



Effect of long-term application of mineral fertilizers and FYM on microbial dynamics, yield and quality of FCV tobacco (*Nicotiana tabacum*) grown in vertisols

D V SUBHASHINI¹ and HARISHU KUMAR P²

ICAR-Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533 105, India

Received: 3 November; Accepted: 24 April 2019

ABSTRACT

A long term manurial experiment (Permanent Manurial Trial) with the objective to study the effect of continuous application of organic manure (FYM) and inorganic fertilizers (NPK) on microbial diversity, yield and quality of Flue-cured Virginia (FCV) tobacco (*Nicotiana tabacum* L.) as monocrop was carried out at CTRI Farm, Katheru, for 53 years commencing from 1956-57 to 2008-09. The results obtained before the closing of the experiment (2003-2008) are discussed in this paper. The microbial dynamics expressed in terms of culturable microbial populations, i.e. bacteria, fungi, actinomycetes, *Azotobacter* and PSB were measured after 53 years of continuous application of mineral fertilizers and organic amendments to vertisols. The population of bacteria, actinomycetes, *Azotobacter* and PSB were maximum in plots treated with mineral fertilizers and FYM while fungal population was maximum in mineral fertilizers treated plots. The population of bacteria, actinomycetes, *Azotobacter* and PSB increased in the treatments with the addition of P and K fertilizers alone or in combination. The fungal population decreased with the addition of NPK along with the FYM. *Bacillus*, *Pseudomonas* species of bacteria and *Trichoderma*, *Aspergillus* and *Rhizopus* species of fungi, *Streptomyces* species of actinomycetes were the main dominating culturable microorganisms in all the treatments. The FYM amended plots sustained crop productivity and microbial populations at higher levels than rest of the mineral fertilizer treatments. The average cured leaf yields indicated that only farm yard manure and inorganic nitrogen application has exerted pressure in increasing the cured leaf yields by 24. 52% and 26. 97% respectively in VT-1158 variety of tobacco in vertisols. The nitrogenous fertilizers alone had the most deleterious effect on crop productivity and the biological soil environment. The results of the present study support the concept of balanced fertilization and integrated nutrient management practices for improving the soil quality and sustained crop productivity

Key words: FYM, Microbial dynamics, Mineral fertilizers, Tobacco, Vertisols

Nearly one-third of the country's total tobacco production comprises of FCV tobacco which is utilized in the manufacture of cigarettes. It is the principal export-oriented type and as much as 50% of the total production of this tobacco is exported to foreign countries. Cigarette tobacco (*Nicotiana tabacum* L.) is grown in the black soils of Andhra Pradesh from October to March as a dry crop on the conserved moisture in the soil. The soils are low in nitrogen, medium in phosphorus and high in potash. The crop is grown year after year without any rotation (Subhashini 2014). Input of mineral fertilizers to soil can affect the biomass and composition of soil microflora (Subhashini 2013). Microorganisms are indispensable for key soil functions like decomposition of soil organic matter, degradation of xenobiotics, and formation of soil aggregates

(Li *et al.* 2010). They can also act as a source and sink for plant nutrients (Simon and Czako 2014). Contradictory results have been reported on the effect of fertilization on biomass and activity of soil microflora (Hartmann *et al.* 2015) and are due to various environmental variables like soil texture and carbon content (Simon *et al.* 2013) and the period of fertilization (Liu *et al.* 2007). Input of fertilizer can stimulate way from dormant to active microbial cells (Simon and Czako 2014) which can affect nutrient cycling in soil. Fertilizers can have both direct and indirect effects on soil microflora (Subhashini 2016). The direct effects can include enhanced nutrient availability, accumulation of toxic metals (Simon *et al.* 2013), and shift in soil pH with negative effects on soil microflora (Liu *et al.* 2010). Indirect effects are connected with the stimulation of plant growth, with increases in rhizodepositions and plant residues input to soil and thus stimulation of growth and activity of microorganisms of fertilized soil (Chu *et al.* 2007). However, too high amount of fertilizer can have negative effects by stressing soil microorganisms (Geisseler and

Present address: ¹Principal Scientist (Microbiology) (dv_subhashini@rediffmail.com), ²Former Principal Scientist (Agronomy).

Scout 2014). The chemical fertilizers are one of the key factors contributing to increase in agricultural production of farming system, but these are known to exhibit deleterious effect on soil environment, if used injudiciously (Zhao *et al.* 2014). The continued low and imbalanced use of nutrients is one of the prime areas of concern for crop sustainability

because in tobacco the ultimate product is the leaf that is consumed unlike other crops. Besides keeping the production level low, the low and imbalanced use of nutrients led to the soil quality deterioration (Christopher *et al.* 2015). Therefore, the objectives of this study were to assess the effect of long-term (53 years) use of farmyard manure and inorganic fertilizers in vertisols on microbial population dynamics, yield and quality of tobacco.

Table 1 Soil Analysis – Initial Soil Samples (1955)

Character	0-9"	9-18"
pH	8.4	8.4
Organic Carbon	0.59	0.57
Available P (kg/ha)	229	313
Available K (kg/ha)	High	High
Coarse sand (%)	2.6	2.5
Fine sand (%)	19.8	19.3
Silt (%)	20.5	21.4
Clay (%)	57.0	51.2
Dyer's method:	< 196 kg P/ha	Low
	196-264 kg P/ha	Medium
	> 264 kg P/ha	High

MATERIALS AND METHODS

The experiment was conducted in traditional black soils (vertisols) under monocropping from October to March as a rainfed crop with the conserved soil moisture. The mechanical and chemical analysis of the initial soil samples from two depths, i.e. 0-9" and 9-18" are presented in Table 1. The soil was clayey with a moderately alkaline pH, medium inorganic carbon, phosphorus and high in potassium. The treatments in the experiment comprised of 2 levels each of farm yard manure (FYM), nitrogen, phosphorus and potash. The layout was factorial split plot, with FYM in the main plot treatments and fertilizers $2 \times 2 \times 2$ in the sub-plot treatments confounding N, P and K interaction (Table 2). The varieties Harrison Special, Delcrest, CTRI Special, Jayasree (MR)

Table 2 Treatment details of long term fertilizer experiment

Treatment No.	Treatment		Treatment details
	FYM	Inorganic fertilizers	
T ₁	No FYM	Nil	No external inputs of nutrients
T ₂	No FYM	N	N through ammonium sulphate @22.4 kg N/ha
T ₃	No FYM	P	P through single super phosphate @112 kg P ₂ O ₅ /ha
T ₄	No FYM	K	K through potassium sulphate @56 kg K ₂ O/ha
T ₅	No FYM	NP	N through ammonium sulphate @22.4 kg N/ha and P through single super phosphate @112 kg P ₂ O ₅ /ha
T ₆	No FYM	NK	N through ammonium sulphate @22.4 kg N/ha and K through potassium sulphate @ 56 kg K ₂ O/ha
T ₇	No FYM	PK	P through single super phosphate @112 kg P ₂ O ₅ / ha and K through potassium sulphate @ 56 kg K ₂ O/ha
T ₈	No FYM	NPK	N through ammonium sulphate @22.4 kg N/ha, through single super phosphate @112 kg P ₂ O ₅ / ha and K through potassium sulphate @ 56 kg K ₂ O/ha.
T ₉	FYM @ 7.5 t/ha	Nil	FYM @ 7.5 t/ha
T ₁₀	FYM @ 7.5 t/ha	N	FYM @ 7.5 t/ha and N through ammonium sulphate @22.4 kg N/ha
T ₁₁	FYM @ 7.5 t/ha	P	FYM @ 7.5 t/ha and P through single super phosphate @112 kg P ₂ O ₅ /ha
T ₁₂	FYM @ 7.5 t/ha	K	FYM @ 7.5 t/ha and K through potassium sulphate @ 56 kg K ₂ O/ha
T ₁₃	FYM @ 7.5 t/ha	NP	FYM @ 7.5 t/ha, N through ammonium sulphate @22.4 kg N/ha and P through single super phosphate @112 kg P ₂ O ₅ /ha
T ₁₄	FYM @ 7.5 t/ha	NK	FYM @ 7.5 t/ha, N through ammonium sulphate @22.4 kg N/ha and K through potassium sulphate @ 56 kg K ₂ O/ha
T ₁₅	FYM @ 7.5 t/ha	PK	FYM @ 7.5 t/ha, P through single super phosphate @112 kg P ₂ O ₅ / ha and K through potassium sulphate @ 56 kg K ₂ O/ha
T ₁₆	FYM @ 7.5 t/ha	NPK	FYM @ 7.5 t/ha, N through ammonium sulphate @22.4 kg N/ha, through single super phosphate @112 kg P ₂ O ₅ / ha and K through potassium sulphate @ 56 kg K ₂ O/ha.

N: 22.4 kg N/ha P: 112 kg P₂O₅/ha K: 56 kg K₂O/ha. FYM applied had 60% moisture and 1.03, 0.27 and 0.42% of N, P, K, respectively.

and VT-1158 were tested in the experiment. A spacing of 33" × 33" (83.87 × 83.87 cm) with a plot size of 15.10 m × 4.18 m (63.12 m²), was maintained uniformly in all the five decades of the trial. Other cultural operations were the same as followed for the general crop of the farm. The crop yield data for cured leaf, bright leaf and grade index from all the individual plots were collected in all the seasons and statistically analyzed. At the end of every 10 years, the data were pooled and combined analysis was carried out. In this article, combined analysis of yield for the last five year before completion of the experiment (2003-08) is presented using the variety VT-1158.

Soil samples were collected from two depths 0-9" and 9-18" initially before the start of the experiment and were analyzed for pH, TSS, organic carbon, available phosphorus and available potassium as per the methods in vogue. In the present context microbiological analysis of soil, yield and quality of tobacco before closing of the experiment (2007-08) is presented.

However, the biological environment of soils was never looked into this particular experiment. The microorganisms present in the soil carry out a wide range of activities like organic matter decomposition, nitrogen fixation and translocation of immobile elements (Hartmann *et al.* 2015). The microbial community structure can provide a sensitive reflection of soil quality. The surface soils (0-0.15 m) had a pH of 5.8 (1:2.5 soil: water ratio). The soil characteristics, details on cultural practices, irrigation and fertilizer application schedule followed at the beginning of the experiment was same till the end of the experiment.

For biological soil environment, samples were collected from a depth of 0-0.15 m. Each sample was compounded by pooling five sub-samples collected in 5 × 3 m² area and a total of 64 soil samples were collected, representing the four replications and 16 treatments. These samples were sieved (< 2 mm) and were then stored in polythene bags and kept in refrigerator at 4-8°C. The serial dilution technique was used for all soil sample counts. The data on cured leaf yield, soil reaction and microbial populations were subjected to standard analysis of variance and the means of the treatments were tested for significant differences at 5%.

RESULTS AND DISCUSSION

Microbial population: The population of bacteria, actinomycetes, *Azotobacter* and PSB were maximum in plots treated with mineral fertilizers and FYM while fungal population was maximum in mineral fertilizers treated plots (Table 3). The population of bacteria, actinomycetes, *Azotobacter* and PSB increased in the treatments with the addition of N, P and K fertilizers alone or in combination. Treatment (T₁₆) recorded highest population of bacteria (29.56 × 10⁵ Cf/g) *Azotobacter* (264.67 × 10³ Cf/g) and PSB (186.67 × 10³ Cf/g). The fungal population decreased with the addition of of NPK along with the FYM. Treatment (T₁₅) recorded highest population of actinomycetes (87.39 × 10³ Cf/g). *Bacillus*, *Pseudomonas* species of bacteria and *Trichoderma*, *Aspergillus* and *Rhizopus* species of fungi,

Table 3 Effect of continuous application of chemical fertilizers and amendments on soil reaction, crop productivity (t/ha) and microbial population (cfu × 10ⁿ/ g soil)

Treatment	Microbial population				
	Bacteria (cfu × 10 ⁵)	Fungi (cfu × 10 ⁴)	Actino- mycetes (cfu × 10 ³)	<i>Azoto- bacter</i> (cfu × 10 ³)	PSB (cfu × 10 ³)
T ₁	8.12	4.53	15.15	134.67	87.77
T ₂	4.97	6.91	18.30	86.67	110.40
T ₃	5.81	5.36	20.50	146.67	83.77
T ₄	5.96	5.00	21.65	155.00	118.03
T ₅	9.58	6.62	21.10	152.00	115.53
T ₆	9.91	6.13	24.13	158.67	120.07
T ₇	11.07	5.17	30.00	168.67	125.17
T ₈	12.85	6.76	27.23	171.00	124.83
T ₉	20.75	5.07	46.85	198.67	152.03
T ₁₀	17.83	5.81	43.18	186.33	130.67
T ₁₁	22.66	4.70	49.52	212.33	124.90
T ₁₂	22.83	4.67	54.70	221.00	141.53
T ₁₃	24.50	6.15	63.18	216.67	150.40
T ₁₄	23.80	6.57	72.51	231.67	159.13
T ₁₅	24.57	3.83	87.39	245.67	148.87
T ₁₆	29.56	4.18	83.40	264.67	186.67
SEm ±	0.28	0.23	0.67	4.39	3.11
CD (P= 0. 05)	0.80	0.68	1.93	12.55	8.99
CV (%)	3.02	7.25	2.73	4.09	4.13

Streptomyces species of actinomycetes were the main dominating culturable microorganisms in all the treatments. The FYM amended plots sustained crop productivity and microbial populations at higher levels than rest of the mineral fertilizer treatments.

pH: FYM application did not influence soil pH. However, after 53 years of continuous application of FYM @ 7.5 t/ha, showed decline in pH values. Among N, P and K application, P application alone significantly reduced soil pH from 7.83 to 7.77.

T.S.S.: Total soluble salts did not get influenced by organic matter or direct application of nutrients. However FYM × K and FYM × P × K showed significant effect.

Organic carbon (%): Various treatments, viz. FYM, N, P and K showed statistical variation with respect to organic carbon build up in soil. Continuous application of FYM improved soil organic carbon by 0.1%. Though N and P application did not show any significant variation, K application reduced organic carbon content by 0.04% significantly. The interactions of N × P × K were significant.

Available phosphorus (kg/ha): FYM and NPK application showed significant effect on soil available P status. Farm Yard manure application significantly improved soil available phosphorus. Among nutrients, N and P did not show any significant variation on soil available P. However,

K application reduced soil available K content. It may be due to replaced calcium by potassium application formed into unavailable calcium phosphates, thereby reduction in the available P content in the soil. The interaction of N $\times$ P $\times$ K were significant.

Available potassium (kg/ha): FYM application did not bring statistical difference over control with respect to available soil K content. N and P application did not bring any significant changes in soil available K over no application. However, soil application of K continuously, increased soil available K by 37.4 kg/ha over no application. The interaction effects were not significant.

Soil chlorides (%): Neither FYM nor NPK application did bring any significant impact on soil chlorides over their respective controls. Application of FYM and Nitrogen recorded significantly improved cured leaf, bright leaf and grade index over control. Due to FYM application, nicotine and reducing sugars increased significantly.

Cured leaf: The average cured leaf yields indicated that only farm yard manure and inorganic nitrogen application has exerted pressure in increasing the cured leaf yields by 24.52% and 26.97% respectively of VT-1158 variety of tobacco in vertisols. The increment due to P application did not show any prominent improvement in the cured leaf yields (-0.85%). However, K had some effect on pushing up the leaf yields to an extent of 4.35%.

Bright leaf: The average bright leaf yields over the five years indicated that only farm yard manure and inorganic nitrogen application could improve the values to an extent of 29.21% and 24.27% respectively over their controls. P application improved only to an extent of 1.56% while K application improved it by 2.73% over their respective control.

Grade index: Grade index also showed similar pattern over five years mean value. The increment for FYM and Nitrogen application was 29.52% and 24.66% respectively.

A significant reduction in bacterial population was noticed with different NPK fertilizer treatments (Table 3). This reduction may be attributed to the changes in chemical properties of soil brought out by long term application of mineral fertilizers (Moeskops *et al.* 2012). The application of FYM in addition to NPK fertilizer (T_{15}), showed maximum counts, which might be due to the stimulatory effect of organic matter (Sharma *et al.* 2010) on the growth and multiplication of bacteria. Genus *Pseudomonas* (*Pseudomonas* species; *P. fluorescens*) was the predominant among culturable bacteria in all the treatments. The lowest bacterial activity was noticed in plots receiving only nitrogen through Ammonium sulphate (T_2) which might be due to more acidification of soil by accumulation of various toxic elements and mining of some important macronutrient (Subhashini 2013).

With changes in mineral fertilizers, fungal population varied with different fertilizer treatments. This is probably due to the acid producing nature of nitrogenous fertilizers (Subhashini 2013) which has affected the soil pH (Table 3), and thereby led to the elevation in the fungal population.

The lowest fungal counts (3.83×10^4 /g soil) were observed in case of FYM and potassium (sulphate of potash) treated plots (T_{15}) which is in accordance with the studies of Sharma *et al.* (2010). FYM addition along with NPK brought the soil to about neutrality by raising the pH to 7.79 from the initial value of 7.70, thereby affecting the fungal population (Table 4). In case of fungi *Trichoderma*, *Aspergillus* and *Rhizopus* were main culturable fungi observed in all the treatments. *Mucor* and *Penicillium* were also observed in some of the treatments. The presence of *Trichoderma*, a bio-control agent against many soil borne pathogens (Subhashini 2014) is an interesting observation which probably reflects the acquisition of natural control mechanism against certain pathogens observed in the present experimental. The highest population of actinomycetes (87.39×10^3 /g soil) was observed in FYM and potassium (sulphate of potash) treated plots (T_{15}) followed by NPK + FYM (T_{16}). High count of actinomycetes in the FYM treated plots is in accordance with the findings of Subhashini (2012), reported more proliferation of these organisms in soils containing high organic matter content. A significantly higher population of actinomycetes was seen on application of FYM along with mineral fertilizers as compared to the plots receiving no FYM. This might be due to the depletion of available nutrients as a result of continuous cropping (Subhashini 2011) which has probably affected the microbial activity. Maximum counts of *Azotobacter* were observed in treatment where FYM was applied in addition to mineral fertilizers. It may be due to large amount of C source provided by FYM (Subhashini 2013) which is required for proliferation of

Table 4 Effect of long term application of manures and fertilizers on soil properties at the termination of the experiment (2008-09) in vertisols of Katheru Farm, CTRI, Rajahmundry. (Soil depth 0-15cm)

	pH	TSS	OC	Av P	Av K	Chlorides
<i>Organics</i>						
No FYM	7.79*	0.17	0.29	35.25	154.56	37.00
FYM @ 7.5 t/ha	7.67	0.19	0.33	27.50	184.81	45.62
CD (P= 0.05)	0.05	NS	NS	NS	NS	NS
CV (%) (A)	1.17	43.87	41.87	49.15	42.74	49.06
<i>Inorganics</i>						
N0	7.73	0.18	0.30	33.03	172.44	39.06
N 22.4 kg/ha	7.73	0.18	0.32	29.72	166.94	43.56
P0	7.76*	0.17	0.30	29.72	166.94	43.56
P 112 kg/ha	7.71	0.19	0.32	29.09	175.94	38.12
K0	7.76*	0.17	0.33*	33.91	160.75	42.25
K 56 kg/ha	7.70	0.19	0.29	28.84	178.62	40.39
SEm $\pm$	0.01	0.01	0.01	2.51	12.82	3.03
CD (P= 0.05)	0.14	NS	0.04	NS	NS	NS
CV (%) (B)	1.16	21.28	23.06	14.17	25.37	41.49
Sig int	NS	NS	NS	NS	NS	NS
Grand mean	7.73	0.18	0.31	31.38	169.69	41.31

Table 5 Effect of long term application of manure and fertilizers on tobacco leaf yields (kg/ha) and quality (variety VT 1158)

	Cured leaf	Bright leaf	Grade Index	Nicotine (%)	Reducing sugars	Chlorides (%)
<i>Organics</i>						
No FYM	1423	859	1154*	1.39	21.84	2.11
FYM @ 7.5 t/ha	1974	1198	1629	1.91	18.48	2.25
CD (P= 0.05)	210	176.5	210.1	0.01	0.89	NS
<i>Inorganics</i>						
N0	1528*	929*	1255	1.67	20.45	2.22
N 22.4 kg/ha	1869	1128	1528	1.63	1.87	2.14
P0	1659	1014	1368	1.62	20.44	2.16
P 112 kg/ha	1738	1043	1414	1.68	19.88	2.20
K0	1698	1072	1417	1.67	20.37	2.15
K 56 kg/ha	1699	985	1366	1.64	19.95	2.21
SEm ±	35.85	33.8	28.4	0.06	0.45	0.04
CD (P= 0.05)	99.38	93.57	78.74	NS	NS	NS
CV (%) (B)	11.94	18.57	11.55	18.95	12.65	10.47
Significant interactions	AB AD	-	BD AD	NP, FYM, FYM × N, FYM × K FYM×P×K	FYM×K	-
Grand mean	1698	1029	163.92	1.65	20.16	2.18

Azotobacter. The *Azotobacter* count was lowest in 100% N treated plots, which is in accordance with the findings of Mahajan *et al.* (2007) who reported that long term inorganic N application decreases organic matter, biological activity and limits N fixation. The increased *Azotobacter* population in this experiment is also probably responsible for augmenting crop productivity (Subhashini *et al.* 2016). Present study revealed that microbial population significantly increased the cured leaf yield of tobacco (Table 5) which is in confirmation with studies of Subhashini (2013). These results are in agreement with the studies of Sharma *et al.* (2010) who also recorded a significant increase in the bacterial, fungal, actinomycetes, and phosphorus solubilizing microorganism counts (0–0. 15 m soil depth) compared to control. The most important variable contributing to crop yield was soil organic carbon followed by bacteria and fungi. The continued low and imbalanced use of nutrients degraded the soil quality and produced a deleterious effect on biological soil environment.

The integrated use of organic amendment (FYM) with recommended levels of inorganic fertilizers increased the soil organic matter content, resulting in more microbial proliferation and thereby sustained soil health. The results of the present study thereby support the concept of balanced fertilization and integrated nutrient management practices for improving the soil quality and sustained crop productivity.

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