

ORIGINAL RESEARCH ARTICLE

Herbicides Roundup (Glyphosate) and Paraquat (Gramoxone) as chemical ripeners for sugarcane variety SP70-1143 (A case study at Debel Khozaie Agro-Industrial Co.)

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ABSTRACT

In the Khuzestan province of Iran, sugarcane harvesting is normally carried out between the early November and March periods, when rain and frost risks occur. This results in delayed harvesting and reduced profitability due to cane deterioration and a reduction in cane sucrose content. Management strategies such as the application of chemical ripeners were hypothesized to tackle such challenges by harvesting the crop earlier when the natural ripening condition was poor. Consequently, paraquat and roundup herbicides were applied as chemical ripeners to investigate the effect of their application time and rate on accelerating the ripening quality of the sugar cane variety SP70-1143 using a split-plot design. The treatments were control (unsprayed) [T0]; 1.0 Lha⁻¹ Paraquat (250 ppm) applied 8 weeks before harvesting (8WBH) [T1]; 0.8 Lha⁻¹ Roundup (200 ppm) applied 8WBH [T2]; 1.0 Lha⁻¹ Paraquat (250 ppm) applied 5 weeks before harvesting (5WBH) [T3], and 0.8 Lha⁻¹ Roundup (200 ppm) applied 5WBH [T4]. The results indicated that the glyphosate application treatments T2 and T4 induced a significant improvement in the sugarcane juice quality through an increase in Brix%, Pol%, %purity, and commercial cane sugar (CCS%) starting 1 week after their application. The results also confirmed the feasibility of an earlier harvesting operation on November 1st, one month before normal harvesting time in the study area, without significant loss of sucrose by application of 0.8 Lha⁻¹ Glyphosate (roundup), 8WBH.

Keywords: chemical ripener; paraquat herbicide; Roundup herbicide; sugarcane

1. Introduction

Sugarcane (*Saccharum* spp.) is harvested when its sucrose content is at its peak so that industrial profitability can be maximized with a concomitant reduction in the amount of cane required to produce each ton of sugar. In Khuzestan province, Iran, harvesting is carried out starting from early November to March, and harvesting starts by crushing variety CP57-1014 and continues with variety SP70-1143 starting from early December, during which rain and frost risks prevail. This results in delayed harvesting and low profitability due to cane deterioration and a decrease in cane sucrose content. Consequently, it becomes mandatory to search for earlier harvesting options for the problem. In this regard, Marin et al.^[1] reported the possibility of earlier harvesting management strategies to avoid rainy winter and frost risks, although earlier harvesting comes with a reduction in profitability due to the reduction in cane sucrose content from the prevailing non-optimal

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ripening condition during early November.

The ripening process in sugarcane (i.e., accumulation of sucrose in the internodes) is stimulated by several climatic factors, including high incident sunlight, low temperature, low soil nutrient content, and water stress^[2,3]. Since managing climatic conditions is not fully feasible in the Khuzestan production system, chemical ripeners were proposed to be used to induce ripening and enhance sucrose content in the early period of the harvest season. The application of these chemicals improves the quality of raw materials to be processed and improves the profitability of the sugarcane industry^[4]. Chemical ripeners have been tested in several countries since the 1920s^[5] and have received much attention since the 1970s^[6] when ethephon (Ethrel) and glyphosate-based compounds appeared^[4]. Today, a number of chemical ripeners, including Ethrel (ethephon), glyphosate (Roundup, etc.), Fusilade Super (Fluazifor-p-butyl), Gallant Super (Haloxypol), sulfometuron methyl (Curavial), Moddus (Trinexapac-ethyl), maleic hydrazide, paraquat, and imazapyr, have been used in the sugarcane industry^[2,4,6-8].

The reaction of sugarcane to various chemicals can be different under different climatic conditions^[9]. Chemical ripeners increase sucrose accumulation mainly by causing growth suppression and lowering the need for cane growth to sucrose sink. This, in turn, increases stalk sucrose content and juice purity^[7,10]. van Heerden^[11], reported a much more rapid suppression of stalk and leaf growth under chemical ripeners compared to the natural ripeners mentioned above. The same results were reported by other studies in Louisiana and Brazil^[12,13]. The ripening responses to chemical ripeners have been reported to be variable and inconsistent in many studies^[4,14,15].

A number of factors have been identified to affect the ripening process, including application rate, time of application, type of ripener, ripener combinations, crop maturity, and treatment to harvest interval^[5-7,16,17]. It has been shown that the use of glyphosate with different formulations has promoted the sucrose content of cane stalks and sugar production compared to controls^[18,19]. However, in some studies, the application of glyphosate resulted in a negative effect when applied at a higher rate, including side shoots on a stem, which leads to lower cane profitability^[20]. However, there are also other studies that have proven to have no detrimental effects from the application of glyphosate at sub-lethal doses on sugarcane yield and quality^[21].

The strong influences of dosage of application, climatic conditions, the time of its application before harvesting, and the cane variety on the effectiveness of ripeners highlight the need for further studies^[4]. The aim of this study was to evaluate the effect of two herbicides as chemical ripeners, including Roundup (a glyphosate compound) and Paraquat (gramoxone), on sugarcane sucrose content and other juice quality factors of variety SP70-1143. The study selected these two chemicals with a premise to explore if and how a systemic herbicide (Roundup) compared to a contact herbicide (Paraquat) could be used to enhance the sucrose content as well as the profitability of early-harvested sugarcane under the climatic conditions of Khuzestan province, Iran. The other part of the study was to evaluate the time of application of ripeners (5 and/or 8 weeks) before harvesting. It was hypothesized that a systemic herbicide (Roundup), which kills the plant gradually, may act differently as compared to the contact herbicide (Paraquat), which kills the plant soon after its application.

2. Materials and method

2.1. Study site and trial design

The study was performed in applied research center of Debel Khozaie Agro-Industrial sugarcane company, Khuzestan province, Iran (31°8' N, 48°39' E). The mean annual precipitation and temperature (1970–2010) of the area were 177 mm and 24 °C, respectively. Mean of min and max temperature in the early harvesting season (i.e., November) were 14.7 and 27.1, respectively (**Table 1**). The soil was clay loam which

is classified as Calciorid according to the U.S. Soil Taxonomy System^[22]. At 0 to 25 cm depth, the soil contains 35% clay (<2 μm), 30% silt (2–20 μm), 34% sand (20–2000 μm), and 0.85% organic matter.

Table 1. Minimum, maximum and mean temperature ($^{\circ}\text{C}$) in studied area during study period (Autumn 2013).

Sampling date	Temperature $^{\circ}\text{C}$		
	Min	Max	Mean
2013/09/25	17.6	37.0	27.3
2013/10/04	20.0	40.6	30.3
2013/10/11	10.8	30.0	20.4
2013/10/18	16.2	37.0	26.6
2013/11/01	18.6	29.5	24.1
2013/11/18	16.0	23.2	19.6
Mean 2013/09/23–2013/10/22	15.2	35.4	25.4
Mean 2013/10/23–2013/11/22	14.7	27.1	20.9

In this study a split-plot experiment^[23] with 3 replications was employed. Each plot consisted of 44 cane rows, each 30 m long and spaced 1.83 m apart ($\approx 2415 \text{ m}^2$). Chemical ripeners [including Roundup (glyphosate) and Paraquat (gramoxone) herbicides] were assigned to the main plot and times of applications [including 8 weeks before harvest (8WBH \approx 16 Sep.) and 5 weeks before harvest (5WBH \approx 5 Oct.)] were assigned to the subplots. The experiment consisted of five treatments viz., T_0 , control treatment (unsprayed); T_1 , 1.0 L ha^{-1} Paraquat (250 ppm) applied 8WBH; T_2 , 0.8 L ha^{-1} Roundup (200 ppm) applied 8WBH; T_3 , 1.0 L ha^{-1} Paraquat (250 ppm) applied 5WBH, and T_4 , 0.8 L ha^{-1} Roundup (200 ppm) applied 5WBH. The application rates and spray-to-harvest intervals were based on climatic conditions and previous studies^[7,10,19]. The ripener treatments were applied from the ground using a turbine sprayer on 16 September and 5 October 2013 (**Figure 1**). Two selected fields of first ratoon crop of variety SP70-1143 were used for the study. The variety SP70-1143 is an early maturing variety has been selected so as to explore the feasibility of allocating this variety for early season harvesting schedule.



Figure 1. The turbine sprayer used for the application of chemical ripeners.

2.2. Weather conditions

Table 1 provides information about min, max and mean monthly temperature in the experimental site during the study period. During the study period (in autumn), the mean temperature ranged in between 20.9 and 25.4 $^{\circ}\text{C}$.

2.3. Crop management

The trial was planted and grown according to the industrial recommendations, with irrigation and fertilization practices for commercial sugarcane production. Irrigation was applied using furrow system up to 4 weeks before harvesting. Urea (350 kg ha^{-1}) was applied in all plots in three splits (2013/05/26 = 100 kg

ha⁻¹, 2013/06/19 = 150 kg ha⁻¹ and 2013/07/03 = 100 kg ha⁻¹). Harvesting of the first ratoon crop of sugarcane variety SP70-1143 started on 1 December 2013.

2.4. Sampling and measurements

From each plot, a sample consisting of 20-stalks was drawn randomly at seven (for 8WBH) and six (for 5WBH) different time intervals from 8 weeks before harvest (8WBH) and 5 weeks before harvest (5WBH) treatments, respectively. Sampling started after the application of ripeners to determine Brix% and Pol% in cane juice extracted using a 2-roller small mill. The sampling dates were: 2013/09/25, 2013/10/04, 2013/10/11, 2013/10/18, 2013/11/01, 2013/11/18, and 2013/12/11. Brix% is the same as degrees (°) brix (in g solute per 100 g solution) is a measure of dissolved solids in sugar juice using a refractometer (Index Instrument GPR 11-37). Pol% (in g solute per 100 g solution) is the apparent sucrose content measured by the optical rotation of polarized light, passing through a sugar solution using a polarimeter (Optical activity, using the dry lead acetate method with undiluted solutions). Two other quality factors including apparent purity (%PTY) and refined sugar (%RS) were calculated from these two measurements as described by Chen and Chou^[24] as:

$$Purity(\%PTY) = (pol \div brix) \times 100 \quad (1)$$

where, sugarcane purity is the percentage of sucrose in total solids in the juice. A higher purity is a result of a higher sucrose content in the total solids present in juice.

To calculate the refined sugar (%RS), a quality ratio factor was first derived by dividing the purity factor (from a standard table) by pol^[24]. Quality ratio is defined as the amount of yellow sugar produced from the amount of fresh cane processed in a sugar factory. Refined sugar was then estimated by multiplying the value of the quality ratio by a constant factor (0.83). This was based on previous experiences and studies, where only 83 percent of yellow sugar could be converted to white (refined) sugar.

2.5. Statistics

All statistical analyses were performed using IBM SPSS Statistics version 17.0. Means were calculated for each plot and used in the calculation of mean and standard error. The averages were also used as input in an ANOVA model to test for treatment effects. The chemical ripener treatment was considered as a main effect, with time of application treatment as a split-plot effect. Duncan's Test was used to calculate the estimated means, standard errors, and difference between means for the treatment effects.

3. Results

3.1. Brix (%)

Brix (total solids), showed a gradual increase from the time of treatment application (**Figure 2**). Although the increasing effect of T2 (200 ppm roundup treatment, 8WBH) was obvious in the early stages of the study when compared with the other treatments, there was no difference between the control (T0) and T2 in the last two sampling times viz., 11/01 and 11/18 (**Figure 2**). The other roundup treatment (T4), however, showed the same effect as T2 and T0 at the last sampling time. Paraquat treatments (T1 and T3) at both times of application (TOA) caused the least degrees of brix% compared to the control and roundup treatments in all sampling times (**Figure 2**). The interaction effect of times of application (TOA) and ripener treatments on brix% was shown to be significantly different ($p < 0.05$) indicating that at different TOA, sugarcane responded to ripeners differently. As shown in **Figure 2**, roundup treatment (T2) applied 8 weeks before harvest (8WBH) induced the highest brix degree as compared to the control and ripener treatments applied 5WBH.

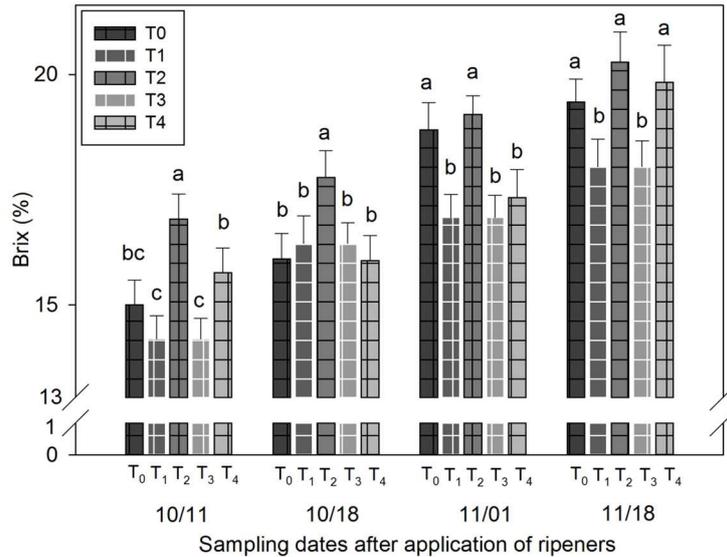


Figure 2. Comparison of the effects of two treatments of Roundup and Paraquat 8 weeks before harvest on Brix (%). T0, control treatment (unsprayed); T1, 1.0 L ha⁻¹ Paraquat (250 ppm) applied 8WBH; T2, 0.8 L ha⁻¹ Roundup (200 ppm) applied 8WBH; T3, 1.0 Lha⁻¹ Paraquat (250 ppm) applied 5WBH, and T4, 0.8 Lha⁻¹ Roundup (200 ppm) applied 5WBH.

3.2. Pol (%)

Ripener treatments and times of their application (TOA), significantly affected pol (p values were <0.01 and <0.05 , respectively). There were also significant ripeners by TOA interaction for pol, indicating that sugarcane responded differently to ripener treatments at different TOA. **Figure 1** shows that a significantly higher pol was found under the application of 200 ppm of roundup 8WBH treatment (T2) in all sampling dates. Lower pol% in T3 (250 ppm paraquat applied 5WBH) until one week before harvesting (sampling date: 11/18) compared to other treatments was a surprise in that it was significantly lower than the rest treatments (**Figure 1**). At this date, both roundup (glyphosate) treatments (T2 and T4) resulted in the highest pol as compared to other treatments. At this sampling date, however, paraquat treatments (T1 and T3), did not affect pol differently when compared with the control treatment (T0) (**Figure 3**). **Figure 3** indicates that the largest pol% was attained under roundup treatment 8WBH (T2) as compared to treatments applied 5WBH and paraquat treatment applied 8WBH.

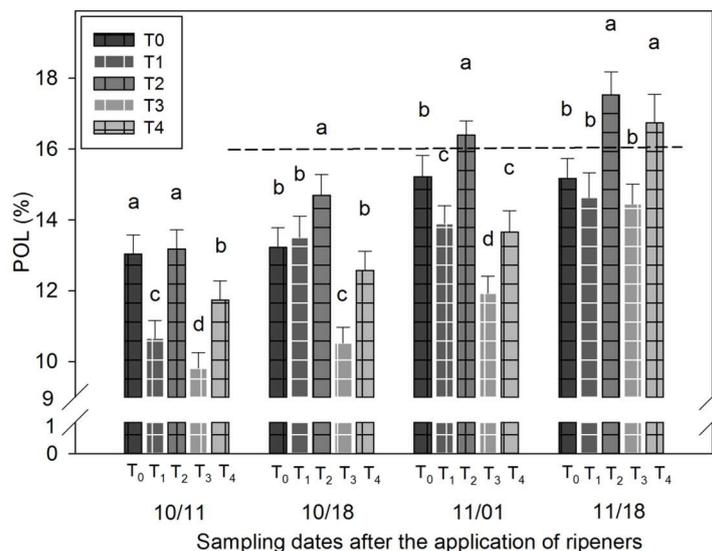


Figure 3. Comparison of the effects of two treatments of Roundup and Paraquat 8 weeks before harvest on Pol percentage. Dashed line presents the least proper limit for POL%^[25,26]. T0, control treatment (unsprayed); T1, 1.0 L ha⁻¹ Paraquat (250 ppm) applied

8WBH; T₂, 0.8 L ha⁻¹ Roundup (200 ppm) applied 8WBH; T₃, 1.0 L ha⁻¹ Paraquat (250 ppm) applied 5WBH, and T₄, 0.8 L ha⁻¹ Roundup (200 ppm) applied 5WBH.

3.3. Purity (%)

The percentage of sucrose content in total solids of the juice (purity) in general showed a similar result as pol. Purity % was affected by ripeners and times of their application (TOA) ($p < 0.01$). In general, the application of 200 ppm roundup ripener applied 8WBH (T₂) in all the sampling dates outperformed all the remaining treatments (Figure 4). However, paraquat ripener applied 8WBH (T₁) didn't show a clear trend (Figure 4). While it was similar to T₂ during the second sampling date (10/18 = three weeks after their application), but fell below control and roundup treatments at the last sampling date—the week before harvesting- (Figure 4). A significant interaction was observed between the type of ripener used and the timing of application (TOA), significantly impacting purity levels ($p < 0.01$). This finding emphasizes that the effect of ripeners on purity varies depending on when they are applied. Specifically, the treatment T₂, applied 8 weeks before harvest (8WBH), was found to notably improve purity percentages. This outcome contrasts with the application of another treatment, T₄, which was applied 5 weeks before harvest (5WBH), and other treatments, as illustrated in Figure 4. This highlights the critical importance of choosing the right time for ripener application to enhance crop purity.

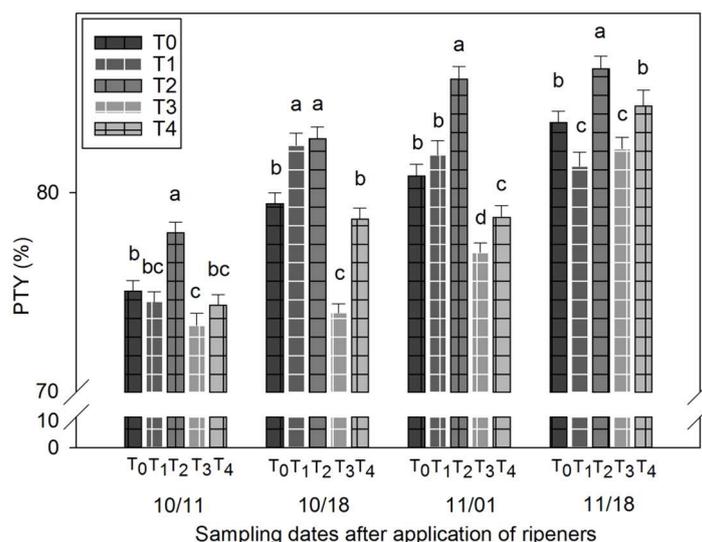


Figure 4. Comparison of the effects of two treatments of Roundup and Paraquat 8 weeks before harvest on PTY percentage. T₀, control treatment (unsprayed); T₀, control treatment (unsprayed); T₁, 1.0 L ha⁻¹ Paraquat (250 ppm) applied 8WBH; T₂, 0.8 L ha⁻¹ Roundup (200 ppm) applied 8WBH; T₃, 1.0 L ha⁻¹ Paraquat (250 ppm) applied 5WBH, and T₄, 0.8 L ha⁻¹ Roundup (200 ppm) applied 5WBH.

3.4. RS (%)

Estimated refined sugar content showed a different result as compared to previous juice quality parameters (Figure 5). Although, roundup treatments appeared to be the best ripening agent compared to paraquat, T₄ (roundup treatment applied 5WBH) boosted RS at the last sampling date (the week before harvesting) compared with all other treatments (Figure 5). In case of RS%, again the interaction effects between TOA and ripener types were significant ($p < 0.01$), indicating that at different TOA, RS was affected by the type of ripeners dissimilarly (Figure 5). T₄ applied 5WBH enhanced RS compared with other ripener treatments and the control.

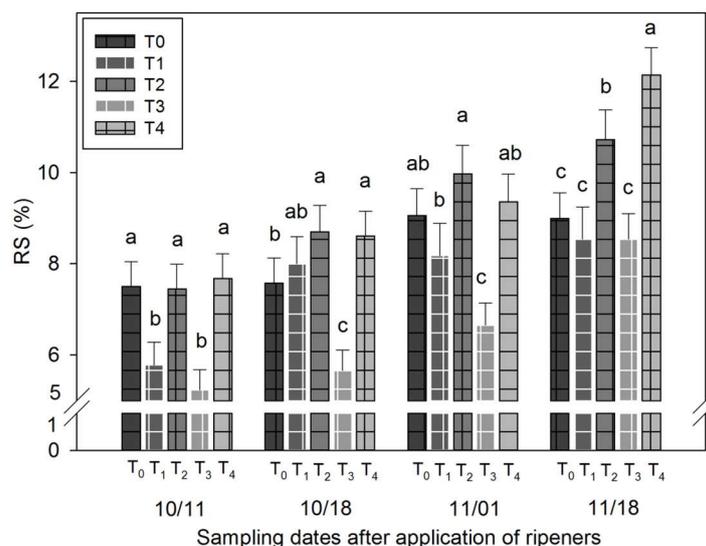


Figure 5. Comparison of the effects of two treatments of Roundup and Paraquat 8 weeks before harvest on RS percentage. T0, control treatment (unsprayed); T0, control treatment (unsprayed); T1, 1.0 L ha⁻¹ Paraquat (250 ppm) applied 8WBH; T2, 0.8 L ha⁻¹ Roundup (200 ppm) applied 8WBH; T3, 1.0 Lha⁻¹ Paraquat (250 ppm) applied 5WBH, and T4, 0.8 Lha⁻¹ Roundup (200 ppm) applied 5WBH.

4. Discussion

The results showed that there was a significant raise in the sugarcane juice quality through an increase in Brix%, Pol% and %purity from 1 week after the glyphosate (roundup) application treatments (T2 and T4, especially T2) as chemical ripener (**Figures 2–4**). Our findings are corroborated by several studies indicating that the application of chemical ripeners enhances sucrose accumulation within the sugarcane. This enhancement is primarily attributed to the ripeners’ ability to inhibit the plant’s growth, thus reducing the demand for resources between growth processes and sucrose storage, or the ‘sink’. Consequently, this redirection of metabolic priorities results in an increase in both stalk sucrose concentration and the purity of the extracted juice. This mechanism of action has been supported by the research conducted by Viator and Viator^[10,19] which collectively reinforce the effectiveness of chemical ripeners in promoting sucrose accumulation by regulating the growth dynamics of sugarcane. The results also agree with Karmollachab et al.^[14] who reported a pronounced effect of glyphosate on Brix and Pol%, 30–40 days after the application of treatments on sugarcane variety CP57-614.

The maturation stage of sugarcane can be identified by using the Pol%. According to Fernandes^[25] and Vasconcelos^[26], Pol% values below 16% and above 18% are characteristic of an immature and over matured sugarcane, respectively. Therefore, despite the possible analytical error, harvesting operation can be started as soon as Pol% attains 16%. Accordingly, considering other quality characteristics such as Brix% and %Purity (**Figures 2–4**), the results suggest the harvesting operation to be carried out on 1 November, i.e., one month before normal harvesting time in the study area. Using a simple factor calculated from previously measured Pol% and Brix% data, assisted to depict the economic benefits of ripeners application. Commercial cane sugar (%), (CCS %), estimates recoverable sucrose from harvestable cane at the sugar mill and can be calculated from the Pol% and Brix% in juice, and cane fiber content, using the CCS formula detailed below^[27,28].

$$\begin{aligned}
 CCS = & \left((39 \times B \times (95 - C)) \div (99.82 + (0.415 \times A)) \right) - (0.5 \times (A \\
 & + \left((0.00137 - (0.00003 \times A)) \times T^2 \right) + \left((0.00172 \times (A - 0.0044)) \times T \right) \quad (2) \\
 & - (0.0224 \times A) - 0.46) \times (97 - C) + 200 \div 100
 \end{aligned}$$

where A = Brix; B = Pol; C = Fiber content, assumed to be 13%; T = temperature, is set at 20 °C.

However, to make it less complicated, the CCS formula can be simplified as described by BSES^[29].

$$CCS = \text{pol in cane} - (\text{impurities in cane} \div 2) \quad (3)$$

where impurity in the cane is calculated as: “Brix in cane – Pol in cane”. Impurities can be dirt, ash, and inverted sugars, which affect crystallization and therefore sucrose recovery^[27]. CCS which is the basis of payment in the sugar industry of some countries, is not a direct measure of sucrose content but is appropriate for estimating recoverable sucrose from fresh cane transported to the sugar mill^[28]. **Table 2** shows the effects of treatments on CCS%. Compared to other treatments, T2 enhanced CCS rapidly from 18 October. On 1 November, the CCS% of canes under T2 treatment reached 15.0 compared to 13.4 for T0 (control treatment). As the average yield of the first ratoon crops of sugarcane variety SP70-1143 in 2013 was 67.8 tons ha⁻¹ (personal communication), recoverable sucrose content of T2 and T0 would be 10.17 and 9.09 tons ha⁻¹, respectively. This indicates a significant increase ($\approx 12\%$) in recoverable sugar as a result of roundup (glyphosate) application 8 weeks before the normal harvesting time. This corresponds to 2448 tons of extra recoverable sugar, if all first ratoon fields receive glyphosate ripener (T2), 8 weeks before harvesting time (the whole fresh cane harvested in first ratoons of sugarcane variety SP70-1143 in 2013 was 20400 tons) (personal communication). Rostron^[6] also found a beneficial effect of 1.0 ton per hectare extra estimated recoverable sugar (ers) and also reported enhanced pol and Brix% values under POLADO (glyphosate) as compared to the control treatment, though, his formula for estimating recoverable sugar was different (ers = sucrose – 0.485 non-sucrose – 0.056 fiber). CCS results once again confirmed the feasibility of an earlier harvesting operation on Nov. 1st, one month before normal harvesting time in the study area, without significant loss of sucrose.

Table 2. CCS% in different treatments of the study calculated from Equation (3).

Sampling time	T0	T1	T2	T3	T4
11 Oct.	12.1a	8.9b	11.3a	7.6c	9.8b
18 Oct.	11.8b	12.1b	13.2a	7.6c	10.9b
1 Nov.	13.4b	12.4c	15.0a	9.4d	11.8c
18 Nov.	13.1b	12.9b	16.2a	12.7b	15.2a

The effect of T4 (another glyphosate treatment) applied 5 weeks before normal harvesting time, compared to T0, showed significantly lower CCS% on 1 November, but significantly higher CCS%, on 18 November (**Table 2**). This indicates an 18-day time-lapse compared to T2, implying the need for an earlier application of glyphosate ripener to get the expected benefits of an earlier harvesting time.

The application of Paraquat ripener did not indicate any positive effect in either 5 or 8 weeks before harvesting times (**Figures 2–4** and **Table 2**). Although both Paraquat ripeners did not show an enhancing effect on cane quality compared to other treatments, however, their application 8 weeks before harvesting time indicated a better performance compared to 5 weeks before harvesting. This in turn offered the feasibility of an earlier application of ripener, i.e., 8 weeks rather than 5 weeks before normal harvesting time (**Figures 2–4** and **Table 2**).

This study also confirmed previous results that indicated the efficacy of Glyphosate as a sugarcane ripener^[30,31] applied glyphosate at 210 g a.i./ha and obtained 10%–28% increase in theoretical recoverable sugar compared with the non-treated. Kouamé et al.^[30] reported that Glyphosate ripener increased sucrose content in sugarcane stalks especially in the 5th internode, sevenfold when compared with the control plot as early as 5 days after their application.

Furthermore, the study results also coincided with an approximate 2 months (8 weeks) time lapse required for a successful ripening after the application of ripeners as highlighted in previous studies^[31,32]. Averaged

across all treatments for the first ratoon, glyphosate herbicide increased CCS% by 12% as compared to the control. Furthermore, it appears that the physiological state of the cane during the application, significantly impacts the ripening activity^[30]. Consequently, the specific timing of ripener application within the growth season plays a crucial role in determining the efficacy of the ripener, as highlighted by Alexander^[33].

5. Conclusions

- The application of 0.8 L ha⁻¹ (200 ppm) glyphosate (roundup), eight weeks before harvesting induced a significant rise in the sugarcane juice quality through an increase in Brix%, pol%, %purity, and commercial cane sugar (CCS%).
- The calculations indicated a significant increase ($\approx 12\%$) in recoverable sugar percent in the first ratoons of sugarcane variety SP70-1143 as a result of roundup (glyphosate) application eight weeks before normal harvesting time.
- The interaction effect of times of application and ripener treatments on sugarcane juice quality factors (Brix%, pol%, and purity%), was shown to be significantly different, indicating that at different times of application, sugarcane responded to ripeners differently. Therefore, the roundup treatment, applied eight weeks before harvest, induced the largest Brix degree, pol%, and purity% compared with control and ripener treatments applied five weeks before harvesting.
- The results also confirmed the feasibility of an earlier harvesting operation on 1 November (one month before normal harvesting time) in the study area without significant loss of sucrose after application of glyphosate (roundup), eight weeks before harvesting.

Author contributions

Conceptualization, FY and MAK; methodology, FY; software, LA and MAK; validation, FY, MMM and LA; formal analysis, FY, MAK and MMM; investigation, FY and MAK; resources, FY and MMM; data curation, LA and MAK; writing—original draft preparation, LA and FY; writing—review and editing, FY and LA; visualization, MAK; supervision, FY; project administration, FY; funding acquisition, MMM. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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