Mysore J. Agric. Sci., 50 (3) : 569-575, 2016

Impact of Green Leaf Manuring on Changes in Microbial Counts and Enzyme Activity in Soil Grown with Groundnut

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Abstract

The effect of application of green leaf manures and farm yard manure in groundnut cultivation was studied for their influence on microbial counts and enzyme activity in soil. These parameters were studied at three different crop stages *i.e.,* 15 days after incorporation in to the soil, at 50 per cent crop flowering and at harvest. The nutrient analysis of different green leaf manures showed that they have more NPK and micronutrient compared to FYM. Among the green leaf manures glyricidia followed by sunhemp and dhaincha had more nutrients and their application to soil has encouraged both general and beneficial micro flora, soil enzyme activity and improved the soil nutrient status.

In the past three decades, chemical fertilizers have been the most commonly used source of plant nutrients in crop cultivation. In developing countries like India, fertilizer prices have been subsidized which encouraged the farmers to apply more chemical fertilizers in their crop production. In the modern agriculture application of organic manures including green leaf manures in crop cultivation has been neglected, which resulted in multi nutrient deficiencies and overall decline in the productive capacity of soils. Organic matter play a vital role in buffering soil pH and in improving physico chemical properties, water holding capacity of soil, decreases soil erosion and encourages microbial activity. Microorganisms play an important role in soil building up process and in nutrient transformations for making easy availability to plant growth (Schulz *et al.,* 2013). Green leaf manuring is one of the low cost agriculture technology helps in minimizing the investment cost of chemical fertilizers and are highly useful in reclamation of alkaline soils, builds up the soil fertility status, controls weed proliferation, root knot nematodes and diseases (Larkin *et al.,* 2011). Busse *et al.* (2009) reported that addition of plant residues to soil helps in soil carbon sequestration and bring changes in fungal and bacterial biomass (Jin *et al.,* 2010). In this study the macro and micronutrient content of different green leaf manure crops was analyzed and the effect of their incorporation to soil on soil microbial population, enzyme activity and on nutrient status of soil was studied in groundnut cultivation.

Material and Methods

In this study the macro and micro nutrient content of different green leaf manures *viz.,* sunhemp, glyricidia, dhaincha, pongamia, neem, eupatorium, cassia and Farm Yard Manure (FYM) were analyzed. A field experiment on groundnut was conducted by using different green leaf manures and FYM during *kharif* 2012 to study their influence on soil microbial population, enzyme activity and on nutrient status of soil. The experiment had 8 treatments and 3 replications laid out in Randomized Complete Block Design. The soil was red sandy loam having a pH of 6.25, low in available nitrogen (250 kg / ha), medium in available phosphorus (19 kg / ha) and high in potassium (316 kg / ha). The experiment was conducted in plots of 6.00 x 3.25m using groundnut variety JL-24. A crop spacing of 30x10 cm was maintained. The recommended dose of chemical fertilizers as per university package of practices for groundnut cultivation is 25:50:25 NPK kg / ha and the recommended FYM was 7.5 t / ha. The required quantity of nitrogen (25 kg N/ha) for groundnut cultivation was supplemented through green leaf manures and FYM based on N equivalent basis. The green leaf manures and FYM were incorporated in to the soil fifteen days prior to sowing of groundnut seeds. Phosphorous and potassium was supplied in the form of single super phosphate and muriate of potash at the time of sowing. The soil samples from the experimental plots were collected at three stages *viz.,* at the time of sowing, at 50 per cent crop flowering and at harvest and were analyzed for microbial population by serial dilution plate count technique. The bacterial population was enumerated on soil extract agar, fungi on martins rose bengal streptomycin sulphate agar and actinomycetes on kustars agar. The phosphorus solubilizing bacteria was enumerated on Pikovskaya's medium, *Rhizobium* on yeast extract mannitol agar and *Azotobacter* on waksman 77 medium. The soil enzyme activity *viz.,* Dehydrogenase, urease and Phosphatase (acid and alkaline phosphatase) were determined as per the method given by Casida *et al.* (1964). The organic carbon percentage was determined by Walkley black chromic acid wet oxidation method (Mcleod, 1973). The available nitrogen in soil after crop harvest was estimated by alkaline permanganate method (Subbaiah and Asija,1956), phosphorus by Brays extract method (Jackson,1973) and potassium by flame photometer method (Muhr *et al*., 1965). The available micronutrient content in soil was estimated by using atomic absorption spectrophotometer .

RESULTS AND DISCUSSION

In this study both macro and micronutrient content of seven green leaf manure crops *viz.,* sun hemp, glyricidia, dhaincha, pongamia, neem, eupatorium, cassia and FYM were analyzed for their nutrient contents and the results are presented in Table I. The highest nitrogen (3.08%) and phosphorus (0.26%) was found in glyricidia followed by sun hemp (2.95% N and 0.25% P). The potssium was found high in eupatorium leaves (2.02%) followed by glyricidia (1.95 %) and neem leaves (1.93 %). Compared to green leaf manure crops the NPK percentage was found less in FYM (1.00% N, 0.20% P and 0.52% K). Micronutrient analysis showed that Zn (38 ppm) and Fe (680 ppm) was highest in dhaincha followed by pongamia (29 ppm Zn & 481 ppm Fe) and glyricidia (28 ppm Zn & 460 ppm Fe). Manganese was found highest in the leaves of sun hemp (242 ppm) followed by neem (202 ppm), eupatorium (175 ppm) and dhaincha (133 ppm). Copper was rich in glyricidia (105 ppm) followed by neem (101ppm) and pongamia (98ppm). FYM had moderate levels of micronutrients.

The soil microbial population was estimated at different crop growth stages of groundnut cultivation *i.e.,*15 days after incorporation of green leaf manures and FYM to soil, at 50 per cent crop flowering and at harvest and the data is presented in Table II. The initial soil microbial count showed that application of different green leaf manures and FYM to soil has influenced

TABLE I

Nutrient composition of different green leaf manure crops and FYM

Green manure crops / FYM	N (%)	P (%)	K (%)	Z (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Qty. used on N Equivalent basis (t/ha)
T_1: Sunhemp	2.95	0.25	1.75	27	235	242	80	3.38
T_2: Glyricidia	3.08	0.26	1.95	28	460	101	105	2.53
T_3: Dhaincha	2.78	0.23	1.73	38	680	133	92	3.59
T_4: Pongamia	2.30	0.25	1.72	29	481	108	98	4.34
T_5: Neem	2.55	0.18	1.93	15	308	202	101	3.92
T_6: Eupatorium	2.18	0.19	2.02	25	271	175	85	4.58
T_7: Cassia	2.43	0.17	1.62	28	302	97	96	4.11
T_8: FYM	1.00	0.20	0.52	22	351	141	108	7.50

TABLE II

Population of soil microorganisms as influenced by application of green leaf manures in groundnut cultivation

Treatment	At initial stages (15 days imcorporation)						At 50% flowering						At harvest					
	Bacteria (Nox10^5 cfu/g soil)	Fungi (Nox10^3 cfu/g soil)	Actin mycetes (Nox10^3cfu/g soil)	PSB (Nox10^3 cfu/g soil)	Rhizobium (Nox10^3 cfu/g soil)	Azotobacter (Nox10^3 cfu/g soil)	Bacteria (Nox10^5 cfu/g soil)	Fungi (Nox10^3 cfu/g soil)	Actin mycetes (Nox10^3 cfu/g soil)	PSB (Nox10^3 cfu/g soil)	Rhizobium (Nox10^3 cfu/g soil)	Azotobacter (Nox10^3 cfu/g soil)	Bacteria (Nox10^5 cfu/g soil)	Fungi (Nox10^3 cfu/g soil)	Actin mycetes (Nox10^3 cfu/g soil)	PSB (Nox10^3 cfu/g soil)	Rhizobium (Nox10^3 cfu/g soil)	Azotobacter (Nox10^3 cfu/g soil)
T_1	45.5	16.5	8.3	9.0	11.0	7.0	91.5	26	14.3	25.0	16.0	11.3	63.3	15.1	12.0	16.0	13.3	9.0
T_2	51.0	19.3	9.0	10.0	12.0	9.0	93.1	30	15.5	27.0	18.0	12.0	65.0	16	14.0	19.0	14.1	11.0
T_3	42.0	15.1	8.0	8.5	10.0	6.5	86.3	21	12.1	21.3	13.0	9.3	54.3	13	10.3	14.0	12.5	8.0
T_4	39.3	12.0	7.8	7.0	9.3	6.1	83.0	19	10.0	20.5	14.0	10.0	50.5	14	9.1	11.0	12.3	8.0
T_5	18.1	9.0	5.0	4.3	3.0	4.5	25.1	13	9.0	10.5	5.0	8.0	20.0	10	5.3	5.0	4.0	6.3
T_6	36.3	10.0	7.0	5.2	4.0	7.5	76.3	15	9.2	16.0	7.3	12.5	43.1	11	7.5	7.1	5.0	9.3
T_7	38.0	11.3	7.3	6.0	6.1	10.0	75.0	18	9.5	18.0	8.0	14.0	44.3	12	7.6	8.2	6.5	12.0
T_8	33.1	13.0	6.7	6.5	10.0	12.0	80.0	22	8.8	15.3	12.1	16.3	46.0	12	7.6	8.0	11.0	14.0
F. test	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SEm ±	0.67	0.35	0.34	0.28	0.33	0.32	0.95	0.56	0.40	0.48	0.42	0.34	0.76	0.48	0.35	0.38	0.30	0.32
C.D at 5%	2.01	1.04	1.00	0.86	0.98	0.97	2.83	1.62	1.10	1.37	1.22	1.00	2.10	1.40	1.02	1.15	0.87	0.93

Note : PSB Phospate solubilizing bactera : T_1 : Sunhemp, T_2 : Glyricidia, T_3 : Dhaincha, T_4: Pongamia, T_5: Neem, T_6 : Eupatorium, T_7 : Cassia, T_8 : FYM

TABLE III

Soil enzyme activity as influenced by application of green leaf manures in groundnut cultivation

Treatments	At initial stages (15 days after incorporation)				At 50% flowering				At harvest			
	DHA (µg TPF / g.soil / 24h)	Urease (µg NH4-N/g.soil / 2hr)	Phosphatase (µg p-nitro phenol / g.soil / hr.		DHA (µg TPF / g.soil / 24h)	Urease (µg NH4-N/g.soil / 2hr)	Phosphatase (µg p-nitro phenol / g.soil / hr.		DHA (µg TPF / g.soil / 24h)	Urease (µg NH4-N/g.soil / 2hr)	Phosphatase (µg p-nitro phenol / g.soil / hr.	
			Acid	Alkaline			Acid	Alkaline			Acid	Alkaline
T_1: Sunhemp	115	26	151	45	136	38	195	53	107	28	160	49
T_2: Glyricidia	124	32	159	51	145	39	197	60	123	29	170	51
T_3: Dhaincha	100	25	150	44	100	37	192	48	97	26	160	45
T_4: Pongamia	94	22	150	40	94	35	190	46	97	25	152	40
T_5: Neem	81	18	141	31	81	29	160	35	73	22	145	35
T_6: Eupatorium	85	19	145	36	85	30	180	41	78	23	147	40
T_7: Cassia	93	20	150	37	93	32	181	43	86	24	151	41
T_8: FYM	65	17	140	30	65	27	149	33	60	22	145	32
SEm±	0.82	0.47	1.21	0.61	0.85	0.58	1.65	0.66	0.69	0.53	1.29	0.63
CD (5%)	2.40	1.40	3.59	1.80	2.51	1.72	4.83	1.94	2.04	1.60	3.85	1.87

the soil microbial population differently. Significant differences were noticed between the treatments. The population of soil bacteria (51×10^5 cfu.g^{-1}soil), fungi (19.3 ×10^3 cfu.g^{-1}soil) and actinomycetes (9.0 ×10^4 cfu.g^{-1}soil) and beneficial bacteria like Phosphate solubilizing bacteria (PSB) (10x10^{5}cfu.g^{-1} soil), *Rhizobium spp* (12 × 10^5 cfu.g^{-1} soil) and *Azotobacter spp* (9 × 10^{5}cfu.g^{-1}soil) were found highest in soils incorporated with glyricidia. The next highest soil microbial population was recorded in soils incorporated with sun hemp followed by dhaincha. Among the different treatments the lowest microbial population *viz.,* soil bacteria (18.1x10^5 cfu.g^{-1}soil), fungi (9.0 ×10^3 cfu.g^{-1}soil), actinomycetes (5.0 ×10^4 cfu.g^{-1}soil) and beneficial bacteria like PSB (4.3×10^{5}cfu. g^{-1}soil), *Rhizobium spp* (3.0 × 10^5 cfu.g^{-1} soil) and *Azotobacter spp* (4.5 ×10^5 cfu.g^{-1}soil) were recorded in soils incorporated with neem leaves.

At 50 per cent crop flowering stage a spurt of microbial activity was noticed in all the treatments. Significant differences were noticed between the treatments. Similar to initial stages the population of soil bacteria (93.1 ×10^5 cfu.g^{-1}soil), fungi (30 ×10^3 cfu. g^{-1}soil), actinomycetes (15.5×10^4 cfu.g^{-1}soil) and beneficial bacteria like PSB (27 ×10^{5}cfu.g^{-1} soil), *Rhizobium spp* (18 × 10^5 cfu.g^{-1} soil) and *Azotobacter spp* (12 × 10^{5}cfu.g^{-1}soil) were found high in soils incorporated with glyricidia. Soils applied with FYM recorded moderate levels of microbial population (bacteria 80 ×10^{5}cfu.g^{-1}soil, fungi 22 × 10^3 cfu.g^{-1} soil and actinomycetes 8.8 × 10^4 cfu.g^{-1} soil) and beneficial bacteria like PSB (15.3 × 10^{5}cfu.g^{-1} soil), *Rhizobium spp* (12.1 × 10^5 cfu.g^{-1} soil) and *Azotobacter spp* (16.3 ×10^{5}cfu.g^{-1}soil). The lowest microbial population *viz.,* bacteria (25.1 × 10^5 cfu.g^{-1}soil), fungi (13.0 × 10^3 cfu.g^{-1} soil), actinomycetes (9.0 ×10^4 cfu.g^{-1}soil), PSB (10.5 ×10^{5}cfu.g^{-1}soil), *Rhizobium spp* (5 ×10^5 cfu.g^{-1} soil) and *Azotobacter spp* (8x10^{5}cfu.g^{-1}soil) were recorded in soils applied with neem leaves. The findings of this study support the views of Drenovsky *et al.* (2004) who reported that soil moisture and organic carbon has a profound influence on the proliferation of soil micro flora especially during luxuriant crop growth stage since, at that time there will be better availability of moisture, amelioration of crop nutrients from organic residues and more root exudates which favors the growth of microorganisms in soil.

A similar trend in microbial population was noticed at crop harvest though the population was found slightly declined compared to 50 per cent crop flowering stage. At harvest, microbial population differed significantly with the treatments. The maximum bacterial population (65 $\times 10^5$ cfu.g^{-1} soil), fungi (16 $\times$ 10^3 cfu .g^{-1} soil), actinomycetes (14 $\times$ 10^4 cfu.g^{-1} soil) and beneficial bacteria like PSB (19x10^5 cfu.g^{-1} soil), *Rhizobium sp* (14.1 $\times$ 10^5 cfu.g^{-1} soil) and *Azotobacter sp* (11 $\times 10^5$ cfu.g^{-1} soil) was recorded in soils incorporated with glyricidia. The lowest bacterial population (20 $\times 10^5$ cfu.g^{-1} soil), fungi (10 $\times 10^3$ cfu.g^{-1} soil), actinomycetes (5.3$\times 10^4$ cfu.g^{-1} soil), PSB (5$\times 10^5$ cfu.g^{-1} soil), *Rhizobium* (4x10^5 cfu.g^{-1} soil) and *Azotobacter* (6.3 $\times 10^5$ cfu.g^{-1} soil) was recorded in soils incorporated with neem leaves. At all the three stages of crop growth soils incorporated with glyricidia showed higher population of bacteria, fungi, actinomycetes and beneficial bacteria like PSB, *Rhizobium*, *Azotobacter* followed by sunhemp and dhaincha. In general soils incorporated with nodulating green manuring crops like glyricidia, sunhemp, dhaincha and pongamia showed more rhizobium population. Similarly soils applied with neem leaves showed lower number of soil bacteria, fungi, actinomycetes and beneficial bacteria which may probably due to antimicrobial property of Azadirachtin of neem. These findings are in accordance with the findings of Rajaratna reddy *et al*. (2013) and Mahmoud *et al.* (2011) who reported that neem has both antibacterial and antifungal properties. Pandey *et al.* (2014) also reported that *Azadirachta indica* (neem) leaves has phytochemical, anti bacterial and radical scavenging properties.

The soil enzyme activity differed significantly with the incorporation of different green leaf manures and FYM (Table III). Initially *i.e.,* 15 days after incorporation of green leaf manures and FYM, the enzyme activity in soils was found low in all the treatments. At initial stages the Dehydrogenase activity (124µg TPF/ g. soil / 24 h), Urease (32 µg NH4-N / g. soil / 2h), acid phosphatase (159 µg p-nitrophenol /g. soil / h) and alkaline phosphatase activity(51 µg p-nitrophenol /g. soil / h) was found highest in soils incorporated with glyricidia followed by sunhemp and dhaincha. The lowest soil enzyme activitiy was recorded in soils applied with FYM (Dehydrogenase 65µg TPF/ g. soil / 24 h, Urease 17 µg NH4-N / g. soil / 2h, acid phosphatase 140 µg p-nitrophenol /g. soil / h and alkaline phosphatase activity 30 µg p-nitrophenol /g. soil / h).

The soil enzyme activity was found increased in all the treatments at 50 per cent crop flowering. However, significant differences were noticed between the treatments. The highest Dehydrogenase activity (145µg TPF/ g. soil / 24 h), Urease (39 µg NH4-N / g. soil / 2h), acid phosphatase (197 µg p-nitrophenol /g. soil / h) and alkaline phosphatase (60 µg p-nitrophenol /g. soil / h) was recorded in soils incorporated with glyricidia. The next highest soil enzyme activity was recorded in soils incorporated with sunhemp and dhaincha. The lowest soil enzyme activitiy was recorded in soils applied with FYM (Dehydrogenase 65µg TPF/ g. soil / 24 h, Urease 27 µg NH4-N / g. soil / 2h, acid phosphatase 149 µg p-nitrophenol /g.soil/ h and alkaline phosphatase activity 32 µg p-nitrophenol /g. soil / h) followed by soils incorporated with neem leaves (Dehydrogenase 81 µg TPF/ g. soil / 24 h, urease 29 µg NH4-N / g. soil / 2h, acid phosphatse 160 µg p-nitrophenol /g. soil / h and alkaline phosphatase 35 µg p-nitrophenol /g. soil / h).

The soil enzyme activity was found decreased at crop harvest compared to enzyme activity at 50 per cent crop flowering stage. The differences between the treatments were found statistically significant. The highest soil dehydrogenase (123µg TPF/ g soil / 24 h),Urease (29 µg NH4-N / g. soil / 2h), acid phosphatase (170 µg p-nitrophenol /g soil / h) and alkaline phosphatase (51 µg p-nitrophenol /g soil / h) was recorded in soils incorporated with glyricidia. The lowest soil enzyme activitiy was recorded in soils applied with FYM (Dehydrogenase 60µg TPF/ g. soil / 24 h , Urease 22 µg NH4-N / g. soil / 2h , acid phosphatase 145 µg p-nitrophenol /g. soil / h and alkaline phosphatase activity 32 µg p-nitrophenol /g

TABLE IV

Soil enzyme activity as influenced by application of green leaf manures in groundnut cultivation

Treatments	pH	EC (dSm^{-1})	OC (%)	Nutrient Status Kg.ha			Micronutrient status (ppm)			
				N	P_2O_5	K_2O	Zn	Fe	Mn	Cu
T_1: Sunhemp	5.4	0.030	0.35	205.3	72.5	140.2	1.20	28.3	18.78	0.69
T_2: Glyricidia	5.8	0.050	0.50	220.1	75.0	145.0	1.25	35.2	20.02	1.00
T_3: Dhaincha	5.3	0.027	0.30	190.4	71.2	138.5	1.16	30.2	17.63	0.68
T_4: Pongamia	5.7	0.040	0.46	180.6	65.4	117.6	0.92	28.0	16.04	0.85
T_5: Neem	5.6	0.038	0.45	182.3	67.3	120.8	0.88	31.9	15.49	0.78
T_6: Eupatorium	5.5	0.035	0.42	185.5	68.2	126.1	0.72	33.5	16.21	0.72
T_7: Cassia	5.4	0.030	0.40	178.2	64.0	112.4	0.96	33.0	15.13	0.70
T_8: FYM	5.7	0.050	0.48	175.0	63.5	100.5	0.68	29.5	15.12	0.67
F test	NS	NS	*	*	*	*	*	*	*	*
SEm±	-	-	0.02	9.11	1.60	4.93	0.04	1.17	0.96	0.05
CD (5%)	-	-	0.06	27.64	4.86	14.79	0.12	3.52	2.89	0.14

soil / h). These results uphold the views of Chaitanya *et al.* (2013) who reported that enzyme activities in soil can be correlated with crop physiological stage, organic carbon content in soil and integrated nutrient management practices.

The soil analysis after crop harvest showed no significant differences in soil pH and EC due to incorporation of green leaf manures. However, significant differences were noticed with respect to soil organic carbon percentage, major and micronutrient content (Table IV). The highest soil nitrogen (220.1 kg/ha), phosphorus (75 kg P_2O_5 / ha), potassium (145 kg K_2O / ha) and micronutrient content the Zn (1.25 ppm), Fe (35.2 ppm), Mn (20.02 ppm) and Cu (1.00 ppm) was recorded in soils incorporated with glyricidia. The lowest soil nitrogen (175.0 kg / ha), phosphorus (63.5 kg P_2O_5 / ha), potassium (100.5 kg K_2O / ha) Zn (0.68 ppm), Fe (29.5ppm), Mn (15.12ppm) and Cu (0.67ppm) was recorded in soils applied with FYM.

The findings of this study has clearly elucidated that incorporation of green leaf manures particularly leguminous green manuring crops like glyricidia, sunhemp and dhaincha has an added advantage to soil for enhancing the microbial population , enzyme activity and soil nutrient status.

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(***Received :*** January, 2016 ***Accepted :*** July, 2016)