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Impact of chemical treatments on *Leuconostoc* bacteria from harvested stored cane/stale cane

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ABSTRACT

Post-harvest sucrose losses are always a critical problem for sugar industries. A predominant factor which is causing these post-harvest losses that affects sugar recovery is the bacterium *Leuconostoc* spp. This study aims to check the efficacy of certain chemical treatments in reducing the proliferation of this bacterium. Our study based on a *Leuconostoc*-specific media revealed that application of 0.5 % aqueous solution of benzalkonium chloride and sodium metasilicate (BKC + SMS), formaldehyde, glutaraldehyde, sodium chloride and pine oil showed significant reduction in zone of proliferation. Considering formaldehyde and glutaraldehyde as control, the most effective treatments were chemical formulations of benzalkonium chloride along with sodium metasilicate, pine oil and sodium chloride in checking the proliferation of this bacterium. The application of these treatments has an immense potential in the sugar industry for reducing post-harvest sugar losses.

1. Introduction

Post-harvest sucrose deterioration in sugarcane is the most crucial problem of Indian sugar industries. It causes heavy loss in economy of sugar mills. Crushing of stale canes in sugar mills causes loss in sugar recovery by 12–50% [1]. As per a study, a heavy loss of Rs. 1600 crores has to be faced by sugar mills due to supply of sucrose deteriorated canes in mills [2]. Various causes are responsible for post-harvest sugarcane deterioration out of which storage conditions and time lag between harvesting and crushing (staling) is mainly accountable for microbiological sucrose losses [3]. Bio-deterioration due to microbial invasion and proliferation in harvested canes leads to loss of 62 % [1]. As per general practice, fresh harvested canes are allowed to left in open fields in piles or in transport vehicles for a long duration of time which paves way for ample invasion, growth and proliferation of micro-organisms that causes loss in weight and sucrose content [3,4]. Such a condition also occurs when canes are stored in cane centers or in mill yards [5]. This leads to deterioration in juice quality, thereby lowering recovery [6].

Of all the micro-organisms invading in harvested sugarcane stalk, the most crucial and devastating one is *Leuconostoc* bacterium belonging to lactic acid bacteria group [7,3]. *Leuconostoc* is a soil borne bacterium and expresses freely on sugarcane tissue, syrups of lower brix and cane juice [6]. These invade sugarcane through cut ends or through cracks and enter into the juice rich region, where it gets favorable conditions for its proliferation [8]. This bacterium consumes sucrose as their energy source and converts sucrose into various other compounds like organic acids, reducing sugars, ethanol and polymers with long and complex chains [9,10]. Mishandling of canes during mechanical harvesting, burning, chopping into billets aggravates the inactivation of the phenol oxidase enzymes present on cane stalks which acts as a protective or anti-bacterial layer [11]. In harvested/stale sugarcane stalks, most of the times, a slimy layer is seen which is formed due to presence of this microbe [12]. Dror et al. [13] had showed that *Leuconostoc mesenteroides* bacteria spoil food commodities by secreting slimy fluid. Under favorable condition, this bacterium undergoes multiplication to form nodular colonies. Harvested canes have been reported to be more infected with this bacterium [6,14]. Studies have indicated that this bacterium and dextran formation is more in stale canes than in freshly harvested canes [4,15]. This is so as dextrasucrase enzyme is secreted by *Leuconostoc mesenteroides/dextranicum* and is responsible for production of dextran [16,17]. It is this enzyme that catalyses glycosyl residues to transfer to polymer of dextran [18]. Difference in formation of dextran in stale and freshly harvested canes is due to the time lag between harvesting and crushing. Moreover, in case

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when abiotic stress conditions occur (particularly under water logging and drought), production of dextran enhanced which further accentuate the losses [9,10]. Association of bacterial count with dextran production and sugar recovery is well known [6]. A large number of problem arises such as elongated sugar crystals, increase in viscosity of juice, blockage of mill filters (due to dextran and other insoluble solids), *etc*., and in sugar mills during cane processing as this bacterium gets washed off into the cane juice where it causes conversion of sucrose (by process of hydrolysis) into polysaccharide termed dextran [19]. This results in low sugar recovery [20]. Another factor which contributes in proliferation of this bacterium is the change in acidity of sugarcane due to increase in time duration from cutting to crushing of cane. High sugar content (about 15 %) and initial pH ranging between 5.0–5.5 of cane juice make a perfectly congenial environment for this bacterium to occur [21]. The pH range for its growth lies between 2.0–7.5 [22] and can even grow at a very low pH [23]. This change in pH also affects the quality of harvested cane leading to change in sucrose level [4,15].

Although a number of measures have been taken to prevent these sucrose losses by microbes but not much success has been achieved in completely eradicating it. Thus, the present study aims to know the anti-inhibitory effect of various chemicals on *Leuconostoc* spp., whose application on sugarcane could help in minimizing the post-harvest sucrose losses in sugarcane. In addition to this a parallel study was also conducted, to know the change in various juice quality parameters (Commercial cane sugars %, pH, titrable acidity index, reducing sugars, total soluble solids, dextran, acid invertases) after 10 days of harvest.

2. Materials and methods

2.1. Site of experiment

Research farm of ICAR-Indian Institute of Sugarcane Research, Lucknow (26°56' N; 80°52'E; 111 m amsl).

2.2. Sample preparation

Harvested sugarcane of variety, CoLk 94184, was kept in open fields for 2–3 days in the month of March (average temperature ranges between 31–33 °C) when there is very likelihood of invasion of the bacterium that causes post-harvest sucrose losses. The juice was extracted from it by use of hand crusher. The juice was collected in clean sterile beakers.

Parallel to this, four piles of harvested canes were made in which one pile was left untreated (Control) while the three piles were sprayed with three respective treatments, *viz*., salt water (NaCl), benzalkonium chloride and sodium metasilicate (BKC + SMS), pine oil with help of sprayer. The spray of each treatment was done on to the pile of harvested whole stalks of canes including the cut ends and growth cracks which is considered to be the site of microbial invasion. All the piles of harvested canes were left in field conditions in month of March. The canes were crushed with roller crushers and juice was extracted from each pile control at 0, 2, 4, 6 and 10 days after harvest, for the analysis of juice quality. Clean sterile beakers were used for collecting the juice of each pile. The concentration of each treatment was 0.5% while in terms of pile, each treatment was of 1 L/pile.

Considering the possibility of toxicity of any chemical on humans, minimum concentration of benzalkonium chloride, sodium metasilicate and pine oil are used in our study so that no toxic effect on the end product, *viz*., sugar formed while higher effect on *Leuconostoc* bacteria could be seen. Besides, the chemicals used are known to be removed/ washed during upstream processing stages such as clarification and evaporation at high temperatures during milling which will further not interrupt in the sugar quality and maintain its non-toxicity.

2.3. Bacterial growth media

For bacterial growth, *Leuconostoc* spp. specific media was prepared using (tryptone (10 g), yeast extract (5 g), sucrose (100 g), agar (20 g) for 1000 mL of media [12]). The media was sterilized at 121 °C for 15 min and prior to use this media, 0.005 % of sodium azide was added. The juice (100 μL) was poured on to the sterile petri dish and was spread with the help of spreader. The plate was incubated at 30 °C for 24–48 h for bacterial growth.

2.4. Identification of Leuconostoc bacteria

Morphological, biochemical and cultural characteristics were studied for identification of *Leuconostoc* spp. bacteria. Morphological characteristics were identified by gram staining as per the standard protocol and motility test by SIMs media (sulphide indole motility media). Biochemical characterization was performed by carbohydrate fermentation test and catalase test (as per Bergeys manual) [24]. Growth at different pH was also evaluated. Cultural characteristics were studied by viewing the isolated colony of this bacterium under high magnifying glass (10X).

2.5. Antimicrobial activity assay

After identification of *Leuconostoc* spp. bacteria, the agar diffusion method was used for analysis of its inhibitory effect on various treatments that might help in minimizing the post-harvest sucrose losses. The identified bacterial broth (200 μL) was spread onto the growth-specific media with help of spreader and was left for 2–3 min. The spreading was done properly by rotating the plate each time so as to ensure that bacteria were distributed evenly onto the media plate. After 2–3 min, disc (size - 5 mm) dipped in treatments were carefully kept onto the plate and was incubated at 30 °C for 24–48 h. The discs were kept equidistant from each other. After incubation, antibacterial activity was expressed in terms of zone of inhibition (diameter in mm). Efficacy of chemicals on *Leuconostoc* spp. was checked by combination of benzalkonium chloride and sodium metasilicate (BKC + SMS), pine oil and sodium chloride (commonly known as salt), considering formaldehyde and glutaraldehyde as chemical controls.

2.6. Evaluation of juice quality parameters

The juice extracted from separate treatments was used for evaluation of sucrose % in juice, Commercial Cane Sugars % (CCS), purity coefficient, reducing sugars, pH, titrable acidity index, °Brix, dextran and soluble acid invertase activity (E.C. 3.2.1.26) for checking the post-harvest sucrose losses by this bacterium. pH was evaluated using pH meter (Systronics). Titrable acidity index (TAI) was determined by NaOH phenolphthalein method. Reducing sugars were evaluated by Nelson Somoygi method [25]. The dextran was evaluated by Haze method [26] and soluble acid invertase activity by Rosario and Santisoparsi method [27]. Proteins were estimated by Lowry method [28]. Sucrose (%) in juice was estimated by polarimetry (HORIBA polarimeter). CCS % was calculated by the formula CCS% = 1.022*S - 0.292*B (S - sucrose and B - °Brix) [29]. Purity coefficient was also estimated as per Solomon et al. [30]. The cane weight was also measured before crushing of canes of different treatments.

2.7. Statistical analysis

Analysis of variance (ANNOVA) was performed using statistical software, CropStats 7.2 [31], where the experiment was conducted in randomized block design with three replications. Furthermore, linear regression was also applied in the experimental results.

3. Results and discussion

3.1. Identification of Leuconostoc bacteria

After 24–48 h of incubation of extracted juice on specific *Leuconostoc* spp. media, the colonies obtained were mucous rich having characteristics of convex with flat edges, smooth, shiny and semi-transparent (Fig. 1). The isolated colonies were tested for identification and were revealed to be gram positive and non motile. Preliminary identification test revealed the bacteria to be catalase negative. For further identification, this bacterium were grown at different pH (4.8 and 6.8) which showed that at pH 6.8, the growth of bacteria occurred while at pH 4.8, it was not. However, when 3% sodium chloride or 6.5% sodium chloride were used at both the pH, the growth of bacteria was not favorable. For confirmatory identification, the carbohydrate fermentation profile revealed that bacterium obtained from incubation of 24–48 h utilizes glucose, mellibiose, maltose, sucrose, fructose, dextrose, lactose, trehalose as carbon sources and evolved oxygen. However, they did not utilize starch, salicin, D-ribose, raffinose and aesculin. All these characteristics established the identity of *Leuconostoc* spp. in sugarcane juice (as per Bergeys manual) [24].

Fig. 1. Colonies of *Leuconostoc* bacteria: Mucous colonies of *Leuconostoc* sp. on sucrose containing medium with 0.005 % sodium azide.

3.2. Antimicrobial activity

Application of aqueous solutions at different concentrations of chemical treatments which were tested showed anti-*Leuconostoc* bacterium susceptibility (Fig. 2a). 0.5 % of aqueous solutions of various chemicals showed variable results on *Leuconostoc* spp. that are summarized in the Fig. 2b. None of the chemicals tested against formaldehyde (zone of inhibition of 20 mm) showed superior results, however, combination of formulation of BKC + SMS along with sodium chloride showed zone of inhibition of 17 mm and 16 mm, respectively, close to formaldehyde. Though pine oil also showed zone of inhibition (of 10 mm) against this bacterium but it is not as effective as the other two chemicals applied (Fig. 2). However, it could also be used as an alternative to minimize the post-harvest sucrose losses. Numerous chemicals efficacy have been tried on to harvested sugarcanes to minimize the sucrose deterioration occurring with the increasing time interval after harvest, however, many were turned down on commercial viability basis [32]. One such is formaldehyde. Use of bactericide like formaldehyde is useful against cane deterioration [33]. It interacts with protein present in cell membrane of the bacteria that caused protein denaturation leading to disruption of cell [34]. However, the use of formaldehyde is considered to be a carcinogen for humans that causes naso-pharyngeal cancer and leukemia [35] and so it is banned in number of countries like U.S., Canada and European Union [36,37].

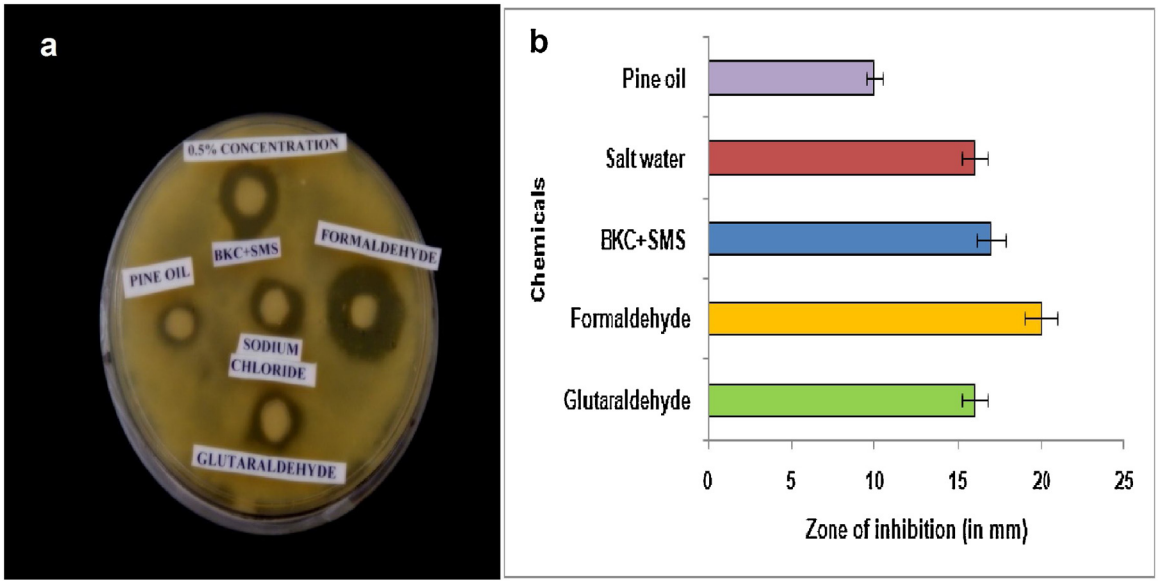

Fig. 2. Effect of various chemical treatments (0.5 % concentration) on *Leuconostoc* sp. a. Different chemicals showing zone of inhibition on petri plate against *Leuconostoc* colonies b. Measurement of zone of inhibition (in mm) of different chemicals.

Comparing the zone of inhibition of the tested chemicals with glutaraldehyde (another chemical as Control), it was revealed that BKC + SMS showed strong 17 mm zone of inhibition relatively higher than Control (zone of inhibition of 16 mm) while aqueous solution of sodium chloride showed same zone of inhibition of 16 mm against *Leuconostoc* spp., however, the application of pine oil on this bacterium showed 10 mm zone of inhibition lesser than the glutaraldehyde. Due to application of glutaraldehyde and benzalkonium chloride on to harvested canes, reduction in *Leuconostoc* load by 26.08 % was evident [38]. Its mode of action is similar to formaldehyde [34]. However, it is strong irritant and causes harm to various parts of human like skin, eyes and respiratory tract, *etc*. [39] which is the reason behind of not using as a biocide over harvested canes. In regards to benzalkonium chloride, Sodium metasilicate and pine oil, a large number of studies on post-harvest losses on fruits have used it as management measure. It has been reported that sodium metasilicate as post-harvest dips has been used to lessen the losses occurring in avocado fruit [40]. Similarly, it was also applied on peach fruit to manage brown rot occurring after harvest considering its non toxic nature [41]. Likewise, formulations using Benzalkonium chloride such as dodecyl sulphate with BKC have also been used due to its disinfectant property [6,38].

Considering results against controls, BKC + SMS formulation and sodium chloride was found to be as effective as formaldehyde and glutaraldehyde. Similar results were also observed by this chemical formulation in another study on freshly harvested canes under normal conditions [30] and under drought conditions [16] due to their synergistic effect of anti-bacterial and anti-inversion property. This might be the reason behind the strong zone of inhibition in our study against this bacterium. Besides, BKC + SMS formulation, sodium chloride was another chemical that showed higher efficacy against both controls in our study. Sodium chloride has been used as preservative in number of food products as it inhibits and kills food borne pathogens like bacteria by withdrawing water from bacterial cell and causes it to dehydrate and die [42]. Sodium chloride also reduces microbial growth as it causes hindrance with cellular enzymes and even compels cells to expel out sodium ions thereby resulting in lessening in microbial growth [43]. Furthermore, microbial cells on application of salts suffer with osmotic shock which in turn looses water from cells resulting in cell death and in this way also minimizes growth of microbes in stored food [44]. In addition to BKC + SMS and sodium chloride, pine oil (obtained from *Pinus sylvesteris*) also revealed to control this bacterium to moderate level as per results revealed in our study. Its phenolic disinfectant, mild antiseptic and anti-bacterial property is also known [45]. Reduction in growth of *Leuconostoc* due to use of this oil in our study may also be attributed to reduction in mannitol production [46] as *Leuconostoc* produces mannitol as its bio-degradable product [47–49].

Thus, this study showed that formulation of benzalkonium chloride and sodium metasilicate is as effective as quaternary ammonium chlorides compounds, *viz*., formaldehyde and glutaraldehyde, followed by sodium chloride and pine oil in terms of controlling agent of *Leuconostoc* bacterium.

3.3. Juice quality parameters favouring Leuconostoc *growth and proliferation*

3.3.1. pH

Leuconostoc is an acidiophillic bacteria and it prefers an initial medium pH of 6.5 for its growth [49]. It can grow at temperature ranging between 10°-30 °C and between 2–7.5 pH range [22,50] but they are sensitive towards extreme high and low temperature [50]. It has been reported that when the internal pH of this bacterium reaches 5.4–5.7 [51], the growth of this bacterium is stopped. High sugar content (about 15 %) and initial pH ranging between 5.0–5.5 makes cane juice a perfect environment for this bacterium to occur [21]. It is well known that cane juice is rich in sugar content (basically sucrose) and has a pH ranging between 5.2–6.8. The juice pH decreases with the delay in crushing making an environment rich condition for *Leuconostoc* to invade and proliferate [9,10]. Other quality parameters are correlated with increase in acidic nature of cane juice and so we evaluated various quality aspects related to it to check the effect of *Leuconostoc* on post-harvest sugarcane deterioration.

Change in pH levels with the delay in crushing/milling has been illustrated in Fig. 3. At initial level, decline in cane juice pH was initiated after two days of staling in treated and control canes after which variation in pH decline pattern was clearly evident in control and treated canes. In control canes, a steep decrease in juice pH was seen after 4 days of staling which later on becomes a gradual decrease up to 10 days of staling. In BKC + SMS treated canes, there was a gradual decrease in pH up to 4 days of staling after which there was steep decrease in pH up to 6 days of staling with a steady decrease up to 10 days of staling. The difference in pH in control and BKC + SMS treated canes varies from 2 days of staling. In salt water treated canes, there was a gradual drop in pH from 0 day to 10 days after harvest. In pine oil treated canes, there was a gradual decrease in pH up to 4 days of staling after which a steady decrease in pH was observed for up to 10 days of staling. The difference between pine oil treated cane and control varies after 2 days of staling. This implies that percentage change in decrease in pH was highest in control canes (8.32 %) followed by salt water treated canes (7.92 %), pine oil treated canes (6.93 %) and BKC + SMS treated canes (6.34 %). This showed that as time increases after harvest, pH of the juice starts decreasing, thus making juice more acidic in nature which facilitates the growth of *Leuconostoc* bacterium. This has been stated by other studies before [4,6]. The pH values of all cane juice samples that were subjected to all the treatments were plotted against time (Fig. 3a). The slope of the regression line was used as a measure of the rate of decline in pH in cane juice samples subjected to different treatments. Juice from control canes showed the highest rate of decline in pH where slope of regression line (m) was -0.075. This was followed by juice obtained from salt water treated sugarcane and pine oil treated canes having slope of regression line (m) as −0.081 and −0.065, respectively, while BKC + SMS treated canes had rate of decline in pH (m= −0.075).

3.3.2. Titrable acidity index (TAI)

There was increasing trend in titrable acidity index after 10 days of harvest in all harvested canes though increasing pattern of TAI differs from control to treated canes. In control canes, zig-zag pattern of increase in TAI was observed. A gradual increase in TAI was seen up to 4 days of staling after which a steep rise was seen up to 6 days of harvest followed by gradual smooth rise in TAI up to 10 days of harvest in control canes. The path of rise in TAI was much higher than the treated canes. In BKC + SMS treated canes, there was a gradual steady increase in TAI was observed for 10 days after harvest. The difference in increase path of TAI in BKC + SMS treated canes and control canes varied after 2 days of harvest. In salt water treated canes, there was a marginal difference in rise of TAI as compared to BKC + SMS treated canes up to 6 days of harvesting but thereafter this rise was much higher than BKC + SMS treated ones. The pine oil treated canes had higher rise in TAI than both the treated canes but the rise was much lower in comparison to control canes. This indicated that change in percentage increase was highest in control canes (58.70 %), followed by salt water treated canes (34.32 %), pine oil treated canes (34.25 %) and BKC + SMS treated canes (17.52 %), revealing BKC + SMS formulation to be superior of all treatments, in respect to TAI. The TAI values of all cane juice samples that were subjected to all the treatments were

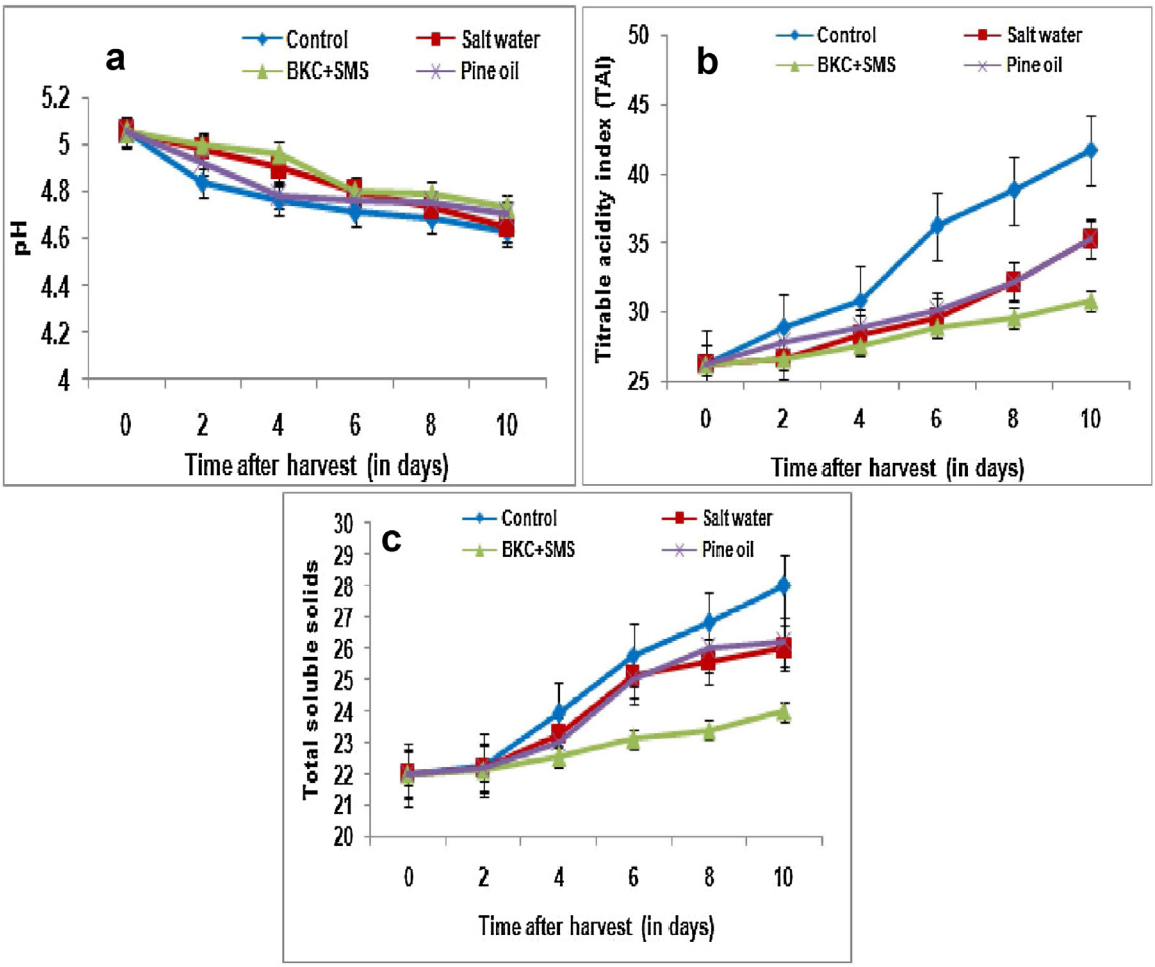

Fig. 3. Stale juice quality parameters favouring *Leuconostoc* growth at 0, 2, 4, 6 and 10 days after harvest in Salt water, BKC + SMS, Pine oil treated canes against untreated canes (Control). A. pH b. Titrable acidity index (TAI) c. Total soluble solids (Brix).

plotted against time (Fig. 3b). Juice from control sugarcane showed the highest rate of incline in TAI (m= +3.201). This was followed by juice from salt water treated canes (m= +1.701), pine oil treated canes (m= +1.701) and BKC + SMS treated canes (m= +0.954). This indicated that juice from BKC + SMS treated cane represented the lowest rate of increase of titrable acidity with time.

It has been reported that during storage or delay in transportation of canes, juice becomes more acidic in nature by reduction in pH value [1]. This combination of high acidity and low pH levels may be attributed to production of lactic and acetic acid [52] that could be correlated with *Leuconostoc* bacterium growth and proliferation. This has been justified in our *in vitro* and field experiment results of various treatments.

3.3.3. Total soluble solids (Brix)

With the increase in cane staling increase in Brix was revealed after 10 days of harvest. There was no variation in increase in brix in control as well as treated canes up to 2 days of harvest. The pattern of increase in brix varies thereafter. A gradual increase was seen in BKC + SMS treated canes in brix while a steepy increase in brix was seen in control canes. In pine oil and salt water treated canes, zig-zag pattern of incarese in brix was seen with marginal difference. On an overall, after 10 days of harvest, highest percentage change in increase in Brix was observed in control canes (27.27 %) followed by salt water treated canes (18.18 %), pine oil treated canes (19.09 %) and BKC + SMS treated canes (9.09 %). This implies that total soluble solids were least increased in BKC + SMS treated canes after 10 days of harvest. Furthermore, the Brix values of all cane juice samples that were subjected to various treatments were plotted against time (Fig. 3c). Juice from control canes showed the highest rate of incline in total soluble solids (Brix) (m= +1.299) followed by juice from pine oil treated canes (m= +0.984), salt water treated canes (m= +0.915) and BKC + SMS treated canes (m= +0.411). This implies that juice from BKC + SMS treated canes showed the least rate of incline in total soluble solids (Brix). Total soluble solids describes the amount of sugars and non-sugars. Rise in brix in stale canes is attributed to formation of high reducing sugars and dextran. This has been supported by results of other studies [53,54]. Similar results has also been achieved in our study.

3.4. Change in quality parameters affecting sugar recovery at mills due to Leuconostoc

3.4.1. Dextran evaluation

Our study showed that although the treated canes have less dextran value in compared to control canes left open in the fields for 2–3 days yet there is difference in dextran value of different treatments. Although all the treatments started with a similar path of increase (but a completely different path as that of control) from 0 day of harvest till 2 days of staling after which all of them showed different patterns of increase. This revealed that on an overall, after 10 days of harvest, highest increase in dextran was observed in control canes (29.38 folds) followed by salt water treated canes

(28.13 folds), pine oil treated canes (26.0 folds) and BKC + SMS treated canes (25.0 folds). In respect to control canes, change in folds were least in BKC + SMS treated canes (0.85 folds) followed by pine oil treated canes (0.88 folds) and salt water treated canes (0.96 folds). This implies that in BKC + SMS treated canes, the amount of dextran increased were least after 10 days of harvest. The dextran values of all cane juice samples that were subjected to all the treatments were plotted against time (Fig. 4a). Juice from control canes showed the highest rate of incline in dextran (m= +134.80) followed by juice from salt water treated sugarcane (m= +123.6), pine oil treated canes (m= +127.5) and BKC + SMS treated canes (m= +103.6). Juice from BKC + SMS treated cane represented the lowest rate of increase of dextran with time.

Our results showed similarity with the earlier studies results which had revealed that with the increase in time after cane harvest, proliferation of bacteria increases. It was reported that during cane processing, action of microbes is usually related with sucrose deterioration and dextran formation (solely due to the presence of *Leuconostoc* bacteria as this bacterium secretes enzyme dextransucrase which inverts sucrose into dextran) [55]. Production of dextran in cane juice causes many troubles during processing of sugar [56] such as increase in viscosity, crystal shape alteration, *etc.* [3]. It has been found out that a loss of 0.0025 pounds of raw sugar takes place from one ppm unit of dextran [57]. Correlation of increase in viscosity of juice and molasses as well as the sugar crystal elongation with increase in dextran value of juice has been observed [58,59]. This might have been seen as a result of microbial contamination especially L. *mesenteroides* that might have entered into the cane either in fields or in sugar mills. Many studies have showed that the best way to overcome the problems caused by this polysaccharide is by the use of dextranase that helps to break down the dextran molecule into smaller ones [60–63]. In many of the early cane varieties high dextran value has been observed after 48 h of harvest without any treatment [54]. A study illustrated that dextran content was reduced to 125 % when use of pine oil over harvested cane in variety CoSe 92,423 was performed [46]. Furthermore, our results of trial of anti-*Leuconostoc* sensitivity may also be associated with dextran values. Our *in vitro* study showed that least zone of inhibition against *Leuconostoc* in juice of BKC + SMS, pine oil and salt water treated canes. There was even less dextran formation in the same treatments in our field study which may be correlated to imply that less growth of *Leuconostoc* and so less dextran production.

3.4.2. Invertase activity

In harvested canes, presence of acid and neutral invertases has been reported in north Indian states where cane deterioration is uncontrollable [64]. Acid invertase activity in harvested canes is a quality decreasing indicator [30]. Increase in acid invertase activity as well as endogenous and exogenous microbial activity with increase in delay in crushing has been evident in number of studies for sucrose losses occurring in harvested canes [65,30]. Our study showed that increase in invertase activity in control and treated canes did not vary up to 2 days of harvest while thereafter variation begins. The control canes showed highest increase (5.57 folds) in invertase activity followed by salt water (4.82 folds) and pine oil treated canes (4.24 folds). In continuation to this, BKC + SMS treated canes showed least increase in acid invertase activity (3.97

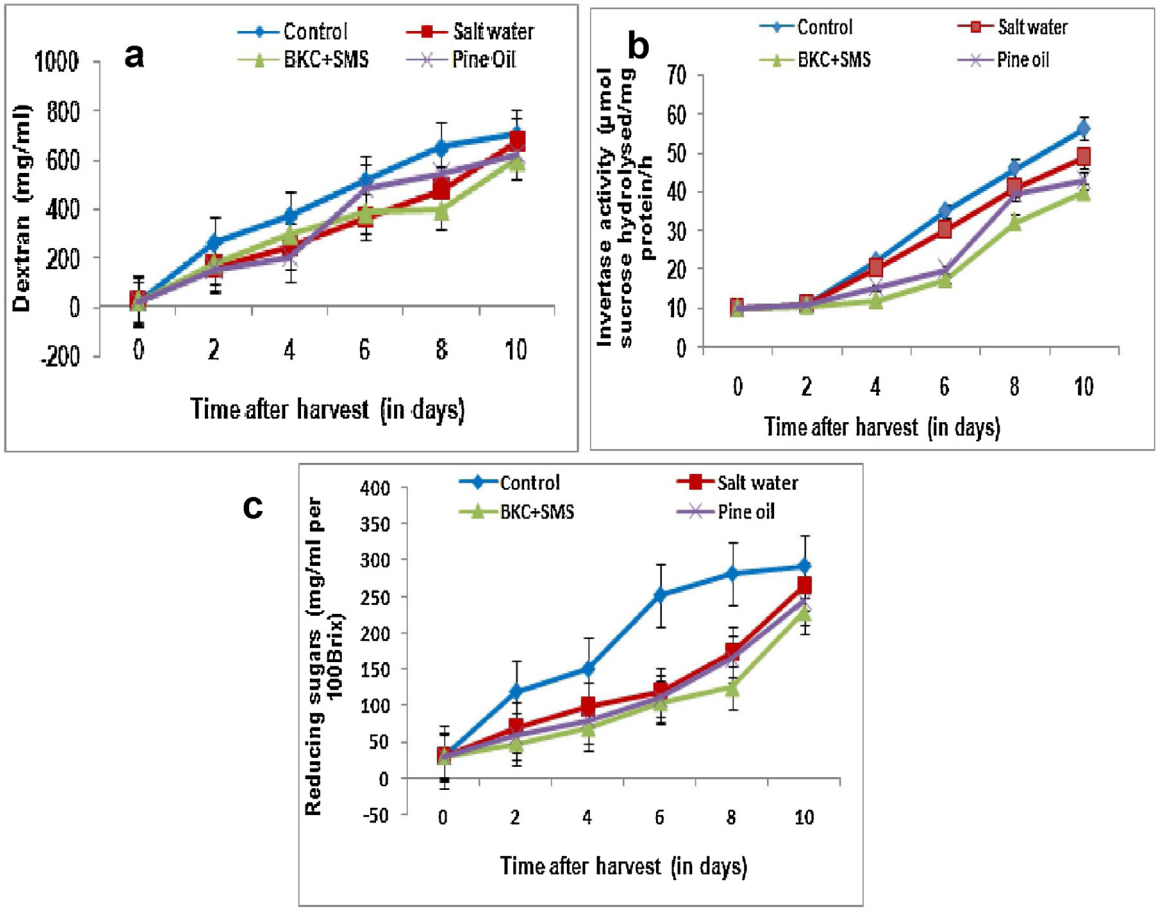

Fig. 4. Changes in juice quality parameters due to *Leuconostoc* growth at 0, 2, 4, 6 and 10 days after harvest in Salt water, BKC + SMS, Pine oil treated canes against untreated canes (Control). a. Dextran b. Inveratse activity c. Reducing sugars.

folds) after 10 days of harvest. Furthermore, considering the change in folds in respect to control canes, increase in invertase activity in salt water treated canes was 0.87 folds while in pine oil and BKC + SMS treated canes were 0.76 and 0.71 folds, respectively. The invertase values of all cane juice samples that were subjected to all the treatments were plotted against time (Fig. 4b). Juice from control canes showed the highest rate of incline in invertase activity (m= +9.97) followed by salt water treated canes (m= +8.35), pine oil treated canes (m= +7.26) and BKC + SMS treated canes (m= +6.30). Results suggested that juice from BKC + SMS treated canes represented the lowest rate of increase of invertase activity with time. It has been noticed that soon after harvest of cane endogenous invertases gets activated due to several reasons, one of them is the growth of *Leuconostoc* in harvested canes [6] as it has the capability of inversing sucrose into fructose and glucose. Exo-invertase activity of the bacterium as well as host invertase activity both plays a role in loss of recoverable sugars in harvested canes [32].

3.4.3. Reducing sugars

It is one of the important factors for determining the post-harvest cane deterioration as it may provide information of estimation of the sugars produced as well as quality parameters in its manufacturing process [66]. In our study, there was an increase in reducing sugars in canes after 10 days of harvest. This is attributed to loss in sucrose content due to delay in crushing as stale canes causes inversion of sucrose into glucose and fructose. Many studies had reported rise in reducing sugars with increasing time interval after harvest [15,16]. The variation in increasing pattern of reducing sugars with the increase in time started from 2 days of staling. The control canes showed a complete different path of increase in reducing sugars with a rise of 87.5 % after 10 days of harvest. The pattern of increase in reducing sugars was similar in all treatments. Salt water treated canes had 80.73 % rise in reducing sugars while in pine oil and BKC + SMS treated canes, rise was 71.48 % and 66.39 % after 10 days of harvest. This implies that reducing sugars was least increased in BKC + SMS treated canes after 10 days of harvest. A gradual increase was seen in BKC + SMS treated canes up to 8 days of staling followed by a steeply increase up to 10 days of harvest indicating that up to 8 days of staling rise in reducing sugars is limitedly increased. In pine oil treated canes, a uniform rise in reducing sugars is seen up to 10 days of staling. A marginal difference in increase in reducing sugars was seen in pine oil and BKC + SMS treated canes up to 6 days of staling. In salt water treated canes, gradual rise in reducing sugars was seen up to 6 days of staling after which a rapid increase in reducing sugars was observed up to 10 days of staling. The reducing sugars values of all cane juice samples (including the treated canes) were plotted against time (Fig. 4c). Juice from control canes showed the highest rate of increase in reducing sugars (m= +54.37) followed by salt water treated canes (m= +43.09), pine oil treated canes (m= +40.61) and BKC + SMS treated canes (m= +36.02). This indicated that juice from BKC + SMS treated cane represented the lowest rate of increase in reducing sugars with time.

3.5. Juice quality parameters affecting economy of sugar mills

3.5.1. Sucrose (%) in juice

The main component of sugarcane is sucrose which is mainly responsible for sugar production [16]. This content has been reported to deteriorate with increase in time duration from harvesting to crushing in several studies [67,30]. Sucrose decline may be up to 2.0 units within 72 h of staling which is dependent on genotypes and season of harvest [2]. Similar results had also been revealed in our study where sucrose (%) in juice started declining the most in control canes (1.17 units) followed by salt water treated canes (0.48 units) after 10 days of harvest. Pine oil treated canes and BKC + SMS treated canes also had decrease in sucrose content after 10 days of harvest but variation in decrease between them is marginal (0.24 units and 0.16 units, respectively). This revealed that the canes treated with BKC + SMS had lowest decrease in sucrose (%) in juice after 10 days of harvest. The values of sucrose (%) in juice in all the samples that were subjected to all the treatments were plotted against time (Fig. 5a). Juice from control canes (m= −0.220) showed the highest decline in sucrose (%) in juice followed by salt water treated canes (m= −0.088), pine oil treated canes (m= −0.043) and BKC + SMS treated canes (m= −0.035). This implies that juice from BKC + SMS treated canes represented the lowest rate of decrease in sucrose (%) in juice with time.

3.5.2. Cane weight

There was a decrease in cane weight after 10 days of harvest. There was a sharp decline in cane weight in control canes after 10 days of harvest while in the treated canes there was a gradual decrease in cane weight over the time. All the treatments applied on to harvested canes showed better results in comparison to control canes from the time of harvest till 10 days after harvest. The difference of treatments on cane weight begins after 2 days of harvest in control and treated canes. Furthermore, all the treated canes showed similar path of decline in cane weight while BKC + SMS treated canes showed a marginal difference in decreasing pattern after 6 days of harvest. The loss in cane weight in control canes were highest with 1.45 Kg after 10 days of staling followed by salt water (0.46 Kg) and pine oil (0.40 Kg) treated canes. The BKC + SMS treated canes showed least decrease in cane weight after 10 days of harvest (0.35 Kg). The cane weight values of all cane samples (including the values of treated canes) were plotted against time (Fig. 5b). Control canes showed highest rate of decline in cane weight (m= −0.251) followed by salt water treated canes (m= −0.094), pine oil treated canes (m= −0.082) and BKC + SMS treated canes (m= −0.064). This indicated that loss in cane weight was least in canes with BKC + SMS treatments. This means that storing of harvested canes with use of BKC + SMS followed by pine oil and salt water will help in reducing the cane weight loss as storage condition is directly correlated to loss in moisture content of canes. Cane weight is an important aspect in India as well as some Asian countries as cane growers are paid on the basis of the cane weight. An increase in time lag between cutting and milling could cause huge economical losses to the cane growers [68]. Several studies have reported that harvested cane starts losing its moisture with the increase in time. This loss in moisture rate depends on the various factors like climatic conditions, cane variety as well as storage method, *etc*. [32].

3.5.3. Commercial cane sugars % (CCS)

Decrease in commercial cane sugars % was observed in all canes after 10 days of harvest. A rapid decrease in CCS% is seen in control canes after 10 days of harvest while in treatments, gradual increase was seen in BKC + SMS followed by zig–zag pattern of decrease in pine oil and salt water treated canes after 10 days of harvest. Higher reduction was seen in control canes with the increase in time after harvest in CCS% by 2.95 units followed by salt water treated canes (1.66 units) and pine oil treated canes (1.47 units). BKC + SMS treated canes had least reduction in CCS% (0.75 units) after 10 days of harvest. The CCS% values of all cane juice samples that were subjected to all the treatments were plotted against time (Fig. 5c). Juice extracted from control cane showed the highest rate of decline in CCS% (m= −0.604) followed by salt water treated sugarcane (m= −0.750), pine oil treated canes (m= −0.332) and BKC + SMS treated canes (m= −0.516). This implies that juice extracted from BKC + SMS treated canes represented the lowest

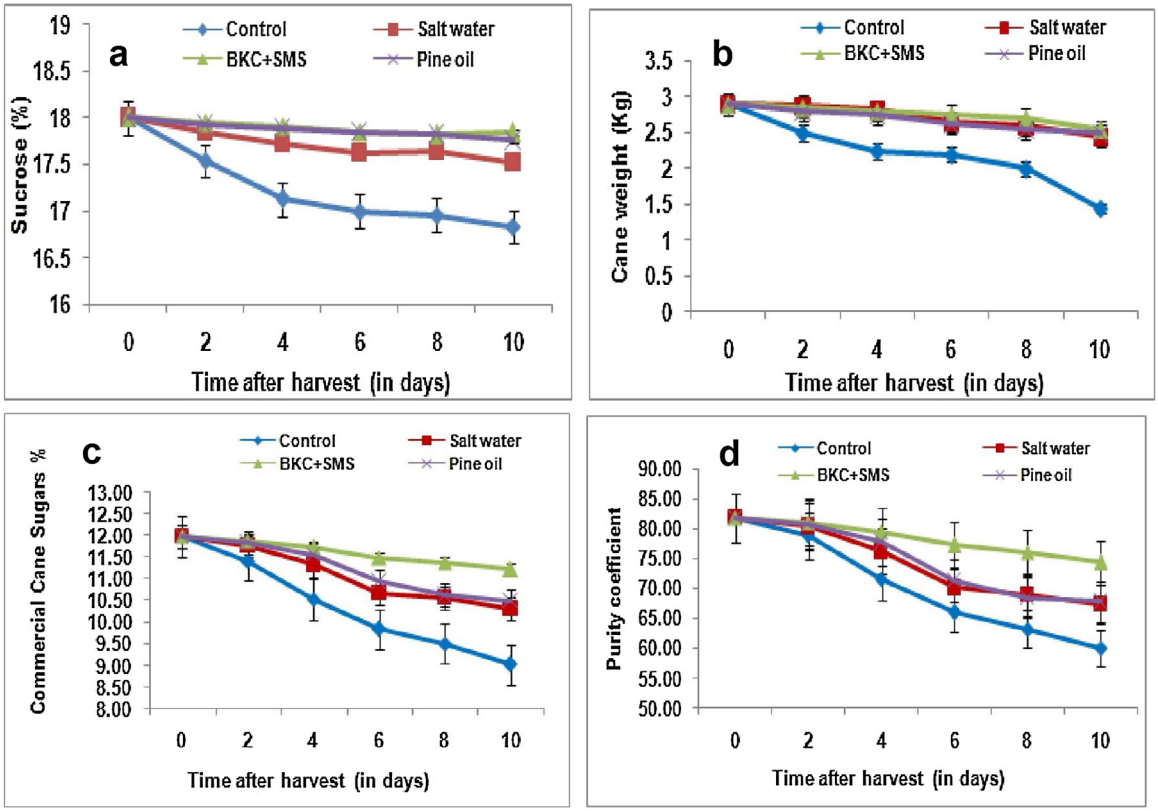

Fig. 5. Changes in juice quality parameters affecting economy of sugar mills due to *Leuconostoc* sp. at 0, 2, 4, 6 and 10 days after harvest in Salt water, BKC + SMS, Pine oil treated canes against untreated canes (Control). a. Sucrose b. Cane weight c. Commercial cane sugars (CCS) d. Purity Coefficient.

rate of decrease of CCS% with time. The interrelationship between sucrose content and CCS% on basis of fresh cane weight has been studied [69] and effective results of pine oil over harvested sugarcane have also been reported [46].

3.5.4. Purity coefficient

Purity coeffcient started declining the most in control canes from the very beginning but the canes that were subjected to treatments showed reduced loss in purity coefficient after 10 days of harvest. In control canes there was a loss of 21.71 units followed by loss in purity coeffcient in salt water treated canes of 14.43 units, pine oil treated canes of 14.03 units and BKC + SMS treated canes of 7.48 units. This indicated that BKC + SMS treated canes stands first in reduced decrease in purity coefficient after 10 days of harvest. The purity coefficient values of all cane juice samples (including the treatments) were plotted against time (Fig. 5d). Juice from control canes showed the highest rate of decline in purity coefficient (m= −4.593) followed by salt water treated canes (m= −3.216), pine oil treated canes (m= −3.326) and BKC + SMS treated canes (m= −1.559). Juice from BKC + SMS treated cane represented the lowest rate of decrease of purity coefficient with time. Similar to our results, studies have shown that as the time period increases after harvest the quality of cane starts deteriorating and so does the purity coeffcient [1,9,10,4,2].

Hence, this showed that as compared to control and other treatments BKC + SMS treated cane showed the best results in minimising the post-harvest sucrose losses. The juice obtained from BKC + SMS treated canes was less acidic with the increase in time after harvest along with relatively lesser amount of reducing sugars and dextran. Also soluble acid invertase activity was reduced with the increase in time after harvest. This might be correlated to the reduction in sucrose (%) in juice, CCS% and purity coefficient with the increase in time after harvest in such canes. Besides, loss in cane weight with the increase in time after harvest was also reduced in BKC + SMS treated canes as compared to other treatments and control. This proved that BKC + SMS was relatively more effective against minimizing the post-harvest sucrose losses as it has a dual effect, *viz.*, inhibition of *Leuconostoc sp.* and controller of sucrose inversion process. However, salt water treatment and pine oil treatment also did not lack behind in

Table 1
Mean performance of post-harvest deterioration parameters.

Treatments	pH	TAI	Brix (%)	Dextran (mg/mL)	Invertase	RS (mg/mL per 100Brix)	Sucrose (%)	CW (Kg)	CCS (%)	Purity (%)
Salt Water	4.85	29.69	24.03	326.50	26.91	125.99	17.70	2.71	11.08	74.14
BKC + SMS	4.89	28.15	22.86	312.50	20.52	100.88	17.89	2.76	11.61	78.35
Pine oil	4.83	30.08	24.06	340.33	23.14	114.55	17.87	2.68	11.24	74.68
Control	4.78	33.76	24.79	420.67	30.17	187.69	17.24	2.21	10.38	70.26
CD at 5 per cent	0.07	1.20	0.79	37.05	2.36	17.39	0.50	0.17	0.42	1.99
CV (%)	2.04	5.92	4.96	15.82	14.03	19.64	4.17	9.94	5.68	4.00
SE (±)	0.02	0.42	0.28	13.05	0.83	6.12	0.17	0.06	0.14	0.70

TAI: Titrable acidity index; RS: Reducing sugars; CW: Cane weight & CCS: Commercial cane sugar.

Table 2
Analysis of variance for different parameters, durations and treatments after harvest.

Source of Variations	Mean Sum of Square (MSE)									
	pH	TAI	Brix (%)	Dextran (mg/mL)	Invertase	RS (mg/mL per 100Brix)	Sucrose (%)	CW (Kg)	CCS (%)	Purity (%)
E	0.23**	157.97**	35.48**	634415.7**	2827.16**	81210.64**	0.45	0.65**	5.67**	429.84**
T x E	0.04**	0.07	1.89	508.85	0.72	85.15	0.42	0.05	0.17	6.01
T	0.09**	101.67**	11.47**	42274.33**	322.53**	26456.08**	1.66*	1.15**	1.97**	197.36**

*E: Time interval; T x E: Interaction & T: Treatments (Control, Salt Water, BKC + SMS, Pine oil).

minimizing the post-harvest sucrose losses of sugarcane. But intake of food products where salt act as a preservative is now being cause a threat for human health [42].

3.6. Statistical analyses

Experimental mean of parameters indicated that treatments have significant difference at certain time interval for all the characters under study (Table 1). ANOVA showed that effect of cane deterioration with increasing time for all the parameters studied were significant except in case of sucrose while, the treatments were found significant in all the quality parameters. Furthermore, interactions (Time interval x Treatment) have no significant difference for all the characters except pH of juice (Table 2).

4. Conclusions

An imperative problem of sugar industries and farmers is the sucrose losses occurring after canes are harvested. In general, these losses are initiated once the cane is cut. Illogical delays in crushing of harvested canes either due to delay in transportation or due to delay in loading of canes in trucks, carts, *etc.*, act as fire to fuel for these losses. This favors the microbial invasion in harvested canes through cut or damaged ends which causes heavy loss in sugar recovery.

Our study concluded that growth of *Leuconostoc* sp. (responsible for low sugar recovery) could be controlled by the use of certain chemicals and eco-friendly compounds which might help in enhancing the recovery of sugars to some extent. BKC + SMS, pine oil and salt water treated canes exhibited a better juice quality profiles as compared to juice from normal untreated canes. Of all the treated canes, juice obtained from BKC + SMS had lowest rate of inclination in dextran and acid invertase activity along with high sucrose (%) in juice, commercial cane sugars (%), purity coefficient along with relatively lesser losses in cane weight. In addition to, juice obtained from canes treated with BKC + SMS had lowest rate of decline in pH and had highest rate of inclination in titrable acidity index and reducing sugars. Use of these treatments over canes would not only helps in controlling this bacterium but even able to control the dextran as well as rate of soluble acid invertase activity to some extent that rapidly increases after harvest. This would lead to relatively lesser loss in sugar recovery on an overall basis, thus, helping in minimization of post-harvest sucrose losses.

Author statement

This is original work and has not been communicated or published to any other journal.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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