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Bio-associative influence of secondary metabolites of turmeric on microbial and biochemical changes in rhizosphere and non-rhizosphere soils under various nutrient management practices

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Abstract
Field experiment was conducted using turmeric as test crop at farmer's field, Byadamudlu village, Chamarajanagara district, Southern Dry Zone of Karnataka during 2016-2017 to determine the bio-associative effect of specific nutrient management practices recommended by different institutions and farmers' practice. In addition to above recommendations, two microbial consortia *i.e.*, Microbial consortia (MC) and Arka Actino Plus (AAP) were evaluated for their influence on microbial and biochemical changes in soils at three levels *i.e.*, 100, 75 and 50% of nutrients recommended by University of Agricultural Sciences, Bengaluru. Both rhizosphere and non-rhizosphere soils were sampled to compare the associative influence on soil microbial and biochemical changes. Higher microbial population, enzymatic activity and soil microbial biomass carbon and nitrogen was observed in non-rhizosphere soils than rhizosphere soils of turmeric, due to its antimicrobial properties, secondary metabolites like curcumin. Nutrient management practice according UAS (B) recommendation ($N_{150}P_{125}K_{250}$) + MC + AAP resulted in maximum bacteria, fungi, actinomycetes, phosphorus solubilising bacterial and N-fixers population (14 x 10^6, 11 x10^3, 31 x 10^4, 19 x 10^4 and 18 x 10^3 cfu g^{-1}, respectively) compared to other treatments in non- rhizosphere soils, similarly higher enzymatic activity and soil microbial biomass carbon and nitrogen was recorded in same treatment.

Keywords: turmeric, curcumin, microbial population and soil enzymes

Introduction
Soil is home to a large number of microorganisms. Soil enzyme activities are often used as indices of microbial activity and soil fertility. Plant rhizosphere is the seat of action of microbial communities and enzymatic activity.

Enzyme activity and microbial biomass are closely related because of soil organic molecules transformation mediated by microorganisms. Soil enzymes play an essential role in catalysing reactions necessary for organic matter decomposition and nutrient cycling. Microbial biomass is the labile portion of the organic fraction in soils and depends on microbial activity.

Dehydrogenases, urease and phosphatases are recognized as very important soils enzymes causing major soil nutrient changes. Dehydrogenase activity provides an index of microbial activity of a soil. Urease and phosphatase are involved in transformations of nitrogen and phosphorus. Activities of these enzymes have often been used as an index of microbial activity (Moorhead and Sinsabaugh, 2000) [5].

Rhizosphere provides suitable environment for microbes and their bio-associative interaction. Usually, rhizospheric soils encourages growth of soil microorganism compared to non-rhizospheric soils resulting from physical and chemical changes and the contribution of excretions and organic debris of roots. The rhizosphere of turmeric is variant in terms of microbial and enzymatic activity.

Turmeric (*Curcuma longa*) is known for its natural antimicrobial activity (Shraddha *et al*., 2017) [8]. Recently many authors have cited antimicrobial property of curcumin, secondary metabolite of turmeric developed in rhizomes. In this context, a study was conducted to understand the influence of secondary metabolites on microbial and biochemical changes in both rhizosphere and non - rhizosphere soils after harvest of turmeric.

Material and Methods
In order to examine the influence of microbial consortia along with different nutrient management practices on soil microbial and biochemical changes, a field experiment was conducted in farmer's field at Byadamudlu village,

Chamarajanagar (district), located in the Southern Dry Zone (Agro-climatic zone VI) of Karnataka. Soil samples were collected from the rhizosphere and non-rhizosphere soils of turmeric before harvesting. Specific nutrient management practices and two microbial consortia were imposed to know the influence in the study. Microbial consortia (MC) containing N-fixing, P & Zn solubilizing and plant growth-promoting microbes used as bio-fertilizers and Arka Actino plus (AAP) containing three *Streptomyces* sp., used as bio-pesticide, and were obtained from ICAR-Indian Institute of Horticultural Research, Bengaluru. Fourteen treatments were imposed with three replications in randomised block design at Byadamudlu village, Chamarajanagar district (Karnataka) in turmeric. The treatments imposed were T_1: Absolute control, T_2: Farmers practice (180:150:120 kg N, P_2O_5, K_2O ha^{-1}), T_3: UAS (B) Rec. (150:125:250 kg N, P_2O_5, K_2O ha^{-1}), T_4: ICAR-IISR Rec.(60:50:120 kg N, P_2O_5, K_2O ha^{-1}), T_5 : TNAU Rec. (125:60:108 kg N, P_2O_5, K_2O ha^{-1}), T_6: Soil test based Rec. (for N, P_2O_5, K_2O), T_7: 50% UAS (B) Rec. (75:63:125 kg N, P_2O_5, K_2O ha^{-1}), T_8: 75% UAS (B) Rec. (113:94:187 kg N, P_2O_5, K_2O ha^{-1}), T_9: 50% UAS (B) Rec. (75:63:125 kg N, P_2O_5, K_2O ha^{-1}) + MC, T_{10}: 75% UAS (B) Rec. (113:94:187 kg N, P_2O_5, K_2O ha^{-1})+ MC, T_{11}: 100% UAS (B) Rec. (150:125:250 kg N, P_2O_5, K_2O Kg ha^{-1})+ MC, T_{12} : 50% UAS (B) Rec.(75:63:125 kg N, P_2O_5, K_2O ha^{-1})+ MC+ AAP, T_{13}: 75% UAS (B) Rec. (113:94:187 kg N, P_2O_5, K_2O ha^{-1}) + MC+ AAP, T_{14}: 100% UAS (B) Rec. (150:125:250 kg N, P_2O_5, K_2O ha^{-1}) + MC+ AAP. Both the microbial cultures were applied to soil at 20 g per litre by soil drenching at two intervals *i.e.,* during land preparation and before second topdressing according to treatments.

Soil microbial population were determined in soil samples (0-15 cm) at intervals and at harvest of turmeric. Total bacteria, fungi, actinomycetes, PSB's and N-fixers were determined using standard dilution plate count technique and plating on specific nutrient media. The media used were nutrient agar for bacteria, Martin's rose bengal agar with streptomycin sulphate for fungi, Kuster's agar for actinomycetes, Pikovskaya's medium for PSB's and Nitrogen - free medium for N-fixers. The number of colonies were recorded and multiplied by the dilution factor for the specific group of microorganisms and expressed as number of colony forming units (cfu) per gram of soil. Methods followed for soil microbial microbial count were according to Carter (1991). Soil biochemical parameters including the enzymatic analysis and soil microbial biomass carbon & nitrogen were determined using standard protocols.

Results and Discussion

Rhizosphere soils are dynamic with respect to nutrient transformations and play a key role in plant nutrition. Soil microbial population was enumerated in non- rhizosphere soils of turmeric after harvest to evaluate the anti - microbial properties of turmeric in soils. Data regarding influence of different nutrient management practices on soil microbial population in rhizosphere and non-rhizosphere soils of turmeric are presented in Table 1. It is observed that treatment T_{14} ($N_{150}P_{125}K_{250}$ -100% UAS(B) rec. +MC+AAP) hosted the maximum bacterial, fungal, actinomycetes, phosphorus solubilising bacterial and N-fixers population (14 x 10^6, 11 x10^3, 31 x 10^4, 19 x 10^4 and 18 x 10^3 cfu g^{-1}, respectively) in non- rhizosphere soils (Fig. 1). Where as in rhizosphere soil of turmeric, the same treatment hosted 84 x 10^5, 48 x10^2, 66 x 10^3, 94 x 10^3 and 74 x 10^2 cfu g^{-1}, respectively. This difference is attributed to phyto-release of secondary metabolites like curcumin which associated with anti-microbial property. However Treatment T_{11} ($N_{150}P_{125}K_{250}$ - 100% UAS (B) rec. +MC) performed followed to T_{14}. The population of soil microorganisms was enhanced in treatments containing microbial inoculants in both rhizosphere and non-rhizosphere soil. Several authors opined that turmeric has antimicrobial activity, due to its secondary metabolites like curcumin produced during rhizome growth. Soil microbial population is dependent on the biogeographical source of the microbes provided by plant and soil (Callaway *et al.*, 2004) [2].

Soil bacteria in both rhizosphere and non-rhizosphere soils of turmeric was significantly influenced by different treatments. Treatment T_{14} recorded maximum population of soil bacteria followed by T_{13}, T_{11}, T_{10}, T_5 and T_3. Soil fungal population ranged from 6 to 11 × 10^3 cfu g^{-1} in non-rhizosphere soils and 25 to 48 × 10^2 cfu g^{-1} in rhizosphere soils. Population of actinomycetes in treatments T_{14} (100% UAS(B) rec. +MC+AAP), T_{11} (100% UAS(B) rec. +MC) and T_3 (100% UAS(B) rec.) were 31 × 10^4, 29× 10^4 and 21 × 10^4 cfu g^{-1} in non-rhizosphere soils and 66 × 10^3, 66× 10^3 and 45 × 10^3 cfu g^{-1}, respectively in rhizosphere soils. The data clearly indicates the influence of microbial inoculants on population. Maximum Population of PSB's and N-fixers were recorded in T_{14}, followed by T_{13} and T_{12} in both rhizosphere and non-rhizosphere soils. Irrespective of group of microbes, minimum population was observed in absolute control in both rhizosphere and non-rhizosphere soils. The negative influence on microbial load due to turmeric in rhizosphere soils is attributed due to its natural antimicrobial properties of in metabolites released. Similar results were reported by Shraddha *et al*. (2017) [8]. Curucmin is a secondary metabolite of turmeric and has an antimicrobial activity (Sakey *et al.* 2012 and Hatamie *et al.* 2012) [7, 4], which reduced microbial population in rhizosphere soils compared to non-rhizosphere soils of turmeric.

Enzyme activity and microbial biomass are closely related because nutrient transformations in soil are mediated by microorganisms. Soil enzymes play an important role in catalysing reactions necessary for organic matter decomposition and nutrient cycling. Microbial biomass is the labile portion of the organic fraction in soils.

Data on soil enzyme activity as influenced by different nutrient management practices during 45, 145 days and at harvest in rhizosphere and non-rhizosphere soils of turmeric are presented in Table 2. The activity of soil enzymes *viz*., dehydrogenase, urease, acid phosphatase and alkaline phosphatase significantly differed among treatments. The assay on activity revealed that non-rhizosphere recorded maximum activity compared to rhizosphere soils. Among different treatments, treatment T_{14} ($N_{150}P_{125}K_{250}$ -100% UAS (B) rec. +MC+AAP) recorded significantly higher enzyme activities and was on par with the treatment T_{11} ($N_{150}P_{125}K_{250}$ -100% UAS (B) rec. +MC).

Enzymes activity revealed that non-rhizosphere recorded maximum activity compared to rhizosphere soils. Among the different treatments, treatment T_{14} ($N_{150}P_{125}K_{250}$ -100% UA S(B) rec. +MC+AAP) recorded significantly higher enzyme activities and was on par with treatment T_{11} ($N_{150}P_{125}K_{250}$ - 100% UAS(B) rec. +MC) in both the rhizosphere and non-rhizosphere soils. The maximum enzyme activity among treatments recorded 40.93 & 31.17, 56.38 & 39.9, 101.50 & 80.11 and 57.78 & 43.74 in T_{14} in case of dehydrogenase (µg of TPF g^{-1} 24 h^{-1}), urease (µg NH_4^+- N g^{-1} h^{-1}), acid and alkaline phosphatase (µg PNP g^{-1} h^{-1}) in non-rhizosphere and rhizosphere soils respectively. Treatments containing

balanced nutrients and microbial cultures were superior with respect to soil enzymes due to enhanced microbial population. It is evident from the data that non-rhizosphere soils recorded comparatively more enzymatic activity than rhizosphere soils (Fig. 2), wherein minimum activity was observed in absolute control. Difference in enzymatic activity was numerically less in rhizosphere soils compared to non-rhizosphere soils.

Results of soil microbial biomass carbon and nitrogen is presented in the Table 3. Soil microbial biomass carbon and microbial biomass nitrogen in non-rhizosphere soils ranged from 80.6 to 382.3 and 9.4 to 44.6 µg g^{-1} in non-rhizosphere soil and 43.1 to 278.6 and 5.0 to 32.5 µg g^{-1} in rhizosphere soil, respectively. Significantly higher soil microbial biomass carbon of 382.3 µg g^{-1} soil was recorded in T_{14} in non-rhizosphere soil compared to rhizosphere soils (278.6 µg g^{-1}). Similarly, higher soil microbial biomass nitrogen of 44.6 µg g^{-1}soil was recorded in non-rhizosphere soil compared to rhizosphere soils (32.5 µg g^{-1}). However farmers' practice (T_2) and UAS(B) recommendation chemical fertilizers only (T_3) maintained soil microbial biomass carbon & nitrogen of 137.2 & 16.0, 225.6 & 26.3 µg g^{-1}, respectively in non-rhizosphere soil. Since soil microbial biomass is directly dependent on the soil biological load and their activity, it is apparent that biomass was more in non-rhizosphere soil in present study.

Rai and Yadav (2011) [6] reported that balanced nutrition of crop, influenced better proliferation of root (rhizosphere) and increased microbial population, microbial activity and enzyme activities in soil. Bader *et al*. (2016) [1] evaluated the antimicrobial activity of turmeric methanolic extracts, attributed to lower microbial activity as is the case in the present study.

Conclusion

Soil microbial population is influenced by rhizosphere of turmeric compared to non-rhizosphere soils. Bio-associative influence *viz*., soil enzymatic activity and soil biomass carbon & nitrogen were directly influenced by changes in soil biota. Hence in the present study addition of external microbial inoculants helped to maintain soil population and their additive bio-associative activity in soils of turmeric.

Table 1: Influence of different nutrient management practices on soil biota (cfu g^{-1}) in rhizosphere and non-rhizosphere soils of turmeric.

Treatments	Soil bacterial (cfu g^{-1})		Soil fungai (cfu g^{-1})		Soil actinomycetes (cfu g^{-1})		Phosphorus solubilising bacteria (cfu g^{-1})		N-fixers (cfu g^{-1})	
	(x 10^5)	(x 10^6)	(x 10^2)	(x 10^3)	(x 10^3)	(x 10^4)	(x 10^3)	(x 10^4)	(x 10^2)	(x 10^3)
	RS	NRS	RS	NRS	RS	NRS	RS	NRS	RS	NRS
T_1	20	6	25	6	31	17	51	8	35	7
T_2	41	9	29	6	39	19	56	9	42	10
T_3	49	10	32	7	45	21	58	10	44	11
T_4	37	6	27	7	48	18	61	11	48	11
T_5	56	10	25	6	43	21	57	10	45	10
T_6	59	11	35	10	47	22	62	12	52	12
T_7	42	7	26	6	42	21	59	12	45	11
T_8	44	8	25	7	47	20	42	9	46	10
T_9	61	9	37	8	50	23	83	14	62	15
T_{10}	78	11	37	8	61	26	85	14	67	16
T_{11}	80	13	40	11	66	29	88	15	69	17
T_{12}	62	7	39	7	50	26	85	14	70	15
T_{13}	73	12	42	9	62	27	91	16	69	17
T_{14}	84	14	48	11	66	31	94	19	74	18
SEm±	8.55	1.67	6.02	0.72	6.71	1.69	7.32	1.76	5.80	1.59
CD (p=0.05)	22.04	4.75	17.82	2.06	17.80	4.85	19.42	5.08	15.64	4.74

RS-Rhizosphere Soil, NRS-Non-Rhizosphere Soil

Table 2: Influence of different nutrient management practices on soil enzyme activity at 45, 145 days and at harvest in in rhizosphere and non-rhizosphere soils of turmeric

Treatments	Dehydrogenase (µg of TPF g^{-1} 24 h^{-1})		Urease (µg NH_4^+- N g^{-1} h^{-1})		Acid phosphatase (µg PNP g^{-1} h^{-1})		Alkaline phosphatase (µg PNP g^{-1} h^{-1})	
	RS	NRS	RS	NRS	RS	NRS	RS	NRS
T_1	6.62	8.69	8.47	11.97	17.02	21.56	10.21	13.49
T_2	15.54	20.40	25.01	35.34	50.21	63.62	26.9	35.53
T_3	16.23	21.31	20.77	29.35	41.7	52.83	28.39	37.50
T_4	10.95	14.38	10.9	15.40	17.87	22.64	10.9	14.40
T_5	12.25	16.08	15.68	22.16	31.49	39.90	19.21	25.38
T_6	18.54	24.34	23.73	33.53	47.66	60.39	26.07	34.44
T_7	13.91	18.26	17.8	25.15	35.74	45.28	21.8	28.80
T_8	14.57	19.13	18.65	26.35	37.44	47.44	22.84	30.17
T_9	27.27	35.81	34.91	49.33	70.08	88.79	38.27	50.55
T_{10}	28.08	36.87	35.94	50.78	72.17	91.44	39.41	52.06
T_{11}	29.84	39.18	38.2	53.98	76.68	97.15	41.87	55.31
T_{12}	27.72	36.40	35.48	50.13	71.23	90.25	38.89	51.37
T_{13}	28.66	37.63	36.68	51.83	73.66	93.33	40.22	53.13
T_{14}	31.17	40.93	39.9	56.38	80.11	101.50	43.74	57.78
SEm±	3.42	3.87	4.56	5.05	6.03	4.42	5.05	5.34
CD (p=0.05)	10.26	10.78	13.65	14.97	16.23	18.39	13.55	15.46

RS-Rhizosphere Soil, NRS-Non-Rhizosphere Soil

Table 3: Influence of different nutrient management practices on soil microbial biomass carbon and soil microbial biomass nitrogen at 45, 145 days and at harvest in rhizosphere and non-rhizosphere soils of turmeric

Treatments	Soil microbial biomass carbon (µg g^{-1}soil)		Soil microbial biomass nitrogen (µg g^{-1}soil)	
	RS	NRS	RS	NRS
T_1	43.1	80.6	5	9.4
T_2	138.9	137.2	16.2	16.0
T_3	145.1	225.6	16.9	26.3
T_4	88.8	112.4	10.3	13.0
T_5	99.3	180.7	11.6	21.1
T_6	150.3	180.7	17.6	21.2
T_7	112.8	136.5	13.1	15.9
T_8	118.2	144.5	13.8	16.9
T_9	243.7	250.9	28.4	29.2
T_{10}	251.0	322.5	29.3	37.6
T_{11}	266.7	364.4	31.1	42.5
T_{12}	247.8	304.7	28.9	35.5
T_{13}	256.1	310.6	29.9	36.3
T_{14}	278.6	382.3	32.5	44.6
S.Em±	26.75	29.32	3.06	3.72
CD (p=0.05)	79.38	84.92	8.34	10.71

RS-Rhizosphere Soil, NRS-Non-Rhizosphere Soil

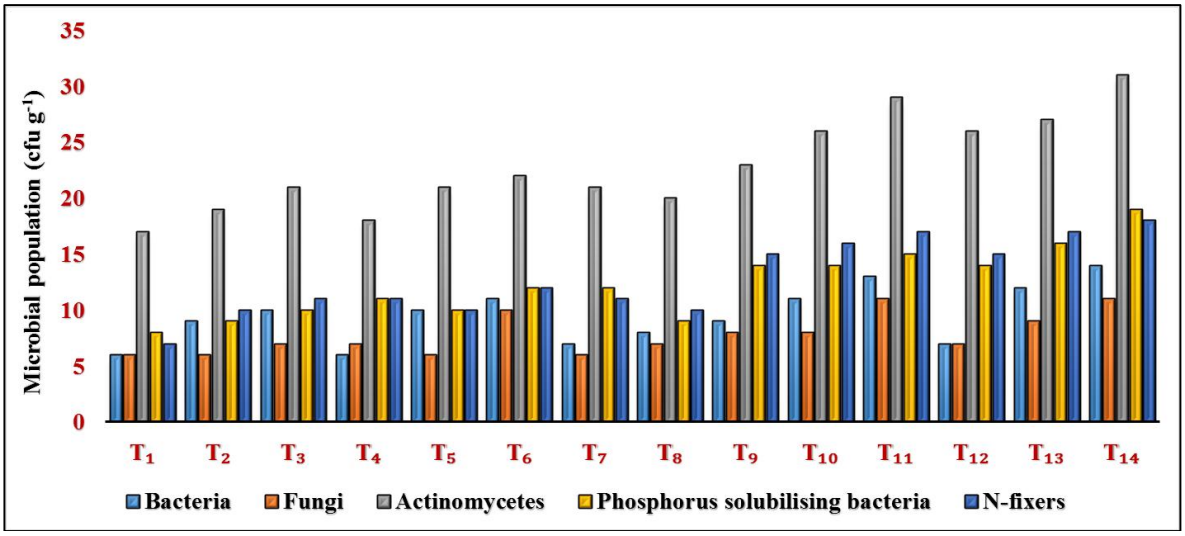

Fig 1: Influence of different nutrient management practices on soil bacteria(x 10^6), fungi (x 10^3), actinomycetes (x 10^4), Phosphorus solubilising bacteria (x 10^4) and N-fixers (x 10^3) in non-rhizosphere soils of turmeric

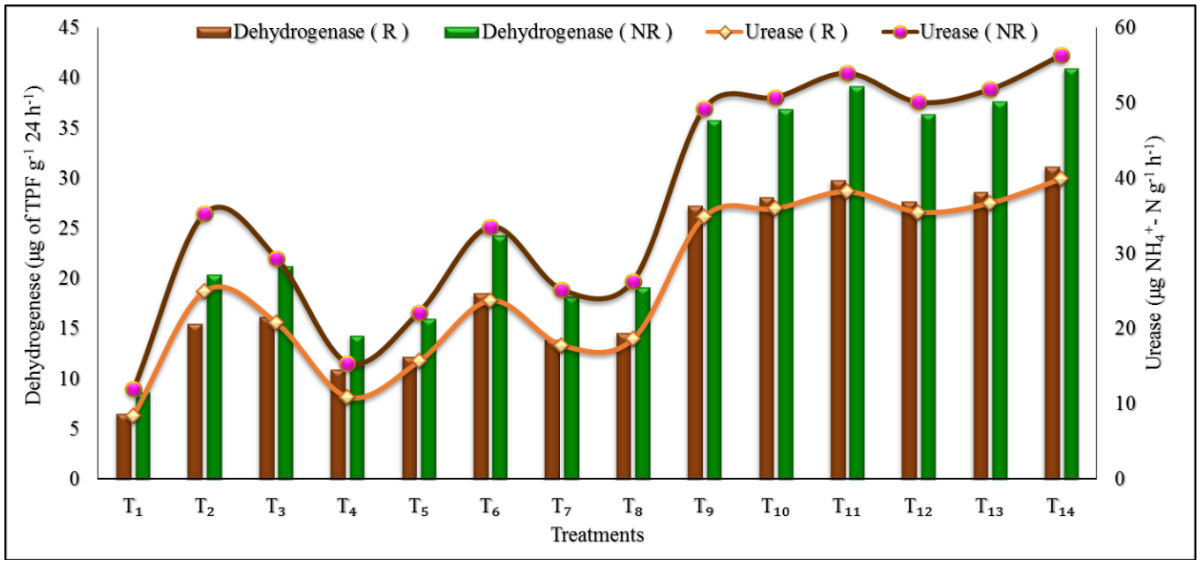

Fig 2: Influence of different nutrient management practices on dehydrogenase & urease activity in rhizosphere (R) and non-rhizosphere (NR) soils at harvest of turmeric

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