom to top at each nitrogen level. The starch content decreased by 34.48, 18.66 and 55.69% in top, middle and bottom position leaves, respectively with increase in nitrogen level from 300 to 1000 kg/ha. At 300 kg N/ha, the top leaves contained 30.04 and 40.51% of starch compared to middle and bottom leaves, respectively. Decrease in starch accumulation with increase in nitrogen application in FCV tobacco was reported by Chandrasekhara Rao *et al.* (2014). The starch content increased significantly with increase in leaf position from bottom to middle on the stalk and decreased from middle to top position. It was also reported that with increase in nitrogen from 120 to 200 kg/ha, the starch content decreased and the lowest content was observed in 200 kg N/ha (Chandrasekhara Rao *et al.*, 2013). In FCV tobacco with starch content below 5% is regarded as a good quality character.

Nicotine content varied from 2.89 to 5.13 % among the leaf positions and nitrogen levels (Table 2). Nicotine content decreased with increase in nitrogen levels from 300 to 1000 kg/ha and at each leaf position. Nicotine content increased significantly with increase in leaf position from bottom to top. Top position leaves contained 21 and 36.6% higher levels of nicotine compared to middle and bottom leaves, respectively. The nicotine content decreased by 10.9% in the top leaves with increase in nitrogen levels from 300 to 1000 kg/ha. The interaction between nitrogen levels and leaf position was significant. Reducing sugars (RS) content varied from 0.46 to 1.41% among the leaf positions and nitrogen levels (Table 2). RS content increased significantly with increase in the leaf position from bottom to top. Top leaves contained 1.78 and 2.07 times higher levels of RS over middle and bottom positions respectively. Except in the middle position, RS content decreased with increased levels of N. Chloride content varied from 0.23 to 0.47% among the leaf positions and nitrogen levels. Chloride content decreased with increase in nitrogen levels (Table 2). Top leaves showed maximum chlorides compared to the lower position leaves. In FCV tobacco, generally, carbohydrates fraction was more (reducing sugars; 8.6 – 27.0%) when compared to the air-cured burley tobacco, which contains lower levels of free sugar. Nicotine, sugars, sugar/nicotine and chlorides in the leaf are important chemical quality parameters in FCV tobacco.

The major polyphenols in tobacco are chlorogenic acid and rutin which play an important role in the quality of tobacco. Chlorogenic acid content increased by 17.68% with increased levels of nitrogen from 300 to 1000 kg/ha (Table 3). Top position leaves contained significantly higher chlorogenic acid content

when compared to bottom and middle leaves. Chlorogenic acid content in the top leaves was 36.79 and 40% higher than the middle and bottom leaves, respectively. Rutin content increased significantly (14.76%) with increase in nitrogen application from 300 to 1000 kg/ha (Table 3). There were no specific trends in the rutin content among the leaf positions and rutin content was significantly higher in the top leaves compared to the middle and bottom leaves at 1000 kg N/ha. Top position leaves showed significantly higher levels of phenols compared to middle and bottom position leaves. Phenolic constituents in the tobacco are affected by genotypes, increase in fertilizers, curing method and leaf position on the stalk (Siva Raju *et al.*, 2003; Chandrasekhara Rao *et al.*, 2013). Lower levels of polyphenols are preferred in FCV tobacco as more attention has been diverted towards their role as precursors of dihydroxybenzene compounds of tobacco smoke

Table 2: Effect of nitrogen and leaf position on nicotine, RS and chlorides in *Lanka* tobacco

Leaf position	Nicotine (%)			Reducing sugars (%)			Chlorides (%)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	5.13	4.57	5.13	1.41	0.81	1.11	0.47	0.32	0.39
Middle	4.25	3.82	4.03	0.46	0.78	0.62	0.31	0.23	0.27
Bottom	3.62	2.89	3.25	0.57	0.50	0.54	0.45	0.27	0.36
Mean	4.52	3.76		0.813	0.70		0.41	0.27	
	SEm(±)CD (P=0.05)			SEm(±) (P=0.05)			SEm(±) CD (P=0.05)		
Nitrogen	**0.04**	**0.12**		**0.05**	**0.16**		**0.06**	**0.18**	
Leaf position	**0.11**	**0.36**		**0.02**	**0.06**		**0.15**	**0.48**	
Nitrogen x Leaf position	**0.16**	**0.51**		**0.03**	**0.09**		**0.02**	**0.07**	

Table 3: Effect of nitrogen levels and leaf position on phenols and nitrate nitrogen in *Lanka* tobacco

Leaf position	Chlorogenic acid (mg/g)			Rutin (mg/g)			Nitrate nitrogen (mg/g)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	3.02	5.08	4.05	7.60	8.92	8.26	2.52	4.60	3.56
Middle	2.72	2.40	2.56	6.94	8.26	7.60	2.45	3.63	3.04
Bottom	2.56	2.30	2.43	7.59	8.23	7.91	1.46	3.35	2.40
Mean	2.77	3.26		7.38	8.47		2.14	3.86	
	SEm± CD (P=0.05)			SEm(±) CD (P=0.05)			SEm± CD (P=0.05)		
Nitrogen	**0.03**	**0.14**		**0.107**	**0.35**		**0.05**	**0.18**	
Leaf position	**0.08**	**0.29**		**0.117**	**0.36**		**0.19**	**0.62**	
Nitrogen x Leaf position	**0.1**	**0.36**		**0.17**	**0.49**		**0.03**	**0.09**	

(Snook and Schlolzheuer, 1988). Catechol, one of the most potent co-carcinogens found in cigarette smoke condensate is a major pyrolitic product of chlorogenic acid.

Nitrate nitrogen content increased significantly with increase in nitrogen levels and leaf positions from bottom to top on the stalk (Table 3). The maximum content of nitrate nitrogen (4.60 mg/g) was in the top position leaves at 1000 kg N/ha. Nitrate nitrogen content increased by 82.53, 48.16 and 129.45% respectively in top, middle and bottom position leaves with increase in nitrogen, levels from 300 to 1000 kg/ha. Wide variation in nitrate content of cured tobacco as influenced by genotype, cultural practices and curing method was reported (Siva Raju *et al.*, 2005). Nitrate nitrogen in tobacco has a great influence on the levels of tobacco specific nitrosamines. Chandrasekhara Rao *et al.* (2013) reported that nitrate nitrogen content increased with increase in nitrogen level up to 180 kg N/ ha in all leaf positions whereas there was a marginal decrease at 200 kg N/ha in FCV tobacco. Raja Rao and Suryanarayana (1988) reported accumulation of higher levels of nitrate nitrogen in air-cured burley tobacco genotypes and it was considered to be a genetic factor.

Petroleum ether extractives (PEE) content varied from 6.02 to 9.43% among the leaf positions and nitrogen levels (Table 4). With increased application of nitrogen from 300 to 1000 kg/ha, the PEE content decreased by 10.4%. Top and middle position leaves contained significantly higher levels of PEE over bottom leaves. At each leaf position, the PEE content decreased non-significantly with increase in nitrogen levels. Acid value increased with increase in leaf position from bottom to top and decreased with increase in nitrogen levels from 300 to 1000 kg/ ha (Table 4). Chandrasekhara Rao *et al.* (2013) reported increase in PEE content with increased application of nitrogen from 40 to 120 kg/ha and decreased with further increase in nitrogen levels. Siva Raju and Sarala (2013) reported lower levels of crude lipids in bottom leaves which increased with increase in leaf position on the stalk (bottom to top) and higher levels of PEE are positively correlated with aroma in FCV tobacco.

Proline content varied from 0.99 to 2.88 mg/ g among the leaf positions and nitrogen levels (Table 4). Proline content decreased significantly (79%) with increase in nitogen levels from 300 to 1000 kg/ha whereas, an increase with increase in leaf position from bottom to top was observed. Top position leaves contained significantly higher levels of proline compared to middle and bottom leaves and the middle position leaves contained significantly higher levels over the lower leaves. In FCV tobacco an increase in proline content

Table 4: Effect of nitrogen and leaf position on PEE, acid value and proline in *Lanka* tobacco

Leaf position	PEE (%)			Acid value (%)			Proline (mg/g)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	9.43	8.36	8.89	0.235	0.167	0.201	2.88	1.19	2.04
Middle	8.48	7.51	7.99	0.151	0.162	0.157	1.78	1.14	1.46
Bottom	6.25	6.02	6.13	0.133	0.151	0.142	1.25	0.99	1.12
Mean	8.05	7.29		0.173	0.16		1.97	1.10	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Nitrogen	**0.005**	**0.015**		**0.02**	**0.06**		**0.12**	**0.37**	
Leaf position	**0.35**	**1.04**		**0.001**	**0.004**		**0.21**	**0.68**	
Nitrogen x Leaf position	**0.49**	**1.36**		**0.002**	**0.005**		**0.03**	**0.09**	

with increase in nitrogen application from 40 to 120 kg/ha and with increase in leaf position on the stalk from bottom to top was reported (Chandrasekhara Rao *et al.*,2013). Transformation of leaf proteins into free amino acids and ammonia during curing contribute significantly to tobacco quality. Higher levels of free amino acids are preferred in FCV tobacco as they react with free sugars at high temperatures to form Amadori compounds which are responsible for flavour.

Thus in the present study, leaf position and nitrogen levels showed variation in the chemical and biochemical quality constituents in *Lanka* tobacco. In general, the total quality constituents were more in 300 kg N/ha treatment when compared to 1000 kg N/ha. Middle and top position leaves showed nearly similar contents of quality constituents compared to bottom position leaves. Chemical constituents which are positively related to quality (nicotine, RS, starch, proline, and PEE) decreased whereas constituents negatively related to quality (chlorophyll a, chlorogenic acid, rutin and nitrate nitrogen) increased with an increase in nitrogen level from 300 to 1000 kg/ha. All the quality constituents increased with increase in leaf position from bottom to top except rutin. The recommendation of 300 kg N/ha for *Lanka* tobacco was found to be optimum for higher yields with maximum quality constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).

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INFLUENCE OF INSECTICIDE FORMULATIONS AND DISCHARGE RATE ON SPRAY CHARACTERISTICS AND INCIDENCE OF MAJOR INSECT PESTS ON FCV TOBACCO

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Investigations were conducted to determine the influence of insecticide formulations on spray characteristics and insect pest incidence in a replicated field trial on flue-cured Virginia (FCV) tobacco during 2013-15. Spray characteristics indicated that at 25 days after planting (DAP), imidacloprid 200 SL emitted through Hi-tech sprayer at a discharge rate of 450 ml/min at 40 PSI pressure with a walking speed of 3.6 - 4 kmph were superior with higher droplet density (67/sq cm), lower uniform coefficient (1.51) and higher coverage (0.66) on plant canopy. At 45 DAP, spraying with novaluron 8.8 SC, 450 ml/min through Hi-tech sprayer exhibited superior spray characteristics of higher droplet density (62), lower uniform coefficient (1.52) and higher coverage (0.65). The treatment comprising initial two sprays with imidacloprid 200 SL and subsequent two sprays with novaluron 8.8 SC applied through Hi-tech sprayer at a discharge rate of 450 ml/min at 40 PSI showed lower uniform coefficient and higher spray coverage on plant canopy which resulted in significant reduction in infestation of aphid, leaf eating caterpillar and budworm with higher cured leaf yield.

Key words: FCV tobacco, Insecticide formulations, Insect pests, Spray characteristics

INTRODUCTION

Success in pest management depends not only on the choice of the insecticide and time of application but also on the type of formulation aids *viz.* solvents, emulsifiers, dispersants, wetting agents, stickers and softeners, and method of application, which can have a profound influence on the performance of active material. Increased coverage and uniform deposition will results in significant reduction in pest infestation. The physical properties of the spray liquid have a substantial effect on spray formation such that changes in formulation type can give changes in spray characteristics that would be equivalent to doubling the flow rate through conventional hydraulic flat fan nozzles (Miller and Ellis, 2000). In the present investigation, attempts were made to study the impact of dicharge rate on spray characteristics and incidence of aphid, *Myjus nicotianae*, leaf eating caterpillar, *Spodoptera litura* and budworm, *Helicoverpa armigera* on FCV tobacco.

MATERIALS AND METHODS

Field experiment was conducted to determine the influence of insecticide formulations on spray characteristics and insect pest incidence on FCV tobacco with imidacloprid (70 WG and 200 SL @ 0.005%) and novaluron (8.8 SC and 10 EC @ 0.01%) during 2013-15. They were evaluated by applying through Hi-tech and compression sprayers at a nozzle discharge rate of 250 and 450 ml/min at 40 PSI with an operator speed of 3.6 to 4 kmph. The experiment comprised of three replications and nine treatments (Table 1). Spray spectrum emitted through the sprayers was collected on photographic paper strips of 5 x 2 cm size on leaf surface at top, middle and bottom canopies of tobacco plant and were analysed using an image analyser with Prog. Res CT3 soft- ware. Spray characteristics *viz.*, droplet density/ cm^2, number median diameter (NMD), volume median diameter (VMD), uniform coefficient (UC) and spray coverage were computed. Spray characteristics were determined at 25 and 45 DAP. Need based sprays were applied for the management of insect pest infestation. Observations on aphid infestation was recorded at 35 DAP, whereas leaf eating caterpillar, *S. litura* and budworm, *H. armigera* infestation was recorded at 45 and 60 DAP.

RESULTS AND DISCUSSION

Influence of insecticide formulations and discharge rate on spray characteristics

Spray characteristics indicated that at 25 DAP, imidacloprid 200 SL emitted through Hi-tech sprayer, at a discharge rate of 450 ml/min (T8) was characterized by superior spray characteristics of higher droplet density (67 per cm^2), lower uniform coefficient (1.51) and higher coverage on plant canopy (0.66) as against of lower droplet density (49/cm^2), higher uniform coefficient (2.39) and lower coverage (0.42) in control (T9). The ratio between NMD and VMD is an indicator of the range of size, thus more uniform the size of droplets, nearer is the ratio to unity. In contrast, imidacloprid 70 WG emitted through compression sprayer at a discharge rate of 250 ml/min through compression sprayer (T1)

Table 1: Treatments

Treatments	
T1	Initial 2 sprays with imidacloprid 70 WG and subsequent 2 sprays with novaluron 8.8 SC @ 250 ml/min through compression sprayer,
T2	Initial 2 sprays with imidacloprid 70 WG and subsequent 2 sprays with novaluron 8.8 SC @ 450 ml/min through compression sprayer
T3	Initial 2 sprays with imidacloprid 200 SL and subsequent 2 sprays with novaluron 10 EC @ 250 ml/min through compression sprayer
T4	Initial 2 sprays with imidacloprid 200 SL and subsequent 2 sprays with novaluron 10 EC @ 450 ml/min through compression sprayer
T5	Initial 2 sprays with imidacloprid 70 WG and subsequent 2 sprays with novaluron 10 EC @ 250 ml/min through Hi-tech sprayer
T6	Initial two sprays with imidacloprid 70 WG and subsequent 2 sprays with novaluron 10 EC @ 450 ml/min through Hi-tech sprayer
T7	Initial 2 sprays with imidacloprid 200 SL and subsequent 2 sprays with novaluron 8.8 SC @ 250 ml/min through Hi-tech sprayer
T8	Initial 2 sprays with imidacloprid 200 SL and subsequent 2 sprays with novaluron 8.8 SC @ 450 ml/min through Hi-tech sprayer
T9	Control

Table 2: Mean spray characteristics on plant canopy as influenced by insecticide formulations, discharge rate and sprayers

Treat-ments	Droplet density /cm^2		NMD		VMD		UC		Coverage	
	25 DAP	45 DAP	25 DAP	45 DAP	25 DAP	45 DAP	25 DAP	45 DAP	25 DAP	45 DAP
T1	32	44	137.4	175.5	383.1	394.8	2.79	2.25	0.35	0.44
T2	43	60	221.0	150.2	415.5	310.1	1.88	2.05	0.52	0.44
T3	29	42	192.5	161.2	421.4	393.6	2.19	2.44	0.45	0.48
T4	45	57	209.6	194.9	379.7	358.2	1.81	1.85	0.55	0.54
T5	38	45	145.7	168.2	303.2	385.1	2.08	2.29	0.48	0.44
T6	61	58	130.2	197.2	255.2	357.2	1.96	1.81	0.51	0.55
T7	30	39	131.9	322.4	249.8	382.5	1.88	1.92	0.53	0.45
T8	67	62	178.9	206.8	270.8	317.5	1.51	1.52	0.66	0.65
T9	49	42	201.6		480.3		2.39		0.42	

UC: Uniuform coefficient

showed inferior spray characteristics *viz.*, lower droplet density (32/cm^2), higher uniformity coefficient (2.79) and lower coverage (0.35) on plant canopy (Table 1). At 45 DAP spraying with novaluron 8.8 SC, 450 ml/min through, Hi-tech sprayer (T8) exhibited superior spray characteristics of higher droplet density (62), lower uniform coefficient (1.52) and higher coverage (0.65) and was superior over the rest of the treatments (Table 2). It may be mainly attributed to the increased droplet size with emulsions over other formulations evaluated. Butler Ellis and Tuck (1999) reported that sprays from hydraulic pressure nozzles operating with emulsions generally give sprays that are coarser than those produced when spraying surfactant solutions. Further, Butler Ellis *et al.* (1997) demonstrated that emulsions cause rapid fluid sheet disintegration with the formation of large droplets. Similarly, formulation effects were studied by Pasupathy and Venugopal (1986a) on cotton and reported that cypermethrin EC formulations applied through knapsack, mist blower and ULV sprayers showed higher mite population when compared to cypermethrin ED formulation. Further they reported that cypermethrin 3 ED @ 15 ng a.i. recorded very low resurgence followed by other ED formulations.

Influence of insecticide formulations and discharge rate on insect pest infestation

Infestation of aphid *M. nicotianae*: At 35 DAP, imidacloprid 200 SL at a discharge rate of 450 ml/min through Hi-tech sprayer (T8) showed significantly lower infestation (6.8%) and it was at a par with other treatments except imidacloprid 70 WG (T1) and 200 SL (T3) at a discharge rate of 250 ml/min through compression sprayer. It was evident from the results that application of imidacloprid 200 SL at a discharge rate of 450 ml/min through Hi-tech sprayer (T8) was effective in minimizing the aphid infestation (Table 3)

Infestation of *S. litura*: At 45 DAP, the infestation of *S. litura* was low (16.8%) in the plots treated with novaluron 8.8 SC at a discharge rate of 450 ml/min applied through Hi-tech sprayer (T8) as against 22.7% in control (T9). It was significantly lower than all the other treatments. The infestation was significantly higher in the plots treated at a discharge rate of 250 ml/min (Table 3).

Infestation of *H. armigera*: The infestation of *H. armigera* in different treatments varied significantly at 45 and 60 DAP. At 60 DAP, the infestation was significantly lower in the plots treated with novaluron 8.8 SC at a discharge rate of 450 ml/min applied through Hi-tech sprayer (T8) and differed from other treatments indicating the superior performance in minimizing the infestation (Table 3). Similar studies on influence of insecticide formulations and spray systems were conducted by Pasupathy and Venugopal (1986b) and reported that cymbush ED formulations were more effective than EC formulations applied through knapsack, mist blower and ULV sprayers for the control of bollworms *Earias vitella, H. armigera* and *Pectinophora gossypiella.* In the present findings, the treatment comprising initial two sprays with imidacloprid 200 SL and subsequent two sprays with novaluron 8.8 SC applied through Hi-tech sprayer at a discharge rate of 450 ml/min at 40 PSI showed lower uniformity coefficient and higher spray coverage on plant canopy which resulted in significantly lower infestation of aphid, leaf eating caterpillar and budworm.

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Table 2: Insect pest incidence as influenced by nozzle discharge rate

Treatment	Aphid infestation DAP (%)		*S. litura* infestation DAP (%)	*H. armigera* infestation DAP (%)	
	25	**35**	**45**	**45**	**60**
T1: 2 spr Imida 70 WG + 2 spr novaluron 8.8 SC - 250 cc/min-CS	4.2 (11.7)	3.6 (10.6)	11.9 (20.2)	6.5 (14.7)	8.9 (12.8)
T2 :2 spr Imida 70 WG + 2 spr novaluron 8.8 SC- 450 cc/min-CS	2.9 (9.8)	2.4 (8.7)	10.7 (19.1)	5.3 (13.2)	7.7 (12.7)
T3: 2 spr Imida 200 SL + 2 spr novaluron10 EC - 250cc/min-CS	2.4 (8.7)	4.2 (11.7)	11.9 (20.2)	7.7 (16.1)	9.5 (13.0)
T4: 2 spr Imida 200 SL + 2 spr nova10 EC - 450cc/min-CS	2.4 (8.7)	2.4 (8.7)	10.1 (18.5)	5.9 (14.1)	7.1 (12.5)
T5: 2 spr Imida 70 WG, + 2 spr novaluron -10 EC250 cc/min Hi- tech	4.2 (11.7)	2.9 (9.8)	8.3 (16.7	7.1 (15.5)	8.3 (10.1)
T6: 2 spr Imida 70WG + 2 spr noval. 10 EC –450 cc/min Hi-tech	4.1 (11.7)	2.4 (8.7)	8.3 (16.7)	4.2 (11.7)	5.9 (10.2)
T7: 2 spr Imida 200 SL + 2 spr noval8.8 SC -250cc/min-Hi-tech	2.9 (9.8)	3.0 (9.8)	10.7 (19.1)	6.5 (14.8)	3.1 (8.9)
T8: 2 spr Imida. 200 SL + 2 spr Noval. 8.8 SC - 450 cc/min Hi-tech	3.6 (10.6)	2.9 (6.8)	6.5 (14.8)	3.5 (10.9)	3.5 (5.3)
T9: Control	4.7 (12.5)	7.7 (16.1)	14.8 (22.7)	11.3 (19.6.)	13.5 (14.8)
SEm±	**1.05**	**1.12**	**0.66**	**0.66**	**0.68**
CD (P=0.05)	**NS**	**3.37**	**2.00**	**1.99**	**2.04**
CV (%)	**17.3**	**18.6**	**6.20**	**7.0**	**7.04**

Figures in parenthesis are arc.sin transformations

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EFFECT OF GENOTYPE AND LEAF POSITION ON PIGMENTS AND THEIR DEGRADATION DERIVATIVES IN BURLEY TOBACCO

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Leaf pigments and their derivatives play an important role in the colour and aroma of tobacco. Chemical and biochemical quality constituents of tobacco (*Nicotiana tabacum* L.) are influenced by leaf position on stalk, cultural practices, genotypes and curing methods. Leaf curing is an important and essential post-harvest process in tobacco production which is likely to have a bearing on leaf quality, particularly in terms of pigments. The present study, therefore, assessed the effects of genotype and leaf position on pigments and their derivatives in air cured burley tobacco. The pigments identified in cured burley tobacco leaf included chlorophylls, carotenoids, pheophytin, chlorophyllide, protoporphyrin and magnesium protoporphyrin. Total chlorophyll (Tchl) content varied from 50.9 to 130 µg/g among the leaf positions and varieties. Tchl content was significantly higher in the variety Banket A1 compared to YB4 and middle position leaves (3rd pick) showed significantly higher levels of Tchl compared to the lower and top position leaves. Carotenoid content varied from 131 to 199 µg/g among the leaf positions and genotypes. The variety YB4 showed higher levels of carotenoids but lower levels of more polar carotenoids (MPC) and less polar carotenoids (LPC) than variety Banket A1. The carotenoid content increased with increase in leaf position with exception at 2nd pick and top position leaves showed maximum carotenoids. The variety Banket A1 showed significantly higher levels of Pheophytin a (Phe a) compared to YB4. Pheophytin b content was 2.3 times higher in the variety Banket A1 compared to YB4. Variety Banket A1 showed higher levels of MgPP and protoporphyrin compared to variety YB4. Chlide a content between the two varieties ranged from 0.06 to 1.73 mg/g whereas chlide b content varied from 1.06 to 2.80 between the varieties and among the picks. The variety YB4 showed significantly higher content of Chlide b compared to Banket A1. Pheophorbide compounds were not detected in both the varieties. The end product of the chlorophyll degradation may be chlide in tobacco and the degradation of chlorophyll pigments after curing was greater in YB4 compared to variety Banket A1. Thus, the advanced breeding line YB4 showed lower levels of chlorophyll pigments indicating the higher breakdown which may be leading to the formation of higher levels of aroma compounds.

Key words: Breakdown compounds, Burley tobacco, Genotypes, Leaf position, Pigments

INTRODUCTION

Chemical and biochemical quality parameters responsible for aroma and quality of tobacco are influenced by manures, leaf position on stalk, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew, 1981). The chemical composition of the final product is strongly influenced, not only by genetic factors and growth conditions, but also by post harvest processing, eg. sun, air, smoke, flue, pit curing etc. The objective of curing the tobacco is to maintain and enhance the potential quality embodied in the harvested leaf and also to provide an environment conducive to the transformation of the leaf into a high quality cured tobacco product. This is achieved through control of the chemical and biochemical conversions and removal of moisture during curing. Burley tobacco, leaves are harvested by priming 3-4 leaves at a time when the leaves are matured and are shade cured. These processes lead to the creation of typical tobacco aroma and the most important part in the generation and transformation of various biochemical compounds. Air-curing is different from flue-curing as there is no control intemperature and moisture. Hence, formation of degradation products in tobacco during air-curing is entirely different from flue-curing. The rate of decline in all the leaf pigments, but especially

chlorophyll, which are observed during maturation of tobacco in the field are greatly accelerated during flue-curing (Long and Weybrew,1981). The breakdown products of pigments during the curing have been reported to give rise to numerous flavor components (Weeks, 1986). Burley, a light air-cured, cigarette tobacco type used for blending purpose in cigarette manufacture and is grown in Andhra Pradesh, Telangana, Karnataka and Orissa states in India. In the present paper, effect of leaf position and genotypes on the chlorophyll pigments and their degradation derivatives in burley tobacco reported.

MATERIALS AND METHODS

Cured leaf samples of variety the Banket A1 and the advanced breeding line YB4 were collected during 2012 season from experimental plots at Vinukonda. Leaf samples from different leaf position on the stalk (bottom, middle and top) were collected separately and the mid-ribs were removed and dried in the hot air oven at 60°C for 3 to 6 h, powdered and passed through 40 micron mesh sieve. Leaf powder (250 mg) was extracted with 5 ml of 80% acetone, centrifuged at 1,500 g for 5 min. The supernatant was separated and the absorbance was measured at 663.6, 646.6 and 440.5 nm, the major absorption peaks of chlorophyll a and b and carotenoids (Car), respectively. Content of the pigments was calculated using the equations of Porra *et al.* (1989) and Holm (1954), respectively and the values obtained were reported as µg/g dry weight.

Chl a =12.25 A663.6-2.55 A646.6 (µg/ml)
Chl b= 20.31 A646.6-4.91 A663.6 (µg/ml)
Chl a+b = 17.76 A646.6 +7.34 A663.6 (µg/ml)
Car = 4.69 A440.5 – 0.267Chl a+b (µg/ml)

Supernatant obtained was mixed with equal volume of hexane, vortexed and kept undisturbed until the interface appeared. The upper phase contains less polar compounds dissolved in hexane and the lower phase contains more polar compounds dissolved in acetone. The upper and lower phases were separated. The lower fraction was used to measure the absorbance at 575, 590 and 628 nm. The equation of Kahn *et al.* (1976) was used to estimate the content of protoporphyrin IX (PPIX) and magnesium-protoporphyrin IX (MgPP).

PPIX = 196.25 A575 - 46.6 A590 - 58.68 A628 (nmole)
MgPP = 61.81 A590 – 23.77 A575 - 3.55 A628 (nmole)

Chlorophyllide (Chlide a and b) content was estimated by the method of McFeeters *et al.* (1971) in acetone fraction at 667, 650 and 440.5 nm and more polar carotenoids (MP Car) were measured by the equation of Holm (1954) and Porra *et al.* (1989).

Chlide a = A667/74.9 (mM)
Chlide b = A650/47.2 (mM)
MP Car = 4.69 A440.5 – 0.267 Chl a+b (µg/ml)

The upper hexane fraction was dried with nitrogen and the pellet was dissolved in 80% acetone. The chlorophyll molecules were destroyed with 50 µl of 12.5% HCl. The absorbance was measured at 665.4, 653.4 and 470 nm which are the major absorption peaks of pheophytin a, b and less polar carotenoids (LP Car), respectively. The content of pheophytin a, b and LP Car were calculated according to the equation of Lichtenthaler (1987)

Phe a = 22.42 A665.4 – 6.81 A653.4 (µg/ml)
Phe b = 40.17 A653.4 – 18.58 A665.4 (µg/ml)
LP Car = (1000 A470 – 4.28 A665.4 – 4.78 A653.4)/ 164 (µg/ml)

RESULTS AND DISCUSSION

The total chlorophyll (TChl) content varied from 43.57 to 130 µg/g among the leaf positions and varieties (Table 1). The total chlorophyll content significantly increased with increase in leaf position (3rd pick) and decreased with further increase in leaf position. The variety Banket A1 showed maximum Chl compared to the variety YB4. The variety Banket A1 showed maximum total chl (130 µg/g) in 3rd pick compared to other picks in both varieties. Chlorophyll a (Chl a) varied from 20.13 to 50.13 µg/g among the picks and varieties (Table 1). The 3rd pick showed maximum Chl a content in both the varieties. Chl a content increased significantly with increase in leaf position from 1st to 3rd pick and decreased with increase in leaf position on the stalk. Banket A1 showed significantly higher contents of Chl a compared to YB4. The Chl a content was at a par

Table 1: Effect of genotype and leaf position chlorophyll pigments in burley tobacco (µg/g)

Pick	Chl a		Mean	Chl b		Mean	Total chl		
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	22.84	20.82	21.83	29.73	22.52	26.12	56.85	43.57	50.21
2	27.79	34.42	31.11	31.81	44.03	37.92	51.25	81.45	66.35
3	29.93	50.13	40.03	44.26	58.80	51.53	51.58	130.00	91.16
4	27.43	30.80	29.11	31.81	44.98	38.39	50.93	77.20	64.06
6	20.13	23.46	21.80	31.60	28.80	30.20	68.86	50.53	59.70
Mean	25.62	31.93		33.84	39.82		55.89	76.69	
	SEm±	CD (P=0.05)		SEm±	CD (P=0.05)		SEm±	CD (P=0.05)	
Varieties	**1.04**	**3.10**		**1.06**	**3.15**		**1.6**	**4.8**	
Pick	**1.65**	**4.90**		**1.60**	**4.90**		**2.5**	**7.6**	
Varieties x pick	**2.30**	**6.94**		**2.30**	**7.04**		**3.65**	**10.8**	

among 2, 3 and 4th picks of YB4 whereas there was a significant variation among the leaf position of Banket A1. The chl b content significantly increased with increase in leaf position from 1st to 3rd pick and thereafter it decreased with increase in leaf position (Table 1). In both the varieties the chl b content was increased upto 3rd pick and decreased with further increase in leaf position. The variety Banket A1 showed 17.67% higher chl b content compared to YB4.

Chlide a content between the two varieties ranged from 0.53 to 1.73 µg/g whereas chlide b content varied from 1.06 to 2.80 µg/g between the varieties and among the picks (Table 2). Both Clide a and Chlide b contents increased up to 3rd pick and decreased on further increase in leaf position on the stalk. The variety YB4 showed significantly higher content of Chlide b compared to Banket A1 (Table 2). PPIX content varied from 16.98 to 33.46 µg/g and there was no variation between the varieties and among the picks 1, 2 and 4 (Table 2).

Pheophytin a (Phe a) content increased significantly with increase in leaf position from bottom to middle position (1st to 3rd pick) and decreased with further increase in leaf position (Table 3). The middle position leaf contained significantly higher content of Phe a compared to other leaf positions. The variety Banket A1 showed significantly higher content of Phe a compared to YB4. The middle position leaves of Banket A1 contained significantly higher level of (34.5 µg/g) Phe a compared to other leaf positions in both the varieties (Table 3). The pheophytin b (Phe b) content also increased significantly with increase in leaf position from bottom to middle and decreased with further increase in leaf positions (Table 3). Middle position leaves contained significantly higher content of Phe b. Banket A1 showed 2.3 times higher Phe b compared to YB4. The middle position leaves of Banket A1 showed maximum and significantly higher content of Phe b over the other leaf positions in both the varieties.

MgPP content varied from 2.99 to 6.99 µg/g between the varieties and among leaf positions (Table 3). The variety Banket A1 showed significantly higher levels of MgPP compared to variety YB4. MgPP content increased significantly with increase in leaf position i.e. from Ist to 3rd pick and thereafter it decreased significantly with increase in leaf position.The variety YB4 showed significantly higher levels of MgPP copared to Banket A1 in each pick except 6th pick.

The carotenoid content varied from 131 to 199 µg/g among the leaf positions and varieties (Table 4). Carotenoid content increased with increase in leaf position with exception at 2nd pick and top leaf (6th pick) showed maximum content of carotenoids. The variety YB4 showed non-significantly higher levels of carotenoids over the variety Banket A1. The more polar carotenoids (MPC) content decreased with increase in leaf

Table 2: Effect of genotype and leaf position on chlide and protoporphirin in burley tobacco (µg/g)

Pick	Chlide a		Mean	Chlide b		Mean	PPIX		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	0.86	0.86	0.86	1.60	1.60	1.60	24.04	23.30	23.67
2	1.20	1.00	1.10	2.13	1.46	1.80	24.00	22.65	23.47
3	1.73	1.26	1.50	2.80	1.70	2.25	28.95	33.46	30.88
4	0.80	0.86	0.83	1.53	1.56	1.55	25.30	26.95	26.13
6	0.60	0.53	0.56	1.13	1.06	1.10	16.98	20.31	18.64
Mean	1.04	0.90		1.84	1.48		23.72	25.39	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Varieties	**0.07**	**NS**		**0.1**	**0.3**		**0.59**	**NS**	
Pick	**0.11**	**0.34**		**0.16**	**0.49**		**0.9**	**2.7**	
Varieties x pick	**0.16**	**NS**		**0.23**	**NS**		**1.32**	**NS**	

Table 3: Effect of genotype and leaf position on MgPP and pheophytin in burley tobacco (µg/g)

Pick	MgPP		Mean	Phe a		Mean	Phe b		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	4.47	3.21	3.84	7.81	22.1	14.98	8.39	17.32	12.85
2	5.32	3.64	4.48	9.29	23.3	16.31	10.52	19.73	15.13
3	6.99	5.44	6.21	10.4	34.5	22.53	11.06	24.70	17.88
4	4.70	4.65	4.68	8.20	18.9	13.56	7.73	17.10	12.42
6	2.99	3.23	3.10	5.20	20.6	12.93	3.06	14.93	9.00
Mean	4.89	4.03		8.19	23.9		8.15	18.76	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Varieties	**0.15**	**0.46**		**0.5**	**1.6**		**0.37**	**1.11**	
Pick	**0.07**	**0.24**		**0.8**	**2.6**		**0.59**	**1.77**	
Varieties x pick	**0.57**	**1.7**		**1.2**	**3.6**		**0.84**	**2.50**	

position from bottom to top (Table 4). The bottom position leaf (1st pick) showed significantly higher levels of MPC compared to upper position leaves. The variety Banket A1 showed significantly higher levels of MPC compared to YB4. The interaction effect between leaf positions and varieties was non-significant.

Less polar carotenoids (LPC) content decreased with increase in leaf position from bottom to top (1st to 6th pick) (Table 4). LPC content was at a par among the first 3 picks and was significantly higher than the other two picks. The variety Banket A1 showed 93.3% higher levels of LPC compared to the variety YB4. In both the varieties, the bottom position leaves showed maximum and significantly higher content of LPC with exception of 3rd pick of Banket A1. The content of MPC was higher than that of LPC.

Chlorophyll pigments offer an all-oriented network of interception, reflection and protection for plant body, allowing plant to use sunlight and cut off its photodamage. The biosynthesis of plant pigments are very complex processes and more than fifteen biochemical reactions are involved in the biosynthesis of chlorophyll originated from precursor glutamate and eight steps in the degradation pathway from chlorophyll to pheophorbide (Reinbothe and Reinbothe, 1996).

Table 4: Effect of genotype and leaf position on carotenoid in burley tobacco (µg/g)

Pick	Carotenoids		Mean	Less polar carotenoids (LPC)		Mean	More polar carotenoids (MPC)		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	162	155	158	32.4	74.3	53.3	137.6	160.0	148.8
2	140	131	135	30.4	53.2	41.8	124.0	154.3	139.1
3	197	176	186	26.8	60.0	43.4	117.0	144.6	130.8
4	199	190	195	18.9	37.2	28.0	125.6	130.6	128.1
6	199	199	199	18.4	21.0	19.7	99.6	118.6	109.1
Mean	179	170		25.4	49.1		120.8	141.6	
	Em±	CD (P=0.05)		SEm±	CD (P=0.05)		SEm±	CD (P=0.05)	
Varieties	**2.5**	**7.5**		**0.59**	**NS**		**3.0**	**8.9**	
Pick	**4.0**	**11.9**		**0.9**	**2.7**		**4.7**	**14.0**	
Varieties x pick	**5.6**	**NS**		**1.32**	**NS**		**6.7**	**NS**	

In tobacco, leaf pigments play an important role in the quality and colour of tobacco as the chlorophyll pigments were degraded maximum leading to the formation of aroma compounds. The changes of chloroplast pigment and contents of degradation products were studied in leaves at different maturities in flue-cured tobacco (Zhao *et al.*, 2009) whereas Zhao *et al.* (2010) reported the effect of irrigation and fertilizers on degradation of chromoplast pigments in FCV tobacco. In the present study, pigments identified included chlorophylls, carotenoids, pheophytin, chlorophyllide, protoporphyrin and magnesium protoporphyrin. The variety YB4 showed significantly lower levels of chlorophyll pigment and higher levels of carotenoids compared to the variety Banket A1. It may be due to the genetic character of variety YB4. The tobacco varieties with higher contents of carotenoids and their degradation compounds showed a positive correlation with tobacco flavour (Wei *et al.*, 2005).

A number of factors are involved during the curing process including genetic traits which makes one of the important aspects in production of flavored tobacco. Therefore, genotypes have their own variables which influence the final composition of the cured leaf. The major pigments in green tobacco are chlorophyll a, chlorophyll b, lutein, â-carotein, neoxanthine and violaxanthin. Degradation of chlorophylls during yellowing stage of flue-curing follows a first-order reaction but during air-curing there was no control of the temperature and humidity which makes a difference between the flue-cured and air cured tobacco quality. Court and Hendel (1982) reported that chlorophyll a and b concentrations were about 1% of the amount measured at harvest in flue-cured tobacco. Siva Raju and Krishnamurthy (2011) and Siva Raju *et al.* (2012) reported that the pigment contents were higher in smoke and pit-cured chewing tobacco compared to air-cured and flue-cured tobacco.

The degradation products of carotenoids include a number of aroma compounds in tobacco. Total amount of pigment degraded and aroma compounds in the leaf are affected by the type of curing (Shi *et al.*, 2012). Different genotypes had different pigment degradation products and had significantly positive correlations with the contents of volatile degraded aroma compounds in cured leaves, while there were no negative correlations with the contents of pigment residue in cured leaves (Ma *et al.*, 2006). The results revealed that the end product of the chlorophyll degradation may be chlide in tobacco and the degradation of chlorophyll pigments was greater in the variety YB4 compared to Banket A1 as indicated by the lower levels of pigments. Thus, the advanced breeding line YB 4 showed lower levels of chlorophyll pigments indicating higher

breakdown which may be leading to the formation of higher levels of aroma compounds.

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SMALLHOLDER FAMILY FARMING OF FCV TOBACCO IN KARNATAKA – A SOCIO-ECONOMIC EVALUATION

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Livelihood security for the smallholders practicing family farming continues to be a cause of concern in India. Small and marginal farmers (with farm size < 2 ha) account for more than 80% of total farms in India and grow crops in complex and risk prone situations. The Flue-cured Virginia (FCV) tobacco, grown predominantly as a rainfed crop, has been the main source of livelihood for umpteen smallholders in the state of Karnataka. Mysore district of the state, accounting for 84% of FCV tobacco area with an average holding size of 0.7 ha, against the state's average holding size of 0.81 ha, leads in socio-economic development indicators compared to neighbouring non-tobacco districts. Against this backdrop, socio-economic contribution of smallholder family farming of FCV tobacco was critically analyzed. The relevant data were collected through secondary sources and analyzed. Comparative economic evaluation revealed that no crop/cropping system is as remunerative as FCV tobacco crop under rainfed farming situations of Karnataka Light Soils (KLS). Further, FCV tobacco generated remarkably greater number of mandays of employment due to which family labour assumes importance. The cultivation of FCV tobacco has led to an improvement in wealth of Mysore district and depicted significant increase by 3% in 3-5 room houses, 23% in standard bathrooms, 12% in standard kitchens, 8% in television sets and 5% in two wheelers compared to that of non-tobacco district, Chamarajnagar. Literacy rate and social indicators *viz.*, water and sanitation were high in FCV tobacco growing district. This study illustrates that FCV tobacco, a non-food cash crop, can have a huge transformational impact on livelihood security and socio-economic well-being of small holder family farmers in Karnataka.

Key words: FCV tobacco, Karnataka, Socio-economic evaluation

INTRODUCTION

In India, about 65% of the population is living in rural areas and over 85% of them are dependent on agriculture and allied activities for their livelihood. Out of the total 129.22 million landholders in the country, small and marginal farmers (with farm size <2 ha) account for 83.3%. Smallholder farmers are generally those who practice farming using family labour and for whom the farm provides the principal source of income (Ellis, 1988). Small holders in India have been growing crops in situations where the rain fall is scanty and erratic and their holdings constitute 84.97% of the total holdings covering 44% of the total operational area.

Tobacco is one of the important commercial crops grown on 0.46 million ha in the country and is valued more for its potential to generate farm income, employment and revenue. Tobacco crop is grown predominantly by smallholders for reasons ranging from economic, social and strategic. Tobacco crop directly or indirectly supports 38 million people (ASSOCHAM- India, 2014) which includes 6 million farmers and 20 million farm labour, and contributes over Rs. 25000 crores to the national exchequer through foreign exchange earnings and internal excise taxes. FCV tobacco crop is grown on soil of poor fertility, drought prone areas mostly as a rainfed crop and can withstand variations in weather conditions better than other crops especially in the states of Andhra Pradesh and Karnataka. Further, FCV tobacco farmers are getting timely advice from stake-holders on meteorological, marketing & management information and about investments to improve resource use efficiency.

FCV tobacco crop is predominantly grown on light textured soils under rainfed conditions in Mysore and Hassan districts of Karnataka. The FCV tobacco grown in Karnataka Light Soils (KLS)

represents superior quality with high export potential and is regarded as quality filler in global market. The Federation of Karnataka FCV Tobacco Growers Association claimed that the tobacco crop was the main source of livelihood in Mysore district and the crop contributes significantly to rural households' income both as profits and as wages and transformed the lives of farmers in this district.

Against this backdrop, socio-economic contribution of smallholder family farming of FCV tobacco in the state of Karnataka was critically analyzed with the objectives: 1. To analyse the economic contribution of FCV tobacco vis-à-vis other crops. 2. To study the impact of FCV tobacco farming on livelihood security and the overall development of the farmers.

MATERIALS AND METHODS

The study was conducted using the experimental design with control and can be illustrated as: Effect of FCV tobacco crop (X) = Indicators of FCV tobacco growing district (Y) – Indicators of non-tobacco district (Y_1). Selected one predominantly FCV tobacco growing district, one comparable adjacent non-tobacco growing district and one district growing different crops with assured irrigation facility in the state of Karnataka. Districts were selected based on compatibility criteria *i.e.* area under FCV tobacco and other comparative crops, dependency on monsoon, irrigation potential, soil condition and returns generated. Respondents were selected from different stake-holders for interaction and elicitation of opinion and to explore the situation of economic, social and development indicators among the selected districts. Data pertaining to returns for FCV tobacco and selected crops were collected from published sources. Quantitative data used for the study were collected from the reports of Directorate of Economics and Statistics. Qualitative data were collected from 100 farmer respondents through in depth interviews and focussed group discussions. Wealth indicators were measured considering inclusive wealth index. The relevant data were analyzed using appropriate statistical methods.

RESULTS AND DISCUSSION

FCV tobacco - A Small holder family farming

Source: Agricultural Census of Karnataka, 2011

Average holding size in Mysore (0.70 ha) and Hassan (0.74 ha) districts was lower than that of Karnataka State (0.81 ha) by 13.58 and 8.64% respectively. Data showed that the size of land holdings in FCV tobacco growing districts is perceptibly lower than that of non-tobacco districts which clearly indicated that the FCV tobacco is a smallholder family farming.

Comparative economic analysis of FCV tobacco and other major crops

Though, there is a call for substitution of FCV tobacco with other crops, economic feasibility of comparative crops is the key to induce tobacco farmers to shift from tobacco cultivation. If alternative crops to FCV tobacco are to be considered, there is a need to look at wider livelihood perspective that includes not only the income provision from a crop but also the institutional support *i.e.* market, extension and credit support. In this backdrop, analysed the published data on comparative economics of FCV tobacco vis-à-vis other crops in KLS.

The data presented in Table 2 revealed that the net returns accrued from FCV tobacco (Rs.36,759/ha) was higher than that of other crops /cropping systems including chillies (Rs.19,415/ ha), cotton (Rs. 16, 413/ha), cotton + soybean (Rs. 20,527/ha) and maize + red gram (Rs.14,299/ha) (Mahadevaswamy *et al.*, 2006). The net returns accrued from various sole crops, maize (Rs.7430/ ha), chillies (Rs. 15,080/ha), groundnut (Rs.8530/ ha), redgram (Rs.4730/ha), cotton (Rs. 9040/ ha) and french bean (Rs.13,410/ha) were not

Table 1: Average holding size in FCV tobacco growing districts vis-à-vis Karnataka

Place	Small holder farms	Area	Av. holding size
Hassan Dist.	387820	286924	0.74
Mysore Dist.	346555	243377	0.70
Karnataka State.	5987042	4870947	0.81

comparable to sole crop of FCV tobacco (Rs.19,720/ha). (Dineshkumar *et al.*, 2010). A study conducted in Mysore district of Karnataka which accounts for 84% of FCV tobacco in the state revealed that the net returns obtained from FCV tobacco was Rs.62,000–90,000/ha and greater than that of other substitute crops *viz.,* cotton (Rs.30,000–35,000/ha), chillies (Rs. 28,000 - 38,000/ha), maize (Rs. 25,000 - 35,000/ha) and finger millet (Rs.8,000 - 10,000/ha) (Ranganadhan, 2014). Comparative analysis of FCV tobacco district and non-tobacco district revealed that the value of money generated from ha of FCV tobacco (Rs. 82,081/ha) outscored that of groundnut (Rs.21,416/ha) and finger millet (Rs. 14,355/ha) by 382 and 572%, respectively. The net returns per unit of land are much higher in FCV tobacco (DES, 2011). Under rainfed conditions, the net returns obtained are Rs.14,522/ha (chickpea), Rs.5,967/ha (rice), Rs.13,845/ha (soybean), Rs.17,099/ha (maize), Rs. 31,885/ha (chillies) and Rs. 35,165/ha (cotton). While, in irrigated situation, the net returns are Rs. 25,062/ha (maize), Rs.52,888/ha (chillies) and Rs.50,713/ha (cotton). Further, man days of employment generated per hectare are, cotton (112), chillies (101), maize (70) and soybean (59) under rain fed situation (Lakshmi and Mundinamani, 2014).

From the above data, it was concluded that no crop/cropping system was as remunerative as FCV tobacco crop under rainfed farming situations

Table 2: Comparative economics of various crops in KLS tobacco growing region

Crops	Gross returns (Rs/ha)	Cost of cultivation (Rs/ha)	Net returns (Rs/ha)	Reference
Maize-Red gram	35,347	21,048	14,299	Mahadevaswamy *et al.*, 2006
Ground nut+ Red gram-Fallow	27,960	15,166	12,794	
Ragi-Red gram	32,310	19,511	12,799	
Cotton-Fallow	34,333	17,920	16,413	
Cotton+ Soybean-Fallow	41,140	20.613	20527	
Green chillies-Fallow	43,315	23,900	19,415	
Bajra+ Red gram- fallow	24,560	9924	14636	
FCV tobacco- Fallow	79373	42614	36759	
Tobacco	43650	23930	19720	Dinesh Kumar *et al.*, 2010
Chillies	28930	13850	15080	
Groundnut	25180	16650	8530	
French bean	29650	16240	13410	
Maize	19460	12030	7430	
Red gram	17430	12700	4730	
Cotton	25530	16490	9040	
Maize	40,000-50,000	15,000	25-35,000	Ranganadhan, 2014
Ragi	18,000-20,000	10,000	8-10,000	
Field bean	25,000-30,000	8,000	17-25,000	
Chillies	50,000-60,000	22,000	28-38,000	
Cotton	45,000-50,000	15,000	30-35,000	
Paddy	30,000-35,000	12,000	18-23,000	
Tobacco	1,37,500-1,65,000	75,000	62-90,000	
Chickpea	39733	25181	14552	Lakshmi and Mundinamani, 2014
Rice	28596	22630	596725	
Soybean	33170	19325	13845	
Maize	38609	21509	17100	
Chillies	61715	29830	31885	
Cotton	63344	28178	35165	

of KLS. Further to state, currently no economically viable alternative crop is available which can be grown under similar climatic conditions and which provides similar socio-economic return as FCV tobacco for farmer. FCV tobacco gives higher returns to the farmers and thus, ensuring a better standard of living. In view of the requirement of more mandays of work in FCV tobacco cultivation, family labour assumes importance in FCV tobacco growing areas. FCV tobacco with well organised marketing system raised farmers' income, improved their economic situations and ultimately helped the farmers to increase house hold food security.

Impact of FCV tobacco on livelihood security of farmers

In the background of available information discussed above, a modest attempt has been made in this study to examine the relative socio-economic development situation in tobacco and non-tobacco growing districts. The basic civil facilities are determining factors for development of a district. For this study, one predominantly FCV tobacco growing district (Mysore), one comparable adjacent non-tobacco growing district (Chamrajnagar) and a district with assured irrigation facility (Mandya) were selected.

All the wealth indicators (Table 3) are significantly conducive in the study domain *i.e.* tobacco growing Mysore district. The wealth indicators *viz.*, owned houses, houses with 3-5 rooms, houses with electricity/standard bathrooms/standard kitchen/television/two wheeler/ four wheeler/ personal computer and availing of banking service in rainfed tobacco growing district (Mysore) are comparable to that of neighbouring irrigated non-tobacco district, Mandya. However, wealth indicators possession in Mysore district is perceptibly superior over rainfed non-tobacco growing district (Chamarajnagar). In general, remunerative and higher net returns from cultivation of crops could motivate farmers towards creation of facilities. Higher per cent of wealth indicators in the FCV tobacco growing district compared to non-tobacco district clearly revealed the prosperity and economic well -being of FCV tobacco farmers.

Education indicator such as literacy is an important component of human resource and a key to over all human development. The higher scale of literacy in FCV tobacco growing district has clearly indicated the remunerative nature of FCV tobacco production.

Farmers' opinion

Eighty seven per cent of the farmers opined that high returns, non-availability of alternatives and availability of credit were the three reasons for their continued tobacco cultivation. Seventy eight percent of the farmers felt that there cannot be any suitable alternative livelihood activity that can supplement agricultural income from alternative crops to the extent of the earnings from tobacco. Forty six per cent of the farmers suggested that the provision of irrigation may

Table 3: Wealth indicators (%) in tobacco and non-tobacco districts

Indicator	Districts		
	Tobacco Mysore	Non-tobacco Mandya	Chamrajnagar
Owned houses	93.98	93.26	94.26
Houses with 3-5 rooms	10.78	11.56	8.29
Houses with electricity	89.75	90.81	85.64
Standard bathrooms	74.41	84.62	50.81
Standard kitchen	89.14	92.25	77.54
Availing banking service	41.27	54.93	34.14
Television sets	49.83	50.93	41.79
Two wheelers	17.89	19.99	12.70
Car/jeep/van	1.41	1.31	0.85
Household computer	4.09	3.30	3.75

Source: Census of India, 2011

Table 4: Education indicator in tobacco and non-tobacco districts

District	Literacy (%)	
	Male	Female
Chamarajnagar (Non-tobacco)	67.93	54.92
Mandya (Non-tobacco)	78.27	62.54
Mysore (Tobacco)	78.46	67.06

Source: Statistical Abstracts of Karnataka State, 2013-14

motivate the farmers toward cultivation of crops like paddy, mulberry, sugarcane, vegetables and ginger. Sixty seven per cent of non-tobacco farmers opined that they are not happy with the different crops being cultivated by them.

All the farmers felt that FCV tobacco is endowed with organised market, credit and institutional support. It is difficult to replace FCV tobacco as a crop unless such facilities are extended to other crops

FCV tobacco growing district, Mysore has depicted better socio-economic development indicators than that of rainfed non-tobacco growing district (Chamarajnagar). Further, socio-economic development of rainfed Mysore district is similar to that of irrigated Mandya district growing other crops. FCV tobacco cultivation is critical for the rural economy in tobacco growing districts and is one of the reasons for continued FCV tobacco cultivation. Further, FCV tobacco enjoys the advantage of institutional and market support that is put in place over the years. It has brought dramatic changes in overall farming, employment, income and socioeconomic balance of the district. Intensive research work has been carried out to identify the remunerative alternative crops to tobacco. None of the alternative crops tested under mono cropping system are as remunerative as tobacco in KLS region. FCV tobacco farmers opined that remunerative nature of FCV tobacco backed by the institutional support encourages them for continuing tobacco cultivation.

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LIPOPHILIC CONSTITUENTS IN HDBRG TOBACCO

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Harvel De Bouxo Rio Grande **(HDBRG) tobacco, popularly known as HD Burley tobacco, which is a high yielding, sun-cured, burley type of tobacco grown under irrigated conditions in the heavy black soils of Andhra Pradesh and used in blend in cigarette manufacture. It has specific characteristics of responding to nitrogen fertilization, producing the highest biomass and seed. Lipophilic constituents play an important role in quality/aroma of tobacco. Representative cured leaf samples of HDBRG tobacco were analyzed for lipophilic constituents by soxhlet hexane extraction, alkaline hydrolysis and fractionation by column chromatography.**

The total hexane extractables in HDBRG tobacco was high (9.94%) and solanesol accounted for 24.24% of the extract. The general lipid profile of fractions indicated the presence of alkanes, fatty acids, sterols, terpenes and fatty alcohols. In the non-polar fraction, the odd-numbered paraffinic homologues were predominant, accounting for ~66% of the fraction. In respect of the combined total of normal and iso alkanes, the relative content of hentriacontane was the highest (~40%) followed by dotriacontane (~19%). In the polar fraction of the hydrolysate from powder, the proportion of saturated fatty acids ($C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$) was ~58%, while that of unsaturated fatty acids ($C_{18:2}$ + $C_{18:3}$) was ~42%. Palmitic acid ($C_{16:0}$: 585 µg/g) was the major fatty acid. In terms of relative contents, stigmasterol (41.6%) was the major phytosterol, followed by campesterol (20.8%), cholesterol (20.4%), β-sitosterol (12.5%) and fucosterol (5.1%). HDBRG tobacco had higher levels of α-tocopherol (vitamin E) (50.7%) and fatty alcohols. The LC-MS analysis of solanesol in the APCI (+) mode revealed abundant stable (M-H_2O+H) ion (m/z at 613.7) with low abundance of other fragmentation ions thus confirming the presence of solanesol in the fractions. The results showed that, higher levels of total hexane solubles and solanesol in the fraction are the characteristic features of this tobacco. Among the lipids, isohentriacontane, palmitic acid, stigmasterol, α-tocopherol and solanesol were the principal constituents in the respective groups.

Key words: HDBRG tobacco, Lipophilic constituents, Non-polar and Polar compounds

INTRODUCTION

Harvel De Bouxo Rio Grande (HDBRG) tobacco, popularly known as HD Burley tobacco, which is a high yielding, sun-cured, burley type of tobacco supposed to be introduced from Brazil. The heavy bodied HDBRG tobacco, which is closely related to burley in chemistry, grown under irrigated conditions in the heavy black soils in Guntur district of Andhra Pradesh and it has specific characteristics of responding to nitrogen fertilization, producing the highest biomass and seed. This leaf is mainly used in the domestic cigarette blends and only a small quantity is exported. The different types of tobacco have distinct physical, chemical and organoleptic characteristics which are primarily governed by the soil, climate, variety and crop husbandry. The important chemical constituents are alkaloids, carbohydrates, nitrogenous compounds, acids, bases and lipids influencing the leaf quality. Some of the compounds have a positive impact on the leaf aroma and smoke flavour, while the others have negative impact.

A wide variety of chemical constituents were reported in different types of tobacco leaf including lipids and sterols. Quantitatively, the non-polar lipids as extracted by petroleum ether exhibit a wide variability and they ranged from 6.51 to 15.30% in flue-cured tobacco germplasm (Chaplin and Miner, 1980). In addition to genetic factors, cultural practices, weather conditions and curing methods determine the quantitative and

qualitative composition of different classes of components comprising the non-polar lipids. The quantitative composition of the hexane extractives were investigated in detail by various workers in FCV tobacco (Ellington *et al.*, 1978), *Lanka* tobacco (Kameswara Rao, 1983), *Natu* tobacco (Nagaraj and Chakraborty, 1977) and burley tobacco (Davis, 1976) by employing sophisticated analytical techniques. Liu *et al.* (2007) observed that Soxhlet extraction failed to quantify the sterol glycosides because of their polarity and also alkaline saponification was insufficient to cleave the acetal bond between the phytosterol and the carbohydrate moiety.

Total ether soluble extractives include almost all the lipid components and estimation of total ESE represents the quality of a particular type of tobacco. In general, it was regarded that higher levels of total ESE will be a positive attribute for tobacco quality. In this paper, an attempt has been made to study the total lipophilic constituents by soxhlet hexane extraction and alkaline hydrolysis methods and their fractionation in HDBRG tobacco grown under irrigated conditions in heavy black soils of Andhra Pradesh.

MATERIALS AND METHODS

HDBRG tobacco was grown in a bulk crop with three replications, at CTRI Research Station, Guntur, Andhra Pradesh with recommended package of cultural practices. Leaf samples were collected during 2009-10 and 2010-11 seasons. Cured leaf samples were collected from all primings. The leaf midribs were removed and resultant lamina portion was dried in the hot air oven at 60°C for 6 hours, powdered, passed through 40 micron mesh and used for chemical analysis. Representative samples were prepared by mixing in relative proportions. For fractionation of total lipids, the representative samples of 2011 season were used.

Hexane extractives

Hexane extractives were extracted from 500 g of tobacco powder with 3000 ml of n-hexane by Soxhlet extraction for 8 h. The solvent was removed using a Buchi flash evaporator at 40°C, and weight of total extractives was recorded. The extraction was carried out in triplicate. Values are expressed in per cent (Grunwald *et al.*, 1977) and results were statistically analysed (Panse and Sukhatme, 1957).

Separation of polar and non-polar lipids

The total hexane extractives thus obtained was dissolved in 600 ml of n-hexane, and successively extracted four times (3 x 250 ml and 1 x 100 ml) with 90% methyl alcohol (MeOH) and pooled. The hexane layer was washed three times successively with a total of 450 ml of distilled water and the water solubles were discarded. Methanol and hexane extracts were concentrated, dried and weights were recorded as per cent of non-polar and polar fractions.

Modified base-hydrolysis procedure for extraction of total lipids

Modified base-hydrolysis procedure was adopted (Ellington *et al.*, 1977) for the quantitative recovery of lipids. Tobacco powder (10 g) was refluxed with 400 ml of 2 N KOH in 85% ethanol for 2 h. The mixture was cooled and adjusted the pH to 2 with conc. HCl after treating with 400 ml of water. The mixture was filtered through a Whatman No. 1 fluted filter paper and the hydrolysis flask and the residue were washed with 30 ml mixture of benzene - 85% ethanol (1:1) five times. Saturated aqueous KCl solution (250 ml) was added and the mixture was extracted with 250 ml of hexane four times. Hexane extract of the hydrolysate was concentrated to dryness on a Buchi flash evaporator at 40°C. Two consecutive 8 ml portions of benzene were added and the mixture was taken to dryness under vacuum after each addition for removal of residual water or ethanol. Simultaneously, modified base-hydrolysis procedure was adopted for the quantitative recovery of lipids in Soxhlet hexane extractives.

Column chromatography

Hexane extract of the hydrolyzate (1g) was layered on the Silica gel (60 – 120 mesh) column (45 2 cm I.D.) in hexane and eluted with 1000 ml of hexane to yield the non-polar lipid fraction (F1). The semi-polar fraction (F2) was then separated with 2500 ml of hexane:benzene (3:1) and the polar lipid fraction (F3) with 1000 ml of

benzene:diethyl ether (3:1). The eluates were evaporated to dryness and weight of each fraction was recorded. The GC-MS analysis was carried out by taking 5 mg of F1 and 10 mg each of fractions F2 and F3 and dissolving in 1 ml of hexane. Hexane extract of the hydrolysate was subjected to silica gel column chromatography with the following solvent eluents successively petroleum ether (SF1), petroleum ether: acetone [95:5 (SF2), 90:10 (SF3), 80:20 (SF4), 60:40 (SF5) and 40:60 (SF6)]. Ten mg each of the two fractions were dissolved in hexane and transferred into a 1 ml volumetric flask and made up to the volume with hexane for GC-MS analysis. Due to the polarity of components in semi-polar and polar fractions, fractions F2 and F3, 40 µl each were derivatised by reacting with BSTFA [N,O-bis (trimethylsilyl) trifluoro acetamide] at 50°C for 30 min.

GC-MS analysis

The GC-MS analysis was carried out on Agilent 6890 GC system equipped with a 5973 N inert mass selective detector and 7863 auto sampler (Agilent Technologies, USA). A ZB-5 MS (5% Phenyl, 95% Dimethyl polysiloxane) (ZebronTM – Phenomenex, USA) column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The oven was programmed from an initial temperature of 50°C (held for 2 min) to the final temperature of 300°C @ of 10°C/min. The final temperature was held for 5 min. Hydrogen was used as the carrier gas with a flow rate of 1.2 ml/min. The inlet and interface temperatures were kept at 270°C. The EI source was operated at 230°C and the quadrupole temperature was 150°C. The MS was scanned from 30 to 600 units for recording full scan spectra. One micro liter of the sample was injected in split-less mode by the auto sampler. The peaks obtained were identified using U S 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 proportion of a particular compound in the total lipid fraction was calculated.

Fatty acids

Hexane extractives (100 mg) were used for extraction and esterification (10% H_2SO_4 in absolute methanol) by the method of Kates (1975). The hexane extractives were made up to 25 ml for GC-MS analysis. The GC-MS analysis was performed 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 oven was programmed from an initial temperature of 100°C (held for 3 min), ramped @ 8°C/min up to 220°C (held for 5 min) to the final temperature of 240°C @ 2°C/min. The final temperature was held for 2 min and the total run time was 35 min. Helium was used as the carrier gas with a flow rate of 0.78 ml/min. The inlet and interface temperatures were kept at 250°C. The EI source was operated at 225°C and the MS was scanned from 50 to 500 units for recording full scan spectra. For calculating fatty acids, the MS was operated in selected ion monitoring (SIM) mode. In the SIM mode, for valid characterization, the following ions were selected as quantifiers and qualifiers for the respective fatty acid (m/z 74, 87 and 55 for $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$; 55, 69 and 74 for $C_{18:1}$; 67, 81 and 95 for $C_{18:2}$; 79, 67 and 95 for $C_{18:3}$). One micro liter of the sample was injected in split mode with the ratio of 1:20 by the auto sampler. Standard fatty acids were purchased from Sigma (St. Louis, MO 63178, USA). Five calibration standard mixtures of fatty acids were prepared by serial dilution with hexane ranging from 0.04 to 96 ppm.

Solanesol

HPLC analysis

Solanesol content in the tobacco powder was estimated (Narasimha Rao *et al.*, 2000) employing Shimadzu LC 8A HPLC with UV-VIS detector, at 210 nm. The mobile phase was HPLC grade isopropyl alcohol: methyl alcohol (60: 40) at a flow rate of 1 ml/min. The retention times and area per cent of different constituents were recorded.

LC–MS analysis

Forty mg each of the polar fractions of the tobacco powder and soxhlet hexane extract hydrolysates were dissolved in IPA and transferred

to 1 ml volumetric flasks and made up to the volume with IPA for identification of solanesol by LC-MS.

LC-MS (Agilent 1100 MSD ion-trap-SL mass spectrometer) coupled with atmospheric pressure chemical ionization (APCI) source in positive ion mode, equipped with a degasser (G1379A), binary pump (G1312A), auto-sampler (G1329A), auto-sampler thermostat (G1329B) and diode array detector (G1315B) of wave length 210 nm was employed for the qualitative and quantitative determination of solanesol in the fractions. Solanesol and other compounds were separated on an Agilent - Eclipse XDB -C18, 4.6 150 mm, 5 µm column using the isocratic mode of elution. For isocratic elution, 50% acetonitrile in isopropanol as mobile phase was pumped at a flow rate of 1.0 ml/min; the sample injection volume was 2 µL with column temperature maintained at ambient conditions. Nitrogen was employed as the nebulizer gas. The ion source conditions were set as follows: temperature, 335 C; nebulizer gas, 35 psi; dry gas, 10.0 l/min; skimmer 40.0 V; capillary exit 128.0 V; trap drive 44.5; max accu time 200 ms; Icc target 20000. The data were acquired and processed using Chemstation 5.3 (Agilent Technologies, Waldbronn, Germany).

RESULTS AND DISCUSSION

Hexane extractables

A large number of components identified in tobacco leaf belong to the broad group of lipids which may be polar or non-polar. The liphophilic constituents *viz.*, paraffins, polyenes, esters, solanesol, sterols, tocopherols and fatty acids are important because they are related to the leaf quality/aroma and smoke flavour. The total hexane extractables or PEE content in HDBRG tobacco was high (9.94%) and solanesol accounted for 24.24% of the extract. Further serial extraction of the total hexane solubles with 90% methanol and water resulted in three fractions *i.e.*, hexane solubles (69.3% of total hexane extractables and 6.9% on the basis tobacco), 90% methanol solubles (17.3% of total hexane extractables and 1.7% on the basis of tobacco (Table 1) and water solubles (13.3% of total hexane extractables and 1.4% on the basis of tobacco). Higher levels of PEE are positively correlated with aroma in FCV tobacco (Grunwald *et al.*, 1977). Even in chewing tobacco, higher levels of PEE are positively correlated with aroma as these extracts contain all lipids and fatty acids (Murthy and Gopalachari, 1984). In sun-cured chewing tobacco varieties grown in Tamil Nadu, PEE levels varied from 5.90 to 7.80% with a mean value of 6.97% (Siva Raju *et al.*, 2012). Gangadhar *et al.* (2011) reported significant positive correlations of nicotine with solanesol and PEE and PEE with solanesol.

The above total lipid extracts were characterized into non-polar and polar fractions employing techniques like GC-MS and LC-MS. Column chromatography of 1 g of the fraction obtained after base hydrolysis of tobacco powder (10 g) yielded non-polar (70 mg), semi-polar (300 mg) and polar (500 mg) fractions accounting for 88% of the total eluates collected. In the case of column chromatography of 30 g of fraction obtained after base hydrolysis of soxhlet hexane extract resulted in non-polar (3.17 g) and polar (24.15 g) fractions, accounting for 91% of the total eluates. The general lipid profile of fractions after column chromatography indicated the presence of alkanes, fatty acids, sterols, terpenes and fatty alcohols.

In the non-polar fraction containing aliphatic alkanes obtained from the hydrolysate fraction of powder, the odd-numbered homologues *viz.*, C_{27}, C_{31} and C_{33} were predominant accounting for ~66% of the fraction (Table 2; Fig.1). In this fraction, the per cent composition of normal (C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32} and C_{33}) and iso (C_{31} and C_{32}) series was 62.8 and 37.2%, respectively. The branched chain hydrocarbon, isohentriacontane was the major compound in the paraffin fraction with a relative content of 26.3%, followed by the linear paraffin n-tritriacontane (20.3%). Straight chain (normal) alkanes, hentriacontane (13.5%), triacontane (8.5%) and dotriacontane (8.2%) accounted for ~30% of the fraction. In respect of the combined total of normal and iso alkanes, the relative content of hentriacontane was the highest (~40%) followed by dotriacontane (~19%).

Similar trends of relative contents were observed in the case of the non-polar fraction resulted from the base hydrolysis of soxhlet hexane

Table 1: Lipid fractions of HDBRG tobacco

Sample	Total hexane extractables		Hexane solubles (after extraction)		90% Methanol solubles		Water solubles	
	(g)	(%)*	(g)	(%)**	(g)	(%)**	(g)	(%)**
2009-10								
S1	37.85	7.57	28.76	75.98	5.50	14.53	3.59	9.49
S2	47.19	9.44	32.78	69.46	5.50	11.66	8.91	18.88
S3	59.72	11.94	41.54	69.56	8.00	13.40	10.18	17.04
S4	49.96	9.99	34.34	68.73	9.00	18.01	6.62	13.25
Mean	**48.68**	**9.74**	**34.36**	**70.93**	**7.00**	**14.40**	**7.33**	**14.67**
SD	**9.00**	**1.80**	**5.34**	**3.39**	**1.78**	**2.68**	**2.89**	**4.17**
CV (%)	**18.49**	**18.46**	**15.53**	**4.77**	**25.42**	**18.62**	**39.47**	**28.43**
2010-11								
S1	54.62	10.92	37.22	68.14	10.00	18.31	7.41	13.56
S2	53.00	10.60	32.65	61.60	10.40	19.62	9.95	18.77
S3	46.50	9.30	33.00	70.97	10.00	21.51	3.50	7.53
S4	48.50	9.70	34.00	70.10	10.50	21.65	4.00	8.25
Mean	**50.66**	**10.13**	**34.22**	**67.70**	**10.23**	**20.27**	**6.22**	**12.03**
SD	**3.79**	**0.76**	**2.08**	**4.24**	**0.26**	**1.60**	**3.04**	**5.24**
CV (%)	**7.49**	**7.47**	**6.08**	**6.26**	**2.57**	**7.91**	**48.85**	**43.55**
Seasons								
Mean	**49.67**	**9.93**	**34.29**	**69.32**	**8.61**	**17.34**	**6.77**	**13.35**
SD	**6.48**	**1.29**	**3.75**	**3.95**	**2.09**	**3.75**	**2.81**	**4.60**
CV (%)	**13.05**	**13.03**	**10.94**	**5.70**	**24.24**	**21.61**	**41.49**	**34.50**

*Per cent of tobacco

** Per cent of total hexane extractables

extract of HDBRG tobacco (Table 2). The odd-numbered paraffins were more (63.7%) in this fraction. The ratio of normal: iso alkanes was 58.8:41.2, isohentriacontane (32%) being the major hydrocarbon, followed by n-tritriacontane (22.7%). The paraffins, hentriacontane (39%) and dotriacontane (19%) were more in respect of the sum of normal and branched chain alkanes.

Nagaraj and Chakraborty (1977) reported that in *Natu* tobacco, the odd-numbered n-paraffins constituted 91% of the total and n-hentriacontane was the major paraffin accounting for 55.6%. Dotriacontane was the major even-numbered compound with a relative content of 7.6%. Devrex and Esnault (1974) reported that in the essential oil from tobacco steam distillate, n-heptacosane ($C_{27}H_{56}$) was the major linear paraffin while isohentriacontane ($C_{31}H_{64}$) was the major branched chain paraffin. According to Chortyk *et al.* (1975), the combined totals for the normal and iso compounds were 37 and 19%, respectively in the case of cigarette and flue-cured tobacco. In *Lanka* tobacco, the odd-numbered alkanes viz., C_{27}, C_{29}, C_{31} and C_{33} were predominant accounting for ~77% of the fraction. The per cent composition of normal, iso and anteiso series was 60, 27 and 13%, respectively. The straight chain hydrocarbon, n-hentriacontane was the major compound in the paraffin fraction with a relative content of 26.5%, followed by n-tritriacontane (9.7%) and n-nonacosane (8.7%) (Kameswara Rao, 1983).The findings emanated from the present study are in consonance with the reported findings.

HDBRG tobacco had higher levels of á-tocopherol (vitamin E) (50.7%) and fatty alcohols *viz.*, decanol (15.8%), hexadecane-4-ol (13.9%),

Table 2: HDBRG tobacco — Composition of lipophilic constituents

Rt (min)	Compound	Powder hydrolysate	Soxhlet hydrolysate
		Relative content (%)	
Non-polar			
22.33	Nonadecane ($C_{19}H_{40}$)	0.42	0.11
23.85	Hexacosane ($C_{26}H_{54}$)	4.34	3.66
25.25	Heptacosane ($C_{27}H_{56}$)	3.57	3.59
25.75	Octacosane ($C_{28}H_{58}$)	2.06	1.02
25.92	Nonacosane ($C_{29}H_{60}$)	1.87	1.58
26.34	Triacontane ($C_{30}H_{62}$)	8.54	9.36
26.58	Hentriacontane (Iso $C_{31}H_{64}$)	26.31	31.99
27.05	Hentriacontane ($C_{31}H_{64}$)	13.45	7.26
27.21	Dotriacontane (Iso $C_{32}H_{66}$)	10.87	9.11
27.64	Dotriacontane ($C_{32}H_{66}$)	8.24	9.66
27.92	Tritriacontane ($C_{33}H_{68}$)	20.34	22.66
Semi-polar			
10.61	Decanol	15.77	-
19.21	Phytol	13.08	78.72
22.35	4-Hexadecanol	13.89	21.28
24.28	Squalene	3.37	-
26.42	á-Tocopherol	50.72	-
28.14	Cycloartenol	3.17	-
Polar			
16.11	Tetradecanoic acid (Myristic acid) - $C_{14:0}$	4.65	3.80
17.10	Pentadecanoic acid $C_{15:0}$	1.77	2.21
18.06	Hexadecanoic acid (Palmitic acid) $C_{16:0}$	39.58	33.55
18.71	Heptadecanoic acid $C_{17:0}$	3.11	3.87
19.55	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid) $C_{18:2}$	7.63	13.05
19.61	9,12,15-Octadecatrienoic acid (Z,Z,Z)(Linolenic acid) $C_{18:3}$	34.51	31.22
19.84	Octadecanoic acid (Stearic acid) $C_{18:0}$	7.26	10.01
21.49	Eicosanoic acid (Arachidic acid) $C_{20:0}$	1.51	2.30
26.40	Cholesterol	20.39	32.25
27.06	Campesterol	20.81	15.97
27.22	Stigmasterol	41.22	28.50
27.63	â-Sitosterol	12.45	13.35
27.73	Fucosterol	5.14	9.94

phytol (13.1%), cycloartenol (3.2%) and a triterpene *i.e.*, squalene (3.4%) (Fig. 2). Squalene was reported as a constituent of burley tobacco (Rodgman *et al.*, 1961). Kameswara Rao *et al.* (1988) reported the presence of the minor terpenes like phytol, squalene, cycloartenol, 24-methylene cycloartanol and beta-amyrin in *Lanka* tobacco and its smoke.

Fatty acids

In the polar fraction of the hydrolysate from powder, the proportion of saturated fatty acids ($C_{14:0}$ + $C_{15:0}$ + $C_{16:0}$ + $C_{17:0}$ + $C_{18:0}$) was ~58%, while that of unsaturated fatty acids ($C_{18:2}$ + $C_{18:3}$) was ~42%. In terms of relative content, palmitic acid ($C_{16:0}$: 39.6%) and linolenic acid ($C_{18:3}$: 34.5%) were the

major acids identified (Table 3). The ratio of unsaturated to saturated fatty acids was 0.72.

In the case of polar fraction of the hydrolysate of Soxhlet hexane extract, a similar trend was observed. The relative contents of saturated and unsaturated fatty acids were 56 and 44%, respectively, with palmitic (33.6%) and linolenic (31.2%) being the principal saturated and unsaturated fatty acids, respectively (Table 2). The ratio of unsaturated to saturated fatty acids was 0.79. In both the cases, oleic acid ($C_{18:1}$) was not detected, may be due to merger with linolenic acid.

Results of the GC-MS analysis (SIM mode) (Fig. 3) using standard fatty acid methyl esters to quantify the fatty acids are presented in Table 3. It is inferred from the mean values of two seasons that palmitic acid ($C_{16:0}$: 585 µg/g) is the major fatty acid in HDBRG tobacco, followed by linolenic acid ($C_{18:3}$: 322 µg/g), Oleic ($C_{18:1}$), linoleic ($C_{18:2}$), myristic ($C_{14:0}$) are the other important fatty acids, with saturated acids accounting for 55.4% while the unsaturated acids accounted for 44.6% of fatty acids. The ratio of unsaturated to saturated fatty acids was 0.80.

Nagaraj and Chakraborty (1979) analyzed fatty acids in *Natu* tobacco by GC and reported the presence of decanoic (6.1%), lauric (8.5%), myristic (14.1%), myristoleic (17.0%), palmitic (10.9%), palmitoleic (6.4%), stearic (9.1%), oleic (8.0%), linoleic (1.6%) and linolenic (18.2%) acids. The saturated acids constituted about 48% of the total whereas the remaining 52% was accounted for by unsaturated fatty acids. Kameswara Rao (1983) reported the following major fatty acids in *Lanka* tobacco leaf, $C_{18:3}$, $C_{16:0}$, $C_{18:2}$ and $C_{18:1}$, the unsaturated acids accounting for 47%. The higher fatty acids (myristic, palmitic, stearic, oleic, linoleic and linolenic) comprised about 0.75 - 1.1% in Virginia tobacco and about 0.5% in Burley, with palmitic being about 25% of these total acids (Leffingwell, 2001).

Sterols

In terms of relative contents, stigmasterol (41.6%) was the major phytosterol, followed by campesterol, cholesterol, â-sitosterol and fucosterol in HDBRG tobacco in the polar fraction separated from the hydrolysate of powder (Table 2). However, differences were observed in the relative content of sterols in the hydrolysate of hexane extract which was obtained by Soxhlet extraction, where cholesterol was the major phytosterol, followed by stigmasterol, campasterol, â-sitosterol and fucosterol (Table 2).

Liu *et al.* (2007) observed that Soxhlet extraction failed to quantify the sterol glycosides because of their polarity and also alakaline

Table 3: Individual fatty acids – Season-wise

Fatty Acid	2009-10				2010-11			
	S1 (µg/g)	S2 (µg/g)	S3 (µg/g)	Mean (µg/g)	S1 (µg/g)	S2 (µg/g)	S3 (µg/g)	Mean (µg/g)
Lauric acid ($C_{12:0}$)	10.33	8.60	11.17	10.03	13.02	8.20	11.00	10.74
Myristic acid ($C_{14:0}$)	118.43	118.15	107.54	114.71	147.44	113.66	142.65	134.58
Pentadecanoic acid ($C_{15:0}$)	67.71	71.77	37.63	59.04	64.19	53.96	50.65	56.27
Palmitic acid ($C_{16:0}$)	585.05	587.84	504.27	559.05	660.07	525.68	648.98	611.58
Heptadecanoic acid ($C_{17:0}$)	38.37	48.06	38.98	41.80	41.15	35.17	42.60	39.64
Linoleic acid ($C_{18:2}$)	171.82	149.18	202.40	174.47	251.18	192.82	261.31	235.10
Oleic acid ($C_{18:1}$)	193.53	151.41	225.18	190.04	279.51	215.00	317.56	270.69
Linolenic acid ($C_{18:3}$)	281.08	194.12	270.01	248.40	406.12	317.78	461.59	395.16
Stearic acid ($C_{18:0}$)	119.04	122.47	118.80	120.10	128.68	109.53	134.12	124.11
Total	1585	1452	1516	1517.67	1991	1572	2070	1877.67

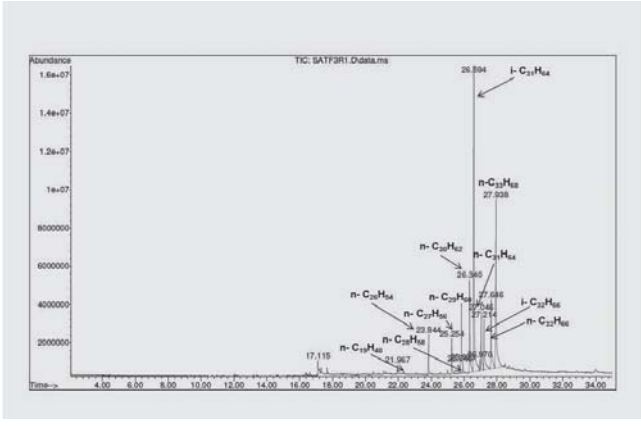

Figure 1. GC-MS chromatogram of non-polar fraction of soxhlet hexane hydrolysate in HDBRG robacco

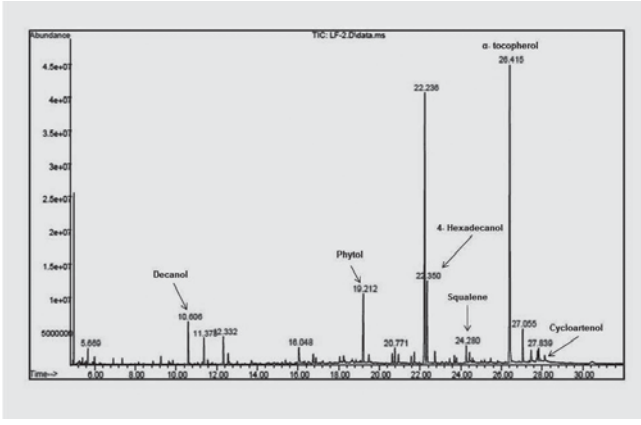

Figure 2. GC-MS chromatogram of TMS derivatives of compounds in the semi-polar fraction of powder hydrolysate of HDBRG tobacco

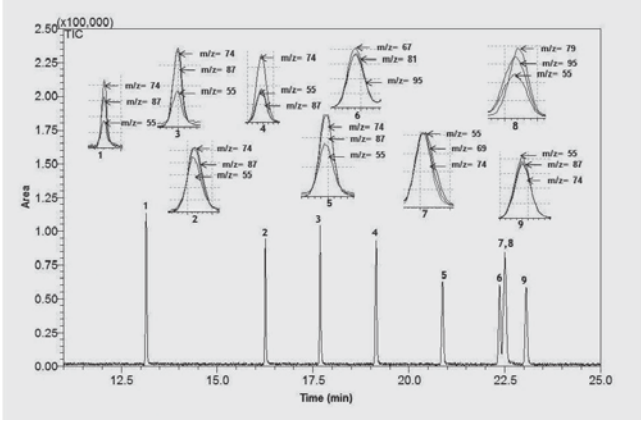

Figure 3. GC-MS chromatogram of methyl esters of FA. The labeled compounds (1-9) are listed in the Table 3.

saponification was insufficient to cleave the acetal bond between the phytosterol and the carbohydrate moiety.

Ellington *et al.* (1977) reported the levels of the four major sterols in FCV tobacco as, cholesterol (0.30 mg/g), campesterol (0.53 mg/g), stigmasterol (0.75 mg/g) and â-sitosterol (0.88 mg/g) totaling 2.46 mg/g. In the two lines of Burley tobacco, the levels of cholesterol, campesterol, stigmasterol and â-sitosterol were, L1: 0.18, 0.23, 0.72 and 0.38 mg/g and L2: 0.21, 0.33, 0.82 and 0.94 mg/g, respectively (Davis, 1976). The contents and forms of distribution of phytosterols in tobacco varied with tobacco cultivar and cultural practices (Grunwald *et al.*, 1977).

Solanesol

Solanesol content in the samples varied from 1.60 to 2.80% with mean values of 1.97 and 2.46% for the seasons 2009-10 and 2010-11, respectively. The LC-MS analysis in the APCI (+) mode revealed abundant stable (M - H_2O+H) ion (m/z at 613.7) with low abundance of other fragmentation ions, confirming the presence of solanesol in the fractions.

The data are consistent with the report of Phani Kiran *et al.* (2008) that HDBRG tobacco grown in the Guntur tract could be a rich source of solanesol (Range: 0.50 – 3.75%; Mean: 1.75%), both the maximum and mean values being the highest among different types of tobacco grown in the country. Solanesol content in various types of tobacco grown in different agro-ecological situations in India ranged from 0.09 to 3.18 % (Narasimha Rao and Prabhu, 2005). Burton *et al.* (1989) investigated the factors influencing solanesol content in burley tobacco and reported that genotype, growing conditions and agronomic practices which have profound influence on the solanesol content in the leaf at various growth stages of the plant.

It is concluded that higher levels of total hexane solubles/petroleum ether extractives and solanesol in the fraction are the characteristic features of this tobacco. Among the lipids, isohentriacontane, palmitic acid, stigmasterol, á-tocopherol and solanesol were the principal constituents in the respective groups.

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GENETIC VARIABILITY FOR SEED AND OIL YIELD IN CHEWING TOBACCO (*NICOTIANA TABACUM* L.) GERMPLASM ACCESSIONS

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The extent of amenable genetic variability, heritability and genetic advance for 19 traits in 59 genotypes of chewing tobacco (*Nicotiana tabacum* L.) were studied at Zonal Agricultural Research Station, Shimoga during *rabi* 2013. Analysis of variance revealed the existence of significant differences among genotypes for all characters studied. The magnitude of PCV and GCV was moderate to high for plant height, days to 50% flowering, leaves per plant, leaf breadth, individual capsule weight, leaf area, internodal length, capsules per plant, chlorophyll content, specific leaf weight, number of branches, capsule weight per plant, seed yield per plant and oil yield per plant. The results showed wide range of variability for all the characters except for oil content. Small differences between GCV and PCV were recorded for all the characters studied which indicated lesser influence of environment on these characters. High heritability coupled with high genetic advance as per cent of mean was observed for leaf area, internodal length, capsules per plant, chlorophyll content, specific leaf weight, number of branches, capsule weight per plant, seed yield per plant and oil yield per plant indicating the role of additive gene in expressing these traits.

Key words: Chewing tobacco, Genetic variablity, Seed oil

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the leading non-food commercial crops in world agriculture. It is an important industrial crop grown in 119 countries of the world. Although tobacco is an important international cash crop, the health hazards associated with tobacco consumption either by smoking or chewing or snuff has led to a worldwide anti-tobacco campaign. So the research efforts need to be geared up for alternative uses of tobacco. Tobacco plant is a prolific producer of seed. The seed oil content in tobacco varies from 35-39% and is comparable to mustard (33%), sunflower (35%), safflower (30%), higher than soybean (17%) and cotton seed (12%) (Patel *et al.,* 1998). Tobacco seed oil is reported to be used in certain pharmaceutical preparations, in alkyd resins and soap manufacture. It is also used as edible oil after suitable refining in Greece, Bulgaria and some other countries. The germplasm needs to be carefully assessed for the prevalence of genetic variability for seed yield, oil yield, yield attributes and other yield limiting characters, as primarily the success of any breeding methodology mainly depends on the prevalence and magnitude of genetic variability and its efficient utilization. Therefore, an attempt was made in the present investigation to assess the variability of 19 characters pertaining to seed yield and its components in chewing tobacco.

MATERIALS AND METHODS

Fifty nine genotypes of chewing tobacco were grown in a randomized complete block design with three replications during 2013-14 of *rabi* season at Shimoga, Karnataka. Each replication consisted of a single row of 10 plants. Spacing from row to row and plant to plant was 60 cm and 30 cm, respectively and the crop was raised as per the recommended package of practices. Observations on plant height, leaves per plant, leaf length, leaf breadth, leaf area per plant, days to 50% flowering, days to maturity, chlorophyll content, specific leaf weight, number of branches, internodal length, capsule length, capsule breadth, individual capsule weight, capsules per

plant, capsules weight per plant, seed yield per plant, oil percentage and oil yield per plant were studied on five random plants on each genotype. Oil content was estimated with NMR (Nuclear Magnetic Resonance) spectrometer at RARS, Raichur. The oil content is expressed as percentage based on dry seed. Estimates of genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) were calculated following the formula suggested by Burton and De Vane (1953). Heritability (broad sense) was calculated following the formula suggested by Hanson *et al.* (1956). Genetic advance as per cent of mean was calculated using the formula suggested by Johnson *et al.* (1955).

RESULTS AND DISCUSSIONS

The results with regard to mean, overall range, genotypic coefficient of variability, phenotypic coefficient of variability, heritability (broad sense) and genetic advance as per cent of mean for all the 19 characters were furnished in Table 1 and Table 2. The phenotypic coefficient of variability and genotypic coefficient of variability were the highest for chlorophyll content followed by internodal length, seed yield per plant, capsules per plant, capsules weight per plant, oil yield per plant and leaf area suggesting that these characters are under the influence of genetic control. Hence, these characters can be relied upon and simple selection can be practiced for further improvement. These results are in consonance with the findings of Patil and Sheriff (1996), Sudhakar *et al.* (2007) in sesamum and Nagesh and Rangaiah (2008) in chewing tobacco.

PCV and GCV were moderate for leaves per plant, leaf length and plant height which is in conformity with the findings of number of leaves per plant by Sastry and Gopinath (1969), leaves per plant by Ibrahim and Avratoscukova (1982). Days to 50% flowering, days to maturity, capsule length, capsule breadth and oil content recorded low PCV and GCV. Similar results were also reported in tobacco for PCV and GCV for days to 50% flowering (Sastry and Gopinath, 1968) and for oil content (Amaranath and Murthy, 1998).

Genotypic coefficient of variability would be more useful for the assessment of inherent or real variability as it exhibits the heritable portion

Table 1: Range, Mean, SEm and CD values of 19 characters

S. No	Character	Range		Mean	SEm±	CD (P=0.05)
1	Plant height (cm)	69.06	123.66	93.64	5.75	16.12
2	Leaves per plant	16.33	30.80	21.24	0.799	2.23
3	Leaf length (cm)	22.08	41.24	34.03	1.63	4.58
4	Leaf breadth (cm)	10.91	22.37	16.83	1.08	3.049
5	Leaf area (cm^2)	186.97	597.76	389.72	16.01	44.84
6	Days to 50% flowering	94.66	162.0	118.11	2.52	7.07
7	Days to maturity	122.66	192.0	147.02	2.80	7.85
8	Chlorophyll content (mg/g fresh wt)	0.36	1.49	0.76	0.0084	0.023
9	Specific leaf weight (mg/cm^2)	4.28	11.48	7.20	0.091	0.25
10	Number of branches	2.20	7.20	4.27	0.37	1.05
11	Internodal length (cm)	1.93	9.31	3.41	0.16	0.46
12	Capsule length (cm)	1.37	2.14	1.68	0.10	0.28
13	Capsule breadth (cm)	0.75	1.21	0.97	0.08	0.22
14	Individual capsule weight (g)	0.145	0.357	0.22	0.014	0.04
15	Capsules per plant	17.73	105.63	58.47	2.64	7.40
16	Capsules weight per plant (g)	2.74	24.85	13.12	1.04	2.92
17	Seed yield per plant (g)	4.66	16.0	9.82	0.69	1.93
18	Oil (%)	38.90	42.06	41.06	0.24	0.69
19	Oil yield per plant (g)	1.93	6.50	4.03	0.28	0.80

Table 2: Estimates of GCV, PCV, heritability and genetic advance for plant characters in tobacco

Sl. No.	Character	GCV (%)	PCV (%)	h^2 (%)	GAM (%)
1	Plant height (cm)	12.09	13.56	79.46	22.20
2	Leaves per plant	11.29	11.90	90.02	22.08
3	Leaf length (cm)	9.81	10.93	80.60	18.15
4	Leaf breadth (cm)	12.67	14.22	79.34	23.25
5	Leaf area (cm^2)	21.43	21.82	96.46	43.35
6	Days to 50% flowering	10.94	11.14	96.33	22.12
7	Days to maturity	9.69	9.87	96.27	19.58
8	Chlorophyll content (mg/g fresh wt)	35.95	35.97	99.91	74.03
9	Specific leaf weight (mg/cm^2)	27.27	27.30	99.78	56.12
10	Number of branches	22.86	24.49	87.15	43.96
11	Internodallength (cm)	34.85	35.19	98.07	71.09
12	Capsule length (cm)	7.32	9.53	58.89	11.57
13	Capsule breadth (cm)	5.56	15.00	13.02	6.38
14	Individual capsule weight (g)	16.72	17.94	86.88	32.10
15	Capsules per plant	28.77	29.12	97.59	58.55
16	Capsules weight per plant (g)	30.93	31.93	93.81	61.71
17	Seed yield per plant (g)	22.00	23.09	90.73	43.17
18	Oil (%)	2.03	2.12	91.83	4.02
19	Oil yield per plant (g)	22.02	23.15	90.50	43.17

only. The estimated GCV for different characters were almost the same, as that of PCV. It is evident therefore, that the influence of environment on the expression of these characters was invariably low in this study.

Heritability estimates revealed that heritable portion of variability present in different characters. The knowledge of heritability enables the plant breeder to decide the course of selection procedure to be followed under a given situation. However, heritability values coupled with genetic advance would be more reliable and useful in formulating selection procedure. In the present study, heritability estimates in broad sense and genetic advance as per cent mean were estimated.

Heritability estimates were high for all the characters studied. This suggested the greater effectiveness of selection and improvement to be expected for these characters in future breeding programme as the genetic variance is mostly due to the additive gene action. Similar results were observed for plant height, days to 50% flowering, number of leaves per plant, leaf breadth and leaf length by Sastry and Gopinath (1968) and for all other characters by Nagesh and Rangaiah (2008) in tobacco.

In the present study, high heritability coupled with high genetic advance as per cent of mean was observed for characters such as capsules per plant, plant height, internodal length, leaves per plant, leaf breadth and leaf area. This indicated the lesser influence of environment in expression of characters and prevalence of additive gene action in their inheritance, hence, these are amenable for simple selection. Similar results were reported for plant height by Ibrahim and Avratoscukova (1982), leaf breadth, leaf number, plant height and internodal length by Dobhal and NageswaraRao (1988), plant height, flowering time, leaf breadth by Rao (1952) and plant height, days to 50% flowering and number of leaves per plant by Chaubey *et al.* (1990).

Hence, selection in early segregating generation for such highly heritable characters like days to maturity, capsule length and leaf length had recorded high heritability and moderate genetic advance and therefore, there

is a scope for improvement through straight selection. Very low value of genetic advance coupled with low genotypic coefficient of variation for oil content indicated the predominance of non-additive genes. Under such situation, recurrent selection would prove useful for improving these traits.

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PRODUCTION AND CHARACTERIZATION OF TOBACCO STALK BIOCHAR

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Pyrolysis temperature and time are known to have profound influence on biochar yield. The optimum parameters of pyrolysis for preparation of biochar from the tobacco stalk biomass are not known. An attempt has therefore been made to find out the optimum pyrolysis process parameters for preparation of biochar from tobacco stalks. The representative tobacco stalk biomass was subjected to thermo-chemical conversion at different temperatures (350 – 500^{0}C) and holding time (60-90 min). Tobacco stalk biochar yield decreased with an increase in temperature and holding time. System operating conditions of 500^{0}C temperature and 90 min holding time were optimum to ensure the complete charring of the biomass and maximum biochar yield. The biochar yield at optimized pyrolysis conditions was around 40% and had 74.9 % fixed carbon, 17.5% ash and remaining 7.6% as volatile matter. Total C and N content of the tobacco stalk biochar was 79 and 1.23%, respectively. While, the total C, N, P, K, Ca, Mg and micronutrient contents were higher in tobacco stalk biochar than in raw biomass. The amount of C conserved in the biochar was 70%. The recovery of total N was the lowest at 55%, while that of other major nutrients remained more or less same at about 80%. Our results indicate the optimum conditions for better biochar recovery from tobacco stalks along with nutrients.

Key words: Biochar, Crop residue, Pyrolysis, Tobacco stalk

INTRODUCTION

Pyrogenic carbonaceous material (PCM) is defined as any carbonaceous residue resulting from pyrolysis. Char is the PCM produced from natural fires. Charcoal is PCM produced from pyrolysis of animal or plant biomass in kilns for use in cooking or heating, including industrial applications such as smelting. Biochar is carbonaceous material produced specifically for application to soil for agronomic or environmental management (Lehmann and Joseph, 2015). International Biochar Initiative, the IBI (2013) defines biochar as a solid material obtained from thermo-chemical conversion of biomass in an oxygen-limited environment. Biochar can potentially play a major role in the long-term storage of carbon in soil, *i.e.*, C sequestration and green house gases (GHG) mitigation. The efficiency of biochar depends upon the material from which it is prepared. Biochar in soils persists against biological and chemical degradation over much longer periods of time because of its recalcitrant nature. The objective of this work is to produce and characterize biochar from the tobacco stalk biomass, an agri-waste having no known economic value.

MATERIALS AND METHODS

Tobacco stalk biomass was manually cut to appropriate size (average 30 cm in length and 1.0 to 1.5 cm in diameter). Fresh samples were stored and left to sundry naturally to moisture content below 10%. Dry bioresidue is a prerequisite to hasten satisfactory and quicker conversion. Representative tobacco biomass samples were tested for chemical composition.

Five experiments with varying operating conditions of temperature (350 – 500^{0}C) and holding time (60-90 min) were conducted at ICAR-CIAE, Bhopal, in order to optimize the annual core biochar reactor operating conditions for producing tobacco stalk biochar. The details of experiment are given in (Table 1).

Table 1: Optimization of tobacco stalk biochar production parameters

S. No	Temperature	Time (min)	Recovery (%)	Condition
Exp. 1	350^{0}C	60	66.0	Torrified
Exp. 2	400^{0}C	60	66.0	Torrified
Exp. 3	450^{0}C	60	60.0	Torrified
Exp. 4	450^{0}C	90	53.3	80% charred
Exp. 5	500^{0}C	90	40.0	Completely charred

The dry biochar was homogenized thoroughly, manually ground to pass through 2 mm sieve prior to analyses. The biochar samples were oven dried at 105^{0}C for 24 h. The pH of the biochar in 1:20 suspension (w/v) was measured using a pH meter (Systronics pH system 362). The electrical conductivity (EC) of biochars was measured at room temperature after suspending biochar in deionised water for 24 h (1:10 biochar to deionised water) using a EC meter. Cation exchange capacity of the biochar was determined by saturating the biochar exchange complex with 1N sodium acetate solution (pH 8.2). One g of biochar sample was leached with sodium acetate solution (pH 8.2) for replacement of exchangeable cations by Na^+ ions. The excess salts were washed down by ethanol and the adsorbed Na^+ ions were released by NH_4^+ ions, using 1N ammonium acetate (pH 7.0) solution. The Na^+ ions so released from the exchange spots were measured by using flame photometer. Total organic carbon (C) content was determined directly by dry combustion on TOC analyzer (Elementar, Germany). Total Nitrogen concentration was estimated using N distillation unit. Concentrations of total P, K, Ca, Mg, Fe, Cu, Zn and Mn in biochar were determined by digesting 0.5 g of each biochar sample in a di-acid mixture (HNO_3:$HClO_4$ in 3:1 ratio).

Biochar yield from the tobacco stalk biomass was calculated by the following equation (Antal and Groni, 2003).

$$\text{Biochar yield (\%)} = (M_{biochar} / M_{biomass}) \times 100 \quad \text{..... eq-1}$$

Where, $M_{biochar}$ is the mass of biochar obtained after conversion and $M_{biomass}$ is the dry mass of the original tobacco stalk biomass loaded into the reactor.

Proximate analyses were conducted for biochar to estimate the percentage volatile matter (VM), ash content and fixed C, on an oven dry-weight basis. The percentages of VM, ash and fixed C were estimated by measurement of weight loss/ mass balance from a sequential muffle procedure. The VM content of the biochar was determined by heating the biochar in a covered ceramic crucible to 700 C ignition for 10 min using laboratory muffle furnace. The samples were withdrawn, weighed and measured weight loss and it was defined as volatile matter (VM), and the residual solid was carbonized biochar. Ash content was determined by heating the carbonized biochar residue of the VM determination in an open crucible via combusting at 700 C for 2 h. The percentage fixed carbon, volatile matter (VM) and ash content of the biochar were calculated using the following equations (Antal and Groni, 2003).

$$\text{Volatile matter (\%)} = (M_{biochar} - M_{cc}) / M_{biochar} \times 100 \quad \text{..... eq-2}$$

Where, $M_{biochar}$ was the initial dry mass of biochar, M_{cc} was dry mass of the carbonized biochar that remained after heating.

$$\text{Ash content (\%)} = (M_{ash} / M_{biochar}) \times 100 \quad \text{.... eq-3}$$

Where, M_{ash} was the dry mass of ash remained after combustion of the carbonized biochar, $M_{biochar}$ was the initial dry mass of biochar.

$$\text{Fixed carbon (\%)} = 100 - \text{VM (\%)} - \text{Ash (\%)} \quad \text{.... eq-4}$$

Where, VM is the volatile matter content of biochar.

All the chemical analyses for different parameters were performed in triplicate and the

results presented as mean ± standard errors

RESULTS AND DISCUSSION

Production of tobacco stalks biochar and optimization of biochar production parameters

Results on the biochar yield from tobacco stalk biomass at different process variables (temperatures and holding time) of the biochar reactor were presented in Table 1. At low temperatures and small holding time charring of the biomass was not complete while, with an increase in temperature and holding time the material was transformed from torrified to charred condition. The optimum conditions for complete charring of tobacco stalk biomass were attained at a temperature of 500 ^{0}C and holding time of 90 minutes with the yield recovery of 40%. Biochar yield tended to decrease with increase in reactor temperature and holding time.

Carbon and nutrient composition of tobacco stalk biomass and biochar

Tobacco stalk biomass contained 45.5% total organic C, 0.9% nitrogen, 0.25% P, 1.9% K, 1.02% Ca and 0.43% Mg (Table 2). The concentration of micronutrients in the biomass varied from 3.8 ppm (Mn) to 82.5 ppm (Fe). The pyrolysis process giving 40% biochar yield lead to enrichment of the carbon and nutrients in the end product (biochar) owing to mass reduction by 2.5 times. This is evident from the higher concentration of C and other nutrients in biochar than in original tobacco biomass (Table 2). The tobacco stalk biochar contained 79% total organic carbon, 1.23% N, 0.51% P, 3.81% K, 2.1% Ca and 0.9% Mg. The concentration of micronutrients in the biochar

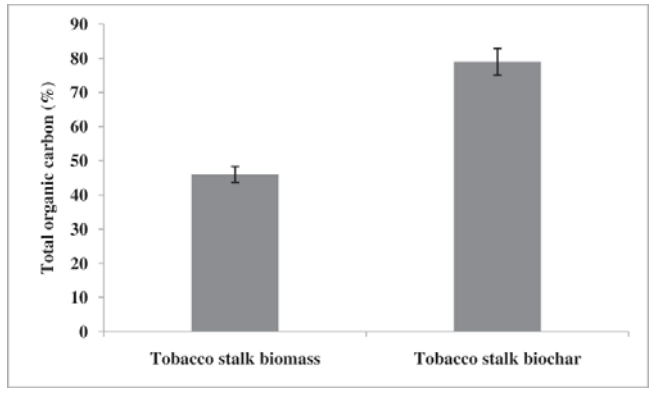

Figure 1: Total organic carbon content of tobacco stalk biomass and tobacco stalk biochar

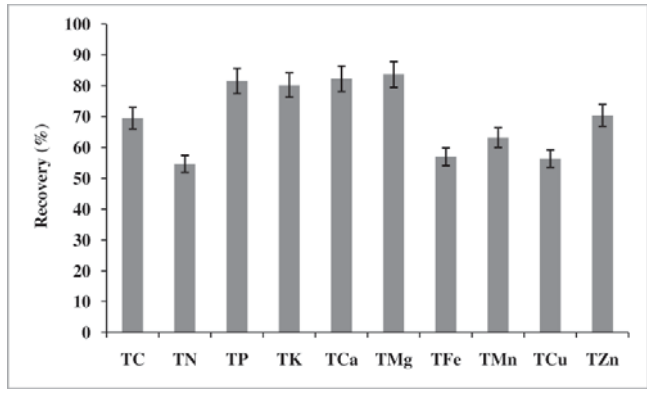

Figure 2: Per cent recovery of nutrients in tobacco stalk biochar (T = total)

Table 2: Average nutrient concentrations of feedstock (tobacco stalk) and biochar (tobacco stalk biochar)

Parameters	Tobacco stalk biomass	Tobacco stalk biochar
Total organic carbon	45.5 ± 1.86	79 ± 3.03
Nitrogen (%)	0.90 ± 0.01	1.23 ± 0.01
C:N Ratio	51:1	67:1
Phosphorus (%)	0.25 ± 0.02	0.51 ± 0.01
Potassium (%)	1.90 ± 0.04	3.81 ± 0.09
Calcium (%)	1.02 ± 0.03	2.10 ± 0.01
Magnesium (%)	0.43 ± 0.03	0.90 ± 0.04
Iron (ppm)	82.50 ± 2.46	117.50 ± 4.49
Manganese (ppm)	3.80 ± 0.10	6.00 ± 0.16
Zinc (ppm)	11.00 ± 0.49	15.50 ± 0.68
Copper (ppm)	4.55 ± 0.18	8.00 ± 0.54

varied from 6 ppm (Mn) to 117.5 ppm (Fe). Quality of feedstock source influences end-product characteristics. In general, most plant based biochar contain elevated C content and the same was also observed in the case of tobacco stalk biochar. The increase in total organic carbon (Fig.1) content of the biochar could be attributed to pyrolysis process where in several chemical reactions takes place resulting in creation of thermally stable fixed C structures (Spokas *et al.,* 2012).

It was clear from the results depicted in Fig. 2, that the amount of carbon conserved from biomass to biochar (% recovery) was 70%. The loss of C under higher temperatures of pyrolysis may be attributed to volatilization of carbon bonded to volatile chemical constituents as reported earlier by Kloss *et al.* (2011). The per cent recovery of nitrogen in the biochar was the lowest (55%) compared to that of all other nutrient elements. The lower N recovery may be attributed to the loss of N compounds during pyrolysis process at higher temperatures. Novak *et al.* (2009) suggested that the decrease in N during higher production temperature can be attributed to aromatization. Further, Gaskin *et al.* (2008) showed that the amount of total N conserved from biomass to biochar ranged from 27.4 to 89.6% in poultry litter and pine chip biochars, respectively. Per cent recovery values in respect of P, K, Ca, Mg (80%) and other micronutrients (60-65%) in the biochar, though relatively greater than that of N, still indicate a substantial loss of nutrients during the process of pyrolysis. During the pyrolysis or oxidation process that generates biochar, heating causes some nutrients to volatilize, especially at the surface of the material, while other nutrients become concentrated in the remaining biochar. Therefore, biochar could act as a soil conditioner and could propel nutrient transformations than serving as a primary source of nutrients (Glasser *et al.*, 2002; Lehmann *et al.*, 2003).

The other important characteristic of biochar was its C: N ratio. The C: N ratio of tobacco stalk biochar produced in the present study was 67:1. In contrast, the C:N ratio of the raw tobacco stalk biomass was 51:1. The C: N ratio of biochar depends not only on relative quantities of the total C and N, but also on relative degree of their loss during the pyrolysis. The wider C: N ratio in the tobacco stalk biochar as compared to that of its biomass indicates preferential volatilization of nitrogen over carbon. This is in agreement with the findings of Shanbagavalli (2012) and Venkatesh *et al.* (2013), who reported wider C/N ratios in maize stover biochar (90:1), groundnut shell biochar (70:1), coir waste biochar (89:1), prosopis wood biochar (83:1) and cotton stalk biochar (40:1 to 62:1).

Physico-chemical characteristics of tobacco stalk biochar

The data on other physical characteristics (Table 3) indicate that the tobacco stalk biochar was alkaline in reaction, with pH of 9.42. The pH of tobacco stalk biochar was greater than that of other reported biochars such as groundnut shell biochar (9.3), prosopis wood biochar (7.57), cotton stalk biochar (8.9 – 9.3) *etc.* (Shenbagavalli, 2012; Venkatesh *et al.*, 2012). The higher pyrolysis temperature was known to have an impact on biochar pH. Higher pyrolysis temperature (500^{0}C) in the present study may have made tobacco stalk biochar more basic. The higher pyrolysis temperatures are reported to remove acidic functional groups and elevate ash content thereby causing biochar to be more basic (Novak *et al.*, 2009). Similar result was observed in the present study where tobacco stalk biochar has recorded alkaline pH and an ash content of 17.47%. Higher temperature during the conversion process had the strongest influence on the biochar pH suggesting that higher temperature might have resulted in higher degree of volatilization, decomposition of surface oxygen groups and dehydroxylation contributing to increased ash residue portion in the biochar (Hass *et al.*, 2012).

Table 3: Characteristics of tobacco stalk biochar

Parameter	Tobacco stalk biochar
pH (1:20)	9.42
EC (dS/m) (1:10)	0.11
CEC (C mol (+)/kg	30.0
Ash (%)	17.47
Volatile matter (VM) (%)	7.61
Fixed carbon (%)	74.92

Cation exchange capacity is also an important characteristic of biochar. Similar to soils, biochar cation exchange capacity (CEC) represents its ability to electrostatically sorb or attract cations. Tobacco stalk biochar had a CEC of 30 C mol (p+) / kg and was well within the range of CEC values reported by Venkatesh *et al.* (2012) for cotton stalk biochar (11.7 to 51.3 C mol (p+) / kg).

The biochar produced from tobacco stalks had 74.9 % fixed carbon, 17.5% ash and remaining 7.6% as volatile matter. Contents of these components in the tobacco stalk biochar appear to be more or less similar in cotton stalk biochar with C 58.7-71, ash 18.1 to 30.2 and 7.9-15.3% as volatile matter. High fixed carbon in tobacco stalk biochar suggests that it is more beneficial as soil amendment, particularly to improve soil physical environment.

The tobacco stalk biochar (having completely charred residue) can be optimally prepared by the pyrolysis at a process temperature of 500^{0}C and a holding time of 90 min. The biochar yield obtained was about 40% of the dry tobacco stalk biomass. Conversion of tobacco stalk biomass to biochar resulted in recovery of carbon and nutrients to the extent of more than 60%, with the exception of nitrogen for which the recovery was 55%. The biochar produced from tobacco stalks was alkaline in nature and had C:N ratio of 67:1. Tobacco stalk biochar also had relatively high proportion of fixed carbon (74.9%) and thereby indicating its suitability as soil amendment for improving the soil physical environment.

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NEUTRAL VOLATILE COMPOUNDS IN ORIENTAL TOBACCO GROWN IN INDIA

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Oriental tobacco also known as 'Turkish tobacco' or 'Aromatic tobacco' possesses typical aromatic smoking quality characterized by sweet and sour taste. The present study was taken up with the objective of assessing the status of neutral volatile compounds (NVCs) in Oriental tobacco grown in the Kurnool tract of Andhra Pradesh. Gas chromatograph-Mass spectrometry (GC-MS) analysis was carried out in the neutral fraction of steam volatiles and 89 NVCs were identified in the samples analysed. The NVCs were classified into eight groups *viz.*, norterpenoids (17%), norlabdanoids (2%), norcarotenoids (9%), cembranoids & thunberganoids (47%), neophytadiene (21%), Maillard reaction products (0.6%), phenylalanine related compounds (1.4%) and carbonyls, alcohols & esters (2%) thus accounting for ~99% of the NVCs identified. Norterpenoids, norlabdanoids and neophytadiene increased by 42, 100 and 31%, respectively in the samples from bottom to top positions on the stalk while cembranoids & thunberganoids decreased by 32%. There was no perceptible variation in the norcarotenoids among the leaf positions. The correlations observed were in tune with the increase of relative proportions in the case of norterpenoids, norlabdanoids and neophytadiene and decrease in the case of cembranoids & thunberganoids from bottom to top position of the plant. Neophytadiene, â-4,8,13-duvatriene-1,3-diol, thunbergol, solanone, epiglobulol, norsolanadione, isomers of megastigmatrienones, (1s,2E,4s,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol, farnesyl acetone, â-damascone, hexahydrothunbergol, 8,13-epoxylabd-14-en-12-one, 8-epoxy-12-norambreinolide, (cis-A/B)-sclareoloxide and sclaral were identified as the important NVCs constituting the volatile profile of Oriental tobacco and their impact on the smoke flavour was documented. Identification of norlabdanoids viz., 8,13-epoxylabd-14-en-12-one, 8-epoxy-12-norambreinolide, (cis-A/B)-sclareoloxide, sclareolide and sclaral in the Oriental tobacco samples analysed in the present study is an important finding as these compounds are responsible for the typical cedar-amber notes of this tobacco.

Key Words: Oriental tobacco, Neutral volatile compounds, GC-MS

INTRODUCTION

A survey of the literature on aroma-bearing constituents reveals that the 300-odd volatile compounds, identified so far do not fall into a single class of compounds but represent a wide array of chemical entities like degradation products of thunberganoids, carotenoids and labdanoids, basic nitrogenous compounds, acidic substances, simple carbonyls and several types of neutral compounds. Wahlberg and Enzell (1987) reported that two major classes of diterpenoids *viz.*, monocyclic cembranoids and the bicyclic labdanoids are found in tobacco. Virginia and Burley tobaccos contain only the cembranoids, while Oriental and Cigar tobaccos contain both labdanoids and cembranoids. It is reported that about 41 compounds are found in substantial quantities to enable the smoker to perceive their impact on smoke flavour. The compounds are broadly classified as acids, alcohols, aldehydes, amides, anhydrides, esters, ethers, hydrocarbons, ketones and lactones. As the aroma-bearing constituents are basically volatile in nature, a micro-analytical technique like gas chromatograph-mass spectrometry (GC-MS) is generally employed as the individual component of the isolates is in minute quantity. 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.

Among the different tobacco types, Oriental tobacco also known as 'Turkish tobacco' or 'Aromatic tobacco' possesses typical aromatic smoking quality characterized by sweet and sour taste. The cheesy-sweety-buttery notes are attributed to the volatile acids *viz.*, â-methylvaleric, isovaleric and 2-methylbutyric acids, while the norlabdanoids *i.e.*, norambreinolide, dehydronorambreinolide, sclaral, ã-bicyclohomofarnesal and ambrox are

reported/purported to be responsible for the cedar-amber notes. The best quality Oriental tobacco is produced in rocky, poor and infertile soils containing minimal amounts of nitrogen and organic matter (Gilchrist, 1999). Very small and bodied leaves are produced with low nicotine and high sugars.

In India, Oriental tobacco is naturally grown in limited area in the Kurnool district of Andhra Pradesh, without application of any of the inorganic fertilizers. Oriental tobacco is characterized by small size, aromatic, flavourful and readily combustible leaf with good filling capacity and used in blending with FCV tobacco in cigarette manufacture. Oriental tobacco leaves are harvested by priming 3-4 leaves at a time when the leaves mature and are air-cured. Air-curing is different from flue-curing where there is no control of temperature and moisture. Hence, formation of degradation derivatives in tobacco during air-curing is entirely different from flue-curing. As a part of comprehensive investigations on the chemical constituents responsible for smoke flavour in Indian tobacco, the present study was taken up with the objective of assessing the status of neutral volatile compounds (NVCs) in Oriental tobacco.

MATERIALS AND METHODS

The cured tobacco samples of Oriental tobacco (variety Izmir) were collected from the field experiments conducted in the Kurnool district of Andhra Pradesh during 2012 season. Leaf samples from different leaf positions on the stalk (bottom, middle and top) were collected separately from three locations as three replications in the experimental field grown with recommended package of practices. The mid-ribs were removed and dried in the hot air oven at 60 °C for 3 to 6 h, powdered and passed through 40 micron mesh. Analysis of nicotine and reducing sugars in the samples was undertaken by employing autoanalyezer (Harvey *et al.*, 1969).

Tobacco powder (10 g) and sodium sulphate (5 g) were taken in 500 ml distilling flask, 250 ml of phosphate buffer (pH 6.8) was added and distillate was collected into 500 ml volumetric flask containing 250 ml methylene chloride (dichlormethane) aqueous and organic layers were separated by using separating funnel organic layer was treated with 50 ml of in tartaric acid. Organic layer thus obtained was passed over anhydrous sodium sulphate. The final solution was made up to 1 ml for analysis (Wu *et al.*, 1992). The GC-MS analysis was carried out using a ZB-5 capillary column (length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm) fixed in a Shimadzu Model QP-2001 Plus GC-MS in Electron Ionisation (EI) mode.

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 (held for 1 min) to 140°C (held for 5 min) @ 6°C/min, from 140°C to 180°C (held 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 minutes. 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 the samples were analysed in scan mode with a mass range of 50 to 500 units at a scan speed of 2500. 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 per cent of a particular compound in the total neutral volatile (NV) fraction was calculated.

RESULTS AND DISCUSSION

The NVCs were classified into eight groups viz., norterpenoids, norlabdanoids, norcarotenoids, cembranoids & thunberganoids, neophytadiene, Maillard Reaction Products, Phenylalanine related compounds and Carbonyls, Alcohols & Esters. The relative proportions of different groups and their composition was estimated. Based on the relative content of about 30 most important NVCs, volatile profile was formulated. Considering the mean

values of all the samples, the results were discussed keeping in view 1) relative proportion of the seven groups of NVCs and the major compounds in each group, 2) volatile profile and 3) correlations among the groups.

In the present study, 89 NVCs were identified in the Oriental tobacco samples (original data not presented for brevity). The proportion of NVCs in the eight groups indicated above is as follows: norterpenoids (17%), norlabdanoids (2%), norcarotenoids (9%), cembranoids & thunberganoids (47%), neophytadiene (21%), Maillard Reaction Products (0.6%), Phenylalanine Related Compounds (1.4%) and Carbonyls, Alcohols & Esters (2%) thus accounting for 99% of the NVCs identified (Table1).

According to Fujimori *et al.* (1978), the medium-range boiling point fraction of Burley tobacco was composed of neophytadiene-related compounds: 46.2%, degradation products of carotenoids (megastigmatrienones, â- damascene etc.): 7.7%, degradation products of thunberganoids (solanone, duvatrienediol *etc.*): 3.6% and mono and sesquiterpenoids (solavetivone *etc.*): 4.7%. Huang *et al.* (2006) reported that 102 volatile compounds among 138 separated peaks were identified and quantified accounting for about 88.9% of the total content. Zhu *et al.* (2009) have identified and quantified 39 volatile components of the tobacco flavour samples accounting for 86.54% of the total content. In the FCV tobacco samples from NLS, KLS and SLS, the identified NVCs accounted for 83.4, 93.2 and 89.4% of the total fraction, respectively (Srihari *et al.*, 2013).

The relative proportion of norterpenoids increased with ascending leaf position on the stalk from bottom to top (14.44 to 20.80%). The increase (~42%) was more pronounced from middle to top positions. Nearly 100% increase was observed in the case of norlabdanoids from bottom (1.32%) to top (2.70%) positions. There was no perceptible variation in the norcarotenoids among the leaf positions (~8.50%). The proportion of cembranoids and thunberganoids was maximum (53.45%) in the leaf from bottom position and ~32% decrease was observed in the samples from top position (36.34%). Relative content of the principal NVC, neophytadiene increased by ~31% from the bottom position (18.16%) to the top position (23.86%). In all the above four groups of NVCs, only marginal differences were observed in the samples from bottom and middle positions. In respect of the other three groups of NVCs *viz.*, Maillard reaction products, phenylalanine related compounds and carbonyls, alcohols & esters, the differences among the plant positions were not significant (Table 1).

Highly significant and positive correlations were observed between norterpenoids and norlabdanoids (0.9767), neophytadiene (0.9768) while the correlation was negative with cembranoids & thunberganoids (-0.9996). Norlabdanoids had a negative correlation with cembranoids & thunberganoids (-0.9824) and a positive correlation with neophytadiene (1.000).

Table 1: Relative proportion (%) of important groups of NVCs, nicotine and reducing sugars in Oriental tobacco leaf from different plant positions

Group	Bottom*	Middle*	Top*	Overall mean
Norterpenoids	14.44	15.40	20.80	16.88
Norlabdanoids	1.32	1.81	2.70	1.95
Norcarotenoids	8.57	8.52	8.57	8.55
Cembranoids & Thunberganoids	53.45	50.37	36.34	46.72
Neophytadiene	18.16	20.18	23.86	20.73
Maillard Reaction Products	0.62	0.59	0.52	0.58
Phenylalanine Related Compounds	1.45	0.98	1.89	1.44
Carbonyls, Alcohols & Esters	1.99	2.14	1.26	1.80
Nicotine (%)	0.53	0.29	0.49	0.44
Reducing sugars (%)	11.69	18.63	11.10	13.81

*Mean of three replications

Neophytadiene had a negative correlation with cembranoids & thunberganoids (-0.9825). The correlations are in tune with the increase of relative proportions in the case of norterpenoids, norlabdanoids and neophytadiene and decrease in the case of cembranoids & thunberganoids from bottom to top position of the plant.

Davis (1976) reported that leaves from the upper stalk position contained greater quantities of neophytadiene and also its content was more in the flue-cured tobacco than air-cured tobacco. According to Grunwald *et al.* (1977) crude lipid, chlorophyll and carotenoids were higher in top leaves than in bottom leaves.

Based on the mean values of the relative proportion of the groups of NVCs, the major compounds in the respective groups are as follows: norterpenoids (epiglobulol, farnesyl acetone, hexahydrofarnesyl acetone and geranyl acetone); norlabadanoids (8,13-epoxylabd-14-en-12-one, 8-epoxy-12-norambreinolide, (cis-A/B)-sclareoloxide and sclaral); norcarotenoids (megastigmatrienone isomers, dihydroactinidiolide and â-damascone); cembranoids and thunberganoids (â-4,8,13-duvatriene-1,3-diol, thunbergol, solanone, norsolanadione and (1s,2E,4s,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol); Maillard reaction products (methylethylmaleimide and indole); phenylalanine related compounds (phenethyl alcohol and phenylacetaldehyde) and carbonyls, alcohols & esters (palmitic aldehyde and 4-vinyl-2-methoxy-phenol). It is inferred from the results that both cembranoids and labdanoids are present in the samples analysed. Wahlberg and Enzell (1987) reported that two major classes of diterpenoids viz., monocyclic cembranoids and the bicyclic labdanoids are found in tobacco. Virginia and Burley tobaccos contain only the cembranoids, while Oriental and Cigar tobaccos contain both the labdanoids & cembranoids.

It is reported in the literature that the compounds responsible for the cedar-amber note of Oriental tobacco are derivatives of labdanoids. It is reported that *N. tomentosiformis*, one of the progenitors of *N. tabacum* produces diterpenoids of the labdane type (Reid, 1974). Schumacher patented norambreinolide (sclareolide) as an important constituent of Oriental tobacco and Kaneko (1971) isolated the compound from cigar tobacco leaf. Giles and Schumacher (1961) have reported the first C_{20} labdanoids in tobacco *i.e.* á- and â-levantenolides. These compounds were identified as the potential precursors of the five C_{16} norlabdanoids (norambreinolide, dehydronorambreinolide, ã-bicyclohomofarnesal, sclaral and ambrox) which contribute to the cedary odour of the Oriental tobacco (Schumacher and Vestal, 1974).

Taking into consideration the mean values of relative content of 28 NVCs in the samples, which accounted for ~93% of NVCs identified, the volatile profile of Oriental tobacco grown in the Kurnool tract of Andhra Pradesh was established (Table 2). It can be inferred that neophytadiene, â-4,8,13-duvatriene-1,3-diol, thunbergol, solanone, epiglobulol, norsolanadione, isomers of megastigmatrienones, (1s,2E,4s,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol, farnesyl acetone, â-damascone, hexahydrothunbergol, 8,13-epoxylabd-14-en-12-one, 8-epoxy-12-norambreinolide, (cis-A/B)-sclareoloxide and sclaral are the important NVCs responsible for the smoke flavour of Oriental tobacco. The other minor compounds detected are listed in Table 3.

The important quality determinants of tobacco are leaf aroma and smoke flavour which refer to the sensory impressions perceived by the nasal passages. These distinct impressions are caused by the volatile constituents. Many biochemical and chemical degradation reactions occur in the leaf during post-harvest processing (curing, fermentation, ageing *etc.*) of tobacco ultimately generating the typical aroma. Many of the green leaf constituents undergo enzymatic, microbial, photochemical and oxidative reactions, the relative importance of which is dependent on the type of curing (flue-curing, air-curing or sun-curing) contributing to typical tobacco aroma.

Impact of some NVCs on the smoking quality has been reported in the literature (Green, 1977; Leffingwell *et al.*, 1972; Demole and Dietrich, 1977): neophytadiene (soothing, smoothing); isomers of megastigmatrienone (spicy, peppery, add body); solanone (smooth, ketonic); geranyl acetone (green, adds body); dihydroactinidiolide (slight cooling); phenylethyl alcohol (floral, rose),

phenylacetaldehyde (intense floral); benzyl alcohol (weak, floral, smoothing), indole (smoothing, floral), duvatrienediol [oxidation products (solanone, oxysolanone, branched chain volatile acids) contribute to smoke flavour]; â-damascone (floral, adds body) and farnesyl acetone (sweet, green, flue-cured note).

Kimland *et al.* (1972) have studied the volatile fraction of the neutral oxygen-containing constituents of sun-cured Greek tobacco and identified 32 compounds (mainly ketones and aldehydes) using GC-MS. The important compounds identified were: â-cyclocitral, benzaldehyde, safranal, 6-methyl-3,5-heptadien-2-one, carvone, 2-acetylpyrrole, hexahydrofarnesyl acetone, phenylethylacetate, dibutylphthalate, benzyl alcohol, phenylethanol,damascenone, farnesyl acetone, 6-methyl-2-heptanone, solanone, geranyl a cetone, 6-methyl-5-hepten-2-one and dihydroactinidiolide.

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). The ionones, megastigmatrienones, damascones and damascenones are considered to be the most

Table 2: Relative content (%) of important NVCs in Oriental tobacco leaf from different plant positions contributing to the volatile profile

Rt (min)	Compound	Bottom*	Middle*	Top*	Mean
8.12	Benzyl alcohol	0.40	0.27	0.71	0.46
8.38	Phenylacetaldehyde	0.54	0.30	0.47	0.44
10.07	Phenethyl alcohol	0.48	0.41	0.70	0.53
12.92	Methylethylmaleimide	0.30	0.25	0.28	0.28
14.50	Indole	0.21	0.10	0.11	0.14
15.01	4-Vinyl-2-methoxy phenol	0.29	0.29	0.29	0.29
16.46	Solanone	4.59	4.10	3.79	4.16
19.58	Geranyl acetone	0.87	0.88	0.92	0.89
20.95	Norsolanadione	3.55	4.06	3.53	3.71
22.58	Dihydroactinidiolide	1.21	1.09	1.30	1.20
23.53	Megastigmatrienone isomers	3.53	2.73	5.09	3.78
25.67	(1s,2E,4s,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	3.70	2.15	4.85	3.57
25.86	Tetrahydroionone	ND	1.37	0.71	1.04
26.73	Hexahydrothunbergol	0.67	1.07	0.93	0.89
27.72	Palmitic aldehyde	0.86	1.33	0.52	0.90
32.28	Neophytadiene	18.16	20.18	23.86	20.73
32.39	Hexahydrofarnesyl acetone	1.80	1.49	1.46	1.59
33.89	(cis-A/B)-Sclareoloxide	0.98	1.33	0.98	1.10
34.72	Farnesyl acetone	2.29	2.08	1.47	1.95
35.49	Isophytol	0.24	0.35	0.29	0.29
35.66	Sclaral (Sclareolide lactol)	0.70	1.03	0.76	0.83
37.56	8-Epoxy-12-norambreinolide	0.89	0.90	0.98	0.92
38.65	Thunbergol	19.89	18.75	3.26	13.97
42.17	Epiglobulol	3.12	2.23	4.58	3.31
44.42	Globulol	1.79	3.01	2.65	2.48
45.66	8,13-Epoxylabd-14-en-12-one	ND	1.02	1.28	1.15
46.13	Duvatriendiol	21.64	20.24	19.98	20.62
46.31	â-Damascone	1.76	1.74	1.21	1.57

*Mean of three replications; ND: Not detected

important carotenoid derivatives found in cigarette smoke. The ionones and damascenones are primary aromatic compounds found in rose oil; therefore, these compounds add floral and woody-like notes to the aroma and taste of cigarette smoke (Roberts, 1988).

The predominant duvane found in tobacco is 4, 8, 13-duvatriene-1,3-diol (DVTol), which accounts for ~50% of the lipids in immature tobacco, produces degradation products by oxidation during senescence and curing. The degradation products like solanone, oxysolanone and branched chain volatile acids are reported to contribute to Burley tobacco flavour. Aasen *et al.* (1975) reported that the volatile degradation products of thunberganoids viz., á- and â-4,8,13-duvatriene-1-3-diols, solanone, norsolanadione etc. are organoleptically important. Neophytadiene has been suggested to be a tobacco flavour

Table 3: Minor NVCs identified in Oriental tobacco samples

Group/Compound	**Group/Compound**
Norterpenoids	**Norcarotenoids**
Dehydroaromadendrene	Dihydro-â-ionone
7,8-Dihydrolinalool	(E,Z-Pseudoionone)
Limonene dioxide 4	3-Oxo-á-ionol
Limonene dioxide 1	3-Oxo-7,8-dihydro-á-ionol
Longiborneol	á-Ionone
trans-Farnesol	Carotol
3,7,11,16-Tetramethyl-hexadeca-2,6,10,14-tetraen-1-ol	Dihydrooxophorone
Nootkatone	Â-Ionone
(Z,E)-Farnesal	Dihydro-á-Ionone
DL-6,7-Dihydro-2,cis-farnesol	Vitamin A Alcohol
Spathulenol	Â-iso Methyl ionone
Widdrol	4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)- 2-butanone
17-Acetoxy-19-kauranal	Tetrahydroactinidiolide
d-Nerolidol	Â-iso Methyl ionone
Globulol	**Maillard Reaction Products**
Â-Cyclo homogeraniol	Furfural
(E)- Citral	Methylvinylmaleimide
8S,14-Cedran-diol	Benzaldehyde
(E)- Geranial	2-Acetylpyrrole
(E)-5-Isopropyl-8-hydroxy-8-methyl-non-6-en-2-one	**Carbonyls, Alcohols & Esters**
Isospathulenol	6-Methyl-5-hepten-2-one
Bicycloelemene	2-trans-6-cis-Nonadienal
3,7,11,16-Tetramethyl-hexadeca-2,6,10,14-tetraen-1-ol	trans-2-Nonenal
Nerolidol-epoxyacetate	4-Vinyl-2-methoxy-phenol
2,3-Epoxy-geranyl acetate	Diethyl phthalate
Citronellyl acetate	Myristaldehyde
Isocaucalol	Isobutyl phthalate
Caryophyllene oxide	Methyl hexadecanoate
Norlabdanoids	Myristaldehyde
Sclereodiol	Ethyl linoleolate
(11e,13z)-Labdadien-8-ol	Ethyl phthalate
13(16),14 Labdien-8-ol	Hendecanal

enhancer and is considered as a flavour carrier by entrapping volatiles in the tobacco smoke aerosol (Leffingwell and Leffingwell, 1988).

It is important to mention that norlabdanoids viz., 8,13-epoxylabd-14-en-12-one, 8-epoxy-12-norambreinolide, (cis-A/B)-sclareoloxide, sclareolide and sclaral were identified in the Oriental tobacco samples analysed in the present study. Even though Oriental tobacco contains many of the volatile compounds present in flue-cured and air-cured (Burley) tobaccos, majority of them are responsible only for the background flavour notes. The predominant flavour characteristics are attributed to the flavour compounds which are derivatives of labdanoids (e.g. norambreinolide, sclaral) possessing the cedar-amber notes.

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EFFICACY OF PLANT EXTRACTS AND INORGANIC SALTS AGAINST CIGARETTE BEETLE *LASIODERMA SERRICORNE* (F).

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Key words: Cigarette beetle, Inorganic salts, Plant extracts

Cigarette beetle, *Lasioderma serricorne* (F.) is primarily a pest of stored tobacco, tobacco seed and tobacco products like cigarettes and cigars. It causes major economic loss to the tobacco industry worldwide. In addition to tobacco, both the adult and larval forms damage other stored commodities both edible and non-edibles like rice, dry pet-food, pharmaceuticals, books, leather, spices and various seeds. Seed is the precious item for the future crop. Seed treatment with insecticides, fumigation and heat treatment are some of the techniques followed by the seed processing units with limited success. Tobacco seed is treated with one or the other insecticide to protect it from damage caused by cigarette beetle. To replace the pesticides, some of the plant extracts and inorganic salts were tested to protect tobacco seed from the damage by cigarette beetle.

Fresh leaf samples (100 g each) of 34 selected plant species were collected separately and allowed to dry under shade for 12 h. Each dry leaf sample was powdered and extracted with acetone for 12 h in Soxhlet extraction unit and the solvent was removed by vacuum evaporator. Crude leaf extracts thus obtained were used in this study by dissolving the extract in acetone to the final concentration of µg/µl. Each crude extract was mixed with tobacco seed (var. Hema) @ 1, 2, 3, 5 and 10 µl/g seed and placed in 15 ml flat bottomed glass culture tubes with screw caps. Ten newly hatched grubs of cigarette beetle were allowed to feed on the treated seed. In another study, stalk solutions (1, 2, 3, 5 and 10 µg/µl) of 13 inorganic salts *viz.*, calcium carbonate, copper sulphate, ferric phosphate, ferrous sulphate, magnesium sulphate, manganese sulphate, potassium chloride, potassium iodide, potassium aluminium sulphate, potassium dihydrogen phosphate, sodium chloride, sodium fluoride and tricalcium phosphate were prepared separately in distilled water. One ml each of the above stock solution is added to 1 gram tobacco seed separately kept in 15 ml flat bottomed glass culture tubes with screw caps. Newly hatched grubs (10) of cigarette beetle were allowed to feed on the treated seed. There were four replications per each treatment. Both the experiments were conducted under laboratory conditions at 25 ± 2°C. Observations on mortality of grub and emergence of adult beetle were recorded at every 48 h interval. Earlier, the stock culture of cigarette beetles was maintained in the laboratory on untreated tobacco seed. The experiment was conducted for two years during 2005-2007 and the data recorded were analysed statistically (Gomez and Gomez, 1984).

The results with 34 extracts at five concentrations were presented in Table 1. The plant extracts differed significantly in causing mortality of the cigarette beetle grubs and the mortality increased with increased concentration of extract. Mortality of grub ranged between 0.0 to 20.0 and 0.0 to 22.0% due to 1 and 2 µg concentration of the extract, respectively. The extracts of *Leucas* sp., *Piper* sp. and *Ocimum* sp. inhibited the development of the grub and caused the highest mortality (48%) of the grubs at 10 µg concentration followed by the extracts of *Nictanthus* sp., *Adiantum* sp., *Myconia* sp. and *Datura stramonium*. The extracts of *Lantena camera* and *Dendrobium* sp. recorded the lowest mortality of the grubs at all concentrations. From the above, it is evident that acetone extracts of *Leucas* sp., *Piper* sp. and *Ocimum* sp. might contain some compounds with insecticidal property that caused mortality to the grubs of cigarette beetle.

Table 1: Mortality of *L. serricorne* by plants extracts*

Name of the plant species	Larval mortality (%)				
	1 µg	**2 µg**	**3 µg**	**5 µg**	**10 µg**
Leucas sp.	18 (25.1)	22 (27.9)	24 (29.3)	26 (30.6)	48 (43.8)
Metastomata malbathricum	00 (00.0)	06 (13.9)	06 (13.9)	08 (16.4)	14 (21.9)
Cleodendron unfortunatum	10 (18.3)	12 (19.9)	12 (20.1)	14 (21.9)	32 (34.4)
Nictanthus sp.	14 (21.9)	18 (25.1)	18 (25.1)	22 (27.9)	42 (40.3)
Piper sp.	20 (26.4)	20 (26.6)	22 (27.9)	26 (30.6)	48 (43.8)
Myconia sp.	14 (21.9)	18 (25.1)	18 (25.1)	18 (25.1)	38 (38.0)
Adiantum sp.	10 (18.3)	18 (25.1)	18 (25.1)	22 (27.9)	40 (39.2)
Thivita nerifolia	10 (18.3)	06 (13.9)	10 (18.3)	12 (20.3)	28 (31.9)
Adhatoda vasica	00 (00.0)	00 (00.0)	06 (13.9)	06 (13.9)	14 (21.9)
Nishinda sp.	00 (00.0)	04 (11.5)	06 (13.9)	10 (18.3)	20 (26.6)
Cyda cordifolia	04 (11.5)	06 (13.9)	06 (13.9)	06 (13.9)	18 (25.1)
Acasia safeda	00 (00.0)	00 (00.0)	04 (11.5)	04 (11.5)	14 (21.9)
Calotropis procera	08 (15.9)	10 (18.3)	14 (21.9)	18 (25.1)	36 (36.8)
Oxalis sp.	08 (15.9)	12 (19.9)	14 (21.9)	14 (21.9)	34 (35.6)
Datura stramonium	12 (19.9)	16 (23.5)	16 (23.6)	18 (25.1)	38 (38.0)
Calamus sp.	04 (11.5)	10 (18.3)	10 (18.3)	14 (21.9)	34 (35.6)
Vinca rosea var. Alba	04 (11.5)	08 (15.9)	10 (18.3)	10 (18.3)	30 (33.2)
Vinca r osea var. Ruby	04 (11.5)	10 (18.3)	10 (18.3)	14 (21.9)	32 (34.4)
Anona reticulata –leaf	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	16 (23.6)
Tagitus erecta var. African	00 (00.0)	04 (11.5)	06 (13.9)	06 (13.9)	14 (21.9)
Terminalia arjun	04 (11.5)	06 (13.9)	10 (18.3)	14 (21.9)	34 (35.6)
Ocimum sp.	00 (00.0)	04 (11.5)	14 (21.9)	24 (29.3)	48 (43.8)
Acacia auriculiformis	00 (00.0)	04 (11.5)	06 (13.9)	10 (18.3)	22 (27.9)
Annona reticulata- seed	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	14 (21.9)
Juniperus sp.	00 (00.0)	00 (00.0)	04 (11.5)	06 (13.9)	20 (26.6)
Juniperus sp. thorny	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	14 (21.9)
Lantena camera	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	06 (13.9)
Dendrobium sp.	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	10 (18.3)
Aegle marmelos	00 (00.0)	00 (00.0)	04 (11.5)	10 (18.3)	34 (35.6)
Dendrobium aphyllum	00 (00.0)	00 (00.0)	00 (00.0)	06 (13.9)	14 (21.9)
Alstonia scholaris	00 (00.0)	04 (11.5)	06 (13.9)	14 (21.9)	30 (33.2)
Cestrum nocturnum	00 (00.0)	00 (00.0)	00 (00.0)	04 (11.5)	12 (20.3)
Cuscuda sp.	00 (00.0)	00 (00.0)	00 (00.0)	06 (13.9)	16 (23.6)
Cajanus cajan (seed coat)	00 (00.0)	04 (11.5)	06 (13.9)	10 (18.3)	26 (30.6)
Control (Malathion)	80 (63.5)	80 (63.5)	80 (63.5)	80 (63.5)	80 (63.5)
SEm±	**2.6**	**2.1**	**1.8**	**1.7**	**1.6**
CD (P=0.05)	**7.8**	**5.9**	**5.4**	**4.6**	**4.4**
CV (%)	**18.1**	**14.7**	**13.2**	**11.8**	**5.8**

* Figures in the parentheses are angular transformed values.

Plant extracts of *Ocimum* sp. is highly toxic to beetles of the species *Sitophilus granarius, S. zeamais, Tribolium castaneum, Prostephanus truncatus* and *Lasioderma serricorne* if admixed to grain. Complete control was achieved after 24 h at a dosage of 0.5 µg/kg or 0.5 mg/kg of grain. Furthermore, it was proved that admixture of these compounds with low quantities of vegetable oils like sunflower seed oil or sesame oil increased toxicity to insects and persistency (Obeng-Ofori *et al.*, 1997). According to Ojimelukwe and Adler (1999), a mixture of ground seeds of brown pepper *Piper guinense* and ground fruits of *Xylopia aethiopica*, controlled 95% of adult *Callosobruchus chinensis* in peas within 48 h.

The current findings indicated that the extracts of *Leucas* sp., *Piper* sp. and *Ocimum* sp. @10 µg/g concentrations inhibited the development of the grub and caused the highest mortality of the grubs. These results are in agreement with the findings of Obeng-Ofori *et al.* (1997) and Ojimelukwe and Adler (1999).

The experiment with 13 inorganic salts at 1, 2, 3, 5 and 10 µg/g concentrations were evaluated for the control of *L. serricorne* and the results were presented in Table 2. The inorganic salts differed significantly in causing mortality to the grubs of cigarette beetle and the mortality increased with the increased concentration of the inorganic salt. The maximum mortality (100%) of the grubs were recorded when the seed (1g) was mixed with 10 µg sodium chloride or potassium chloride or ferrous sulphate (82%).

Davis *et al.* (1984) reported that tricalcium phosphate acted as a legume grain protectant against three bean weevils (Navy beans, Cowpeas and *Vigna unguiculata*) at low levels (dusted at 0.1 and 0.25% by weight). Sodium chloride mixed with turmeric powder and mustard oil to basmati rice protected it from the damage by rice weevil (Jilani and Su, 1983). Magnesium carbonate mixed with wheat seed protected it from the damage by *Trogoderma granarium* (Sharma and Verma, 1971). In the current study, sodium chloride, potassium

Table 2: Mortality of *L. serricorne* influenced by some inorganic salts*

Inorganic salt/Conc.	Mortality (%)				
	1 µg	**2 µg**	**3 µg**	**5 µg**	**10 µg**
Calcium carbonate	18 (50.1)	20 (53.1)	18 (50.1)	14 (43.8)	16 (47.9)
Copper sulphate	30 (65.7)	24 (58.4)	20 (53.1)	16 (46.8)	10 (36.6)
Ferric phosphate	50 (89.9)	42 (80.7)	34 (71.2)	24 (58.4)	18 (50.1)
Manganese sulphate	24 (58.4)	22 (55.8)	20 (53.1)	18 (50.1)	16 (46.8)
Magnesium sulphate	16 (46.8)	14 (42.9)	18 (50.1)	10 (36.6)	10 (36.6)
Potassium aluminium sulphate	32 (68.7)	22 (55.8)	18 (50.1)	18 (50.1)	16 (46.8)
Potassium dihydrogen phosphate	28 (63.8)	18 (50.1)	20 (53.1)	12 (40.5)	12 (40.5)
Potassium iodide	18 (50.1)	10 (36.6)	12 (40.5)	6 (27.9)	8 (32.8)
Sodium chloride	72 (116.1)	82 (129.8)	86 (136.0)	94 (151.9)	100 (179.9)
Sodium fluoride	32 (68.7)	28 (63.8)	26 (61.2)	22 (55.8)	20 (53.1)
Tricalcium phosphate	28 (63.8)	24 (58.4)	26 (61.2)	20 (53.1)	20 (53.1)
Potassium chloride	64 (106.2)	72 (116.1)	82 (129.8)	90 (143.2)	100 (179.9)
Ferrous sulphate	56 (96.8)	64 (106.2)	68 (111.0)	72 (116.1)	82 (129.8)
Control (untreated)	4 (23.0)	4 (23.0)	0	4 (23.0)	0
SEm±	**2.6**	**1.7**	**0.9**	**1.9**	**1.66**
CD (P=0.05)	**8.0**	**5.2**	**2.9**	**5.8**	**5.0**
CV (%)	**10.72**	**7.3**	**3.8**	**11.3**	**6.68**

* Figures in the parentheses are angular transformed values.

chloride and ferrous sulphate protected the tobacco seed and it might be due to inhibiting one or the other growth processes of *L. serricorne* or must be acting as a stomach poison and thereby causing mortality.

Thus the results showed that the crude extracts of *Leucas* sp., *Piper* sp. and *Ocimum* sp (10 mg/kg) or inorganic salts *viz*., sodium chloride or potassium chloride or ferrous sulphate (10 mg/kg) can be used as tobacco seed protecting agents against *L. serricorne*.

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MANAGEMENT OF FROG-EYE SPOT DISEASE IN *BIDI* TOBACCO NURSERY

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Frog-eye spot disease caused by *Cercospora nicotianae* is a dreadful disease in nursery and field crop of *bidi* tobacco. Avoidable loss in yield and nicotine content has been estimated to the tune of 21 and 20 %, respectively and carbendazim is recommended to manage the disease (Patel *et al.*, 1984; Patel *et al.*, 1991; Patel *et al.*, 2001). Now-a-days, a number of new compounds are available in the market. Therefore, present experiment was conducted to study the bio-efficacy of the new compounds in management of the disease.

The experiment was carried out at the nursery of BTRS, AAU, Anand during 2010-11 to 2012-13. Thirty-two beds, each of 1.2 x 1.2 m, were prepared applying manures and fertilizer as per the recommendation. The beds were seeded with susceptible *bidi* tobacco variety Anand 119 @ 5 kg seeds/ha. The experiment was conducted in RBD with four replications and eight treatments *i.e.* carbendazim 12% + mancozeb 63% @ 0.225%, hexaconazole @ 0.01% , propiconazole @ 0.05% , difenoconazole @ 0.05%, propineb @ 0.225% , penconazole @ 0.04% , carbendazim @ 0.025% and control (no fungicide). The fungicides were sprayed at the initiation of the disease and thereafter as and when required at 10 days interval. Observations on frog-eye spot were recorded at appropriate time following 0-5 scale. Similarly, observations on other parameters were recorded at appropriate time following standard procedure. The data were statistically analyzed.

Pooled results (Table 1) revealed non-significant differences for germination count, fresh weight and total surviving seedlings. This indicated no adverse effect of the treatments on the growth of the seedlings. Application of fungicides significantly reduced frog-eye spot disease compared to control and increased healthy transplantable seedlings barring penconazole @ 0.04% and propiconazole @ 0.05%, with minimum disease in the treatment of carbendazim + mancozeb @ 0.225 % and maximum transplants in hexaconazole @ 0.01%. The treatment, carbendazim + mancozeb @ 0.225% was the best in reducing the disease and was at a par with carbendazim @ 0.025%. Affectivity of carbendazim in managing the disease in *bidi* tobacco nursery and field crops are well documented in literature (Patel *et al.*, 1984; Patel *et al.*, 1991; Patel *et al.*, 2001). No information on the combi-product of carbendazim with mancozeb was available in *bidi* tobacco. Therefore, the finding will be of great importance in *bidi* tobacoo where two diseases, frog-eye and brown spots, are occurring in the field crop and can be managed by a single combi-product of carbendazim and mancozeb.

Based on the effectiveness, number of transplants and economics, hexaconazole @ 0.01% or propineb @ 0.225 % or carbendazim + mancozeb @ 0.225 % in alteration to carbendazim @ 0.025% can be recommended. However, economics worked out for hexaconazole, propineb, carbendazim + mancozeb and carbendazim gave net realization of Rs. 2,13,952, 1,99,448, 1,85,070 and 1,80,030, respectively and ICBR 1:50.7, 1:33.9, 1:24.0 and 1:14.5, respectively.

Looking to the effectiveness and ICBR, farmers are advised to apply two sprays of carbendazim + mancozeb @ 0.225 % (1.125 kg. a.i./ ha.; 30 g/10 l water/200 m^2) at 10 days interval starting from initiation of the disease for effective and economical management of frog-eye spot disease in *bidi* tobacco nursery.

For effective and economical management of frog-eye spot disease in *bidi* tobacco nursery,

Table 1: Effect of various treatments in management of frog-eye spot disease in *bidi* tobacco nursery

Treatment	Germination (count/ 25 cm^2)	Fresh weight (g)	Frog-eye spot index (0-5)*		Seedlings/m^2	
			($\sqrt{x+1}$)	Retra.	Transplantable	Total surviving
Carbendazim 12%+ mancozeb 63% @ 0.225%	10	293	1.06	0.01	394	472
Hexaconazole @ 0.01%	10	329	1.28	0.08	434	512
Propiconazole @ 0.05%	11	233	1.45	0.20	350	451
Difenoconazole @ 0.05%	11	293	1.28	0.08	394	462
Propineb @ 0.225%	10	290	1.62	0.38	414	467
Penconazole** @ 0.04%	11	277	1.78	0.61	327	390
Carbendazim @ 0.025%	10	287	1.12	0.01	389	484
Control (no fungicide)	9	273	1.96	0.92	300	409
SEm±	**0.53**	**27.35**	**0.04**		**23.26**	**31.68**
CD (P=0.05)	**NS**	**NS**	**0.10**		**66.40**	**NS**
Year effect.	**Sign**	**Sign**	**Sign**		**Sign**	**NS**
Y X T inter	**NS**	**NS**	**NS**		**NS**	**NS**
CV (%)	**15.31**	**28.85**	**7.63**		**18.59**	**20.85**

*0= Free; 5= Maximum disease intensity; **Not applied

Table 2: Economics of effective treatments

Treatment	No. of transplants/ ICBR 7000 m^2 ('000)	Gross income, (Rs./ha)	Cost of prod. (Rs./ha)	Net realiz. (Rs./ha)	Additional over control		
					Income (Rs./ha)	Expen. (Rs./ha)	
Carbendazim 12% + mancozeb 63% @ 0.225%	2758	2,75,800	90,730	1,85,070	65,800	2730	1:24
Hexaconazole @ 0.01%	3038	3,03,800	89,848	2,13,952	93,800	1848	1:50.7
Propiconazole @ 0.05%	2450	2,45,000	91,394	1,53,606	35,000	3394	1:10.3
Difenoconazole @ 0.05%	2758	2,75,800	96,299	1,79,501	65,800	8299	1:7.9
Propineb @ 0.225%	2898	2,89,800	90,352	1,99,448	79,800	2352	1:33.9
Penconazole** @ 0.04%	2723	2,72,300	92,270	1,80,030	62,300	427	1:145
Carbendazim @ 0.025%	2100	2,10,000	88,000	1,22,000			

Cost of cultivation**:** *bidi* tobacco nursery/ha,Rs.88,000/ha;
Selling price of 1000 seedlings**:** Rs. 100,
Cost of Carbendazim 12% + mancozeb 63% (Sixer 75 WP): Rs. 650/kg
Hexaconazole (Contaf 5 SC)**:** Rs. 660/l, Propineb (Antracol 70 WP): Rs. 560/kg Propiconazole (Result 25 EC): Rs.1212/l, Carbendazim (Bavistin 50 WP): Rs. 610/l
Difenoconazole (Score 25 EC)**:** Rs. 2964/l

farmers are advised to apply two sprays of hexaconazole (5 SC) @ 0.01 % (2 ml/l) or propineb (70 WP) @ 0.225 % (3 g/l) or carbendazim + mancozeb (75 WP) @ 0.225 % (3 g/l) in alteration to carbendazim (50 WP) @ 0.025 % (0.5 g/l).

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YIELD AND ECONOMICS OF ORIENTAL TOBACCO AS INFLUENCED BY TIME OF PLANTING AND METHOD OF CURING

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Oriental tobacco is an important type of tobacco, which is mainly used in blending of premium brands of cigarettes. Apart from the well-known negative effects of tobacco consumption on health, the consumption has not decreased. In recent years due to the increased awareness of the ill effects of smoking, there is considerable shift in the smoker's preference towards the soft blended cigarettes with consistent increase in demand for Oriental tobacco.

The agro-climatic conditions and soils of the southern part of Chittoor and Ananthapur districts of Andhra Pradesh are similar to the areas where the world's finest quality Oriental tobacco was produced. Hence, there is a need to develop suitable agro-techniques for profitable production. To know the feasibility of growing as a rainfed crop, it is important to explore optimum planting time and suitable curing method for obtaining higher yield and good economic returns. In view of the above, the present study was conducted to find out the optimum time of planting and suitable curing method for achieving better results.

A field experiment was conducted during *rabi*, 2013 in farmer's fields of P.T.M. Mandal, Chittoor district, Andhra Pradesh to study the effect of time of planting and methods of curing on yield and economics of Oriental tobacco. The soil of the experimental field was sandy clay loam in texture having low in available nitrogen (263.4 kg/ha), organic carbon (0.42%) and medium in available phosphorus (49.0 kg/ha) and potassium (225.0 kg/ha), alkaline in reaction (pH 8.1) and EC of 0.40 d/Sm. The experiment was laid out in Randomized Block Design with factorial concept and replicated thrice with 12 treatments, consisting combination of four planting dates *viz.*, T_1 (first fortnight of October), T_2 (second fortnight of October), T_3 (first fortnight of November) and T_4 (second fortnight of November) and three curing methods *viz.*, C_1 (open rack sun curing), C_2 (25% ventilated polyhouse curing) and C_3 (50% polyhouse curing). The Oriental tobacco cv. Izmir seedlings were transplanted at a spacing of 35 10 cm. Nutrients 20 kg N, 60 kg P_2O_5 and 60 kg K_2O/ha was applied through urea, single super phosphate and sulphate of potash, respectively as basal at the time of transplanting. Inter culture and weeding were followed as per the requirements. Four primings were done in October planted crop and three primings in November planted crop and curing was done as per the treatments. Observations on cured leaf yield were recorded. Cost of cultivation per hectare was calculated as per the treatments on the basis of cost of inputs. Gross returns (Rs/ha) were computed by multiplying the cured leaf yield with the prevailing market price. Net returns (Rs/ha) were computed by deducting the cost of cultivation from gross returns. Benefit cost ratio was calculated by dividing the gross returns with cost of cultivation.

The green leaf yield of Oriental tobacco was significantly influenced by the time of planting. The highest green leaf yield (3794 kg/ha) was recorded when the crop was planted during second fortnight of October, which was superior to other dates of planting followed by 3245kg/ha (IFN October) and 2334 kg/ha (IFN November). This might be mainly due to enhanced growth stature *viz.*, the higher plant height, larger LAI, more number of leaves/plant and higher dry matter production resulted in higher

green leaf yield. Similar increase in the green leaf yield in the October second fortnight planted crop was also reported by Venkata Ramana Reddy (2000) and Prasada Rao *et al.* (2002). The lowest green leaf yield (1754 kg/ha) was observed in the crop planted during November second fortnight because the crop received lesser amount of rainfall leading to lower moisture availability during the active growth stages of crop coupled with reduced leaf growth duration. Reduced performance of late planted tobacco has been adequately documented by Sinha *et al.* (1984), Kumaraswamy *et al.* (1998) and Sreeramulu *et al.* (2000).

Planting of Oriental tobacco during second fortnight of October recorded the highest cured leaf yield (791 kg/ha), which was significantly superior to either early or later two plantings (Table 1). This might be due to higher level of growth parameters which act as both source and sink. Better performance of crop planted during October second fortnight was due to favourable weather parameters resulting in higher marketable yield. Similar results were reported by Sannibabu *et al.* (1986) in FCV tobacco and Prasad Rao *et al.* (2002) in Oriental tobacco. The lowest cured leaf yield (365 kg/ha) was recorded in the crop planted during November second fortnight might be due to lesser rainfall received and lower moisture availability during the active growth stages of crop. The results were in accordance with the findings of Venkata Ramana Reddy (2000).

Among the different methods of curing, the highest cured leaf yield was recorded in the 25% ventilated curing method which was significantly superior to the other methods. This might be due to availability of better curing conditions *viz.*, the favourable temperature and humidity in the polyhouse which prevent the excess drying of leaves (Bae, 1987). The lowest cured leaf yield was recorded in the open rack curing method due to direct exposure of leaves to sun during curing process. The leaves that were sun-dried had generally greater rate of weight loss indicating moisture release compared to those cured conventionally inside a curing house. Similar results were reported by Dulay *et al.* (1987) in burley tobacco.

The interaction between the time of planting and curing method did not exert any significant effect on cured leaf yield.

Gross returns, net returns and B:C ratio in Oriental tobacco were significantly influenced by time of planting, methods of curing and the interaction between them.

The highest gross returns (Rs.122316/ha) and net returns (Rs.81442/ha) were realized when the crop was planted during second fortnight of October, which was significantly higher than the other dates of planting with a B:C ratio of 2.98. This might be ascribed to higher cured leaf yield with better quality characters resulted in higher market price. The lowest gross returns (Rs.67015/ha) and net returns (Rs.26141/ ha) were obtained in the crop planted during November second fortnight with a benefit cost ratio of 1.63. Delay in planting beyond October deflated the gross returns due to lower cured leaf yield. Similar results were noticed by Venkata Ramana Reddy (2000) and Prasada Rao *et al.* (2002). Among the methods of curing, the highest gross returns (Rs.1,11,580/ha) and net returns (Rs.69,106/ha) were acquired with 25% ventilated polyhouse curing with a benefit cost ratio of 2.63, which was significantly higher than the other curing methods. This might be due to production of better leaf quality with desirable characters and higher flavourable grades with better colour, aroma, taste and flavour which acquire good market price. The lowest gross returns, net returns and B:C ratio were realized with open rack curing method.

The interaction between the planting time and method of curing had shown considerable effect on the gross returns, net returns and B:C. The highest gross (Rs.1,41,883/ha) and net returns (Rs.99,409/ ha) with a B:C ratio of 3.34 were obtained in the crop planted during second fortnight of October and cured under 25% ventilated polyhouse which was significantly higher than the other treatment combinations. This might be due to higher cured leaf yield with better quality obtained from the treatment. The lowest gross returns, net returns and B:C ratio were obtained in the November second fortnight planted crop cured under open rack sun-curing. The results showed that the planting of Oriental tobacco during October second fortnight

Table 1: Influence of time of planting and method of curing on cured leaf yield (kg/ ha) and economics of Oriental Tobacco

Treatments	Cured leaf	Gross returns	Net returns	B:C ratio
Time of planting				
I FN Oct	673	102639	61765	2.81
II FN Oct	791	122316	81442	2.98
I FN Nov	502	88825	47951	2.17
II FN Nov	365	67015	26141	1.63
SEm±	**25.3**	**1243**	**1243**	**0.028**
CD (P=0.05)	**74.2**	**3646**	**3646**	**0.083**
Curing				
Open sun	549	83341	45667	2.21
25% vent.	633	111580	69106	2.63
50% vent.	567	90675	48201	2.14
SEm±	**21.9**	**1077**	**1077**	**0.025**
CD (P=0.05)	**64.2**	**3157**	**3158**	**0.072**
Time of planting Curing				
SEm±	**43.8**	**2153**	**2153**	**0.049**
CD (P=0.05)	**NS**	**7451**	**7451**	**0.170**

with curing in 25% ventilated polyhouse has resulted in higher yields, best quality leaf and higher economic returns.

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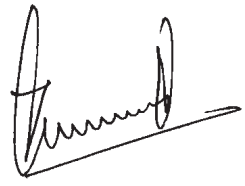

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