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Investigations on Reduction of TSNA in Burley Tobacco

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ABSTRACT

Tobacco Specific Nitrosamines (TSNA) are identified as the major carcinogenic compounds in tobacco. In view of the increasing health consciousness, monitoring and reduction of this group of compounds is attracting attention of researchers all over the world today, more than ever before. Analysis of different types of tobacco produced under varying agro-ecological situations adopting different production practices in India has revealed that Burley tobacco in East Godavari district of Andhra Pradesh has higher levels of TSNA (6-6 to 9.3 ppm). The investigations carried out for developing suitable techniques to reduce the levels of these compounds in Burley tobacco are presented. A significant reduction in TSNA ranging from 11.1 to 28.2% was observed due to removal of midrib and subsequent air-curing. Further, highly significant differences were observed in the levels of TSNA among the treatments due to foliar spray of manganese sulphate @ 0.20% on a 150-day old Burley tobacco field crop and a significant reduction (50.2%) in TSNA could be achieved after 30 days after spray. Similarly, exogenous application of ascorbic acid @ 0.25% on the harvested Burley tobacco green leaves and subsequent air-curing resulted in highly significant reduction (46.5%) in TSNA at 30 days after spray.

INTRODUCTION

India is the third largest producer and fifth largest exporter of tobacco in the world with an annual production of about 750 million kg. Burley tobacco is grown in the Alfisols of East Godavari district in Andhra Pradesh as rain-fed crop and is popularly known as Yeleswaram Burley. The crop is raised in an area of 5,000 ha with a total production of around 5.0 million kg. Tobacco Specific Nitrosamines (TSNA) are identified as the major carcinogens in tobacco. Generally, TSNA are not present in freshly harvested green leaf and they are formed during curing due to nitrosation of tobacco alkaloids. The nitrosating agent is nitrite derived from tobacco nitrate. Bacteria (*Enterobacter; Agrobacterium radiobacter*) and tobacco enzymes take part in the reduction of nitrate. In view of the increasing health consciousness, monitoring and reduction of this group of compounds is attracting attention of researchers all over the world today, more than ever before. Analysis of different types of tobacco produced under varying agro-ecological situations adopting different production practices in India has revealed that Burley tobacco has higher levels of TSNA ranging from 6.6 to 9.3 ppm (Padmavathy, 2007). The objective of the present investigation is to find out suitable techniques to reduce the levels of these compounds in Burley tobacco to make tobacco less harmful.

MATERIALS AND METHODS

Agro-techniques

A field experiment was conducted at CTRI Research Centre, Jeddangi with the objective to identify agro-techniques/post-harvest techniques to reduce TSNA in Burley tobacco with the following treatments: AT1 - Priming and curing with midrib (120 kg N/ha); AT2 - Priming and curing without midrib (120 kg N/ha); AT3 - Priming and curing with midrib (*Azotobacter* + 90 kg N/ha); AT4 - Priming and curing without midrib (*Azotobacter* + 90 kg N/ha); AT5 - Priming and curing with midrib (30kg/ha N in organic form + 90 kg N/ha); AT6 - Priming and curing without midrib (30 kg/ha N in organic +90 kg N/ha); AT7 - Priming and curing with midrib (120kg N/ha+1kg Ammonium molybdate) and AT8 - Priming and curing without midrib (120 kg N/ha + 1kg Ammonium molybdate). The trial was laid out in a Randomized Block Design with three replications planting the variety, Banket A1 adopting the recommended package of practices. Cured leaf samples were collected, midrib was removed, powdered and analysed for TSNA. The pooled data of three replications were statistically analysed.

Foliar application of manganese

In a 150-day old [150 days after planting (DAP)] field crop of Burley tobacco variety Banket A1 grown during the crop season with recommended package of practices (spacing of 80 x 40 cm and 125: 50: 50 kg/ha of N: P_2O_5: K_2O), four rows of 14 plants each constituting four replications were randomly selected and 0.2% $MnSO_4$ (20 g/10 lit) was sprayed. Two rows of 14 plants each were kept as control. Before spray, green leaf samples were collected and immediately dried in a microwave oven and kept for analysis and the remaining leaf was subjected to air-curing. Leaf was harvested at 165 DAP/15 days after spray (DAS) and 180 DAP/30 DAS and air-cured as per the practice in vogue. Both green leaf and cured leaf samples viz., FT1 - 150 DAP/0 DAS (Green leaf); FT2 - 150 DAP & 0 DAS (Cured leaf); FT3 - 165 DAP/15 DAS (Cured leaf); FT4: 180 DAP/30 DAS (Cured leaf) and FT5 (Control) - 180 DAP & No spray (Cured leaf), were powdered and analyses of nicotine, nornicotine, nitrate, polyphenols (chlorogenic acid rutin) and TSNA were carried out adopting the standard procedures. The data of four replications were statistically analysed.

Exogenous application of ascorbic acid and tannic acid

Green leaf (6 kg) harvested from a field crop of Burley tobacco variety Banket A1 raised as per the recommended package of practices (spacing of 80 x 40 cm and 125: 50: 50 kg/ha of N: P_2O_5: K_2O) was collected at 150 DAP and divided in to 3 parts constituting 3 replications. Immediately after harvest, leaves were spread on plastic sheets and 0.25% ascorbic acid, 0.25% tannic acid and water were sprayed uniformly on both sides of leaves separately. Green leaf samples of control (water spray) and sprayed leaves (0 DAS) were quickly dried in microwave oven and kept for analysis. The remaining leaf was subjected to air-curing as per the practice in vogue. Both green leaf and cured samples were powdered after midrib removal nicotine, nornicotine, nitrate, polyphenols (chlorogenic acid and rutin) and TSNA were estimated adopting the standard procedures. The data of three replications were statistically analysed.

RESULTS AND DISCUSSION

Agro-techniques

Non-significant differences were observed in the reduction of TSNA (Table 1 and Fig.1) among the treatments AT3 and AT2 when compared to control (AT1). Highly significant reduction in TSNA of 18.4, 28.2 and 29.3% were recorded in the treatments, AT6, AT4 and AT7, respectively when compared to control (AT1). Except in the case of AT8, a significant reduction in TSNA ranging from 11.1 to 28.2% was observed due to removal of midrib and subsequent air-curing. It is observed that application of ammonium molybdate (AT7) has resulted in the highest reduction of 29.3% in TSNA. According to Hiatt and Ragland (1963), molybdenum deficiency causes reduction in the ascorbic acid. Crawford *et al*. (1992) reported that ascorbic acid is a well-known scavenger of nitrite and hence a potential inhibitor of nitrosamine formation.

Table 1: Effect of agro-techniques on the levels of TSNA in Burley tobacco

Treatment	**Total TSNA (ppm)**
AT1 - Priming and curing with midrib (120 kg N/ha) - Control	9.40
AT2 - Priming and curing without midrib (120 kg N/ha)	8.29
AT3 - Priming and curing with midrib (*Azotobacter* + 90 kg N/ha)	9.30
AT4 - Priming and curing without midrib (*Azotobacter* + 90 kg N/ha)	6.75
AT5 - Priming and curing with midrib (30 kg/ha N in organic form + 90 kg N/ha)	9.27
AT6 - Priming and curing without midrib (30 kg/ha N in organic form + 90 kg N/ha)	7.66
AT7 - Priming and curing with midrib (120 kg N/ha + 1 kg Ammonium molybdate)	6.65
AT8 - Priming and curing without midrib (120 kg N/ha + 1 kg Ammonium molybdate)	8.36
CV (%)	**2.56**
SEm ±	**0.1215**
CD (P=0.05)	**0.3644**

Djordjevic *et al.* (1985) reported that mature green leaves contained small amounts (0.6 to 1.5 ppm) of TSNA that increased (0.9 to 17.8 ppm) during curing. Leaves from higher stalk positions and leaves with increased time in the curing process had greater amounts of TSNA. Chamberlain and Chortyk (1992) observed that lower fertilization levels and careful manipulation of curing parameters could lower nitrosamine levels in cured tobacco. Cui *et al*. (1997) have reported significant decrease in nitrite formation and TSNA accumulation in the treatments viz., primed, primed – midrib – split and primed – midrib – lamina separate when compared to stalk – cured control. According to Deo Singh *et al*. (2002), decrease in inorganic nitrogenous fertilizer and increase in organic manure resulted in the reduction of TSNA in FCV tobacco. According to Burton and Bush (2005) changes in post-harvest practices can result in decreased accumulation of TSNA for Burley tobacco. The significant reduction in TSNA recorded in the present study could be attributed to i) hastening of the curing process due to removal of midrib, ii) substitution of 25% of recommended dose of nitrogen through the biofertilizer, *Azotobacter* and organic manure i.e. Farm Yard Manure (FYM) and iii) application of molybdenum which favours the formation of ascorbic acid which is reported to block the nitrosation.

Foliar application of manganese

It is inferred from the statistical analysis of the data that the differences in nicotine, nitrate and total polyphenols were non-significant among the treatments (Table 2 and Fig.2). However, highly significant differences were observed in the levels of TSNA among the treatments due to foliar spray of manganese sulphate. Significant increase in TSNA was observed between the green leaf sample harvested at 150 DAP (FT1) and the cured leaf sample (FT2). Only a marginal increase was observed in the samples harvested at 165 DAP/15 DAS (FT3) and there was a decline in TSNA in the sample harvested at 180 DAP/30 DAS (FT4) indicating 50.2% reduction in TSNA when compared to the control (leaf harvested at 180 DAP and no spray). Even though, non-significant, the nitrate content was lower in the sample (FT4). The marginal increase in TSNA in the sample (FT3) could be attributed to the higher level of nitrate. According to Maton (1947), increasing the manganese concentration in solution culture for Turkish tobacco increased the ascorbic acid content of tobacco leaves. The ascorbic acid content of leaves and fruits of tomato plants fertilized with 150 g Mn/10m^2 was 12-30% higher than that of control plants (Bronsort, 1950). Laker *et al*. (1981) reported that manganese deficiency in plants could lead to ascorbic acid deficiency which will in turn lead to nitrite accumulation and nitrosamine production.

Table 2: Effect of application of manganese on the levels of TSNA in Burley tobacco

Treatment	Nicotine (mg/g)	Nornicotine (mg/g)	Nitrate (mg/g)	Polyphenols (mg/g)	TSNA (ppm)
FT1 - 150 DAP* & 0 DAS** (Green leaf)	24.36	6.56	9.12	6.89	3.38
FT2 - 150 DAP & 0 DAS (Cured leaf)	25.44	5.68	10.95	8.75	7.92
FT3 - 165 DAP & 15 DAS (Cured leaf)	27.10	7.57	13.94	7.79	9.25
FT4 - 180 DAP & 30 DAS (Cured leaf)	24.60	7.25	8.96	8.39	6.76
FT5 (Control) - 180 DAP & No spray (Cured leaf)	24.86	5.49	11.07	8.45	13.58
CV (%)	**11.72**	**22.38**	**21.98**	**14.98**	**9.24**
SEm±	**1.4810**	**0.7286**	**1.1879**	**0.6032**	**0.3779**
CD (P= 0.05)	**NS**	**NS**	**NS**	**NS**	**1.1388**

** DAP: Days after planting; ** DAS: Days after spray*

Exogenous application of ascorbic acid and tannic acid

It is inferred from the data (Table 3 and Fig.3) that exogenous application of ascorbic acid (ET3) resulted in highly significant reduction (46.5%) in TSNA and significant reduction in nicotine (15.8%) when compared to tannic acid (ET5) (TSNA: 13.9% and nicotine: 3.7%) at 30 DAS. Though non-significant, the levels of nitrate in the treatments ET3 and ET5 were low when compared to the untreated sample (ET6). According to Mirvish *et al*. (1972), ascorbate has been shown to be more than 95% effective in blocking nitrosation of morpholine and piperzine. Chamberlain *et al*. (1986) observed that spraying of 0.1 N solution of ascorbic acid 24 hours prior to last harvest had no effect on the blocking of nitrosation. However, spraying of ascorbic acid solution on harvested NC 2326 leaves resulted in a 76% reduction in TSNA after flue-curing compared to untreated leaves. MacKown *et al.* (1988) have reported that formation of TSNA in a slurry of midrib tissues derived from the air-cured Burley tobacco is prevented, if ascorbic acid is added. Lourenco *et al.* (1994) reported a certain reduction in the pH and alkaloid content and thus possibly preventing the nitrosamine formation by spraying ascorbic acid on the cured leaves. Cui *et al*. (1997) have reported a significant decrease of nitrite and TSNA due to spraying of ascorbic acid. Burton (unpublished results) reported that ascorbic acid could be incorporated into the leaf in large quantities, but that it is readily metabolized. After three days, the level had decreased to that of the untreated leaves. Further, TSNA and nitrite levels are lower in tobacco leaves treated with ascorbic acid after 26 days of curing than in control leaves treated only with water. Thus, it can be concluded from the results that significant reduction (50.2%) in TSNA could be attributed to foliar application of manganese after 30 DAS when compared to control and 15 DAS may not be sufficient.

Table 3: Effect of application of ascorbic acid and tannic acid on the levels of TSNA in Burley tobacco

Treatment	Nicotine (mg/g)	Nornicotine (mg/g)	Nitrate (mg/g)	Polyphenols (mg/g)	TSNA (ppm)
ET1 - Water spray (Green leaf)	24.81	5.76	8.28	7.29	3.32
ET2 - Ascorbic acid @ 0.25% - 0 DAS (Green leaf)	22.46	6.75	5.49	10.19	2.05
ET3 - Ascorbic acid @ 0.25% - 30 DAS (Cured leaf)	24.49	6.06	6.31	8.11	3.93
ET4 - Tannic acid @ 0.25% - 0 DAS (Green leaf)	28.19	6.59	7.97	7.96	2.75
ET5 - Tannic acid @ 0.25% - 30 DAS (Cured leaf)	28.00	6.37	6.37	7.11	6.32
ET6 - Water spray – 30 DAS (Cured leaf)	29.09	8.59	8.37	8.68	7.34
CV (%)	**9.35**	**18.46**	**21.65**	**14.50**	**13.42**
SEm±	**1.4125**	**0.7127**	**0.8913**	**0.6887**	**0.3319**
CD (P= 0.05)	**4.3527**	**NS**	**NS**	**NS**	**1.0226**

** DAP: Days after planting* *** DAS: Days after spray*

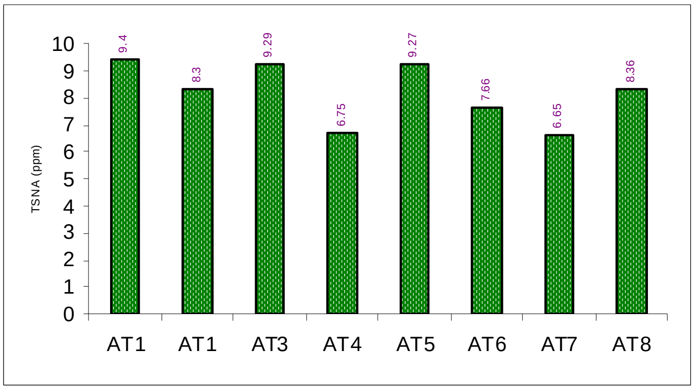

Fig. 1: Effect of agro-techniques on the levels of TSNA in Burley tobacco

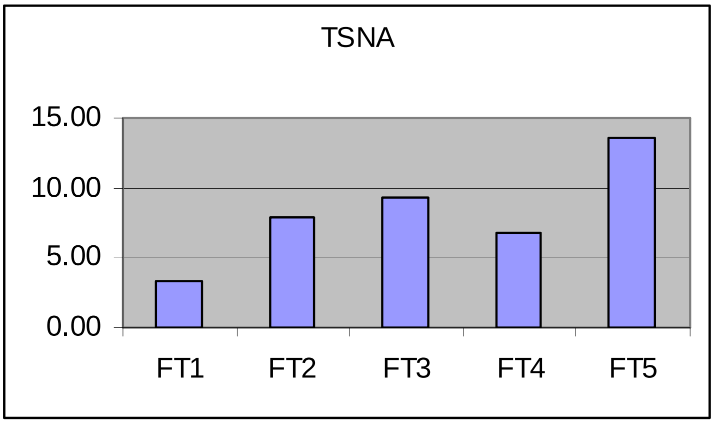

Fig.2: Effect of application of manganese on the levels of TSNA (ppm) in Burley tobacco

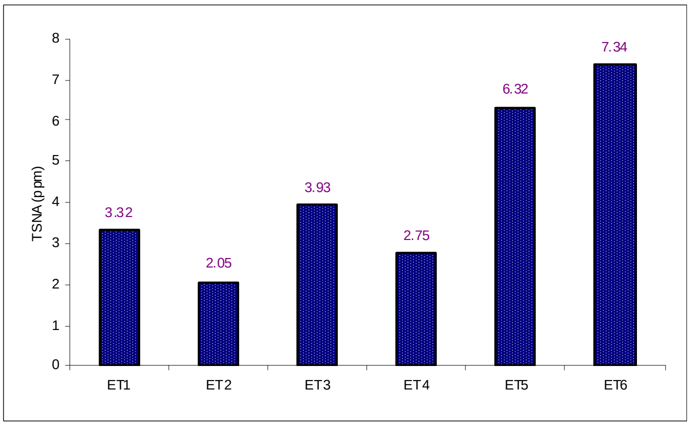

Fig. 3: Exogenous application of Ascorbic acid and Tannic acid

The investigations have established that a significant reduction in TSNA ranging from 11.1 to 28.2% was observed due to removal of midrib and subsequent air-curing. Further, a significant reduction of 50.2% in TSNA could be achieved due to foliar spray of manganese sulphate @ 0.20% on a 150-day old Burley tobacco field crop. Similarly, exogenous application of ascorbic acid @ 0.25% on the harvested Burley tobacco green leaves and subsequent air-curing resulted in highly significant reduction (46.5%) in TSNA.

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