



## Can we not mitigate climate change using seaweed based biostimulant: A case study with sugarcane cultivation in India



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### ABSTRACT

Strategies for sustainably increasing sugarcane productivity without any negative implications to the environment are challenging. In the present investigation, field trials were conducted to demonstrate the potential of an agro-technique involving foliar applications of *Kappaphycus alvarezii* seaweed based biostimulant in combination with recommended rate of synthetic fertilizers (RRF) for sustainably enhancing sugarcane production and mitigating environmental impacts. *Kappaphycus* seaweed extract (KSWE) applied at 5% concentration enhanced cane productivity by 12.5 and 8%, respectively, in plant and ratoon crops. Interestingly, the treatment involving 6.25% KSWE +50% RRF showed yield parity ( $p < 0.05$ ) with control (water+100% RRF) in ratoon while there was 7.9% reduction over control in plant crop with a concomitant savings of 50% RRF. These results revealed that KSWE application in addition to recommended rate of fertilizer application, can reduce gap between potential and real yield which otherwise requires application of incremental inputs in the form of synthetic fertilizers to obtain similar yields. The findings confirmed our hypothesis that the use of KSWE not only results in hypothetical savings in the incremental application of synthetic fertilizers but also can be used for achieving target yields sustainably. The sugar yield too was enhanced thus increasing the returns on investment. The technique is practically feasibility and scalability. The potential of the KSWE in lowering GHGs is manifested by the way of saving at least 260 kg CO<sub>2</sub> equivalents (Mg cane production)<sup>-1</sup> ha<sup>-1</sup> when applied at 5% concentration. This would translate in to savings of ca. 9.3 million Mg of CO<sub>2</sub> equivalents if one assumes employing KSWE for at least 10% of the total cane production in India for the year 2015–16. Therefore, the present study advocates a paradigm shift in policy to encourage use of biostimulants in the context of mitigating adverse effects of global climate change and expecting better returns from sugarcane cultivation.

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**Abbreviations:** mm, millimeter; L, liter; h, hour; y, year; ha, hectare; 000 ha<sup>-1</sup>, thousands per hectare; cm, centimeter; g, grams; nos, number; m<sup>2</sup>, square meter; m<sup>3</sup>, cubic meter; Mg, Mega gram; 1,4- DB Eq, 1,4-Dichlorobenzene equivalents; CFC, Chlorofluorocarbon; NMVOC, non methane volatile organic compounds; m<sup>2</sup>a, square meter times year; PM, particulate matter; N, nitrogen; SWE, seaweed extract.

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### 1. Introduction

Sugarcane is a crop that has high biomass productivity and demands higher amounts of nutrients (Singh et al., 2007) for obtaining target yields. Consequently, the recommended rates are mostly in the range of 150–200 kg N ha<sup>-1</sup> across the globe (Thorburn et al., 2011), except in Brazil where the N application rates are comparatively less (Carmo et al., 2013). As the yield response is commensurate with soil N status (Gilbert et al., 2008) and/or N application rates, it becomes imperative to apply higher rates of fertilizers for obtaining target yields in soils with poor N status. However, the excessive use of synthetic fertilizers,

especially, N and P have been detrimental to the environment (Hartemink, 2008). Recent studies conducted by Wang et al. (2016) in Australia, Garcia et al. (2016) in Mexico, Pryor et al. (2017) in South Africa, Cardozo et al., 2016 in Brazil clearly have highlighted the implications of high N fertilization in sugarcane cultivation and the need for mitigating the same. Thus to address the trade-offs between higher N fertilization and environmental integrity various mitigation strategies have been emphasized for yield improvement in sugarcane. These include N replacement (Thorburn et al., 2011), integrated nutrient management (INM) strategies involving crop rotation with legumes (Gopalsundaram et al., 2012), green manuring (Gilbert et al., 2008) and use of organic residues (Yadav and Verma, 1995). However, they have their own drawbacks. Applications of organic residues in conjunction with synthetic fertilizers have contributed to higher amounts of green-house gas emissions during sugarcane cultivation (Carmona et al., 2013). In addition, transport of organic inputs like farmyard manure or compost involves huge costs. Moreover, yield responses have been shown to vary with the source of organic residues, predominantly on account of differences in their nutrient contents and crop cycle. Production of animal based manures, composting, its transport as well as their mineralization are also associated with significant environmental impacts, especially methane, nitrogen dioxide and nitrous oxide emissions (Hou et al., 2015). N replacement although seems to address the issue will have serious practical challenges when implemented on a larger scale. Thus enhancing crop productivity in a sustainable manner with any practically feasible technology would be desirable to meet the challenges of the demand for increasing sugarcane production for sugar, biofuel and bioenergy. Foliar application of *Kappaphycus alvarezii* seaweed extract (KSWE) in addition to recommended rate of fertilizer application in a wide variety of crops like soybean (Rathore et al., 2009), maize (Singh et al., 2016), rice (Sharma et al., 2017), green gram (Pramanick et al., 2013) has revealed its potential to sustainably increase crop yields, thereby, reducing yield gap (between potential and real yield) which would have otherwise required application of incremental inputs in the form of synthetic fertilizers in order to obtain similar yields. Thus we hypothesize that one can save the incremental application of synthetic fertilizers by using KSWE. KSWE is also reported to be effective against various biotic (Agarwal et al., 2016) and abiotic stresses (Trivedi et al., 2018). There are few research reports evaluating seaweed biostimulant application on sugarcane in India either along the Western plateau and hill region, (Deshmukh and Phonde, 2013); or Southern plateau and hill region (Karthikeyan and Shanmugam, 2017). However, validation across several other agro-climatic regions is essential to evaluate its efficacy for use in sugarcane cultivation. The present experiment was carried out in Upper Gangetic plain which represents a different agro-climatic region of India. Moreover, the possibility of mitigating environmental impacts by use of SWE in sugarcane cultivation has neither been contemplated nor quantified till now. Thus, a detailed study was carried out for two consecutive seasons during 2012–14 with the following objectives: 1) to investigate the efficacy of KSWE and test our hypothesis that the KSWE applied in combination with recommended rate of fertilizers (RRF) would enhance cane productivity in a sustainable manner, 2) to study the economics of KSWE use and 3) to quantify the environmental impacts and benefits resulting from application of both synthetic fertilizers as well as KSWE and to support the hypothesis of sustainability of the seaweed extract by deducing the benefits across various environmental impact categories (Mg of cane production)<sup>-1</sup> ha<sup>-1</sup> as they constitute the only variable inputs during sugarcane cultivation.

## 2. Materials and methods

### 2.1. Experimental site, design and treatment

The field experiment was conducted at Indian Institute of Sugarcane Research, Lucknow, India (26° 56'N, 80° 52'E) during 2012–2014. The altitude of the site is 111 m above mean sea level. The soil was silty loam in texture with an initial pH of 8.6 and organic carbon content of 0.45%, while the available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O contents were at 220, 28.3 and 253 kg ha<sup>-1</sup> respectively. The meteorological data during the experimentation is presented in Table S1 and S2. A total of 10 treatments involving different concentrations (2.5, 5.0, 7.5 and 10%) of *Kappaphycus* and *Gracilaria* SWEs in conjunction with RRF were applied to sugarcane plant crop along with a suitable control (water + RRF). In addition, a lower dose of RRF (50% RRF) in combination with 6.25% K-SWE was also tested. In the present manuscript data pertaining to only KSWE treatments are presented and discussed. The RRF for sugarcane plant crop was 150:60:60 kg ha<sup>-1</sup> N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O and was applied through urea, di-ammonium phosphate and muriate of potash (MOP), respectively. Half dose of N (through di-ammonium phosphate and Urea) and full dose of P and K were applied at the time of planting while the remaining half dose of N was applied in two equal splits, the first at 45 days after planting (DAP) and the second at the onset of monsoon (first week of July). In ratoon crop, only N was applied at 187 kg ha<sup>-1</sup> through urea at two stages; one at ratoon initiation and the other at the onset of monsoon. Three sprays of KSWE, with a spray volume of 800 L ha<sup>-1</sup>, were applied at 60 (early formative), 90 (late formative) and 120 days after planting (grand growth stage) in both plant and ratoon crops. The experiment was set up in a randomized block design with 3 replications. The plot size was 9 m × 8 m and with a row to row spacing of 0.75 m. For sugarcane planting, three budded cane setts were pre-treated with 1% KSWE for 5–10 min and then placed in furrows in overlapping fashion (bud to bud placement) at 55000 setts ha<sup>-1</sup>. Ratoon was initiated after the harvest of plant crop in February 2013 and the cut ends of the stubbles were drenched with 1.0% KSWE.

### 2.2. Preparation of KSWE

KSWE was prepared from *Kappaphycus alvarezii* using a procedure as described earlier in Trivedi et al. (2017) and this was considered 100% concentration. As per the treatments, appropriate dilutions were prepared with water. The chemical composition of the KSWE has been described in Singh et al. (2016). The same batch of KSWE was also used in the present experiment.

### 2.3. Field operations and management

The sugarcane variety Co S94257 was planted following harvest of rapeseed in March 2012. The field was initially ploughed with a disc plough after a pre-planting irrigation. This was followed by two harrowing operations with disc harrow which was eventually followed by planking. Before planting, both the ends of all the setts were dipped in 0.1% Bavistin solution to control sett borne disease. Chlorpyrifos at the rate of 5 L ha<sup>-1</sup> was applied in furrows to control termites. The crops were irrigated during the pre and post monsoon periods with 480 mm of water for plant crop and 400 mm in ratoon crop. Two hoeing operations at 45 and 85 DAP were done to control weeds in the plant crop. In ratoon crop, a single hoeing operation was done at its initiation (March 2013) followed by trash mulching operations. One weeding operation was carried out 60 days after ratoon initiation (DARI).

#### 2.4. Germination, growth and yield measurements

Germination in sugarcane was recorded by counting the plant population at 40 DAP from middle 4 rows of each plot 8 m in length. Germination percentage was calculated as the ratio of plant population at germination stage to the number of buds planted. Tillers were counted in a similar manner for each plot at various growth stages in plant and ratoon crops and presented on hectare basis. However, the tillers that emerged after 150 DAP in plant crop and 120 DARI in ratoon were not included in counting. Plant height at various stages was measured in plant and ratoon crops from 5 randomly selected plants in each plot as the distance from ground level to the last fully expanded leaf. The tillers produced till 60 DAP were considered for selection to record plant height. Periodic changes in the dry matter accumulation (DMA) were determined by destructive sampling of all the plants from a row of 1 m length from 3 randomly selected spots within each plot. Crop growth rate was calculated from DMA using the formula given below as described by Radford (1967) and expressed as  $\text{kg ha}^{-1} \text{ day}^{-1}$ .

$$\text{CGR} = \frac{W_2 - W_1}{t_2 - t_1} \quad (1)$$

Where,  $W_1$  = dry weight of plant at time  $t_1$ ,  $W_2$  = dry weight of plant at time  $t_2$ .

Leaves from the plant samples collected for DMA were detached and their length as well as maximum width was recorded in order to obtain the leaf area. Leaf area was calculated as the product of the leaf length, maximum leaf width and the factor 0.67. Leaf area index (LAI) was calculated as the ratio of total leaf area of plants to the ground area covered by the plants. The plants were harvested manually at ground level. Before harvesting, 10 randomly selected canes from each plot were used for measuring yield attributes namely cane length, cane diameter, number of internodes, cane weight as well as juice analysis. A net plot of 4 lines of 8 m length from each plot was harvested manually and detashed to record the number of millable canes (NMC) and cane yield and presented on hectare basis. The cane juice was analysed for quality parameters such as brix, sucrose (%) and purity (%) according to Meade and Chen (1977). Commercial cane sugar (CCS %) was calculated according to the formula given below:

$$\text{CCS}(\%) = (S - 0.4(B - S)) \times 0.73 \quad (2)$$

Where, S = sucrose % and B = brix in juice.

#### 2.5. Soil and plant analysis

Soil samples were collected at a depth of 0–15 cm from 10 different spots before sowing. These were bulked and sub sampled into 4 portions. One representative portion was kept for analysis. After harvest of the crop, soil samples were collected from 0 to 15 and 15–30 cm depth in each plot. The values of all the parameters were averaged and expressed as the mean of 3 replications. Electrical conductivity was measured by conductivity meter while pH was measured in a 1: 2 soil: water suspension (Jackson, 1973). Organic carbon was determined by Walkley and Black method (Walkley and Black, 1934). CEC, available N, P and S were estimated according to Amma (1989), Watanabe and Olsen (1965) and Williams and Steinbergs (1959). Potassium was determined flame photometrically (Jackson, 1973) while calcium and magnesium were determined by EDTA titration (Black, 1965). N, P, K and S content of the plants were estimated by macro-Kjeldahl (Jackson, 1973), phosphoric acid vanadomolybdate (Jackson, 1973), flame photometric (Jackson, 1973) and turbidimetric (Chesnin and Yien, 1951) methods, respectively.

#### 2.6. Economics

Benefit: cost ratio (B:C ratio), a ratio of gross return on investment to the total cost of cultivation was used to explain the economics of sugarcane cultivation. The prevailing market price of sugar cane i.e., Indian Rupees (INR) 2900  $\text{Mg}^{-1}$  was used to calculate the economic returns from the produce. The common cost of cultivation excluding the fertilizer cost was INR 98,450  $\text{ha}^{-1}$ . The variable cost was attributed to different fertilizer and SWE doses, which varied according to the treatment. The cost of 100% RRF was INR 6119  $\text{ha}^{-1}$ . The cost of KSWE was INR 30  $\text{L}^{-1}$ .

#### 2.7. Life cycle assessment

The goal and scope of the study was limited to determining the impacts resulting from the production, transport and application of the fertilizers (synthetic and KSWE) cumulatively for plant and ratoon crops as they constitute the only variable inputs required for cane production in 1ha for 2y. The study also included determination of the  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions from synthetic N fertilizers subsequent to their application. The functional unit was accordingly defined as the cumulative fertilizer application as per treatments for cane production in 1ha for 2y. We strongly believe that KSWE is not accountable for any  $\text{N}_2\text{O}$  emissions as it contains negligible quantities of N. This was estimated to be 85  $\text{mg L}^{-1}$  (Mondal et al., 2015) which may be directly absorbed by the plants upon their application. All the  $\text{N}_2\text{O}$  emissions were finally expressed in terms of  $\text{CO}_2$  eq. Thus the system boundaries in the present study included the processes involved in the production of fertilizers, KSWE, their transport to the site of sugarcane cultivation in 1 ha area. Other steps of cane production in 1ha 2y<sup>-1</sup> such as land preparation, irrigation, pesticide, fungicide application etc. which are common to all the treatments were not determined and do not fall within the scope of the present study. Emissions of P due to phosphorus fertilization were also not estimated and hence do not fall within the scope of the present study. The boundaries are as depicted in Fig. 1. The modelling was carried out using GaBi software (Version 6.0).

##### 2.7.1. Life cycle inventory

Environmental impacts resulting during production of fertilizers applied as per RRF (a common input for all the treatments) were determined using Eco-invent 3.3 and professional datasets (Think Step). The datasets and databases used for modelling were in compliance with the ISO 14044, ISO 14064 and ISO 14025 standards. Urea and complex fertilizers like di-ammonium phosphate and SSP are manufactured in India. However, due to lack of datasets specific to Indian manufacturing conditions, Eco-invent 3.3 datasets rest of the world (RoW) for production and transport to regional storage of urea as N, di-ammonium phosphate both in terms of N and  $\text{P}_2\text{O}_5$  and MOP as  $\text{K}_2\text{O}$  were used as substitutes in our model. Although MOP is entirely imported into India, its transport was not included as the impacts were less than 1% of the total impacts. Transport of synthetic fertilizers from their storage site to the field was assumed to be carried out in a lorry 3.5–7 t capacity with an assumed distance of 200 km.

Impacts owing to the production of 1000 L of KSWE (Ghosh et al., 2015) have been reported by us earlier and the same was used in the present study. Production of KSWE was modelled using Eco-invent 2.2 and professional databases (Think Step) which included various processes such as seaweed cultivation, transport to factory, processing step during extract preparation and packaging. KSWE was assumed to be transported by rail over a distance of 2500 km in the present study. Five liters each of KSWE was used for either treating the cane setts prior to sowing during plant crop

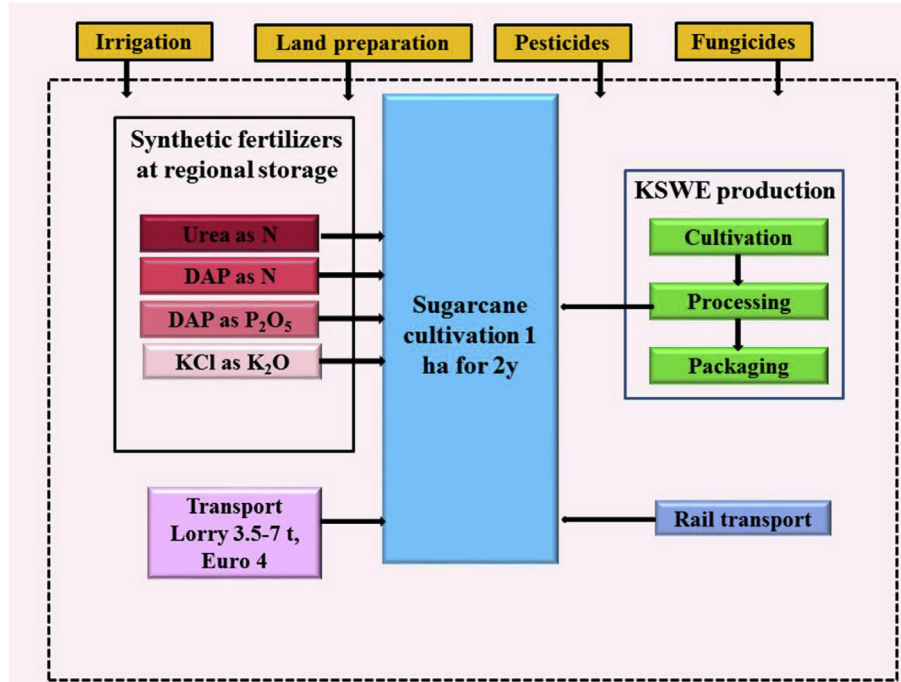


Fig. 1. Flow chart describes system boundaries of the present study. Dotted lines delimit the system boundaries.

or as a drench during ratoon initiation. The amount of KSWE varied according to the concentration applied in each treatment and was 130, 250, 310, 370 and 490 L ha<sup>-1</sup> for 2.5, 5.0, 6.25, 7.5 and 10.0%, respectively.

2.7.2. N<sub>2</sub>O emissions from synthetic N fertilizers

Cumulative N<sub>2</sub>O emissions (direct and indirect) from synthetic N fertilizers applied viz., urea and di-ammonium phosphate were calculated as per tier I methodology of IPCC guidelines (IPCC, 2006). The equations (3)–(5), given below for determining direct and indirect N<sub>2</sub>O emissions were derived from the original equations 11.1, 11.9 and 11.10 of tier I methodology of IPCC (IPCC, 2006). The original equations were abridged as the field was solely fertilized by synthetic fertilizers and neither animal manure nor other organic sources were used. The soils were low in organic carbon content (0.45%) and do not fall under the category of organic soils. Hence, equations (3)–(5) were obtained after considering a value of zero for all the parameters that relate to organic sources.

$$N_2O_{direct} = N_2O_{inputs} - N \times 44/28 \tag{3}$$

where,

$$N_2O_{inputs} - N = F_{SN} \times EF_1$$

where F<sub>SN</sub> was the amount of synthetic N fertilizer applied cumulatively for 2y; EF<sub>1</sub> was the emission factor for N<sub>2</sub>O emissions from N inputs and the value 0.0046 (Neto et al., 2016) was used. Indirect N<sub>2</sub>O emissions were calculated as the sum of N<sub>2</sub>O from atmospheric deposition of N volatilised from managed soils and N leaching/runoff from managed soils (Nunes et al., 2016). The N<sub>2</sub>O from atmospheric deposition was calculated from equation (4) while N leaching/runoff from equation (5).

$$N_2O_{ATD} = F_{SN} \times Frac_{GASF} \times EF_4 \times 44/28 \tag{4}$$

$$N_2O_L = F_{SN} \times Frac_{LEACH} \times EF_5 \times 44/28 \tag{5}$$

where, Frac<sub>GASF</sub> was the fraction of synthetic N that volatilises as NH<sub>3</sub> and NO<sub>x</sub>, expressed in kg N volatilised (kg of N applied)<sup>-1</sup>; EF<sub>4</sub> was the emission factor for N<sub>2</sub>O emission from atmospheric deposition of N on soil with units of kg N-N<sub>2</sub>O (kg NH<sub>3</sub>-N + NO<sub>x</sub>-N volatilised)<sup>-1</sup>; Frac<sub>LEACH</sub> was the fraction of all the N added to soil which lost through leaching/run off and expressed in kg N y<sup>-1</sup>; EF<sub>5</sub> was the emission factor for N<sub>2</sub>O emissions from N leaching/runoff; F<sub>SN</sub> was the amount of synthetic N fertilizer applied cumulatively for 2y and was 337 kg N. IPCC default values of 10 and 30% were considered as the fractions of synthetic N that would undergo volatilisation and leaching, respectively, whereas 0.01 and 0.0075 were used as values for EF<sub>4</sub> and EF<sub>5</sub>, respectively. All the N<sub>2</sub>O emissions were finally converted into CO<sub>2</sub> eq by multiplying with a factor of 298.

2.7.3. CO<sub>2</sub> emission from urea

Carbon dioxide emission in kg ha<sup>-1</sup> y<sup>-1</sup> from urea was calculated using equation (6) (IPCC, 2006).

$$CO_2 = M \times EF \times 1000 \times 44/12 \tag{6}$$

Where, M is the amount of urea added to the soil for fertilization and expressed in t 2y<sup>-1</sup>; EF is emission factor for which a default value of 0.2 was used in the present study. The sum total of all the farm emissions (CO<sub>2</sub> eq of N<sub>2</sub>O + CO<sub>2</sub> from urea) from synthetic fertilizers was added to CO<sub>2</sub> eq deduced for RRF and KSWE production prior to calculating the impacts. The impacts were calculated using ReCiPe Midpoint (H) method and expressed in terms of impacts Mg<sup>-1</sup> of cumulative cane production over two years. No allocation was carried out and the entire burden of impact was apportioned to cane production. Absolute savings in any impact category was determined as the difference between assumed and absolute values. Assumed impact was a hypothetical value calculated as the impact that would result in a particular treatment on

account of obtaining similar yield with incremental synthetic fertilizer inputs. Absolute values were the actual impacts resulting due to the treatments in present study that were estimated from modelling. Control was used as the basis for calculating the assumed impacts.

### 2.8. Statistical analysis

As the experiment was laid in a Randomized complete block design with three replications, a single factor analysis of variance was carried out using the procedure described by Fisher (1953) suitable for the above design for those parameters of growth, nutrients, their uptake, yield and yield attributes which satisfied the assumptions. The hypothesis was tested at 5% level. Post hoc comparison of means was carried out by Least Significant Difference (LSD) at  $p < 0.05$  using MSTATC software (Michigan State University, East Lansing).

## 3. Results

### 3.1. Effect of KSWE on germination, growth and physiological parameters

Pre-planting treatment of setts with 1.0% KSWE resulted in the enhancement of germination percentage in all the treatments relative to control (water + RRF) (Fig. 2).

Maximum germination was observed in the combination involving 6.25% K-SWE and 50% RRF (T6) with an increase of 9.8% as compared to control. Among the various combinations of RRF with KSWE, concentrations greater than 2.5% produced more number of tillers in the plant as well as ratoon crop at different stages of growth relative to control (Fig. 3a and b).

Further, the tiller population in the plant crop increased from 60 to 120 DAP in all the treatments following which it declined (Fig. 3a) while in ratoon the decline was observed after 90 DARI (Fig. 3b). It should be noted that the tiller population in the treatment involving combination of 50% RRF and 6.25% K-SWE (T6) was statistically at par with control at all the stages of growth in plant and ratoon crops, except, at 180 DAP as well as at harvest in the plant crop where the plant population declined due to reduced fertilizer application (Fig. 3a and b).

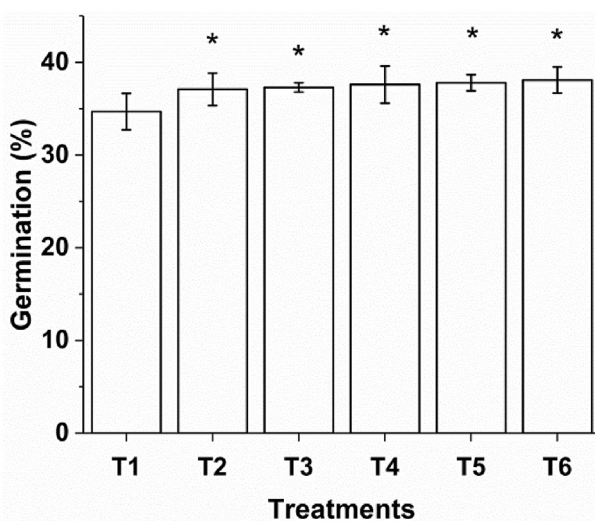


Fig. 2. Influence of foliar application of KSWE in combination with synthetic fertilizers on germination in plant crop. Labels of treatments are the same as in Table 1. Bars (SD) with asterisk are significantly different from control treatment (T1) at  $p < 0.05$  by LSD.

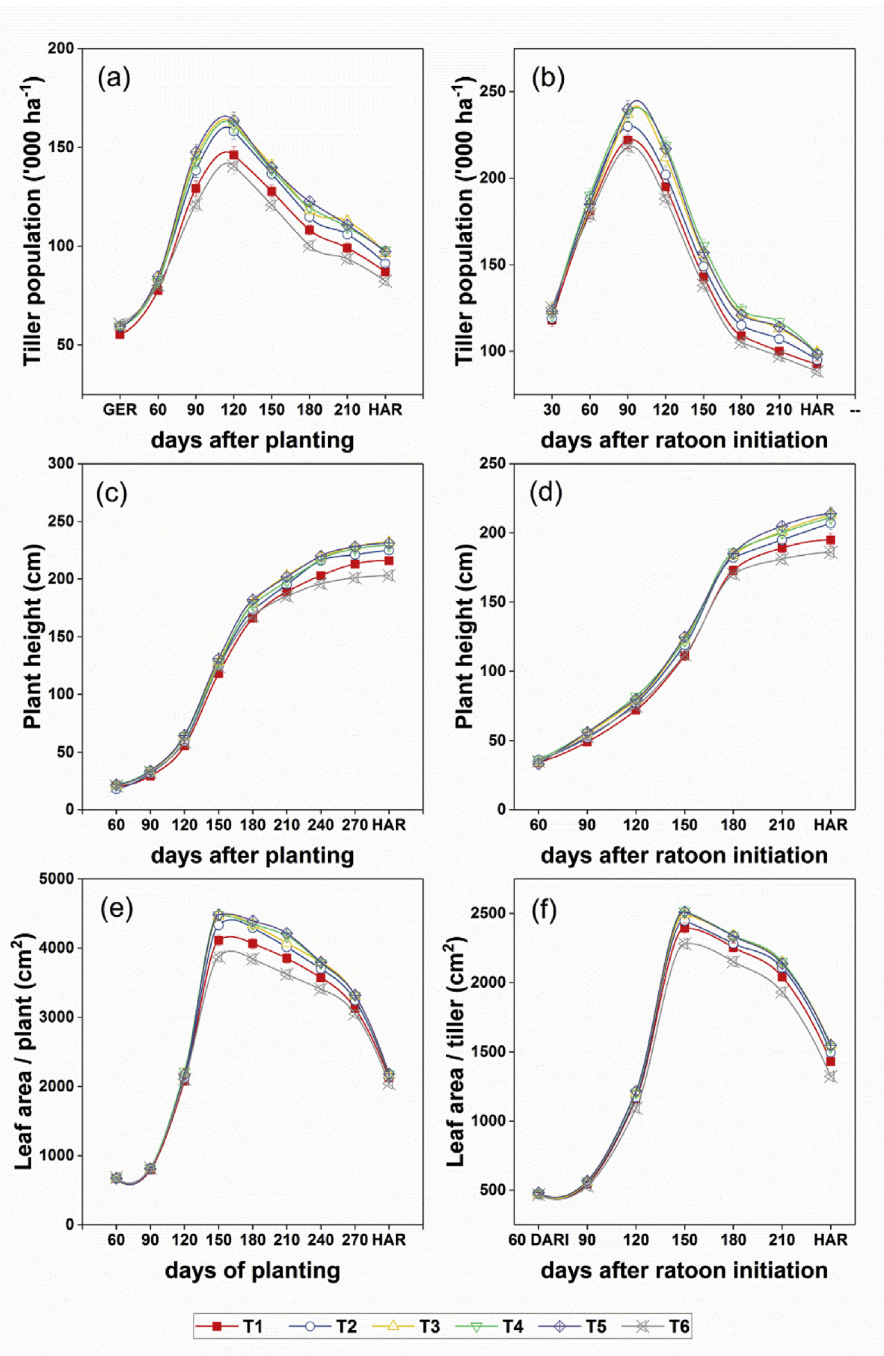
KSWE applied at concentrations of 5% or more along with RRF increased the plant height in comparison to control and T6 after 90 days of planting and ratoon initiation in plant and ratoon crops, respectively (Fig. 3c and d). The average increase in plant height at harvest stage in the plant crop due to KSWE + RRF treatment was 6.1% (Fig. 3c). Similarly, the increase was 8.3% in ratoon crop (Fig. 3d). Further, plant height in T6 was at par with control at all stages in both plant and ratoon crops, except at 270 DAP and at harvest in the plant crop where a marginal decrease was observed.

Leaf area in plant and ratoon crop was enhanced only after the first foliar application of KSWE (Fig. 3e and f). Maximum leaf area per plant was recorded at 150 DAP and DARI, respectively, in plant and ratoon crop (Fig. 3e and f). In the plant crop, leaf area was higher in relation to control in the treatments involving combinations of RRF with KSWE at concentrations exceeding 2.5% from 120 to 270 DAP. However, all the treatments were at par for leaf area measured at harvest. In contrast, in ratoon crop, the leaf area per tiller measured at harvest was significantly higher than control in the KSWE + RRF treatments at concentrations above 2.5% while being at par during earlier growth stages (Fig. 3f). Leaf area per plant in T6 and control did not vary at different growth stages viz., 120, 270 DAP and at harvest in the plant crop while leaf area was consistently lower than control at all the remaining stages of growth in ratoon crop (Fig. 3e and f). Leaf area index (LAI) was enhanced by KSWE treatments at concentrations of 5% or more in combination with RRF at the rapidly growing stages of the plants viz., 120–210 DAP and DARI in both plant and ratoon crops, respectively (Fig. 4a and b). In addition, at any specific crop stage, all the KSWE + RRF treatments were at par with each other for LAI. Highest LAI was measured at 150 DAP and DARI in plant and ratoon crops, respectively (Fig. 4a and b).

The dry matter accumulation (DMA) in all the treatments was at par with each other at 60 DAP in the plant crop. Similarly in ratoon, the treatments did not differ for DMA till 90 DARI (Fig. 4c and d). KSWE + RRF treatments at concentrations above 2.5% increased DMA from 120 DAP till harvest in the plant crop in relation to control (Fig. 4c). In ratoon crop, a similar increase was observed from 150 DARI till harvest (Fig. 4d). Further, KSWE + RRF treatments at concentrations above 5% did not bring about any further enhancement in DMA as compared to that obtained at 5% level. DMA in 6.25% KSWE + 50% RRF treatment (T6) was consistently lower than control from 120 DAP till harvest in the plant crop. In the ratoon crop, DMA was lower than control for T6 only at harvest while being at par at all the other growth stages (Fig. 4c and d). The reduction in DMA in T6 treatment at harvest stage was 10.8% and 8.7%, in relation to control in plant and ratoon crops, respectively. The average increase in DMA at harvest in RRF + KSWE treatments was 13.4% and 7.9% over control in plant and ratoon crop, respectively, (Fig. 4c and d). The highest crop growth rate was during the growth periods of 120–150 DAP and DARI respectively, in plant and ratoon crop (Fig. 4e and f). The increase in CGR during the above period was 12 and 10.1% on account of KSWE + RRF treatments in plant and ratoon crops, respectively.

### 3.2. Effect of KSWE on yield attributes, yield and juice quality parameters

Majority of the KSWE + RRF treatments at concentrations above 2.5%, increased cane length and cane weight over control while being at par with each other in both plant and ratoon crop. Cane diameter remained unaltered by any of the treatments in plant as well as ratoon crops (Table 1). In the plant crop, KSWE + RRF treatments enhanced internode number over control at all the tested concentrations. In ratoon crop KSWE at concentrations only above 2.5% increased internode numbers. Cane weight improved as

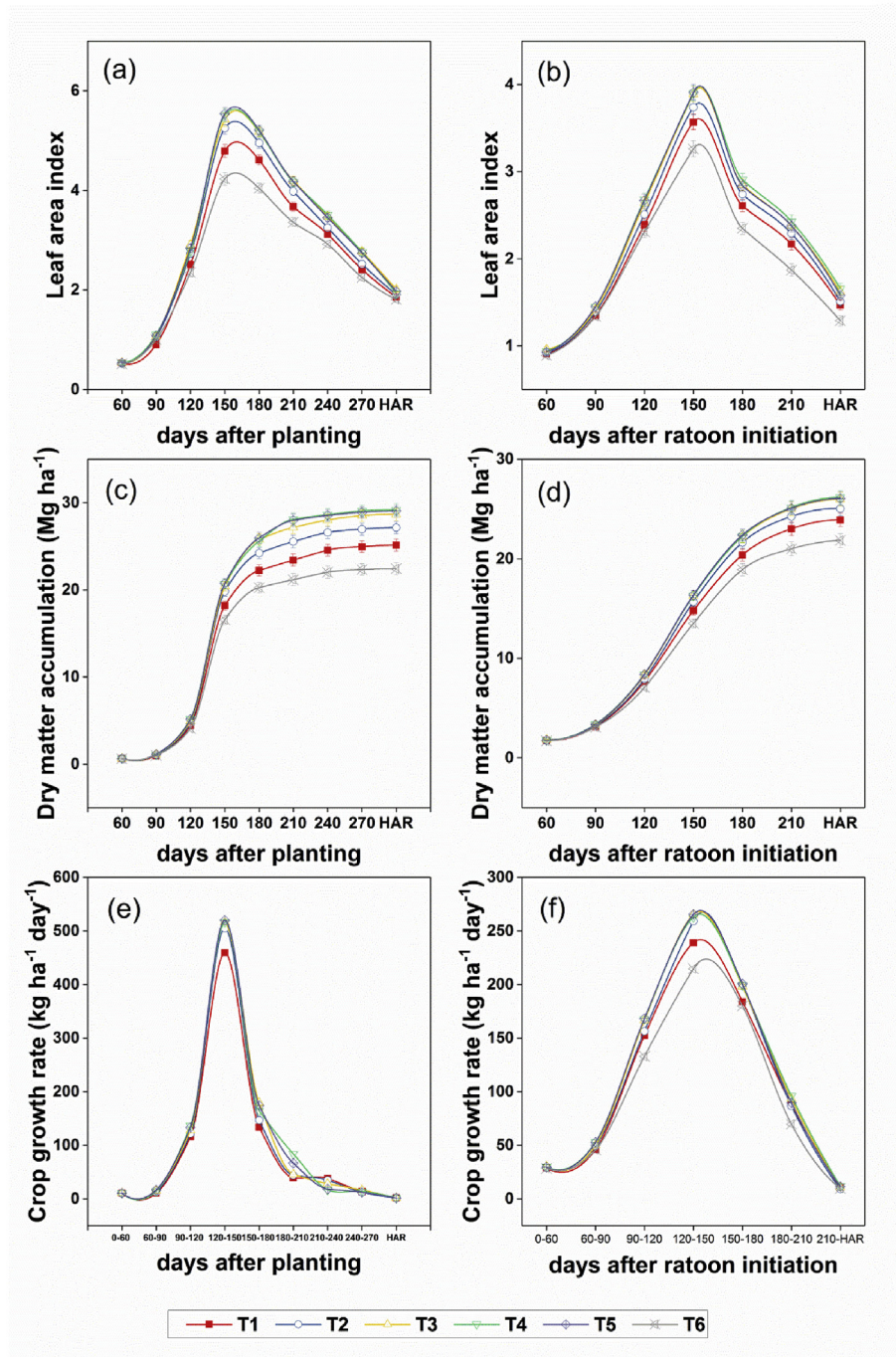


**Fig. 3.** Influence of foliar application of KSWE in combination with synthetic fertilizers on tiller population (a & b), height (c & d) and leaf area (e & f) in plant and ratoon crop respectively. RRF- recommended rate of fertilizers; KSWE- Kappaphycus seaweed extract; HAR-at harvest. Labels T1 to T6 denote treatments as given in Table 1.

a consequence of the increase in cane length. Number of millable canes (NMC) was higher in plant crop when KSWE was applied at concentrations above 2.5% along with RRF. However, this was not true in ratoon where only 5% application of KSWE enhanced NMC (Table 1). Increase in cane length, cane weight and NMC led to increase in cane yield over control by treatments involving combination of RRF with all the concentrations of KSWE in both plant and ratoon crops (Table 1). Application of KSWE at 5% level was found to be the optimum concentration as it significantly enhanced yield over control. At 5% level, cane yield increased by 12.5 and 7.9% over control, in plant and ratoon crops, respectively, owing to KSWE

application. Further, an intriguing fact was that in spite of lowering fertilizer requirement by 50%, the cane yield in T6 treatment was at par with control in ratoon (Table 1) while in plant crop the yield decreased by 7.9% in T6 (Table 1). Commercial cane sugar production or sugar yield ( $\text{Mg ha}^{-1}$ ) followed the trend of cane yield and varied between 6.37 and 7.85  $\text{Mg ha}^{-1}$  in KSWE treatments in plant crop. CCS was a little lower in ratoon and varied between 5.61 and 6.7  $\text{Mg ha}^{-1}$  (Table 1). Further, the CCS yield was similar in control and T6 treatments.

The juice quality parameters like brix, sucrose %, CCS %, however, remained unaltered relative to control in KSWE treatments in plant



**Fig. 4.** Influence of foliar application of KSWE in combination with synthetic fertilizers on growth indices and dry matter accumulation in plant and ratoon crops. RRF- recommended rate of fertilizers; KSWE- Kappaphycus seaweed extract. Labels T1 to T6 denote treatments as given in Table 1.

and ratoon crops (Table S3). Purity which is the amount of pure sucrose in dry matter of juice was enhanced by KSWE treatments only in plant crop (Table S3).

### 3.3. Effect of KSWE application on nutrient content, uptake in plant and its availability in soil after harvest

Nutrient contents (N, P, K, and S) in leaf and cane were estimated. It was found that only N content in plant crop was significantly influenced due to the KSWE treatments compared to control (Table S4, S5, S6, S7). In the plant crop, N content of leaf and cane

was higher in KSWE + RRF treatments at all the tested concentrations as compared to control but were at par with one another (Table S4). Treatments T6 and control were at par with each other during both the seasons with respect to N content of leaf (Table S4 and S5). However, KSWE + RRF treatments at concentrations higher than 2.5% significantly improved the uptake of N, P, K and S elements relative to control. The total N uptake increased by 19–27% over control in KSWE + RRF treatments in plant crop while the increase was 8–14% in ratoon (Table S4, S5, S6, S7). The N content of leaf in treatment T6 was at par with control in plant and ratoon crops while N content of the cane in T6 was similar to

**Table 1**

Influence of KSWE treatment on sugarcane yield, and yield attributes in plant (P) and ratoon crop (R). NMC -Number of millable canes; CCS - Commercial cane sugar; KSWE – Kappaphycus seaweed extract; RRF – recommended rate of fertilizers; Values followed by different superscript alphabets are significantly different at  $p < 0.05$  by LSD.

Treatments	Cane length (cm)		Diameter (cm)		Cane weight (g)		Internode (nos.)		NMC ('000 ha <sup>-1</sup> )		Cane yield (Mg ha <sup>-1</sup> )		CCS (Mg ha <sup>-1</sup> )	
	P	R	P	R	P	R	P	R	P	R	P	R	P	R
<b>T1 - Water spray + RRF (control)</b>	<b>185<sup>b</sup></b>	<b>169<sup>b</sup></b>	<b>2.31<sup>a</sup></b>	<b>2.23<sup>a</sup></b>	<b>760<sup>b</sup></b>	<b>700<sup>b</sup></b>	<b>19.6<sup>bc</sup></b>	<b>17.5<sup>d</sup></b>	<b>87.2<sup>c</sup></b>	<b>92.4<sup>bc</sup></b>	<b>59.9<sup>b</sup></b>	<b>54.3<sup>cd</sup></b>	<b>6.73<sup>b</sup></b>	<b>5.93<sup>cd</sup></b>
T2 - 2.5% KSWE + RRF	194 <sup>a</sup>	174 <sup>ab</sup>	2.35 <sup>a</sup>	2.23 <sup>a</sup>	785 <sup>a</sup>	717 <sup>ab</sup>	20.9 <sup>a</sup>	17.8 <sup>cd</sup>	92.2 <sup>b</sup>	95.9 <sup>abc</sup>	64.6 <sup>a</sup>	55.1 <sup>bc</sup>	7.37 <sup>a</sup>	6.00 <sup>bcd</sup>
T3 - 5.0% KSWE + RRF	198 <sup>a</sup>	178 <sup>a</sup>	2.31 <sup>a</sup>	2.25 <sup>a</sup>	795 <sup>a</sup>	724 <sup>a</sup>	21.1 <sup>a</sup>	18.9 <sup>abc</sup>	96.4 <sup>ab</sup>	99.6 <sup>a</sup>	67.4 <sup>a</sup>	58.6 <sup>ab</sup>	7.85 <sup>a</sup>	6.50 <sup>ab</sup>
T4 - 7.5% KSWE + RRF	197 <sup>a</sup>	177 <sup>ab</sup>	2.36 <sup>a</sup>	2.24 <sup>a</sup>	800 <sup>a</sup>	725 <sup>a</sup>	20.7 <sup>a</sup>	18.9 <sup>abc</sup>	98.0 <sup>a</sup>	98.4 <sup>ab</sup>	68.2 <sup>a</sup>	58.9 <sup>ab</sup>	7.83 <sup>a</sup>	6.40 <sup>abc</sup>
T5 - 10% KSWE + RRF	199 <sup>a</sup>	178 <sup>a</sup>	2.35 <sup>a</sup>	2.27 <sup>a</sup>	801 <sup>a</sup>	729 <sup>a</sup>	20.9 <sup>a</sup>	19.2 <sup>ab</sup>	97.3 <sup>a</sup>	98.3 <sup>ab</sup>	67.7 <sup>a</sup>	58.6 <sup>ab</sup>	7.79 <sup>a</sup>	6.40 <sup>abc</sup>
<b>T6 - 6.25% KSWE + 50% RRF</b>	<b>174<sup>c</sup></b>	<b>160<sup>c</sup></b>	<b>2.32<sup>a</sup></b>	<b>2.21<sup>a</sup></b>	<b>733<sup>c</sup></b>	<b>677<sup>c</sup></b>	<b>19.1<sup>c</sup></b>	<b>17.3<sup>d</sup></b>	<b>82.2<sup>d</sup></b>	<b>89.4<sup>c</sup></b>	<b>55.2<sup>c</sup></b>	<b>50.7<sup>d</sup></b>	<b>6.37<sup>b</sup></b>	<b>5.61<sup>d</sup></b>

control only ratoon.

The KSWE + RRF treatments enhanced the total P uptake by 7–21% over control in plant and ratoon crops (Tables S4, S5). Similarly, total K and S uptake were also improved in KSWE + RRF treatments in both the plant as well as ratoon crops.

Organic carbon content and available P, K and S in soil at crop harvest was not affected by KSWE treatments (Tables S8, S9). In contrast, available N content in 0–15 cm soil at harvest of plant and ratoon crops decreased in most of the KSWE treatments while it remained unaltered in 15–30 cm soil depth, except for T6 where it was lower as compared to control at least in the plant crop (Tables S8).

#### 3.4. Economics of cultivation

It is clearly evident from Table 2 that the B:C ratio was higher when KSWE was used at a concentration of 5% in plant and ratoon crops, implying a higher rate of return to the farmers. In the plant crop, the rate of return was 13% higher than control when KSWE was applied at 5% while the same was 10.9% higher in ratoon.

#### 3.5. Environmental benefits on account of KSWE use

Environmental impacts deduced for the production as well as transport of 1000 L of KSWE from factory gate to the experimental site are given in Table 3 from which values for the appropriate amount of KSWE applied in each treatment was determined. Similarly impacts owing to production and transport of synthetic fertilizers are also given in Table 3. Direct N<sub>2</sub>O emissions in farm due to the application of synthetic N fertilizers (both DAP and Urea) was calculated and found to be 2.436 kg N<sub>2</sub>O 2 y<sup>-1</sup> while the indirect emissions due to atmospheric deposition and leaching amounted to 1.721 kg N<sub>2</sub>O 2 y<sup>-1</sup>. The CO<sub>2</sub> emission from urea was 500 kg CO<sub>2</sub> 2 y<sup>-1</sup>. Various N<sub>2</sub>O and CO<sub>2</sub> emissions from synthetic N fertilizers are presented in Table 4. Among the 18 different impact categories that were deduced, application of KSWE resulted in marked reduction of impacts in at least 9 different environmental categories in comparison to control (Fig. 5 and Table S10). In

contrast, the impacts (Mg cane production)<sup>-1</sup> in all the KSWE treatments pertaining to agricultural land occupation (ALO) and natural land transformation (NLT) were higher than control (Fig. 5a, d). Among the KSWE + RRF treatments, impacts (Mg cane production)<sup>-1</sup> were reduced to the maximum extent in most of the environmental categories when KSWE foliar applied at 5% concentration (Table S10).

For climate change category, application of KSWE at 5% concentration resulted in 7.7% reduction relative to control (Fig. 5b). This would envisage savings of 260 kg CO<sub>2</sub> eq (Mg cane production)<sup>-1</sup> following foliar application of KSWE. Further, it has to be noted that in 50% RRF + KSWE treatment there was a dramatic reduction in impacts under CC (43.7%). This would amount to enormous savings of 1234 kg CO<sub>2</sub> eq (Mg cane production)<sup>-1</sup> with a concomitant reduction of just 7.3% reduction in yield with respect to control. Other impact categories like fossil depletion, freshwater ecotoxicity, human toxicity, metal depletion, ozone depletion, particulate matter formation, terrestrial acidification and terrestrial ecotoxicity too followed the same trend as CC, in that the impacts (Mg cane production)<sup>-1</sup> were consistently lower than control whenever KSWE was applied at 5% level (Fig. 5 and Table S10). In case of water depletion, reductions in impacts were observed when KSWE was applied at 5%. However, in contrast, higher concentrations of KSWE in conjunction with RRF increased water depletion relative to control suggesting that determination of optimum concentration of KSWE for increasing yield is a prerequisite for saving water (Fig. 5f). The absolute savings in water was 21 m<sup>3</sup> (Mg of cane production)<sup>-1</sup> in 5% KSWE treatment. The lower dose combination resulted in reduction of impacts by at least 40% in 9 major environmental categories (Table S10).

## 4. Discussion

It is evident from the results that treatments involving application of KSWE in combination with RRF enhanced the cane yield relative to control in both plant as well as ratoon crops with concomitant increase in sugar yields. Although, all the concentrations of KSWE when applied in conjunction with RRF were able to

**Table 2**

Economics of sugarcane cultivation under different treatments in both plant (P) and ratoon (R) crops. Benefit: cost (B: C) is the ratio of total cost to gross return. INR is Indian Rupees (1US\$ = 67 INR).

Treatments	Total cost (INR ha <sup>-1</sup> )		Gross Return (INR ha <sup>-1</sup> )		Net Return (INR ha <sup>-1</sup> )		B: C ratio	
	P	R	P	R	P	R	P	R
<b>T1 - Water spray + RRF (control)</b>	<b>104569</b>	<b>76550</b>	<b>173710</b>	<b>157470</b>	<b>69141</b>	<b>80920</b>	<b>1.66</b>	<b>2.06</b>
T2 - 2.5% KSWE + RRF	106429	78410	187340	159790	80911	81380	1.76	2.04
T3 - 5.0% KSWE + RRF	108229	80210	195460	169940	87231	89730	1.81	2.12
T4 - 7.5% KSWE + RRF	110029	82010	197780	170810	87751	88800	1.80	2.08
T5 - 10% KSWE + RRF	111829	83810	196330	169940	84501	86130	1.76	2.03
<b>T6 - 6.25% KSWE + 50% RRF</b>	<b>106069</b>	<b>80360</b>	<b>160080</b>	<b>147030</b>	<b>54011</b>	<b>66670</b>	<b>1.51</b>	<b>1.83</b>



**Table 3**

ReCiPe Midpoint (H) environmental impacts owing to production and transport of 1000 L of Kappaphycus seaweed extracts as well as synthetic fertilizers applied at recommended rates for sugarcane. KSWE – Kappaphycus seaweed extract.

Impact categories	KSWE	Synthetic fertilizers
Agricultural land occupation [m <sup>2</sup> a]	77.3	36.2
Climate change [kg CO <sub>2</sub> -eq]	221	1310
Fossil depletion [kg oil eq]	89.6	556
Freshwater ecotoxicity [kg 1,4-DB eq]	1.56	9.4
Freshwater eutrophication [kg P eq]	$8.83 \times 10^{-2}$	$2.94 \times 10^{-1}$
Human toxicity [kg 1,4-DB eq]	31.8	536
Ionising radiation [kg U235 eq]	28.5	73.9
Marine ecotoxicity [kg 1,4-DB eq]	1.45	5.33
Marine eutrophication [kg N eq.]	$3.66 \times 10^{-1}$	1.17
Metal depletion [kg Fe eq]	15.4	94.0
Natural land transformation [m <sup>2</sup> ]	302	$3.46 \times 10^{-1}$
Ozone depletion [kg CFC-11 eq]	$8.4 \times 10^{-6}$	$1.8 \times 10^{-4}$
Particulate matter formation [kg PM10 eq]	$4.44 \times 10^{-1}$	3.44
Photochemical oxidant formation [kg NMVOC]	1.04	3.41
Terrestrial acidification [kg SO <sub>2</sub> eq]	1.05	9.58
Terrestrial ecotoxicity [kg 1,4-DB eq]	$1.8 \times 10^{-2}$	$3.38 \times 10^{-1}$
Urban land occupation [m <sup>2</sup> a]	17.3	49.6
Water depletion [m <sup>3</sup> ]	841	2240

**Table 4**

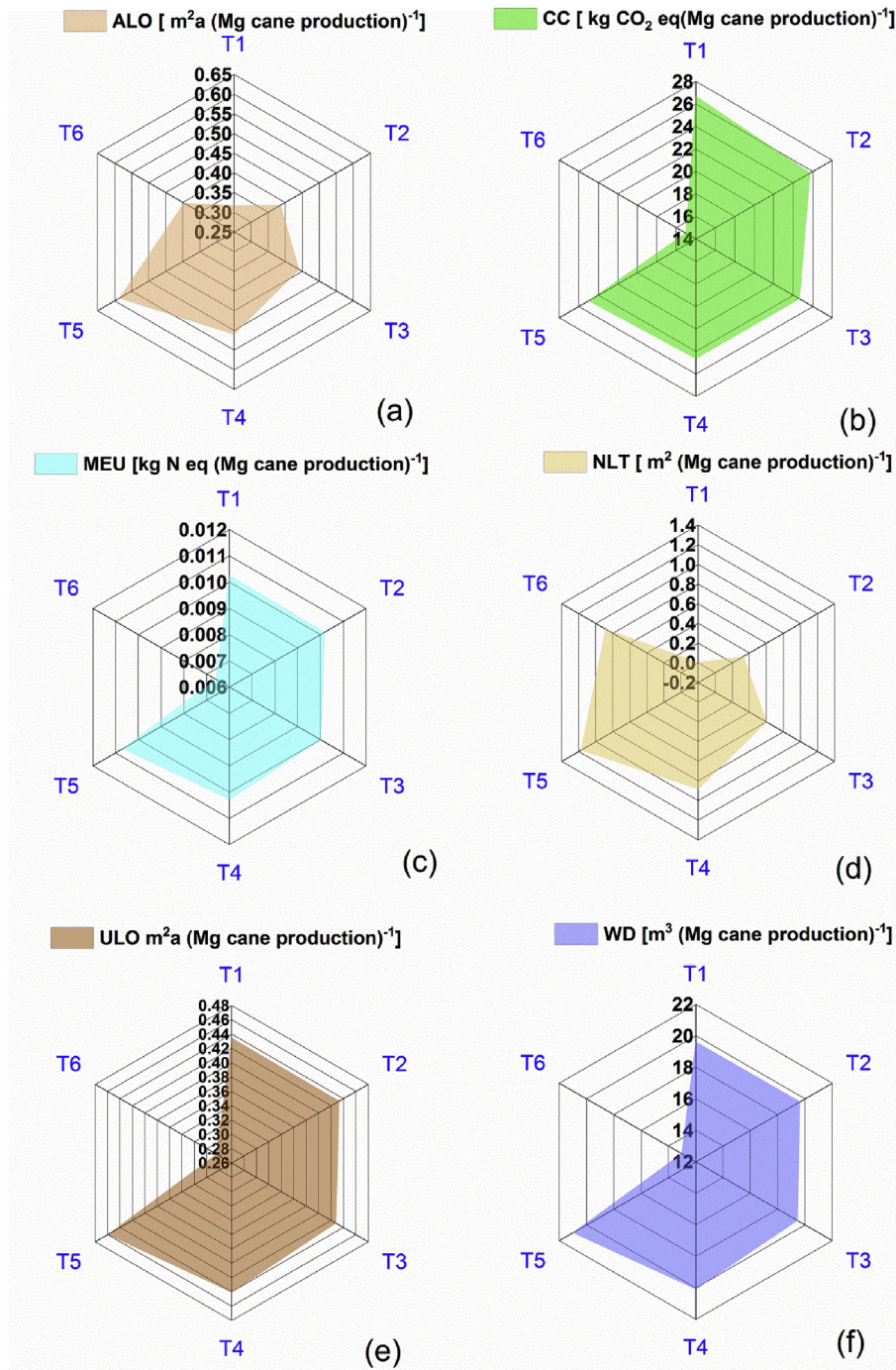
Emissions from synthetic N fertilizers.

Emissions	Values (kg N <sub>2</sub> O 2y <sup>-1</sup> )	CO <sub>2</sub> equivalents (kg CO <sub>2</sub> 2y <sup>-1</sup> )
Direct N <sub>2</sub> O <sub>direct</sub> emissions	2.436	726
Indirect N <sub>2</sub> O <sub>ATD</sub> emissions	0.530	158
Indirect N <sub>2</sub> O <sub>I</sub> emissions	1.192	355
CO <sub>2</sub> emissions from Urea	–	500
Total		1739

increase cane production in the plant crop it was not in true in ratoon wherein a minimum threshold concentration of at least 5% was required for yield enhancement. This enhancement of yield was primarily led by an increase in cane length, cane weight and NMC. This is further supported by the strong correlation between yield and cane length, weight and millable cane numbers (correlation data not shown). Notably, the KSWE treated plants showed improvement in the various growth and physiological parameters relative to control starting right from the moment foliar application was initiated but only at an optimal concentration. This concentration was found to be 5% KSWE when used along with full dose of RRF under the given conditions. Economic analysis too supported the above fact that application of KSWE only at 5% would fetch more returns. Further higher concentrations of KSWE that were tested did not influence growth and productivity relative to the optimal dose as they remained statistically insignificant although these concentrations improved all the tested parameters relative to control. Similar findings were reported in rice (Sharma et al., 2017) where in the yield enhancement relative to control was observed at concentrations equal to or higher than 10% with the optimum concentration being 10%. Pre-treatment of setts resulted in stimulation of its germination leading to early establishment of the tillers. Higher number of tillers in KSWE treatments culminated in the increase in the number of millable canes. The increase in plant height directly resulted in a concomitant increase in the cane length. In addition, KSWE application also resulted in maximizing the leaf area per plant indicating faster canopy development which is critical in determining yield (Sinclair et al., 2004). The higher leaf area formed during active growth stages and the resultant LAI could have increased interception of solar radiation leading to the formation of higher photosynthates consequently leading to higher DMA in KSWE treated plants in both plant and ratoon crops. This relationship between LAI and biomass accumulation in sugarcane is well documented in literature (Robertson et al., 1996; Sandhu et al.,

2012). Further, leaf transcriptome analysis of maize plant (Trivedi et al., 2016), a species belonging to the same family as sugarcane, when subjected to foliar spray of KSWE revealed induction of biological processes with significant changes in photosynthesis and starch biosynthesis related genes which might have led to enhanced biomass production. Similar phenomenon might have also occurred in sugarcane which led to increase in DMA. In addition to enhancing various growth parameters, KSWE in combination with RRF also facilitated the uptake of the nutrients such as N, P, K and S from the soil for supporting the growth. This is evident in the case of N where the N content increased in KSWE + RRF treatments. The pronounced increase in N content and its uptake might have been due to increase in nitrate transporters and nitrate reductase activity in roots as well as increased expression of nitrogen assimilation related genes in leaves similar to those observed in maize plants treated with KSWE (Ghosh, 2016). Although the exact mechanism by which the seaweed extracts enhance growth and productivity still needs to completely deciphered (Arioli et al., 2015) but there is intense speculation that the various active ingredients such as plant growth regulators (Blunden and Wildgoose, 1977), betaines (Trivedi et al., 2018), phenolics (Rengasamy et al., 2015), micro- and macro-nutrients of the pristin extracts and additives such as humic acids either alone or in a synergistic manner may be responsible for the bioactivity.

There is no evidence till date that seaweed based biostimulants or for that matter any plant biostimulant would entirely supplement for N and other mineral requirements for improving productivity of sugarcane. However, the most intriguing result of the present investigation was that KSWE when applied in combination with 50% RRF (T6) showed yield (cane yield) parity relative to control (100% RRF) at least in ratoon crop. Analyses of the results reveal that this has been primarily caused by maintenance of plant height, similar tiller counts and hence the number of millable canes between control and T6 treatments. Although, most of the growth,



**Fig. 5.** Various environmental impacts expressed in tonnes per cane production in the treatments involving KSWE in combination with synthetic fertilizers. Labels of treatments are the same as in Table 3. ALO – agricultural land occupation; CC- climate change; MEU- marine eutrophication; NLT-natural land transformation; ULO- urban land occupation; WD- water depletion.

physiological parameters, yield and yield attributes in the T6 treatment were significantly lower in plant crop, analyses of variance at a higher probability level such as 10% could have easily resulted in parity with control in most of the parameters that were evaluated even in plant crop. Interestingly, the commercial cane sugar production in both plant and ratoon crops were similar in control and lower dose treatment. This relationship between yield and a sustainable combination of input assumes significance as a mere reduction of 7.9% in cane yield results in a 50% savings in synthetic fertilizer inputs and thus can serve as a fertilizer

supplement. Notably, there was no detrimental effect on sugar accumulation.

Agriculture is one of the major contributors of GHGs. Among the various crops, sugarcane flooded rice and potato cultivation contribute to bulk of the GHGs at least in India (Vettera et al., 2017). It is perceived that the former's contribution is predominantly due to high input requirement (Thorburn et al., 2011) increased mechanization etc. (Cardozo et al., 2016). Production of synthetic fertilizers does entail GHG emissions. In addition, synthetic N fertilizers further contribute to field GHGs emissions following their

application (Carmo et al., 2013). On the contrary, KSWE production is much eco-friendly (Ghosh et al., 2015) and does not entail any field emissions. Furthermore, as the biostimulant is of marine origin, it would ease considerable pressure on land use change which contributes to greater proportion of GHGs (Anand et al., 2018; Smith et al., 2013) thus easing supply side pressures. Thus, the use of KSWE in sugarcane cultivation does have a tremendous potential to lower green-house gas (GHG) emissions by supplementing for synthetic fertilizer input. This apparently can be seen with the hypothetical scope of saving 260 kg per Mg cane production in 1 ha of land area while using KSWE at 5% concentration in conjunction with RRF. This would translate to savings of ca. 9.3 million Mg of CO<sub>2</sub> eq if one assumes employing KSWE for at least 10% of the total cane production in India for 2015–16. The impact could have far reaching consequences as adoption of this agro-technique would lessen the environmental burden considering Government of India's voluntary commitment to reduce the GHG emission intensity of Gross Domestic Product (GDP) by 20–25%, with the exception of the agriculture sector (UNFCC, 2015). The reductions in GHGs would be dramatic if one considers lower rate of fertilizer application (50% RRF) and implementation of the strategy at the global scale. The benefits of SWE use is not only restricted to gains in the climate change category but also to other environmental impact categories. The other prominent being water depletion wherein similar estimation would result in savings of 1.12 billion cubic meters of water.

## 5. Conclusion

The results of the study unequivocally prove the benefits of using biostimulant towards sustainably increasing sugarcane productivity by an average of 10% when applied at the optimum rate of 5%. Not only was the cane yield improved but the sugar yield too was enhanced on account of KSWE treatments. Thus we recommend the use of *Kappaphycus* seaweed extracts concomitantly with RRF to bridge gaps between potential and real yield or with a low rate of RRF for obtaining targeted yield so that one can expect a better rate of returns. In addition, use of KSWE in sugarcane cultivation because of its practical feasibility can serve as an excellent mitigation strategy for overcoming the challenges of trade-offs between increasing cane productivity and maintaining environmental integrity by offering sustainable solutions. This mitigation would also ease the pressure of developing countries like India and leverage negotiations at UNFCC for adapting agricultural management strategies for reducing GHGs. It is advocated that any sustainable solution for enhancing crop productivity should weigh on quantifying the environmental benefits prior to their recommendation at the policy level itself so as to allow informed decision by the stake holders. It also prompts a paradigm shift to look at the seaweed based biostimulants for their potential towards mitigating climate change.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jclepro.2018.09.070>.

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