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Diluted Sugar Mill Effluent Application with PGPR Improves the Performance of Maize (*Zea mays* L.) under an Arid Climate

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Abstract: The disposal of sugar mill effluent is a serious matter of concern for the sugar industry. In this regard, the dilution of sugar mill effluent in combination with plant growth promoting rhizobacteria (PGPR) might be a viable option for improving crop growth. In this study, we evaluated the potential of diluted sugar mill effluent (SME) and PGPR to improve maize (*Zea mays* L.) performance. Seeds of a maize hybrid (Pioneer 1543) were sown in 20 kg soil-filled pots. The pots were irrigated with various sugar mill effluent concentrations (viz. 0, 15%, 30%, 45%, 60%, 75% and 100% v/v). The results indicated that application of SME up to a concentration of 75% improved the stay-green, leaf emergence, growth and productivity of maize. However, the application of SME at a concentration of 100% was detrimental for maize plants and decreased the maize growth. The application of PGPR was also beneficial for improvement in stay-green, leaf emergence, growth and productivity of maize as compared with control (no PGPR application). In conclusion, the use of SME at concentration of 75% in combination with PGPR was the most effective method for improvement in stay-green, leaf emergence, growth and productivity of maize.

Keywords: diluted sugar mill effluent; maize; stay-green; leaf emergence; PGPR; arid climate

1. Introduction

Maize (*Zea mays* L.) is an important cereal crop of Pakistan. It is consumed as food, fodder and feed by human beings, animals and poultry birds, respectively. In Pakistan, it is currently cultivated on 0.9 million hectares of land, to producing 1.3 million tonnes of product [1]. At present, the use of industrial effluents for irrigation is gaining momentum. In many areas of the world, treated and diluted industrial effluents have been used for irrigation purposes [2–4], owing to their positive influences on crop growth through the improvement in soil properties [5–7].

Among the various industrial effluents, effluents from the sugar industry are of great agricultural importance as they contain surplus quantities of zinc, manganese, iron, copper, magnesium, potassium, calcium, phosphorus and nitrogen [8]. Owing to the presence of these macro- and micronutrients, effluents from the sugar industry can be used as a substitute for chemical fertilizers [5], especially in the neighborhood areas of the sugar mills where the deposition of sugar effluents in suitable places always remains a serious matter of concern.

The farmers living in areas near the sugar mills utilize the effluents from sugar mills as an irrigation source, neglecting their impacts on crop and soil health [9]. However, the sugar industry effluents may contain some heavy metals, like copper and nickel, which may negatively affect crop performance

when used for irrigation purpose [10]. This indicates that long-term use of effluents from sugar mills for irrigation purposes may deteriorate soil health and the quality of the produce. Therefore, it is vital that measures are adopted for judicious use of sugar mill effluents as an alternate source of irrigation, to allow sustainable crop production.

In this scenario, the use of sugar industry effluents after suitable dilution might be a useful option to reduce the harmful effects of sugar industry effluents on maize growth [3]. The other option is the use of plant growth-promoting rhizobacteria (PGPR) to improve the growth of plants irrigated with sugar mill effluents. The PGPRs consist of multiple groups of free-living soil bacteria that can boost the growth and development of plants in heavy metal-contaminated soils through various mechanisms [11–13].

For this study, we hypothesized that the use of sugar industry effluents after dilution in combination with PGPR might be a useful strategy for improving maize growth and grain yield.

2. Materials and Methods

2.1. Site and Soil

This study was carried out in pots (each containing 20 kg soil) in the greenhouse of College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Punjab, Pakistan. Before filling the pots with soil, the soil was sampled, air dried, crushed and sieved through a 2 mm sieve. These samples were analysed in regard to physico-chemical properties in the soil and in a water-testing laboratory. The experimental soil was sandy loam with a pH of 7.2, total nitrogen content of 0.13 g kg⁻¹, available phosphorous content of 7.6 ppm, electrical conductivity of 3.6 dS m⁻¹, and extractable potassium content of 100 ppm. The lowest temperature recorded during the crop growth period was 11 °C while the highest was 41.6 °C. The climate of Layyah is arid and had very low relative humidity during the experimental period.

2.2. Collection of Sugar Mill Effluents and PGPRs

The sugar mill effluent used in this study was collected once from the Layyah Sugar Mill, located in Layyah city, Pakistan. The effluent samples were collected in plastic containers from the point of disposal of the Layyah Sugar Mill. The sugar mill effluent had an electrical conductivity of 809 µS cm⁻¹, pH of 8.8, Ca + Mg content of 7.88 ppm, sodium content of 0.21 ppm, bicarbonate content of 9.05 ppm, residual sodium carbonate content of 1.17 ppm, sodium adsorption ratio of 0.10, potassium content of 150 ppm, nitrate content of 400 ppm, phosphate content of 150 ppm, cadmium content of 8.18 ppm and copper content of 8.23 ppm, and zinc content of 15 ppm. The sugar mill effluent had no carbonate or chloride. The water chemical analysis was carried out as detailed elsewhere [14,15]. The bacterial strain, *Bacillus* sp. MWT-14 (accession no. KT933232), was used in this study for seed inoculation.

2.3. Seed Source

The certified seeds (uniform size, colour and weight) of maize hybrid (Pioneer 1543) were purchased from Pioneer seed company (private limited) Sahiwal.

2.4. Experimental Design and Crop Husbandry

The experiment was conducted in a completely randomized design with three replications (two pots per replicate). The experiment consisted of six various sugar mill effluent concentrations viz. 15%, 30%, 45%, 60%, 75%, 100% and a control treatment irrigated with tap water. Each pot was irrigated with 800 mL of its respective concentration of test solution every three days. Crops were sown on 20 February 2016 in pots (54 cm × 36 cm) after filling the pots with 20 kg of soil. The experiment was repeated over space to validate the results. Initially, five seeds were sown in each pot which were thinned to four plants per pot after uniform emergence.

2.5. Data Recording

The plant samples were collected 13, 26 and 39 days after sowing to record data on the size of the leaf area per plant, number of leaves per plant, stem diameter, plant height, amount of dry biomass per plant and chlorophyll content index. One plant from each replicate was harvested on each sampling date to record the data on these parameters. One plant was left in each pot to record data on number of grains per cob, 100-grain weight and grain yield. The crops were harvested on 2 June 2016.

2.6. Statistical Analysis

Data collected on all parameters for both experiments was merged and was analyzed statistically using the Analysis of Variance Technique, and Fisher's Least Significantly Difference (LSD) test at a 1% probability level was applied to compare the treatments' means [16].

3. Results

The application of sugar mill effluent significantly affected the size of the leaf area per plant, number of leaves per plant, stem diameter, plant height, chlorophyll content index, and amount of dry biomass per plant at 13, 26 and 40 days after sowing (DAS). Likewise, the application of PGPR also significantly affected the size of the leaf area per plant, number of leaves per plant, stem diameter, plant height, chlorophyll content index, and amount of dry biomass per plant at 13, 26 and 40 DAS, except for plant height at 13 DAS and stem diameter at 26 DAS. The interaction of sugar mill effluent with PGPR was also significant for plant height at 26 DAS, stem diameter at 26 DAS, the size of the leaf area at 26 and 40 DAS and amount of dry biomass per plant and chlorophyll content index at 13, 26 and 40 DAS (Tables 1 and 2).

At 13 and 40 DAS, the highest plant height was recorded with the application of 75% sugar mill effluent which was statistically similar to the application of 60% sugar mill effluent at 13 DAS and the application of 30% and 45% sugar mill effluent at 40 DAS. At 26 DAS, the highest plant height was recorded with the application of 75% water effluent with PGPR which was statistically similar to the application of the same concentration of sugar mill effluent without PGPR, and to the application of 30% sugar effluent with PGPR (Table 1). At 13 and 26 DAS, the highest stem diameter was recorded with the application of 75% sugar mill effluent and that was statistically similar to the application of 60% and 15% sugar mill effluent. The highest stem diameter at 13 DAS was recorded with PGPR. At 40 DAS, the highest stem diameter was recorded with the application of 75% sugar mill effluent with/without PGPR which was statistically similar to the application of 30% sugar mill effluent with PGPR (Table 1). The highest number of leaves was recorded with the application of 75% sugar mill effluent at 13, 26 and 40 DAS which was statistically similar to the application of 60% sugar mill effluent at all sampling dates. The use of PGPR also improved the number of leaves per plant compared to the condition with no PGPR use, at 13, 26 and 40 DAS (Table 1).

At 13 DAS, the greatest leaf area was measured with the application of 75% sugar mill effluent. The use of PGPR also improved the size of the leaf area at 13 DAS. At 26 and 40 DAS, the greatest leaf area was recorded with the application of 75% sugar mill effluent with PGPR and that was statistically similar to the application of 30% sugar mill effluent with PGPR at 26 DAS and to the application of 60% sugar mill effluent with PGPR at 40 DAS (Table 2). The highest amount of dry biomass per plant and the highest chlorophyll content index at 13, 26 and 40 DAS were recorded with the application of 75% sugar mill effluent with PGPR (Table 2).

The highest number of grains per cob, 100-grain weight and grain yield were recorded when the PGPRs were applied as opposed to no PGPRs application. Among the sugar mill effluents, the highest number of grains per cob, 100-grain weight and grain yield occurred with the application of 75% sugar mill effluent which was statistically similar to the application of 75% sugar mill effluent (Table 3).

Table 1. Influence of diluted sugar mill effluent and PGPR on plant height, stem diameter and number of leaves per plant.

	No PGPR	PGPR	Mean (SME)	No PGPR	PGPR	Mean (SME)	No PGPR	PGPR	Mean (SME)
SME (%)	13 days after sowing			26 days after sowing			40 days after sowing		
	Plant height (cm)								
0	18.4	24.2	21.3 ^c	37.0 ^e	45.3 ^{cd}	41.2 ^d	45.0	52.3	48.7 ^c
15	23.8	24.5	24.1 ^{abc}	52.2 ^{ab}	52.5 ^{ab}	52.3 ^{abc}	47.0	53.0	50.0 ^c
30	24.8	25.5	25.1 ^{abc}	50.3 ^{abc}	55.0 ^a	52.6 ^{ab}	59.0	59.3	59.2 ^{ab}
45	23.7	23.8	23.7 ^{bc}	47.5 ^{bcd}	50.5 ^{abc}	49.0 ^{bc}	56.3	57.2	56.7 ^{ab}
60	27.0	24.5	25.8 ^{ab}	49.5 ^{abcd}	47.7 ^{bcd}	48.6 ^c	54.2	56.3	55.3 ^b
75	26.3	29.0	27.7 ^a	52.7 ^{ab}	54.5 ^a	53.6 ^a	60.3	61.5	60.9 ^a
100	21.3	22.0	21.7 ^c	45.8 ^{cd}	43.5 ^{cd}	44.6 ^d	47.3	46.7	47.0 ^c
Mean (PGPR)	23.6	24.8		45.1 ^b	49.9 ^a		52.7 ^b	55.2 ^a	
LSD ($p \leq 0.05$)	PGPR = NS; SME = 3.9; PGPR \times SME = NS			PGPR = 1.35; SME = 3.93; PGPR \times SME: 6.41			PGPR = 1.58; SME = 4.57; PGPR \times SME = NS		
	Stem diameter (cm)								
0	4.2	3.6	3.9 ^b	5.4	6.3	5.9 ^c	7.8 ^d	7.7 ^d	7.7 ^c
15	4.5	5.4	4.9 ^a	8.2	7.9	7.5 ^b	9.2 ^{bcd}	9.8 ^{bc}	9.5 ^b
30	4.2	4.8	4.5 ^b	5.3	7.5	7.8 ^b	7.5 ^d	11.0 ^{ab}	9.2 ^b
45	3.7	4.5	4.1 ^{ab}	7.4	7.6	7.5 ^b	8.0 ^{cd}	8.8 ^{cd}	8.4 ^{bc}
60	4.5	5.1	4.8 ^a	7.6	8.5	8.0 ^b	8.1 ^{cd}	9.3 ^{bcd}	8.7 ^{bc}
75	4.7	5.2	5.0 ^a	9.8	10.1	9.9 ^a	12.8 ^a	12.2 ^a	12.5 ^a
100	4.0	4.7	4.4 ^{ab}	7.6	7.1	7.3 ^b	8.9 ^{cd}	8.8 ^{cd}	8.9 ^{bc}
Mean (PGPR)	4.3 ^b	4.8 ^a		7.5	7.9		8.9 ^b	9.6 ^a	
LSD ($p \leq 0.05$)	PGPR = 0.24; SME = 0.69; PGPR \times SME = NS			PGPR = NS; SME = 1.21; PGPR \times SME = NS			PGPR = 0.41; SME = 1.18; PGPR \times SME = 1.93		
	Number of leaves per plant								
0	3.7	4.4	4.1 ^{bcd}	6.6	7.2	6.9 ^{cd}	6.6	7.6	7.1 ^{bcd}
15	3.3	3.5	3.4 ^d	6.3	6.1	6.2 ^d	6.6	6.6	6.6 ^d
30	4.0	4.3	4.1 ^{bcd}	6.7	7.5	7.1 ^{bc}	7.5	7.7	7.6 ^{bcd}
45	4.0	4.4	4.2 ^{abc}	6.5	7.7	7.1 ^{bc}	8.0	9.3	8.7 ^{abc}
60	4.5	5.1	4.8 ^{ab}	7.5	8.3	7.9 ^{ab}	8.1	9.5	8.8 ^{ab}
75	5.0	5.1	5.0 ^a	7.7	9.0	8.3 ^a	9.7	11.2	10.4 ^a
100	3.7	3.9	3.8 ^{cd}	6.3	7.2	6.8 ^c	6.5	7.3	6.9 ^{cd}
Mean (PGPR)	4.0 ^b	4.4 ^a		6.8 ^b	7.6 ^a		7.6 ^b	8.5 ^a	
LSD ($p \leq 0.05$)	PGPR = 0.29; SME = 0.84; PGPR \times SME = NS			PGPR = 0.30; SME = 0.86; PGPR \times SME = NS			PGPR = 0.29; SME = 0.84; PGPR \times SME = NS		

Means of main effects and interactions sharing the same case letter for a parameter at each sampling date do not differ significantly at $p \leq 0.05$; SME = diluted sugar mill effluent; PGPR = plant growth promoting rhizobacteria; NS = non-significant. LSD, least significant difference.

Table 2. Influence of diluted sugar mill effluent and PGPR on size of leaf area, amount of dry biomass per plant and chlorophyll content of maize.

SME (%)	No PGPR	PGPR	Mean (SME)	No PGPR	PGPR	Mean (SME)	No PGPR	PGPR	Mean (SME)
	13 days after sowing			26 days after sowing			40 days after sowing		
	Leaf area (cm ²)								
0	40.7	51.3	46.0 ^{bc}	90.7 ⁱ	127.3 ^h	109.0 ^e	325.2 ^f	379.5 ^e	352.3 ^e
15	49.7	53.0	51.3 ^b	152.4 ^{gh}	234.6 ^{bc}	193.5 ^c	391.3 ^e	454.8 ^d	423.0 ^d
30	44.7	50.3	47.5 ^{bc}	184.3 ^{ef}	252.7 ^{ab}	1218.5 ^b	343.7 ^{ef}	455.4 ^d	399.5 ^d
45	48.3	51.7	50.0 ^b	157.2 ^{fg}	199.3 ^{de}	178.3 ^{cd}	453.8 ^d	560.0 ^c	506.9 ^c
60	50.7	54.7	52.7 ^b	219.0 ^{cd}	234.3 ^{bc}	226.7 ^{ab}	540.0 ^c	633.1 ^{ab}	586.5 ^b
75	54.0	65.3	59.7 ^a	211.0 ^{cde}	275.7 ^a	243.3 ^a	584.1 ^{bc}	682.8 ^a	633.5 ^a
100	39.7	42.7	41.2 ^c	155.2 ^g	189.0 ^e	172.1 ^d	386.0 ^e	453.8 ^d	419.9 ^d
Mean (PGPR)	46.8 ^b	52.7 ^a		167.1 ^b	216.1 ^a		432.0 ^b	517.1 ^a	
LSD ($p \leq 0.05$)	PGPR = 2.3; SME = 6.8; PGPR \times SME = NS			PGPR = 5.8; SME = 16.9; PGPR \times SME = 27.6			PGPR = 10.6; SME = 30.8; PGPR \times SME = 50.3		
	Amount of dry biomass per plant (g)								
0	0.5 ^g	2.3 ^{bcde}	1.4 ^e	1.9 ^e	1.4 ^f	1.7 ^e	2.9 ^{hi}	3.3 ^{gh}	3.1 ^e
15	2.1 ^{de}	2.3 ^{bcde}	2.2 ^{bc}	1.8 ^{ef}	1.7 ^{ef}	1.8 ^e	5.2 ^d	5.2 ^d	5.2 ^c
30	2.4 ^{bcd}	2.3 ^{bcde}	2.4 ^{bc}	2.6 ^{cd}	2.5 ^{cd}	2.6 ^c	5.2 ^d	4.4 ^{ef}	4.8 ^d
45	2.3 ^{cde}	2.6 ^b	2.4 ^b	2.3 ^{de}	2.6 ^{cd}	2.4 ^{cd}	5.2 ^d	5.0 ^{de}	5.1 ^{cd}
60	2.1 ^e	2.3 ^{bcde}	2.2 ^c	2.9 ^c	3.6 ^b	3.3 ^b	6.2 ^b	5.4 ^d	5.8 ^b
75	2.5 ^{bc}	3.6 ^a	3.1 ^a	3.7 ^b	5.5 ^a	4.6 ^a	6.0 ^{bc}	7.3 ^a	6.7 ^a
100	1.5 ^f	1.7 ^f	1.6 ^d	1.8 ^{ef}	2.5 ^{cd}	2.1 ^e	2.5 ⁱ	3.8 ^{fg}	3.2 ^e
Mean (PGPR)	1.9 ^b	2.5 ^a		2.4 ^b	2.8 ^a		4.7 ^b	4.9 ^a	
LSD ($p \leq 0.05$)	PGPR = 0.07; SME = 0.20; PGPR \times SME = 0.32			PGPR = 0.11; SME = 0.32; PGPR \times SME = 0.53			PGPR = 0.14; SME = 0.41; PGPR \times SME = 0.66		
	Chlorophyll content index (Spade value)								
0	9.0 ^{de}	11.5 ^{bc}	10.3 ^b	7.3 ^{bcde}	6.3 ^e	6.8 ^c	7.9 ^{cde}	6.1 ^f	7.0 ^e
15	11.6 ^{bc}	11.9 ^{bc}	11.7 ^a	7.0 ^{cde}	7.9 ^{bcd}	7.4 ^{bc}	8.0 ^{cd}	9.2 ^{bc}	8.6 ^{bc}
30	10.8 ^{cd}	13.3 ^{ab}	11.8 ^a	7.6 ^{bcde}	7.8 ^{bcde}	7.7 ^{bc}	7.0 ^{def}	8.3 ^{cd}	7.7 ^{cd}
45	9.2 ^{de}	11.5 ^{bc}	10.3 ^b	8.6 ^b	6.6 ^{de}	7.6 ^{bc}	8.9 ^{bc}	6.2 ^{ef}	7.5 ^d
60	7.2 ^{ef}	11.3 ^{bc}	9.3 ^b	7.5 ^{bcde}	8.2 ^{bc}	7.9 ^b	7.9 ^{cd}	10.0 ^b	9.0 ^b
75	10.4 ^{cd}	14.1 ^a	12.3 ^a	8.8 ^b	11.2 ^a	10.0 ^a	9.2 ^{bc}	12.6 ^a	10.9 ^a
100	5.9 ^f	9.8 ^{cd}	7.9 ^c	7.0 ^{cde}	8.3 ^{bc}	7.6 ^{bc}	5.8 ^{cde}	7.8 ^{cde}	6.8 ^d
Mean (PGPR)	9.1 ^b	11.9 ^a		7.48 ^b	8.0 ^a		7.8 ^b	8.6 ^a	
LSD ($p \leq 0.05$)	PGPR = 0.45; SME = 1.29; PGPR \times SME = 2.11			PGPR = 0.32; SME = 0.93; PGPR \times SME = 1.51			PGPR = 0.35; SME = 1.02; PGPR \times SME = 1.66		

Means of main effects and interactions sharing the same case letter for a parameter at each sampling date do not differ significantly at $p \leq 0.05$; SME = diluted sugar mill effluent; PGPR = plant growth promoting rhizobacteria; NS = non-significant.

Table 3. Influence of diluted sugar mill effluent and PGPR on number of grains per cob, 100-grain weight and grain yield of maize.

Treatments	Grains per Cob	100-Grain Weight (g)	Grain Yield (g/plant)
Plant growth promoting rhizobacteria			
PGPRs	270 ^a	26.1 ^a	53.0 ^a
No PGPRs	244 ^b	19.6 ^b	36.4 ^b
LSD ($p \leq 0.05$)	23	3.1	6.6
Sugar mill effluent			
0	199 ^d	18.1 ^c	36.0 ^d
15	231 ^c	22.8 ^b	41.3 ^b
30	248 ^b	23.1 ^b	43.7 ^b
45	258 ^b	23.3 ^a	45.4 ^b
60	308 ^a	24.9 ^a	51.4 ^a
75	333 ^a	25.5 ^a	53.1 ^a
100	222 ^c	22.1 ^b	41.9 ^b
LSD ($p \leq 0.05$)	20	1.20	5.0

Means of main effects and interactions sharing the same case letter for a parameter do not differ significantly at $p \leq 0.05$.

4. Discussion

This study indicated that the use of diluted sugar mill effluent in maize plants can improve the size of the leaf area per plant, the number of leaves per plant, the stem diameter, the plant height, the chlorophyll content index, and the amount of dry biomass per plant. Indeed, the sugar mill effluents consist of surplus quantities of macronutrients (magnesium, potassium, calcium, phosphorus, nitrogen) and micronutrients (zinc, iron, manganese) [3,8,17], which might have resulted in the improvement in growth of maize when used after dilution. The role of macronutrients and micronutrients present in sugar mill effluent in improving the growth of cereals including maize has been reported widely [3,17]. The improvement in the chlorophyll content in this study due to the application of diluted sugar mill effluent might be attributed to the presence of magnesium, manganese, and iron [18] within the sugar mill effluent which are key elements for the synthesis of chlorophyll in crop plants.

The higher concentrations of sugar mill effluent without dilution negatively impacted the growth of maize. This might be due to the presence of a surplus amount of heavy metals (cadmium and chromium) present in the effluent which inhibit seed germination and growth by interfering with metabolic activities [3,19,20], and altering the seed–water interaction which is necessary to trigger enzyme activity [21]. Various studies have reported that long-term application of sugar mill and other industry effluents may enhance the buildup of heavy metals in the soil to a toxic level [3,10,22], thus contaminating the soil and eventually, the growth of crop plants [3].

In this study, the use of PGPR was beneficial for improving the growth of maize irrigated with various concentrations of sugar mill effluent. This indicates that the use of PGPR coupled with the use of sugar mill effluent might be helpful for improving plant growth through conquering the heavy metal toxicity due to the sole use of sugar mill effluent [12,23]. PGPRs colonize around the roots of plants and prevent heavy metal toxicity through the production and secretion of various regulatory compounds (viz. siderophores, phytohormones and heavy metal binding proteins) [24]. PGPR also produce phytohormones (e.g., indole acetic acid) which improves plant growth and retards metal uptake in plants [25]. This study also indicated that the dilution of sugar mill effluents was most useful for growth promotion as indicated in earlier studies [3,26]. Some other studies have also reported that PGPRs are very useful for crop growth promotion in contaminated soils [27–29].

The highest grain yield was recorded when the PGPRs were applied alongside the application of 75% and 60% sugar mill effluents (Table 3). The high grain yield with these treatments was the outcome of better leaf emergence, growth and improved stay-green which ultimately enhanced the number of grains per cob and the 100-grain weight, thus resulting in higher grain yields [6]. Indeed, grain weight

is an important yield-contributing trait which is related to stay-green in cereals [30] under different abiotic stresses.

5. Conclusions

The use of SME at a concentration of 75% in combination with PGPRs was most effective for improving in the stay-green, leaf emergence, growth and productivity of maize under an arid climate.

Author Contributions: M.I. and T.A.Y. conceived and designed the experiments; M.S. performed the experiments; A.Sh. and A.Sa. analyzed the data; A.W. contributed reagents/materials/analysis tools; A.N. wrote the paper.

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Nomenclature

PGPR	plant growth promoting rhizobacteria
SME	sugar mill effluent
DAS	days after sowing
LSD	least significant difference
Kg	kilogram
g	gram
%	percentage

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