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Multi-Location Evaluation of Copper Fertilization on Cane Yield of Sugarcane (Saccharum officinarum L.) under Alkaline-Calcareous Soils of Southern Punjab, Pakistan

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Muhammad Javid Qamar (Corresponding Author)
Soil Fertility (Field), Bahawalpur, Punjab, Pakistan
javedqamarswt@gmail.com
https://orcid.org/0009-0005-7271-0807
Muhammad Rashid Farooq
Soil Fertility (Field), Bahawalpur, Punjab, Pakistan
https://orcid.org/0009-0005-3121-7397
Sehrish Jameel
Soil and Water Testing Laboratory, Bahawalnagar, Punjab, Pakistan
https://orcid.org/0009-0003-2983-9336
Muhammad Bilal
Soil and Water Testing Laboratory, Dera Ghazi Khan, Punjab, Pakistan
https://orcid.org/0009-0003-6512-9446
Aftab Ahmad Sheikh
Agricultural Chemist, Bayer Pakistan, Lahore
https://orcid.org/ 0009-0003-8306-6565
Beenish Butt
Soil and Water Testing Laboratory for Research, Multan, Punjab, Pakistan
https://orcid.org/0009-0000-0741-6875
Muhammad Asif
Soil and Water Testing Laboratory, Khanewal, Punjab, Pakistan
https://orcid.org/0009-0008-9139-7870

Tahir Akbar
Soil and Water Testing Laboratory, Muzaffargarh, Punjab, Pakistan
https://orcid.org/0009-0003-1699-0256
Ali Rizwan
Soil and Water Testing Laboratory, Bahawalnagar, Punjab, Pakistan
https://orcid.org/0000-0002-8248-8016
Samina Hamid
Soil and Water Testing Laboratory, Rawalpindi, Punjab, Pakistan
https://orcid.org/0000-0002-0510-4138
Muhammad Imran
Soil and Water Testing Laboratory, Rahim Yar Khan, Punjab, Pakistan
https://orcid.org/0009-0007-2965-0612
Muhammad Asadullah
Soil and Water Testing Laboratory, Bahawalnagar, Punjab, Pakistan
https://orcid.org/0009-0008-0391-2466

Abstract

Sugarcane (Saccharum officinarum L.) productivity in southern Punjab is constrained not only by macronutrient imbalance but increasingly by latent micronutrient deficiencies on the region's alkaline-calcareous soils, where free $CaCO_3$ and chronically low organic matter immobilize plant-available copper. A multi-location field trial was therefore conducted during the 2023–24 crop season at three contrasting sites of the Bahawalpur–Bahawalnagar tract ; Haroonabad-1, Haroonabad-2, and Bahawalpur; to evaluate the response of spring-planted CPF-246 cane to graded levels of broadcast copper sulphate pentahydrate ($CuSO_4{\cdot}5H_2O$). Five treatments comprising 0, 6, 8, 10, and 12 kg $CuSO_4{\cdot}5H_2O$ ha^{-1}, applied over a uniform NPK basal of 250–175–125 kg ha^{-1}, were arranged in a two-factor randomized complete block design with three replications at each site. Soil analysis confirmed alkaline pH (8.22–8.40), *low organic matter (0.23–0.86%), and contrasting EC (1.31–2.9 dS m^{-1}) and macronutrient status across the three sites. Combined analysis of variance revealed highly significant effects of treatment ($F = 14.34$, $p < 0.01$) and location ($F = 46.36$, $p < 0.05$) on cane yield, while their interaction was marginally non-significant ($F = 2.29$, $p = 0.0825$). Cu fertilization progressively improved cane yield, with pooled mean yields rising from 104,993.8 kg ha^{-1} in the unfertilized control to 112,843.2 kg ha^{-1} at the highest tested dose. The greatest cane yield response over the NPK-only control was recorded at 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1} (+7,849.4 kg ha^{-1}; +7.5%), which was therefore identified as the recommended Cu rate for spring-planted sugarcane on the alkaline-calcareous soils of southern Punjab. The inclusion of 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1} as a basal soil-broadcast input, alongside the standard NPK schedule, is recommended to capture the latent Cu limitation on cane productivity in this agro-ecological zone.*

Keywords: *Sugarcane, copper fertilization, $CuSO_4$, multi-location trial, alkaline-calcareous soils*

I. Introduction

Sugarcane (*Saccharum officinarum* L.) ranks among the most economically important industrial crops of the tropics and subtropics, supplying approximately 80% of the world's sugar and a rising share of bioethanol, bagasse-based bioenergy, and allied by-products (FAO, 2023). The crop is cultivated on about 26 million hectares globally, with Brazil, India, China, Thailand, and Pakistan jointly accounting for the most of world production (FAOSTAT, 2023). In Pakistan, sugarcane occupies roughly 1.19 million hectares and contributes nearly 2.7% to the value added in agriculture and about 0.5% to the national GDP, sustaining an extensive network of sugar mills, growers, and downstream industries (GoP, 2024). Punjab alone accounts for more than 60% of the national cane area, with the Bahawalpur–Bahawalnagar belt of southern Punjab emerging as a major cane-producing tract under semi-arid, canal-irrigated conditions (Afghan et al., 2024). Despite this scale, the national average cane yield of about 68–74 t ha^{-1} remains well below the genetic potential of cultivated varieties and considerably lower than yields achieved in comparable agro-ecologies of India and Egypt (Shahzad et al., 2019). The persistent yield gap has been linked to imbalanced fertilization, poor varietal usage by farmers, marginal land allocation, and, increasingly, to micronutrient deficiencies that remain largely overlooked in routine fertilizer programmes (Majeed et al., 2022; Manzoor et al., 2023).

The soils of southern Punjab are characteristically alkaline, calcareous, and low in organic matter, with pH values commonly ranging from 7.8 to 8.5 and free $CaCO_3$ contents that fix and immobilize several essential micronutrients (Rashid and Ryan, 2004; Imran et al., 2020). Decades of intensive cropping under canal irrigation, combined with NPK-centric fertilizer schedules, have progressively depleted the available pools of zinc, boron, iron, and copper across the Indus Basin (Zia et al., 2007; Majeed et al., 2022). National soil surveys have consistently reported that more than half of Pakistan's cultivated soils are deficient in one or more micronutrients, with copper deficiency being particularly common in coarse-textured, low-organic-matter soils of the southern districts (Manzoor et al., 2023). While the agronomic significance of zinc and boron has received substantial research attention in cereals and cotton, the role of copper in sugarcane productivity under these conditions remains comparatively under-investigated.

Copper is a structural and catalytic constituent of more than thirty plant enzymes, including plastocyanin, cytochrome c oxidase, superoxide dismutase, and polyphenol oxidase, and is therefore central to photosynthetic electron transport, mitochondrial respiration, lignin biosynthesis, and antioxidant defense (Yruela, 2009; Printz et al., 2016). Adequate Cu nutrition has been linked to greater chlorophyll stability, stronger stalk lignification, improved resistance to lodging, and enhanced sucrose accumulation in graminaceous crops (Adrees et al., 2015; Hunter et al., 2023). In sugarcane specifically, copper participates in tiller initiation, internodal elongation, disease resistance and the activity of enzymes regulating sucrose phosphate synthase, all of which directly govern cane stand density and final yield (McCray et al., 2010; Majeed et al., 2022). Field studies on Cu-deficient soils have shown that adequate Cu supply increases the number of millable canes per unit area and reduces premature tiller mortality, thereby translating into measurable yield gains (Naga Madhuri et al., 2013). Deficiency symptoms, often masked under field conditions, typically appear as chlorotic young leaves, reduced tillering, delayed maturity, and weak stalks that lodge under irrigation or wind stress. Despite this physiological centrality, calibrated soil-applied Cu dose–response data for sugarcane on alkaline-calcareous soils of Pakistan are sparse, and most current fertilizer recommendations issued by provincial agriculture departments still do not include copper as a routine input.

The few regional studies that have addressed copper nutrition in sugarcane have generally been confined to single sites or used a single Cu rate, making it difficult to identify an agronomically optimal dose or to determine whether the response is consistent across soils of differing fertility status (Naga Madhuri et al., 2013; Majeed et al., 2022). A multi-location, dose–response evaluation of copper fertilization in sugarcane under the agro-ecological conditions of southern Punjab is therefore warranted. The present study was designed (i) to evaluate the response of sugarcane (cv. CPF-246) to four graded levels of soil-broadcast copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), applied over a uniform NPK basal dose, in terms of cane yield during the 2023–24 crop season at three contrasting sites of the Bahawalpur–Bahawalnagar tract; (ii) to identify the Cu rate that produces the highest yield response over the unfertilized control; and (iii) to test whether the Cu response interacts significantly with location, thereby clarifying whether a single regional recommendation is justified or whether site-specific calibration is required.

2. Materials and Methods

2.1 Experimental sites

The field experiment was conducted during the 2023–24 sugarcane crop season at three sites located in the canal-irrigated, semi-arid tract of southern Punjab, Pakistan: two sites in District Bahawalnagar (Haroonabad-1 and Haroonabad-2) and one site at Bahawalpur. The region is characterized by hot, dry summers, mild winters, low to moderate annual rainfall, and predominantly alluvial, calcareous soils developed on Indus

Basin sediments. Prior to land preparation, composite soil samples were collected from each site at two depths (0–15 cm and 15–30 cm) following standard auger sampling protocols (Ryan et al., 2001). Samples were air-dried, ground, sieved through a 2 mm screen, and analyzed for electrical conductivity of the saturation extract (EC_e) by conductivity meter, pH (1:1 soil-to-water suspension) by glass electrode, organic matter by the Walkley–Black wet oxidation method, available phosphorus by the AB-DTPA extraction procedure, and exchangeable potassium by ammonium acetate extraction followed by flame photometry (Ryan et al., 2001; Estefan et al., 2013). The soil analytical data for the three experimental sites are presented in Table 1.

All three sites carried alkaline, low-organic-matter soils typical of the southern Punjab cropping plain. Haroonabad-1 and Haroonabad-2 represented moderately saline conditions (EC_e 2.4–2.9 dS m^{-1}) with adequate available K (125–177 ppm) and low-to-medium available P (6.2–10.9 ppm), while Bahawalpur represented a non-saline (EC_e 1.1–1.3 dS m^{-1}) but markedly P- and K-deficient soil (P 3.95–5.71 ppm; K 51–90 ppm). Organic matter content was uniformly low across all sites (0.23–0.86%), and surface pH ranged from 8.22 to 8.40, conditions known to depress the plant availability of soil-bound copper.

Table 1. Soil physico-chemical properties of the three experimental sites at two sampling depths.

Soil parameter	EC (dS m^{-1})	pH	OM (%)	Available P (ppm)	Available K (ppm)

Soil parameter	EC (dS m^{-1})	pH	OM (%)	Available P (ppm)	Available K (ppm)
Haroonabad-I					
Depth 0–15 cm	2.5	8.2	0.56	6.3	165
Depth 15–30 cm	2.4	8.1	0.86	6.2	177
Haroonabad-2					
Depth 0–15 cm	2.9	8.4	0.71	10.9	125
Depth 15–30 cm	2.7	8.3	0.77	9.1	177
Bahawalpur					
Depth 0–15 cm	1.31	8.24	0.51	5.71	90
Depth 15–30 cm	1.10	8.22	0.23	3.95	51

2.2 Plant material

A commercial sugarcane variety, CPF-246, was used at all three locations. CPF-246 is a widely cultivated, mid-maturing variety recommended for the southern Punjab cane belt and was selected for its consistent performance under the prevailing semi-arid, canal-irrigated conditions of the region. Disease-free, healthy three-budded setts were obtained from certified seed stock and used for planting at all three sites.

2.3 Treatments and experimental design

The experiment evaluated five treatments comprising a graded series of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) rates applied over a uniform NPK basal dose. The treatments were: T1 = Control (NPK only, 0 kg Cu ha⁻¹); T2 = NPK + 6 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}; T3 = NPK + 8 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}; T4 = NPK + 10 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}; and T5 = NPK + 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}. The uniform basal NPK dose comprised 250 kg N, 175 kg P_2O_5, and 125 kg K_2O ha^{-1}, applied as urea, di-ammonium phosphate (DAP), and sulphate of potash (SOP), respectively. Treatment codes and nutrient compositions are summarized in Table 2.

The experiment was laid out as a two-factor randomized complete block design (RCBD) with locations and Cu fertilization levels as the two factors, with three replications at each site. All five treatments were randomly allocated to plots within each replication at each location, and the same randomization protocol was followed at all three sites.

Table 2. Treatment compositions used to study the response of sugarcane to graded levels of copper fertilization.

Treatment	Code	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	$CuSO_4 \cdot 5H_2O$ (kg ha^{-1})
T1_Control	T1	250	175	125	0

T2_Cu_6	T2	250	175	125	6
T3_Cu_8	T3	250	175	125	8
T4_Cu_10	T4	250	175	125	10
T5_Cu_12	T5	250	175	125	12

2.4 Crop management

Planting was carried out during the recommended spring planting window of 2023 at all three locations. The full doses of P_2O_5, K_2O, and $CuSO_4·5H_2O$ were broadcast uniformly over the prepared seedbed and incorporated into the surface soil through a final shallow tillage operation immediately before sett placement. The total N dose was applied in three equal splits: one-third at planting, one-third at the tillering stage (approximately 60–90 days after planting), and one-third at the grand growth stage (approximately 120–150 days after planting). Setts were placed end-to-end in opened furrows at the recommended depth and covered with moist soil. Irrigation was applied through the canal supply system; the first irrigation was given immediately after planting to ensure uniform sett germination, followed by subsequent irrigations as required by crop stage, evaporative demand, and rainfall. Weeds were controlled by a combination of pre-emergence herbicide application and two manual hoeings during the early tillering phase. Plant protection measures against borers and sucking pests were applied uniformly across all plots whenever scouting indicated treatment thresholds had been reached. All other agronomic and plant protection operations were carried out as per the standard production recommendations of the Punjab Agriculture Department for spring-planted sugarcane.

2.5 Data recording

At physiological maturity, cane yield was estimated by harvesting all canes from the net plot area, weighing the harvested produce in the field using a calibrated spring balance, and extrapolating the plot yield to a per-hectare basis (kg ha^{-1}). All measurements were taken on the net plot area, excluding border rows, to minimize edge effects.

2.6 Statistical analysis

The data were subjected to two-factor analysis of variance (ANOVA) appropriate for a multi-location randomized complete block design, with locations and treatments treated as fixed effects and replications nested within locations. Treatment means were separated using Fisher's protected least significant difference (LSD) test at the 5% probability level (Steel et al., 1997). The significance of the location, treatment, and location × treatment interaction effects was tested at $p \leq 0.05$ and $p \leq 0.01$ levels. The yield response of each Cu treatment over the unfertilized control was computed for each location as the absolute and percentage yield difference from T1. Statistical analyses were performed using Statistix 8.1, and graphical presentations were prepared using R/RStudio and Microsoft Excel.

3. Results and Discussion

3.1 Soil status of the experimental sites

Soil analytical data (Table 1) confirmed that the three experimental sites represented contrasting fertility profiles within the broadly similar alkaline-calcareous matrix of southern Punjab. Surface soil pH varied within a narrow range (8.22–8.40) across all three sites, conditions that strongly limit the plant availability of soil-bound copper through carbonate fixation and adsorption onto calcium-rich exchange surfaces. Electrical conductivity differentiated the sites more sharply: Haroonabad-1 and Haroonabad-2 carried moderately saline surface soils (EC_e 2.5 and 2.9 dS m^{-1}, respectively), while Bahawalpur was non-saline (EC_e 1.31 dS m^{-1}). Organic matter content was uniformly low (0.23–0.86%), with Bahawalpur registering the lowest surface OM (0.51%) and subsurface OM (0.23%). Available phosphorus ranged from 3.95 to 10.9 ppm and was clearly inadequate at Bahawalpur, while available potassium varied from 51 ppm (deficient, Bahawalpur subsoil) to 177 ppm (adequate, Haroonabad subsoils). Collectively, these soil conditions; alkaline pH, low organic matter, and variable but generally limiting macronutrient status, represent the broader fertility constraints under which the cane crop was raised and were expected to amplify the agronomic value of micronutrient supplementation.

These results align closely with the wider characterization of the Indus Basin cane belt, where alkaline pH and free $CaCO_3$ are recognized as the primary chemical drivers of micronutrient immobilization (Rashid and Ryan, 2004; Imran et al., 2020). The low organic matter content recorded across all three sites is particularly relevant for copper nutrition, because soil organic matter is a principal repository of complexed Cu^{2+} available for slow release into the soil solution; its depletion under continuous, NPK-centric cropping systems is a well-documented driver of latent Cu deficiency on Pakistani soils (Zia et al., 2007; Majeed et al., 2022). The site-to-site contrast in salinity and macronutrient status further provided a useful natural gradient against which the consistency of Cu response could be tested.

3.2 Analysis of variance for cane yield

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The combined analysis of variance for cane yield across the three locations revealed highly significant effects of both location and treatment, while their interaction was marginally non-significant (Table 3). The location effect was significant at the 5% probability level ($F = 46.36$, $p = 0.0209$), indicating that the three sites produced significantly different mean cane yields, an expected outcome given their contrasting soil fertility status documented in Table 1. The treatment effect was highly significant at the 1% probability level ($F = 14.34$, $p < 0.0001$), confirming that the graded copper fertilization rates produced statistically significant differences in cane yield over and above the uniform NPK basal dose. The location × treatment interaction was marginally non-significant at the 5% probability level ($F = 2.29$, $p = 0.0825$), indicating that the broad direction of the cane yield response to Cu fertilization was consistent across the three contrasting sites, although location-specific divergences existed at the higher end of the Cu series.

The simultaneous significance of the main treatment effect and broad consistency of the response across sites are particularly valuable for extension-scale interpretation. They imply that the optimum Cu rate identified through this trial can be recommended as a regional input for sugarcane growers across the Bahawalpur–Bahawalnagar tract without requiring detailed site-specific calibration based on soil EC, P, or K status. Comparable consistency of micronutrient response across contrasting soil fertility gradients has been reported for Zn in wheat and rice on Pakistani alkaline soils (Rashid and Ryan, 2004) and for Cu in cereals grown on calcareous soils (Rahman et al., 2022), supporting the generalizability of the present finding within similar agro-ecological zones.

Table 3. Combined analysis of variance for cane yield of sugarcane across three locations and five copper fertilization levels.

Source of variation	DF	Sum of squares	Mean square	F-value	p-value
Replications	2	4,784,794	2,392,397	—	—
Locations (L)	2	1.21×10^8	1.21×10^8	46.36	0.0209*
Error (a)	2	5,228,410	2,614,205	—	—
Treatments (T)	4	3.38×10^8	8.45×10^7	14.34	<0.0001**
L × T interaction	8	5.41×10^7	1.35×10^7	2.29	0.0825ns
Error (b)	30	1.77×10^8	5,892,974	—	—
Total	**44**				

*Note: * = significant at 5% probability level; ** = significant at 1% probability level; ns = non-significant.*

3.3 Cane yield response to copper fertilization

Across the pooled mean of the three locations, soil-applied copper sulphate pentahydrate produced a progressive improvement in cane yield as the Cu rate increased from 0 to 12 kg ha^{-1} (Figure 1). The unfertilized NPK-only control (T1) recorded the lowest pooled mean cane yield of 104,993.8 kg ha^{-1}, while application of 6, 8, 10, and 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1} produced pooled mean yields of 108,139.5, 110,707.9, 112,358.0, and 112,843.2 kg ha^{-1}, respectively (Table 4). The yield response over the unfertilized control therefore rose from +3,145.7 kg ha^{-1} (+3.0%) at T2 to +7,849.4 kg ha^{-1} (+7.5%) at T5, with the largest absolute yield gain recorded at the highest tested rate. The location-wise yield performance is presented in Figures 2, 3, and 4 for Haroonabad-1, Haroonabad-2, and Bahawalpur, respectively.

This dose–response pattern is physiologically coherent and aligns with established understanding of copper nutrition in graminaceous crops. Adequate Cu supply enhances the activity of plastocyanin, an essential electron carrier on the donor side of photosystem I, and thereby supports photosynthetic carbon assimilation through the grand growth phase of the cane crop (Yruela, 2009; Printz et al., 2016). Cu is simultaneously a structural component of Cu/Zn superoxide dismutase, the principal cytosolic antioxidant enzyme protecting expanding tissues against reactive oxygen species generated under high-irradiance and salinity stress, conditions that prevail at all three of the present sites (Adrees et al., 2015). Equally important for cane in particular, Cu activates lignin biosynthetic enzymes including polyphenol oxidase and laccases, thereby strengthening stalk integrity and reducing yield losses through lodging during the late grand growth and maturation phases (Printz et al., 2016; Hunter et al., 2023). The magnitude of the yield gain recorded at the highest Cu rate; +7,849.4 kg ha^{-1} over the unfertilized control on a pooled-mean basis, suggests that copper has been a latent but quantitatively important limitation on cane productivity at all three sites, and that NPK-only fertilization schedules currently in widespread use in the region significantly underexploit the yield potential of CPF-246 on alkaline-calcareous soils.

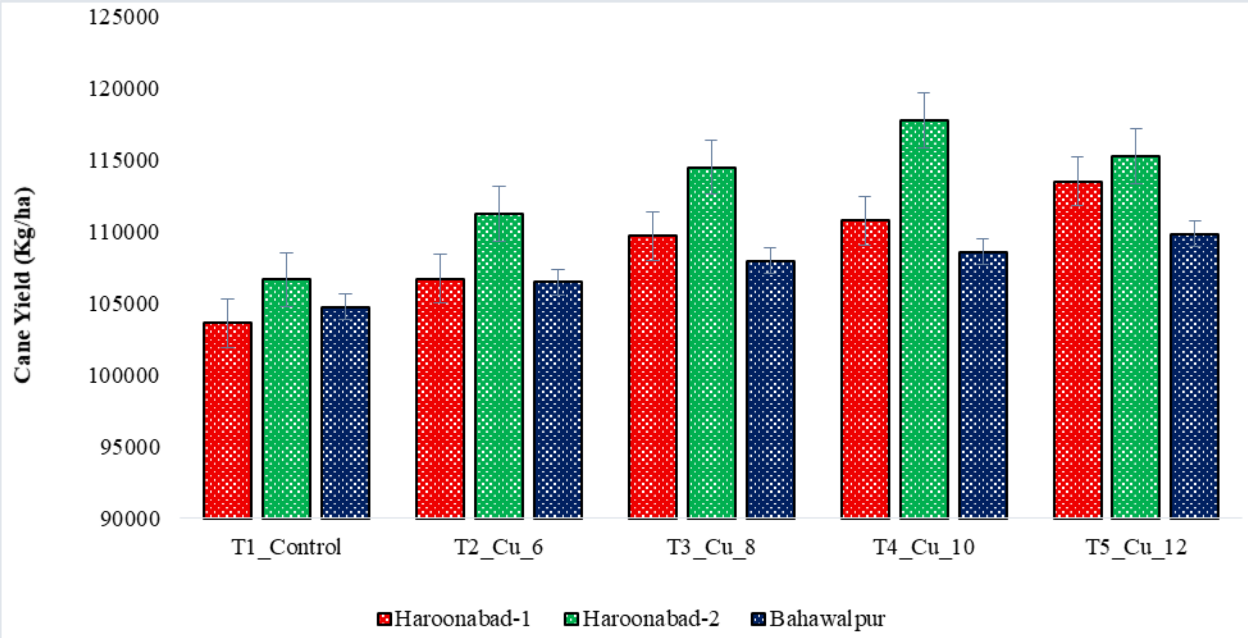

Figure 1 | Performance of sugarcane crop at five different cupper fertilization at three different locations

Table 4. Site-wise and pooled mean cane yield of sugarcane (cv. CPF-246) under five graded levels of copper fertilization across three locations of southern Punjab during 2023–24.

Treatment	Haroonabad-1 (kg ha^{-1})	Haroonabad-2 (kg ha^{-1})	Bahawalpur (kg ha^{-1})	Pooled mean (kg ha^{-1})	Gain over T1 (%)
T1 (0 kg)	103,629.6	106,629.6	104,722.2	104,993.8	—
T2 (6 kg)	106,685.2	111,259.2	106,474.1	108,139.5	+3.0
T3 (8 kg)	109,703.7	114,481.5	107,938.5	110,707.9	+5.4
T4 (10 kg)	110,759.2	117,740.7	108,574.1	112,358.0	+7.0

Treatment	Haroonabad-1 (kg ha^{-1})	Haroonabad-2 (kg ha^{-1})	Bahawalpur (kg ha^{-1})	Pooled mean (kg ha^{-1})	Gain over T1 (%)
T5 (12 kg)	113,481.5	115,222.2	109,825.9	112,843.2	+7.5

3.4 Location-wise response and the location × treatment interaction

When the data were disaggregated by location, two consistent and one site-specific pattern emerged (Figures 2–4 and Table 4). Haroonabad-2 recorded the highest mean cane yields across all treatments, ranging from 106,629.6 kg ha^{-1} at T1 to a peak of 117,740.7 kg ha^{-1} at T4 (10 kg ha^{-1}), followed by a small decline to 115,222.2 kg ha^{-1} at T5 (12 kg ha^{-1}). Haroonabad-1 and Bahawalpur, by contrast, showed monotonically rising responses across the full Cu series, with yield at Haroonabad-1 climbing from 103,629.6 kg ha^{-1} (T1) to 113,481.5 kg ha^{-1} (T5); a gain of +9,851.9 kg ha^{-1}, and at Bahawalpur from 104,722.2 kg ha^{-1} (T1) to 109,825.9 kg ha^{-1} (T5); a gain of +5,103.7 kg ha^{-1}. The absolute yield ranking across locations corresponds closely to the soil fertility status documented in Table 1: Haroonabad-2 carried the highest available P (10.9 ppm) and surface organic matter (0.71%), Haroonabad-1 was intermediate, and Bahawalpur carried the lowest available P (5.71 ppm) and K (90 ppm), explaining its lower baseline yield even under uniform NPK basal fertilization.

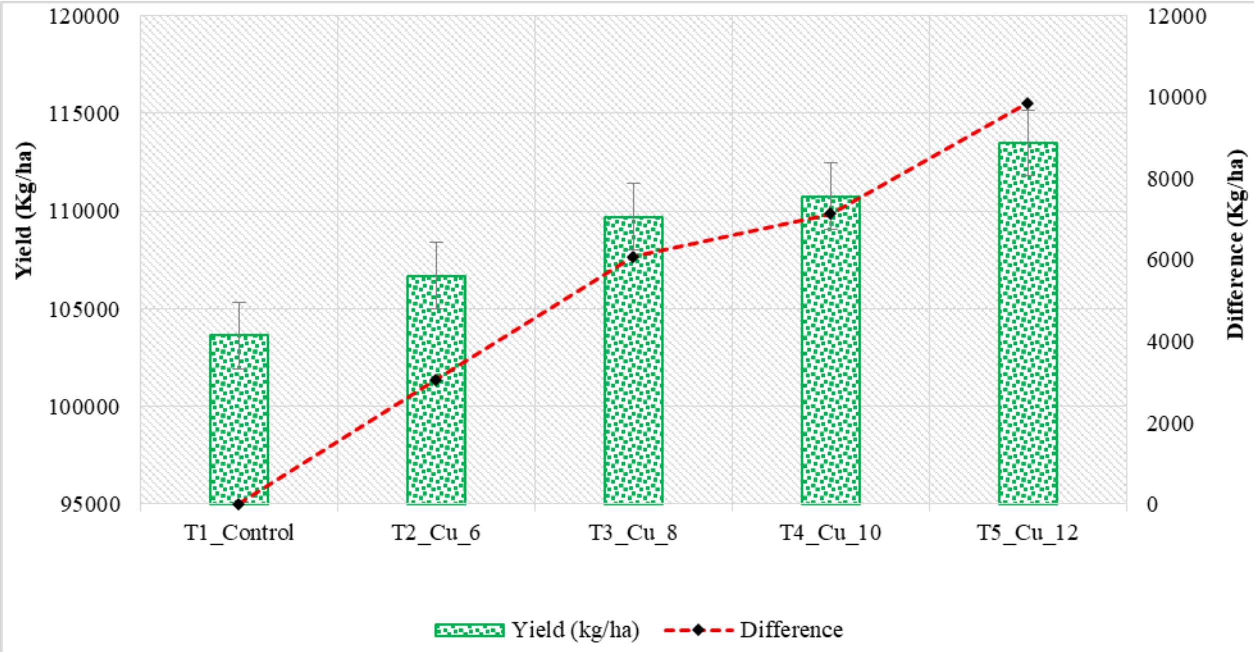

Figure 2 | Yield performance of sugarcane crop at five different cupper fertilization levels at Haroonabad-I location and difference from control

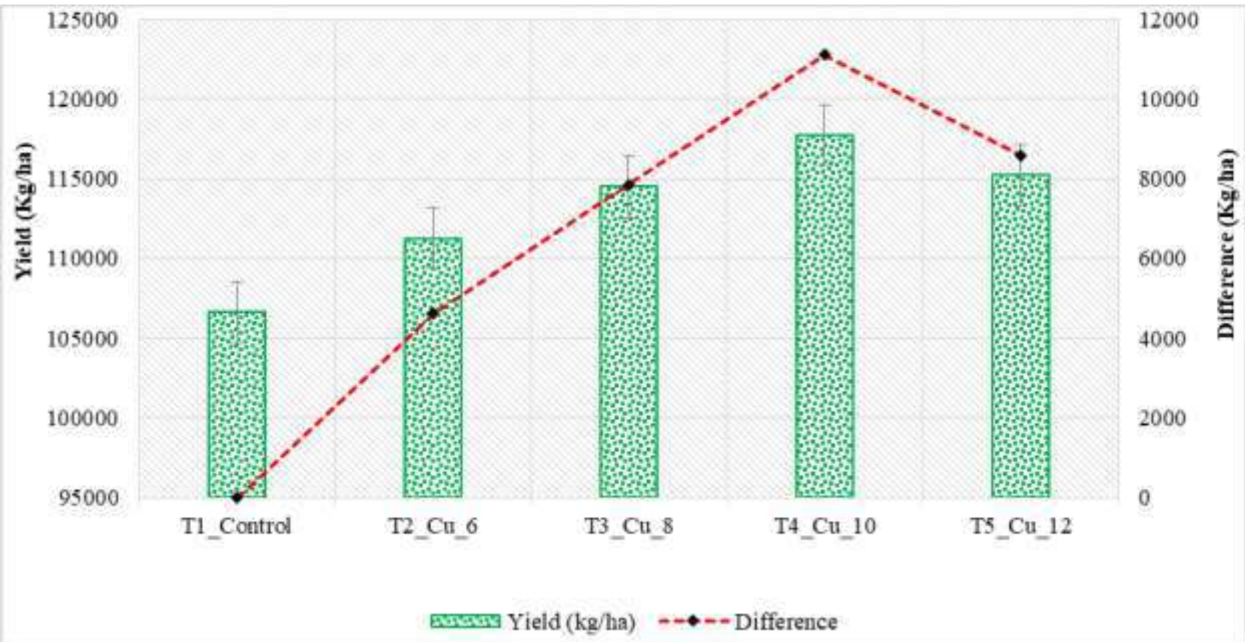

Figure 3 | Yield performance of sugarcane crop at five different cupper fertilization levels at Haroonabad-2 location and difference from control

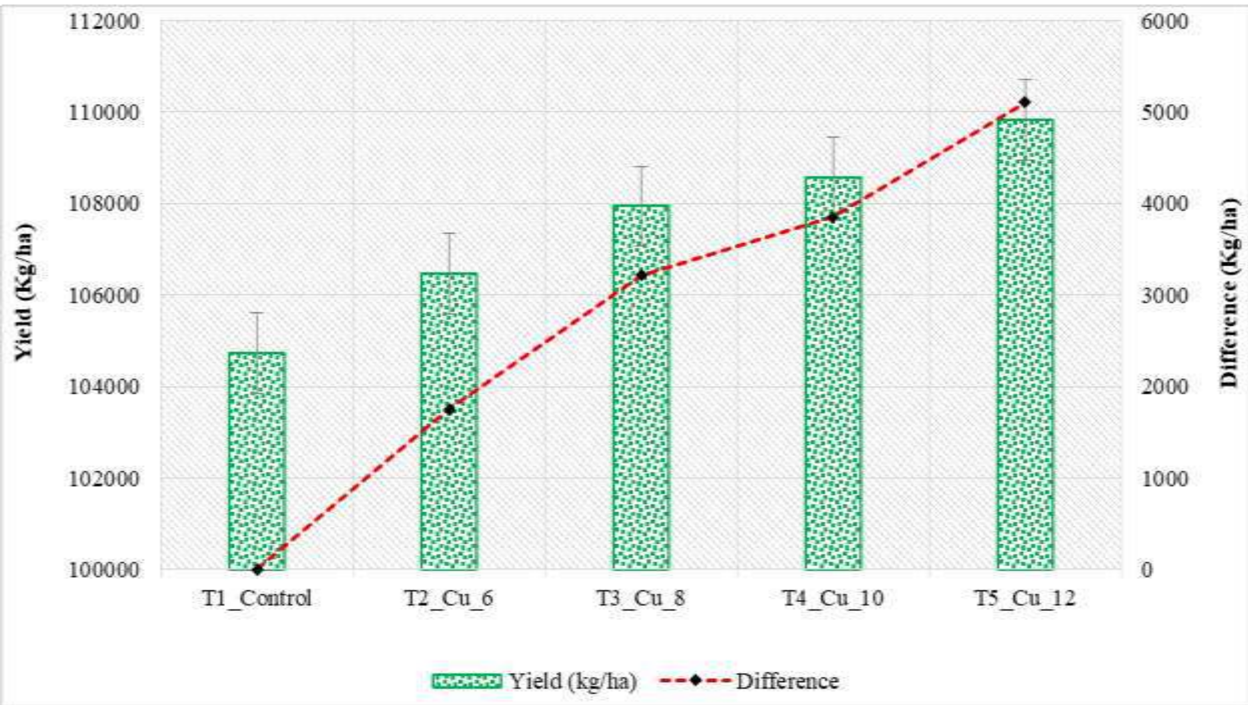

Figure 2 | Yield performance of sugarcane crop at five different cupper fertilization levels at Bahawalpur location and difference from control

The location × treatment interaction (F = 2.29, p = 0.0825) was marginally non-significant at the 5% probability level. The principal driver of this borderline interaction was the divergent yield trajectory at Haroonabad-2, where the response curve plateaued and slightly declined at T5, while the responses at the other two sites continued to rise across the full Cu series. The Haroonabad-2 yield dip at the highest Cu rate may reflect the comparatively better baseline P and OM status at that site, which would have permitted higher tissue Cu loading and brought the crop closer to the sufficiency threshold beyond which further Cu supply ceases to be agronomically beneficial.

Antagonistic interactions of Cu with Zn and Fe uptake at high tissue Cu concentrations are well documented in graminaceous crops (Adrees et al., 2015; Rahman et al., 2022) and provide a plausible mechanism for the modest yield depression observed at T5 on the more fertile of the three sites. From an extension standpoint, however, the formal non-significance of the interaction term, together with the consistency of the broad treatment ranking across all three sites, supports the use of a regional Cu recommendation rather than separate site-specific calibrations.

3.5 Identifying the recommended Cu rate

Taking the pooled and location-wise yield responses together, the T5 treatment (12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}) produced the largest absolute cane yield response over the unfertilized NPK-only control on a pooled-mean basis and is therefore identified as the recommended Cu rate for spring-planted CPF-246 sugarcane on the alkaline-calcareous soils of southern Punjab. At this rate, the pooled mean cane yield reached 112,843.2 kg ha^{-1}, representing an absolute gain of +7,849.4 kg ha^{-1} (+7.5%) over the NPK-only control. The yield gain at T5 was substantial and consistent across all three sites, ranging from +5,103.7 kg ha^{-1} at Bahawalpur to +9,851.9 kg ha^{-1} at Haroonabad-1, indicating that 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1} delivered the largest reproducible cane yield response and represented the upper end of the agronomic response curve within the rates tested. Given the comparatively low cost of $CuSO_4 \cdot 5H_2O$ against the substantial absolute yield response, this rate emerges as both an agronomically and economically defensible recommendation. Final cane yield in sugarcane is jointly determined by stand density and individual cane biomass (Bull and Glasziou, 1975), and the consistent yield gains observed under T5 indicate that copper supply at this level adequately addresses both of these yield components on alkaline-calcareous soils where Cu bioavailability is otherwise constrained by carbonate fixation.

It should be noted that the pooled-mean yield advantage from T4 to T5 was modest (+485.2 kg ha^{-1}; +0.43%), and that the response at Haroonabad-2 specifically peaked at T4 (10 kg ha^{-1}) rather than at T5. The flattening of the response curve at the more fertile Haroonabad-2 site, and the small but consistent additional gains observed at the two lower-fertility sites, together suggest that the true sufficiency threshold for soil-applied Cu under these alkaline-calcareous conditions lies at or near 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}. The marginal yield improvement at T5 also signals that Cu supply beyond this rate is unlikely to deliver additional agronomic benefit and may begin to introduce risks of induced Zn or Fe deficiency, particularly on soils where these micronutrients are already marginal (Rahman et al., 2022). Validation of the present recommendation under ratoon-crop conditions, autumn-planted cane, and a broader soil fertility gradient would further strengthen its applicability across the wider sugarcane cropping systems of southern Punjab.

4. Conclusion

The present multi-location field investigation demonstrated that soil-applied copper sulphate pentahydrate, broadcast at planting alongside a uniform NPK basal dose, produced a statistically significant and agronomically meaningful improvement in cane yield of spring-planted sugarcane (cv. CPF-246) under the alkaline-calcareous, canal-irrigated conditions of southern Punjab. Pooled mean cane yield rose progressively from

104,993.8 kg ha^{-1} in the unfertilized control to 112,843.2 kg ha^{-1} at the highest tested dose, representing an absolute yield gain of +7,849.4 kg ha^{-1} (+7.5%) at 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1}. The yield response was monotonic and still rising at Haroonabad-1 and Bahawalpur, while at the more fertile Haroonabad-2 site the response peaked at 10 kg ha^{-1} and declined slightly at 12 kg ha^{-1}, generating a marginally non-significant location × treatment interaction ($p = 0.0825$). On the strength of the largest absolute yield response over the unfertilized control across the pooled and site-level analyses, the inclusion of 12 kg $CuSO_4 \cdot 5H_2O$ ha^{-1} as a basal soil-broadcast input is recommended as a regional fertilizer practice for sugarcane growers across the Bahawalpur–Bahawalnagar tract, complementing rather than replacing the existing NPK schedule. The findings further argue for a broader re-examination of copper, and of micronutrient nutrition in general, in the fertilizer recommendations issued for sugarcane on alkaline soils of Pakistan, where decades of NPK-centric fertilization have left a latent but evidently corrigible Cu limitation on productivity. Future work should extend the present trial to ratoon-crop performance, to autumn-planted cane, and to wider soil fertility gradients, and should additionally evaluate the response of juice quality parameters such as Brix, sucrose percentage, and commercial cane sugar to graded Cu fertilization, so that a fully integrated micronutrient recommendation for sugarcane in southern Punjab can be developed.

Author Contributions

MJQ; Conceptualization and Execution, Supervision, Planning, Writing – original draft; MRF, SJ; Experimental layout, Field execution and crop management at Haroonabad-1 and Haroonabad-2 sites; MBL, AR; Field execution and crop management at Bahawalpur site, Treatment application; AAS, BB; Data analysis, Statistical computation, Data visualization; MA, TA; Soil sampling, Soil chemical analysis, Soil fertility characterization; SH, MIM, MAU; Yield data recording, Data compilation; MJQ, AAS, MRF; Writing – review & editing.

Conflict of interest

The authors declared absence of conflict of interest.

References

1. Government of Pakistan (GoP) (2024). Pakistan Economic Survey 2023–24, Chapter 2: Agriculture. Finance Division, Economic Adviser's Wing, Islamabad.
2. Afghan, S., Khan, M. E., Verma, K. K., & Nikpay, A. (2024). Economic importance and yield potential of sugarcane in Pakistan. In *Sugarcane cultivation and management* (pp. 263-291). Apple Academic Press.
3. Shahzad, A.N., Qureshi, M.K., Wakeel, A., Misselbrook, T. (2019). Crop production in Pakistan and low nitrogen use efficiencies. Nature Sustainability, 2(12): 1106–1114.

4. Majeed, A., Rashid, I., Niaz, A., Ditta, A., Sameen, A., Al-Huqail, A.A., Siddiqui, M.H. (2022). Balanced use of Zn, Cu, Fe, and B improves the yield and sucrose contents of sugarcane juice cultivated in sandy clay loam soil. Agronomy, 12(3): 696.
5. Manzoor, M., Khan, M.Z., Ahmad, S., Alqahtani, M.D., Shabaan, M., Sarwar, S., Hameed, M.A., Zulfiqar, U., Hussain, S., Ali, M.F., Ahmad, M., Haider, F.U. (2023). Optimizing sugarcane growth, yield, and quality in different ecological zones and irrigation sources amidst environmental stressors. Plants, 12(20): 3526. https://doi.org/10.3390/plants12203526
6. Rashid, A., Ryan, J. (2004). Micronutrient constraints to crop production in soils with Mediterranean-type climates: a review. Journal of Plant Nutrition, 27(6): 959–975.
7. Imran, M., Rehim, A., Sarwar, N., Hussain, S. (2020). Zinc bioavailability in maize grains in response to phosphorus–zinc interaction. Journal of Plant Nutrition and Soil Science, 183(3): 305–311.
8. Zia, M.H., Ahmad, R., Khaliq, I., Ahmad, A., Irshad, M. (2007). Micronutrients status and management in orchards soils: applied aspects. Soil and Environment, 26(1): 13–22.
9. Yruela, I. (2009). Copper in plants: acquisition, transport and interactions. Functional Plant Biology, 36(5): 409–430.
10. Printz, B., Lutts, S., Hausman, J.F., Sergeant, K. (2016). Copper trafficking in plants and its implication on cell wall dynamics. Frontiers in Plant Science, 7: 601.
11. Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M.K., Bharwana, S.A. (2015). The effect of excess copper on growth and

physiology of important food crops: a review. Environmental Science and Pollution Research, 22(11): 8148–8162.

12. Hunter, A.S., Bagniewska-Zadworna, A., Sokolowska, K., Brodribb, T.J. (2023). The effects of copper deficiency on lignification, xylem vessel structure, and hydraulic traits in hybrid poplar. Physiologia Plantarum, 175(5): e14006.

13. McCray, J.M., Rice, R.W., Ezenwa, I.V., Lang, T.A., Baucum, L. (2010). Sugarcane plant nutrient diagnosis. University of Florida IFAS Extension, Document SS-AGR-128. University of Florida, Gainesville, FL.

14. Naga Madhuri, K.V., Sarala, N.V., Hemanth Kumar, M., Subba Rao, M., Giridhar, V. (2013). Influence of micronutrients on yield and quality of sugarcane. Sugar Tech, 15(2): 187–191. https://doi.org/10.1007/s12355-012-0196-3

15. Ryan, J., Estefan, G., Rashid, A. (2001). Soil and Plant Analysis Laboratory Manual, 2nd ed. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

16. Estefan, G., Sommer, R., Ryan, J. (2013). Methods of Soil, Plant, and Water Analysis: A Manual for the West Asia and North Africa Region, 3rd ed. International Center for Agricultural Research in the Dry Areas (ICARDA), Beirut, Lebanon.

17. Steel, R.G.D., Torrie, J.H., Dickey, D.A. (1997). Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw-Hill Book Co., New York.

18. Rahman, N., Peak, D., Schoenau, J. (2022). Antagonistic effect of copper and zinc in fertilization of spring wheat under low soil phosphorus conditions. Canadian Journal of Soil Science, 102(3): 797–809. https://doi.org/10.1139/cjss-2021-0189

19. Bull, T.A., Glasziou, K.T. (1975). Sugarcane. In: L.T. Evans (ed.), Crop Physiology: Some Case Histories. Cambridge University Press, Cambridge, UK, pp. 51–72.

20. FAO (2023). World Food and Agriculture – Statistical Yearbook 2023. Food and Agriculture Organization of the United Nations, Rome.

21. FAOSTAT (2023). Crops and livestock products database. Food and Agriculture Organization of the United Nations. Available at: https://www.fao.org/faostat (accessed 2024).