



Effect of soil nutrient management and land configuration on rhizospheric microbial diversity under cotton (*Gossypium* spp.)-wheat (*Triticum aestivum*) cropping system in semi-arid region

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ABSTRACT

Microbial diversities in the vicinity of root rhizosphere play a crucial role in promoting plant and soil ecosystem health and productivity. They have great potential as key indicators of soil health in agro-ecosystems enhancing the availability of nutrients. Therefore, a study was carried out during 2019–20 and 2020–21 at research farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana to investigate the rhizospheric microbial diversities under cotton (*Gossypium* spp.)-wheat (*Triticum aestivum* L.) cropping system as affected by land configuration and nutrient management. Experiment consisted of 5 nutrient treatments replicated thrice and two land configurations, viz. raised bed (RB) and flatbed (FB) planned in factorial randomized block design (RBD). The results of experiment under RB revealed that farmyard manure (FYM) and FYM with biofertilizer and cow urine formulation (FYM + Org) showed the highest bacterial, fungal, phosphate solubilizing bacteria, azotobacter and actinomycetes population (6.92 to 7.51, 24.63 to 26.80, 7.61 to 7.68, 8.74 to 9.15, 7.27 to 7.66 cfu/g soil, respectively). The decrease in microbial diversity was observed under inorganic fertilizer application through soil test crop response approach with targeted yield of 5.5 and 2.8 mg/ha (STCR_{5.5/2.8}) and 6.0 and 3.2 mg/ha (STCR_{6.0/3.2}) and control for cotton and wheat, respectively. Higher microbial population and soil organic carbon was found in RB compared to FB. The STCR_{5.5/2.8} and STCR_{6.0/3.2} recorded highest cotton and wheat yield followed by FYM and FYM+Org and least in control under cotton-wheat system. Thus, the study suggests that RB planting with an addition of manures along with biofertilizer and cow urine formulation could be a sustainable and feasible practice through enhanced microbial population raising soil fertility leading to increase the crop yields under semi-arid regions.

Keywords: Biofertilizer, Raised and flat bed, Soil test crop, Yield

India is the second-largest producer of cotton (*Gossypium* spp.) covering 13.01 Mha area (Singh 2017) out of which 0.74 Mha is covered by semi-arid region of Haryana, accounting for 1.82 million bales or 5.69% of India's production (Goyal and Singh 2018). Globally, India stands second in area (31.61 Mha) under wheat (*Triticum aestivum* L.) with a production of 109.52 million tonnes (Anonymous 2021). Cotton-wheat cropping system is being followed in Punjab, Haryana and Rajasthan with an area of 1.7 Mha. Managing nutrients through organic sources is the main challenge under an exhaustive cotton-wheat cropping system. Adequate supply of nutrient during the early phase of crop growth is essential for obtaining optimum economic yields (Loganathan and Wahab 2014). The appropriate

land configuration (raised bed, RB and flatbed, FB) helps efficient use of soil moisture thus intensify crop diversity and enhances the productivity. RB planting improves water productivity and increases the nutrient efficiency (Majeed *et al.* 2015). The soil test crop response (STCR) model of nutrient management (Ramamoorthy *et al.* 1967) is a key for precise nutrient application with consideration of crop needs and nutrients available in the soil.

Soil is the habitat for huge diversity of life although soil organisms comprises <1% of its total mass. Rhizosphere exerts the significant impact on soil quality, stability and fertility. The diversity and biomass of soil microbial communities are the major regulators of decomposing organic matter and recycling nutrients. The soil biota populations are most sensitive indices for soil health and ecosystem productivity (Mandal *et al.* 2018).

Soil microbiome and community structure are affected by different agricultural practices, viz. planting system, crop type and edaphic properties. Planting under RB increases seedling emergence and decreases soil crusting (Ahmad *et*

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al. 2009). Organic manures act as a source of energy for proliferation of microbes and also plays a crucial role in crop production (Usman *et al.* 2013). The planting methods and their effect on microbial population under semi-arid cotton-wheat region are of major concern for nutrient management. Thus, the study was carried out to examine the impact of nutrient management techniques on microbial population under raised and flatbed system.

MATERIALS AND METHODS

The experiment was conducted during 2019–20 and 2020–21 at research farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar (29°16'N, 75°7'E, 216 m amsl), Haryana under cotton-wheat cropping system. The average annual temperature of the experimental area was 24.8°C having an average annual rainfall of 443 mm. The soils are categorized as Typic Haplustepts (Goyal *et al.* 2009, Yadav *et al.* 2023).

The experiment consisted of 5 treatments with 3 replications under two planting methods, viz. raised bed (RB) and flatbed (FB) arranged in a factorial randomized block design (RBD) (Table 1). In FYM treatment, manure @15 t/ha was applied during each cropping season. However, in FYM + Org treatment, along with manure, seeds were treated with azophosphotica in addition to FYM along with application of cow urine-based formulation (Jeevamrut), with 1st and 2nd irrigation in each crop.

Full dose of phosphorous and half nitrogen was applied at the time of sowing (basal) in cotton and wheat. The remaining half nitrogen in two equal splits i.e. the vegetative and squaring stages of *Bt* cotton and at crown root initiation (CRI) and flowering stages of wheat in STCR treatments. In cotton crop, full dose of potassium was applied as basal dose. In STCR treatments, fertilizer doses were calculated based on fertilizer prescription equations for specific targeted yield (Ramamoorthy *et al.* 1967) of *Bt* cotton and wheat given as:

Wheat	<i>Bt</i> cotton
FN = 5.22T-1.04SN-0.12FYM(N)	FN = 10.48T-1.60SN-0.13FYM(N)
FP ₂ O ₅ = 2.38T-4.06SP-0.14FYM(P ₂ O ₅)	FP ₂ O ₅ = 4.39T-5.64 SP-0.14FYM(P ₂ O ₅)
	-F(K ₂ O) = 7.64T0.77SK-0.10FYM(K ₂ O)

where FN, FP₂O₅ and FK₂O are fertilizer N, P₂O₅ and K₂O (kg/ha) rates respectively; SN, SP and SK are the initial soil available NPK (kg/ha); FYM (N), FYM (P₂O₅) and FYM (K₂O) are the N, P₂O₅ and K₂O content in FYM (kg/ha); and T is the yield target (mg/ha).

Fertilizer dose equations for K₂O were not developed for the wheat crop as soils are inherently enriched with potash due to existence of illite minerals (Goyal *et al.* 2008) and thus response to potassic fertilizers by wheat was negligible (Goyal and Jhorar 2007, Goyal *et al.* 2020).

Wheat (var. WH 1105) and *Bt* cotton (var. RCH 773)

Table 1 Treatment details

Treatment	Symbol
Control	Ck
FYM at 15 mg/ha	FYM
FYM at 15 mg/ha + biofertilizer + cow urine-based formulation	FYM + Org
Fertilizer dose for 2.8 and 5.5 mg/ha grain yield target of cotton and wheat crop, respectively (kg/ha)	STCR _{2.8/5.5}
Fertilizer dose for 3.2 and 6.0 mg/ha grain yield target of cotton and wheat crop, respectively (kg/ha)	STCR _{3.2/6.0}

were sown in winter (*rabi*) and rainy (*kharif*) cropping season. The net unit plot size was 100 m². The crops were grown by following package of practices for *rabi* and *kharif* crops of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Initial soil samples (0–15 cm and 15–30 cm) collected were found low in soil organic carbon (SOC) (0.28 and 0.15%, respectively). The population of bacteria, fungi, actinomycetes, phosphorous solubilizing bacteria and *Azotobacter* were 5.39, 10.98, 5.15, 5.01 and 5.21 and 2.71, 5.52, 2.09, 2.02 and 3.19, respectively in 0–15 cm and 15–30 cm. The rhizospheric soil samples representing 0–15 cm soil was collected by shaking of excavated plant roots at the time of crop harvesting from each plot of experiment. Part of the soil was air dried, processed and sieved through 0.5 mm for SOC and other part of the moist samples was refrigerated at 4°C for microbial analysis. The SOC was determined by wet oxidation method (Walkley and Black 1934). For estimation of microbial count; serial dilutions were prepared and placed on the media. The plates were incubated at 25 or 30°C (Vieira and Nahas 2005). Number of colonies was counted after 3–5 days. Dilution and growth media were used to estimate the different microbes (Bacteria: Nutrient agar medium: 10⁻⁶–10⁻⁷; Fungus: Rose Bengal medium: 10⁻²–10⁻³; Actinomycetes: Kenknight and Munaiers medium: 10⁻⁴–10⁻⁵; *Azotobacter*: Jensen's N free Medium: 10⁻³–10⁻⁴; PSB: Pikovskayas Medium: 10⁻³–10⁻⁴). Grain and straw yield were recorded by taking yield of whole plot from each treatment.

The data were subjected to analyze the least square difference (LSD) and compare the effect of treatment at *P*<0.05 using 'SPSS' window version 17 (SPSS Inc. Chicago, IL, USA) and ANOVA random effect model was applied. Data in tables are expressed as the average of three repetitions ± standard error of mean. Significance (*P*<0.05) of the treatment means were estimated by employing Duncan's multiple range test. The graphing was done using Origin v.8.5 software (Origin lab corporation, Northampton, United States).

RESULTS AND DISCUSSION

Soil organic carbon (SOC): The treatment effects on SOC under different planting system are given in Fig. 1. There was an increase in SOC under FYM and FYM + Org treatment over the Ck due to enhanced above and below

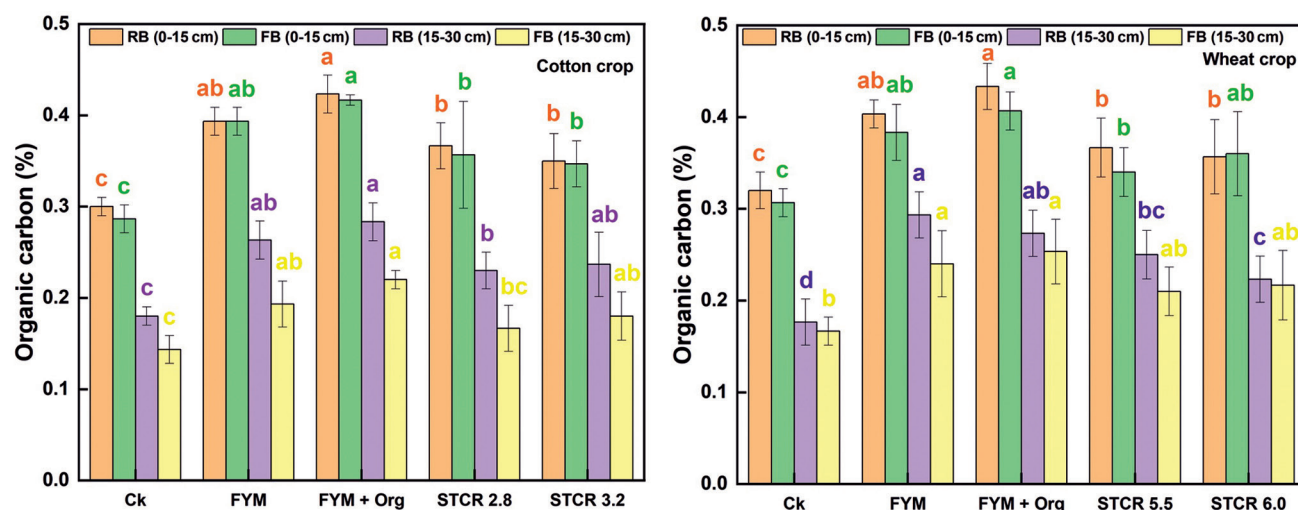


Fig. 1 Effect of planting system and nutrient management practices on the organic carbon of soil under cotton-wheat cropping system (Pooled data of two years).

Values followed by similar letters indicate non-significant difference ($P < 0.05$) according to Duncan Multiple Range test (DMRT) for separation of means, (mean $\pm$ se, $n = 3$).

RB, Raised bed; FB, Flat bed; Ck, Control; FYM, Farmyard manure at 15 mg/ha; FYM + Org, FYM at 15 t/ha + Biofertilizer + cow urine formulation; STCR, Balanced fertilization as per soil test crop response correlation approach.

ground biomass of the crop along with further intensification of bacteria with the addition of biofertilizer and cow urine formulations. The organic carbon was also found higher in fertilizer alone treatments (STCR_{2.8/5.5} and STCR_{3.0/6.0}) as compared to Ck due to higher root biomass with better yields (Singh *et al.* 2015). However, studies also showed the non-significant effect of application of FYM during the initial phase of crop growth (Kumar *et al.* 2020, Rani *et al.* 2022).

Pooled data of the two year showed slightly higher SOC under RB than FB planting. The RB planting resulted in greater root growth that might have substantial impact on increasing the SOC content (Gaurav *et al.* 2018). Planting on RB provides natural drainage to the soil, providing good condition for root development (Alagoz *et al.* 2020). The SOC with only FYM treatments was found at par with STCR treatments. However, the significantly higher SOC were observed in treatments where FYM was applied along with biofertilizer and cow urine formulation (FYM + Org) consecutively for two years under RB and FB planting, compared to control in both crops. Similar results were also obtained in cotton. Above and below ground biomass of plant is probably the major factor for the distribution of SOC with depth as organic matter content generally decreases with increasing depth from the surface due to the availability of more organic matter from the crops at surface (Sharma *et al.* 2022, Wang *et al.* 2022).

Microbial population of soil: Application of organic manures increased the microbial population in FYM and FYM + Org treatment as compared to STCR treatments and control (Xu *et al.* 2018). This might be due to the higher organic carbon content, particularly the biologically active phase of carbon, which served as a source of energy for micro-organisms proliferating in soil (Whitman *et al.*

2016). Feng (*et al.* 2022) showed the significantly positive correlation of SOC with microbial population. The soil amended with organic material showed higher biodiversity and enhanced soil fertility than conventional plots and creates system which shows less dependence on external inputs (Hartmann *et al.* 2015). The increase in microbial count was also observed in STCR_{2.8/5.5} and STCR_{3.0/6.0} where nutrients were applied through fertilizers only as compared to Ck but lower than organic manures treatments

Microbial population (bacterial, actinomycetes, azotobacter and fungal) of the rhizospheric soil (0–15 cm) was found higher in RB than FB in both cotton and wheat. This might be due to creation of more favourable microbial micro habitat during construction of the RB (Zhang *et al.* 2012) resulted in reduced bulk density of soil, especially near the root zone (Kumar *et al.* 2021). Formation of beds can increase soil aggregation and also maintain optimal ratios of solid, liquid and gaseous states in agricultural soils (Garg *et al.* 2022). The enhanced porosity of soil resulted in higher water holding capacity and hydraulic conductivity, thus affecting the microbial count in RB (Patino Zuniga *et al.* 2009). These improvements in soil micro-climate stimulated the decomposition of organic matter and mineralization which further increased the microbial population.

The increase in bacterial count under RB was found to be 4.1 and 2.2% as compared to FB in wheat and cotton crop, respectively. The increment of 22.8, 30.5, 12.8 and 15.9% and 25.6, 30.1, 14.6 and 17.2% under FYM, FYM + Org, STCR_{2.8/5.5} and STCR_{3.2/6.0} as compared to Ck was observed in wheat and cotton crop, respectively for surface soil. Variation in fungal, actinomycetes, Azotobacter and PSB count also followed the similar pattern (Table 2, 3 and Fig. 1).

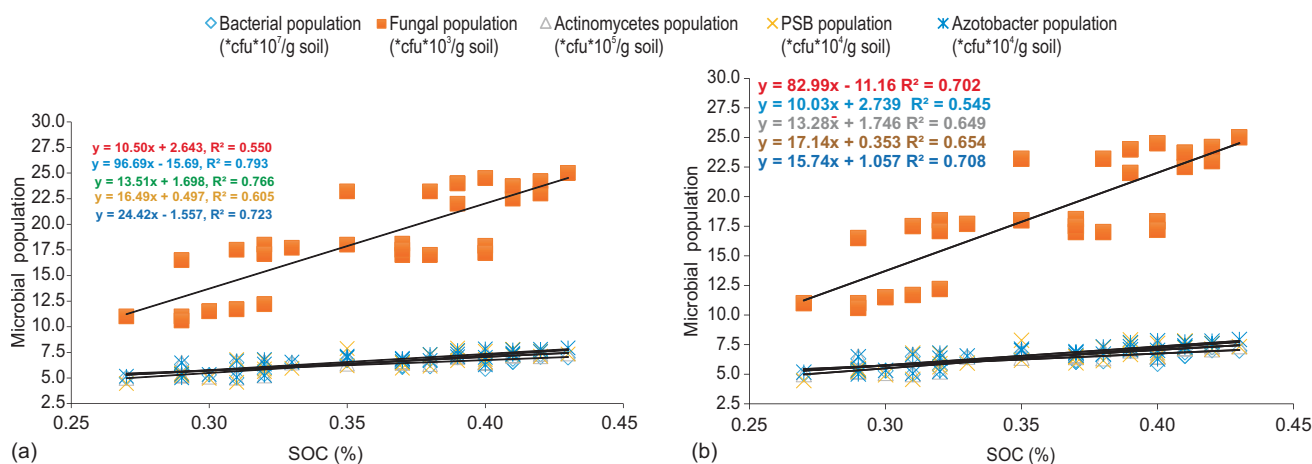


Fig. 2 Relationship of microbial population with SOC (%) in (a) RB and (b) FB planting methods of cotton-wheat cropping system. SOC, Soil organic carbon; PSB, Phosphate solubilizing bacteria; LSD, Least significant difference ($P < 0.05$).

Decrease in microbial count was noticed at 15–30 cm soil due to decline in carbon content. Hao *et al.* (2021) observed the exponential decrease in bacterial population with depth which was mainly due to edaphic factors including soil organic matter, bulk density and length of the time when soil remains saturated with water.

Significantly linear and positive relationship was observed for organic carbon with microbial population in RB and FB system (Fig. 2).

Yield of cotton and wheat crop: The mean grain yield of wheat crop was 1878 and 1850 kg/ha in control treatment

which was increased by 40 and 38% in FYM treatments, and 218 and 215% in STCR treatments under RB and FB planting, respectively. The corresponding increase in seed cotton yield under FYM treatments was 22 and 31% and in fertilizer amended treatments by 99 and 96% compared to the Ck treatment (1539 and 1504 kg/ha) in RB and FB system, respectively (Fig. 3). In organic treatments, the yield was low as compared to inorganic fertilizer treatments. This might be due to slow release of nutrient from FYM and requirement by plant during the initial period of its growth is high (Rani *et al.* 2022). The low compaction and

Table 2 Effect of planting system and nutrient management practices on the rhizospheric microbial population in wheat crop under cotton-wheat cropping system (Pooled data of two years)

	Bacterial population (*cfu*10 ⁷ /g soil)		PSB population (*cfu*10 ³ /g soil)		Actinomycetes population (*cfu*10 ⁵ /g soil)		Fungal population (*cfu*10 ⁴ /g soil)		Azotobacter population (*cfu*10 ⁴ /g soil)	
	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30
	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
<i>Land configuration</i>										
RB	6.66	4.09	6.72	3.40	6.75	3.52	20.48	11.74	7.58	4.51
FB	6.40	4.02	6.63	3.36	6.56	3.45	19.03	10.93	6.76	4.43
SEm±	0.083	0.088	0.108	0.068	0.026	0.029	0.115	0.096	0.049	0.028
LSD	0.250	NS	NS	NS	0.078	NS	0.344	0.287	0.146	NS
<i>Nutrient management</i>										
Ck	5.61	2.80	5.21	2.13	5.42	2.58	12.08	7.12	5.34	3.46
FYM	6.89	4.78	7.59	4.21	7.20	4.33	24.13	13.67	8.01	5.24
FYM+Org	7.32	5.34	7.65	4.34	7.52	4.40	25.65	15.38	8.48	5.54
STCR _{5.5}	6.33	3.65	6.43	3.10	6.54	3.04	18.23	10.00	6.92	4.04
STCR _{6.0}	6.50	3.71	6.49	3.14	6.59	3.08	18.68	10.50	7.09	4.08
SEm±	0.132	0.140	0.171	0.107	0.041	0.046	0.182	0.151	0.077	0.045
LSD	0.395	0.418	0.512	0.322	0.123	0.136	0.544	0.453	0.231	0.134
Land configuration × Nutrient management	NS	NS	NS	NS	0.174	NS	NS	NS	0.327	NS

RB, Raised bed; FB, Flat bed; PSB, Phosphate solubilizing bacteria; Ck, Control; FYM, Farmyard manure at 15 mg/ha; FYM + Org, FYM at 15 t/ha + Biofertilizer + cow urine formulation; STCR, Balanced fertilization as per soil test crop response correlation approach; SEm±, Standard error of mean; LSD, Least significant difference ($P < 0.05$).

Table 3 Effect of planting system and nutrient management practices on the rhizospheric microbial population in cotton crop under cotton-wheat cropping system (Pooled data of two years)

	Bacterial population (*cfu*10 ⁷ /soil)		PSB population (*cfu*10 ³ /g soil)		Actinomycetes population (*cfu*10 ⁵ /g soil)		Fungal population (*cfu*10 ⁴ /g soil)		Azotobacter population (*cfu*10 ⁴ /g soil)	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
<i>Land configuration</i>										
RB	6.44	4.07	6.53	3.35	6.68	3.35	19.94	11.24	7.45	4.36
FB	6.30	4.01	6.41	3.30	6.49	3.30	18.33	9.90	6.68	4.33
SEm±	0.081	0.108	0.124	0.066	0.027	0.016	0.132	0.102	0.045	0.043
LSD	NS	NS	NS	NS	0.080	0.047	0.394	0.305	0.136	NS
<i>Nutrient management</i>										
Ck	5.42	2.74	5.08	2.08	5.32	2.14	11.60	6.28	5.25	3.15
FYM	6.81	4.75	7.29	4.09	7.15	4.19	23.32	12.62	7.89	5.05
FYM + Org	7.05	5.32	7.54	4.29	7.45	4.24	24.78	14.02	8.34	5.50
STCR _{5.5}	6.21	3.68	6.16	3.07	6.46	2.98	17.62	9.63	6.83	3.99
STCR _{6.0}	6.35	3.71	6.26	3.10	6.55	3.07	18.35	10.30	7.01	4.04
SEm±	0.127	0.171	0.196	0.104	0.042	0.025	0.208	0.161	0.072	0.068
LSD	0.382	0.511	0.586	0.311	0.127	0.074	0.623	0.482	0.215	0.204
Land configuration × Nutrient management	NS	NS	NS	NS	0.179	NS	NS	0.681	0.303	NS

RB, Raised bed; FB, Flat bed; PSB, Phosphate solubilizing bacteria; Ck, Control; FYM, Farmyard manure at 15 mg/ha; FYM + Org, FYM at 15 t/ha + Biofertilizer + cow urine formulation; STCR, Balanced fertilization as per soil test crop response correlation approach; SEm±, Standard error of mean; LSD, Least significant difference ($P < 0.05$).

exposure of more surface area to incident solar radiation in RB than FB was the contributing factor for the increased yield (Alagöz *et al.* 2020).

The present work concludes that a two-year cotton-wheat cropping system under application of organic and inorganic sources of nutrient with raised bed and flatbed planting significantly impacted the soil organic carbon and

soil microbial count. The improvement in soil organic carbon of the rhizospheric soil was due to enhancement in soil microbial activities. Also, the raised bed planting providing the congenial eco-physiological environment in terms of increasing the soil organic carbon and porosity of the soil, decrease compactness, thus leading to higher augmentation of soil microorganism resulting in the higher fertility of soil

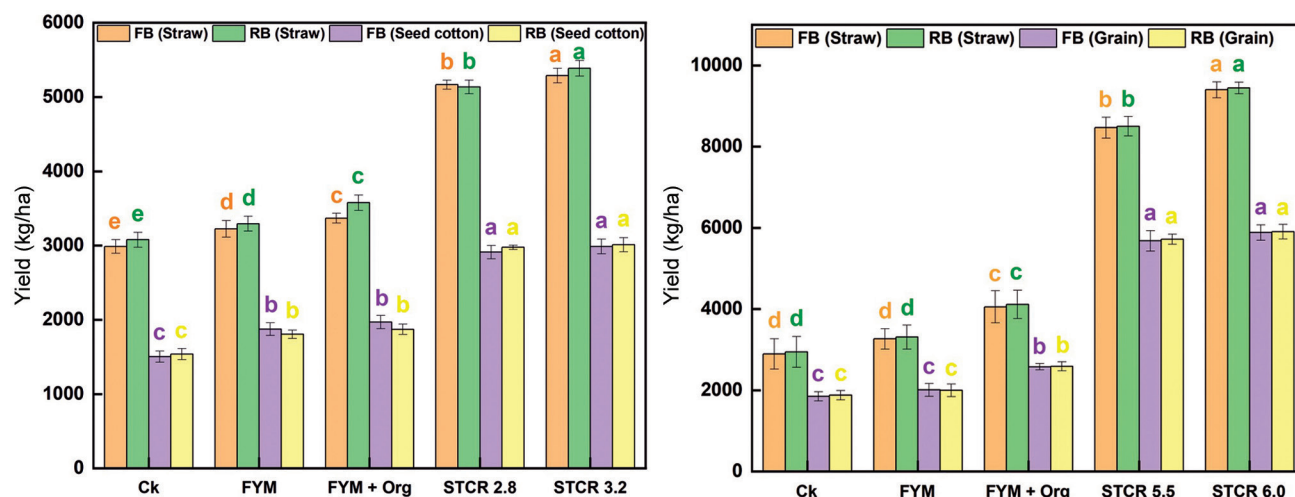


Fig. 3 Effect of planting system and nutrient management practices on yield of cotton and wheat crop under cotton-wheat cropping system (Pooled data of two years)

Values followed by similar letters indicate non-significant difference ($P < 0.05$) according to Duncan Multiple Range test (DMRT) for separation of means (mean±se, $n = 3$)

RB, Raised bed; FB, Flat bed; Ck, Control; FYM, Farmyard manure at 15 mg/ha; FYM + Org, FYM at 15 t/ha + Biofertilizer + cow urine formulation; STCR, Balanced fertilization as per soil test crop response correlation model.

through increased availability of nutrients from the vicinity of plant roots. Adoption of raised bed planting makes crops less prone to lodging and more tolerant to water stress thereby making it more adaptable to unfavorable climate.

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