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Diatomite and Rice Husk Biochar as Silicon Source and its Effect on Yield of Wetland Rice

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Field experiments were carried out during *summer* and *kharif* of 2015 and *kharif* of 2016 with and without application of silicon (Si) under rice cropped and non-cropped condition. Diatomite and rice husk biochar was used as Si source and applied @ 300 kg ha^{-1} and 2 t ha^{-1}, respectively. The continual application of Si increased the straw and grain yield consistently compared to control. Straw and grain Si content and its uptake also increased with application of Si but not significantly differed. Treatment under the cropping situation recorded higher soil pH. Although, there was no significant difference in electrical conductivity (EC) among the treatments, post-harvest soils recorded higher values than initial soil. Plant available Si content in post-harvest soil was significantly higher in control where no crop was grown with or without added Si. However, in the presence of rice crop, soil Si content decreased and/or was on par with the other treatments.

Key words: Diatomite, rice husk biochar, silicon, rice, growth, yield

Silicon (Si) is the second most abundant element on the surface of the earth and silicon dioxide (SiO_2) comprises 50-70% of the soil mass. As a consequence, all plants rooting in soil contain some Si in their tissues. The role of Si in plant growth and development was overlooked until the beginning of the 20th century because of the abundance of the element in nature and because visible symptoms of either Si deficiency or toxicity are not apparent. However, repeated cropping and the constant application of chemical fertilizers have depleted the amount of Si that is available to plants in the soil. An awareness of Si deficiency in soil is now recognized as being a limiting factor for crop production, particularly in soils that are deemed to be low or limiting in plant available Si and for known Si accumulating plants such as rice and sugarcane. Silicon is not recognized as an essential element for plant growth but the beneficial effects on the growth, development, yield and disease resistance have been observed in a wide variety of plant species (Ma *et al.* 2006). Among the crops, rice is considered as a typical Si accumulator and absorbed by plants as monosilicic acid (H_4SiO_4). Silicon can be taken up by rice at a rate as high as 500 kg Si ha^{-1} yr^{-1} (Makabe *et al.* 2009). Production of 5 t ha^{-1} of grain yield of rice is estimated to remove about 230-470 kg elemental Si from soil, depending upon soil and plant factors. Absorption of Si will be about 108 per cent more than the nitrogen (N) content. Adequate supply of Si to rice from tillering to elongation stage increases the number of grains per panicle and the percentage of ripening (Korndorfer *et al*. 2001).

Most of the traditional rice fields of the world are low in plant available Si and addition of Si is reported to improve the rice yield (Liang *et al.* 2015). This emphasize the need for identifying an ideal Si source for field application, which can improve soil Si status, plant available Si and sustain rice production. Silicon sources for agricultural use range from chemical products to natural minerals to byproducts of steel and iron industries. All these products are shown to be effective in improving crop growth and yield. In general, their usefulness depends on the reactivity rather than total Si content (Haynes 2014). But, for field application an ideal Si source should possess attributes like local availability, cost effectiveness, easy to handle, improve plant available

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Si and its bioavailability, environment friendly and improve crop growth and yield. Also, for Si sources to be most effective as a fertilizer, it is essential to acquire knowledge of physical and chemical characteristics of Si sources and to understand the extent of bioavailability from sources for adequate plant uptake.

Most commonly used Si source is silicate slag mainly because of its cost effectiveness and small amount of easily soluble Si content. These slags may contain hazardous levels of heavy metals. On the other hand, silicate minerals such as wollastonite ($CaSiO_3$) are characterized by high price and limited mineral reserves and don't have the ability to combine beneficial effects other than Si amendments such as the increase of soil fertility and productivity (Houben *et al.* 2014). Hence, naturally occurring material and/or plant derived Si sources are necessary for sustainable agriculture.

Diatomite or diatomaceous earth (DE) refers to sedimentary rocks that result from the deposition of Si rich unicellular life forms known as diatoms. Diatoms are aquatic algae and found in both salt and fresh water. Fresh water DE deposits are rich source of Si. Silicon is main constituent of the diatom frustules and material has high plant available Si, highly porous in structure, low density, high surface area and cation exchange capacity (CEC) for retaining nutrients in soil. Rizwan *et al.* (2012) showed that DE application might reduce the toxicity of Cd in wheat and attributed this effect to Si, which induced a decrease in available Cd in the soil. At the plant level, a decrease of Cd translocation to shoots was reported. In a study conducted by Priya *et al.* (2015) on potato by using DE as Si source recorded significantly higher results for growth and yield parameters of the crop at 60 days after planting. Similar study was also carried out by Kumbargire *et al*. (2016) on banana highlighted significant effect of DE on increasing the nutrient content and thereby improving the yield and quality of the crop. Crooks and Prentice (2016) and Pati *et al*. (2016) showed that application of DE as Si fertilizer significantly increased the yield of rice crops in India. Pyrolyzed crop residues are found to contain appreciable amount of plant available Si (Xin *et al*. 2014). Biochar is any source of biomass previously heated in the absence or at low concentration of oxygen with the purpose of application to the soil (Maia *et al.* 2011). Interestingly, depending upon feedstock and burning temperature, biochar may contain significant amount of Si. Liu *et al*. (2014) demonstrated role of biochar to improve silicon availability and uptake by rice in acid and slightly acidic soil. Varying with site condition, plant available Si content of soil increased significantly with biochar application. Significant increase in rice shoot Si was detected in four out of six sites, which was well correlated to concurrent increase in soil pH and direct addition from biochar. Xin *et al*. (2014) studied transformation and dissolution of Si from Si-rich biochar. Biochars are prepared from rice straw at different pyrolysis temperature ranging from 150 to 700 °C. Available Si content in soil released in the form of silicic acid, is an important nutrient for growth of silicophilic plants such as rice. Among the biochars, rice husk biochar can be a good source of Si.

The continual application of any Si source should not limit the growth and performance of rice crop and soil quality. In this view, field experiments were conducted to know the effect of diatomite and rice husk biochar as Si sources on growth and yield of rice for three consecutive seasons in same experimental field, besides understanding long-term effects of their application.

Materials and Methods

The soil of experimental plot was neutral in reaction with sandy loam in texture. Acetic acid and calcium chloride extractable Si was medium in range. Available N and potassium (K) content of the soil was medium in range whereas available phosphorus (P) was very high. Secondary nutrients were higher than the critical limit (Table 1).

Execution of the experiment

Field experiments were carried out at C- Block, ZARS, V. C. Farm, Mandya, Karnataka, India during *summer* (March-June) and *kharif* 2015 (August-

Table 1. Initial properties of experimental soil

Parameters		Content
pH (1:2.5 soil : water)		7.10
EC (dS m^{-1}) (1:2.5 soil : water)		0.22
Organic carbon (g kg^{-1})		11.7
Particle size distribution (%)	Sand	76.5
	Silt	6.6
	Clay	16.9
Textural class		Sandy loam
0.01*M* $CaCl_2$ – Si (mg kg^{-1})		42.0
0.5*M* Acetic acid – Si (mg kg^{-1})		73.8
Available N (kg ha^{-1})		340
Available P_2O_5 (kg ha^{-1})		203
Available K_2O (kg ha^{-1})		348
Exch.Ca [cmol(p^+)kg^{-1}]		4.75
Exch. Mg [cmol(p^+)kg^{-1}]		2.25

November) and *kharif* 2016 (August-November). Land was ploughed twice followed by puddling and leveling under saturated moisture content, layout of the field was carried out as planned dimensions. Each plot size was 20 m^2 (4 m × 5 m) demarcated by 0.5 m bunds and 1 m furrow to prevent lateral entry of water from one plot to another. Flow of water from one treatment plot to another treatment plot was avoided to a greater extent by irrigating to each plot separately with pipes. Without disturbing the layout of the experiment second and third experiments were carried out during *kharif* 2015 and 2016 by adopting same set of treatments in same plots by ploughing and leveling of the individual plot manually.

Details of the treatments and fertilizer application

Experiment was conducted with combination of with (Si_1) and without Si (Si_0) and with rice plant (RP) and without rice (RP_0) (having four treatments *viz.,* Si_0 + RP_0 (Bare); Si_0 + RP (rice); Si_1 + RP_0 (Bare + Si); Si_1+ RP (rice + Si) replicated four times. Twenty one-days-old rice seedlings of variety Tanu (KMP-101 130-135 days duration) were transplanted with a spacing of 20 cm ×10 cm and standing water was maintained to achieve submergence condition. Recommended dose of fertilizers (125: 62.5: 62.5 $N:P_2O_5:K_2O$ kg ha^{-1} in *summer,* 100: 50: 50 $N:P_2O_5:K_2O$ kg ha^{-1} during *kharif*) were applied as per package of practice. Recommended dose of P_2O_5 (single superphosphate, SSP), half dose of recommended K_2O (muriate of potash, MOP) and N (urea) were applied prior to transplanting. Remaining N and K_2O were given as two splits at 30th and 60th day after transplanting.

Two Si sources used in the study were diatomaceous earth or diatomite (DE) and rice husk biochar (RHB). Diatomite is a sedimentary rock which was used for field experiment having 30% Si and RHB was produced at relatively lower temperature at around 400 °C and contains 31% Si. Diatomite was applied @ 300 kg ha^{-1} during *summer* and *kharif* 2015, whereas RHB @ 2 t ha^{-1} during *kharif* 2016. Silicon sources were applied as basal dosage along with the fertilizers. Compositions of DE and RHB are provided in table 2.

Grain and straw yield was recorded at the time of harvesting. Plant samples were collected from field after the harvest of crop and washed with deionised water and were dried in an oven at 70 °C, powdered and analyzed for total Si content. The Si uptake by the crop was computed using Si content and biomass yield and expressed as kg ha^{-1}. After the harvest of the crop, surface soil samples were collected plot-wise using screw auger, air-dried, powdered, sieved with 2-mm sieve and analyzed for pH, EC, 0.5 *M* acetic acid and 0.01*M* $CaCl_2$ extractable Si.

Table 2. Composition of Si sources

Properties	DE	RHB
pH (1:2.5 soil : water)	9.21	7.39
EC (dS m^{-1}) (1:2.5 soil : water)	0.72	1.62
Cation exchange capacity [cmol(p^+)kg^{-1}]	52.0	38.6
N (%)	0.03	0.78
P (%)	0.02	0.24
K (%)	0.40	0.96
Si (%)	30.0	31.0
Ca (%)	2.70	0.36
Mg (%)	3.25	0.31
S (%)	0.17	0.05
Al_2O_3 (%)	15.3	n.d
Fe (mg kg^{-1})	2.00	0.077
Mn (mg kg^{-1})	0.02	0.055
B (mg kg^{-1})	6.00	8.36
Zn (mg kg^{-1})	19.0	63.0
Cu (mg kg^{-1})	20.0	31.0
Mo (mg kg^{-1})	0.10	n.d

DE – Diatomite; RHB – Rice husk biochar; n.d – not determined

Analysis of soil and plant samples

Soil analysis

Soil pH and EC was measured in a suspension of 1:2.5 soil: water ratio. Soil textural class was determined by following International Pipette method (Jackson 1973), available N was determined by alkaline potassium permanganate method (Subbiah and Asija 1956). Estimation of available P_2O_5 of soil was by following Olsen method (Olsen *et al*. 1954). Available K and exchangeable Ca, Mg were extracted by using 1*N* ammonium acetate. Potassium was analyzed by using flame photometry (Jackson 1973) and exchangeable Ca, Mg was determined by complexiometric titration method (Baruah and Barthakur 1997).

0.01M $CaCl_2$ extractable Si

Soil sample (2 g) was taken in a 50 mL centrifuge tube and 20 mL of 0.01*M* $CaCl_2$ was added. After continuous end to end shaking in a mechanical shaker for 16 h, the solution was centrifuged at 2000 rpm for 10 min and then filtered.

Silicon in the extracting solution was determined by transferring 1 mL of filtrate into plastic centrifuge tube and then 2.5 mL of 0.5 *M* sulphuric acid and 2.5 mL of ammonium molybdate solution (pH 7) was added. After vortex stirring for 5 min, 1.25 mL of tartaric acid solution was added. After allowing for additional 2 min, 0.25 mL reducing agent (amino napthol sulphonic acid, ANSA) was added. After 5 min, but not later than 30 min following addition of the reducing agent, absorbance was measured at 820 nm using UV visible spectrophotometer (Shimadzu). Simultaneously, Si standards (0, 0.5, 1, 2, 3, 4, 5 and 6 mg L^{-1}) prepared in the same matrix were also measured using UV visible spectrophotometer (Haysom and Chapman 1975).

0.5 M acetic acid extractable Si

Available Si in soil was extracted using 0.5 *M* acetic acid with the soil to extractant ratio of 1:2.5 as outlined by Korndorfer *et al.* (2001). After shaking continuously for a period of 1h, solution was centrifuged at 3000 rpm for 3 min and then filtered. The filtrate was then used for Si determination. Silicon in the extracting solution was determined by adopting the procedure of Narayanaswamy and Prakash (2009).

An aliquot of 0.25 mL filtrate was taken into a plastic centrifuge tube and then added with 10.5 mL of distilled water, plus 0.25 mL of 1:1 hydrochloric acid, and 0.5 mL of 10% ammonium molybdate solution. After allowing for 5 min, 0.5 mL of 20% tartaric acid solution was added. After allowing for additional 2 min, 0.5 mL reducing agent (ANSA) was added. After 5 min, but not later than 30 min following addition of the reducing agent, absorbance was measured at 630 nm using UV-visible spectrophotometer (SHIMADZU Pharma spec, UV-1700 series) with auto sample changer (ASC-5). Simultaneously, Si standards (0, 0.2, 0.4, 0.8, 1.2 and 1.6 mg L^{-1}) prepared in the same matrix were also measured using UV-visible spectrophotometer.

Silicon analysis in straw and grain

The powdered grain and straw samples were dried in an oven at 70 ºC for 2-3 h prior to analysis. The sample (0.1 g) was digested in a mixture of 7 mL of HNO_3 (70%), 2 mL of H_2O_2 (30%) and 1 mL of HF (40%) using microwave digestion system (Milestone-start D) with following steps: 1000 watt for 17 min, 1000 watt for 10 min and venting for 10 min. The digested samples were diluted to 50 mL with 4% boric acid.

The Si concentration in the digested solution was determined by transferring 0.5 mL of digested aliquot to a plastic centrifuge tube, to this 3.75 mL of 0.2 *N* HCl, 0.5 mL of 10% ammonium molybdate $(NH_4)_6Mo_7O_2)$ and 0.5 mL of 20% tartaric acid and 0.5 mL of reducing agent (ANSA) was added and the volume was made up to 12.5 mL with distilled water. After 1h, the absorbance was measured at 600 nm with spectrophotometer. Similarly, standards (0, 0.2, 0.4, 0.8 and 1.2 mg L^{-1}) were prepared by following the same procedure.

Statistical analysis

Data generated were statistically analyzed using XLSTAT software via one-way ANOVA by using Fisher test at $p \leq 0.05$ with multiple observations.

Results and Discussion

Straw and grain yield

Application of DE as Si source @ 300 kg ha^{-1} increased straw (11.67 ± 0.75 t ha^{-1}) and grain (6.98 ± 0.3 t ha^{-1}) yield over control but found to be significant only with grain yield during *summer* 2015. During *kharif* 2015, a significantly higher straw yield recorded (10.30 ± 0.71 t ha^{-1}) compared to control (7.96 ± 0.77 t ha^{-1}). However, there was no significant difference in grain yield among the treatments. Application of RHB as Si source @ 2 t ha^{-1} recorded higher straw (10.44 ± 1.72 t ha^{-1}) and grain (6.47 ± 0.40 t ha^{-1}) yield compared to control during *kharif* 2016. Interestingly, there was a sustainable increase in grain and straw yield in the plots (Fig. 1) with continuous application of Si sources (DE and RHB). These results corroborate with the findings of Singh *et al.* (2006) who have shown that Si affects floral fertility and as such an increase in the number of filled spikelets and consequently number of grains and grain weights. According to Agarie *et al.* (1992) maintenance of photosynthetic activity due to Si fertilization could be one of the reasons for the increased dry matter production. Increase in the crop growth and yield attributes by the application of Si was also in agreement with many workers and in accordance with the present investigation. Similarly, Prakash *et al.* (2007) reported that rice-hull ash can be used to improve the plant-available Si status of soil for enhanced growth and yield of rice in coastal soils of Karnataka.

Straw and grain Si content

Data pertinent to the straw and grain Si content as affected by application of DE and RHB as Si source is given in fig. 2. Diatomite and RHB application increased the Si content of rice straw and grain.

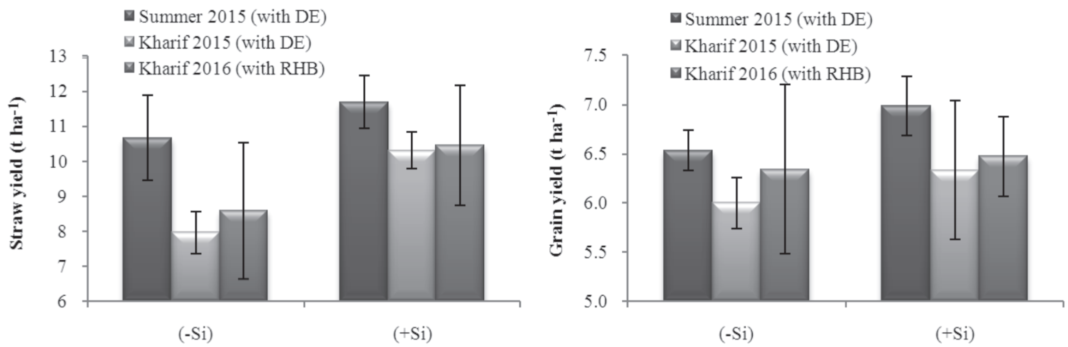

Fig. 1. Effect of silicon sources on straw and grain yield of rice (diatomite during *summer* 2015 and *kharif* 2015 and rice husk biochar during 2016)

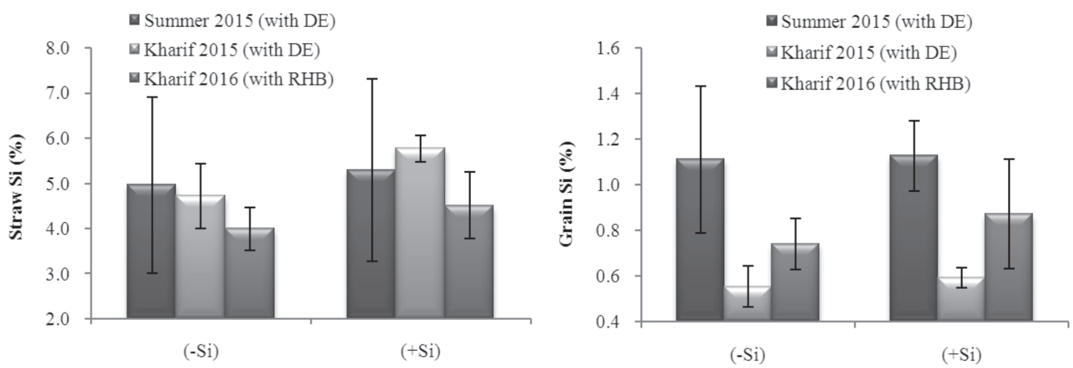

Fig. 2. Effect of silicon sources on straw and grain silicon content of rice (diatomite during *summer* 2015 and *kharif* 2015 and rice husk biochar during 2016)

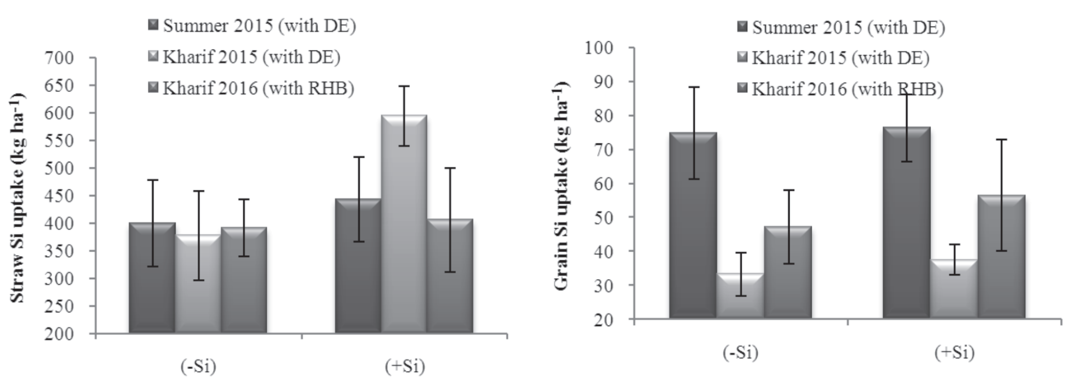

Fig. 3. Effect of silicon sources on straw and grain silicon uptake of rice (diatomite during *summer* 2015 and *kharif* 2015 and rice husk biochar during 2016)

However, straw (3.99-5.77%) and grain (0.55-1.13 %) Si content varied between the seasons which might be due to unreasonable extraneous factors.

Straw and grain Si uptake also increased with application of DE and RHB compared to control (Fig. 3). Rice is a typical Si-accumulating plant and Si can make up for 10 per cent of the shoot dry weight, which is several fold higher than those of essential macronutrients such as N, P and K (Ma and Takahashi 2002).

A significant variation in content and uptake of Si in rice straw in the present investigation may be due to distribution of silicic acid in the shoots in relation to transpirational termini and deposition in the plant parts. Deren *et al.* (1994) noticed increased Si concentration in plant tissue with increasing rate

Table 3. Effect of diatomite and rice husk biochar on pH (1:2.5 soil: water) and electrical conductivity of post-harvest soil

Treatments	*Summer 2015		*Kharif 2015		**Kharif 2016	
	pH	EC (dS m^{-1})	pH	EC (dS m^{-1})	pH	EC (dS m^{-1})
Bare	7.62 ± 0.18 (b)	0.24 ± 0.02 (a)	7.59± 0.29 (a)	0.12± 0.01 (c)	7.57± 0.67 (a)	0.24± 0.05 (b)
Rice	7.90 ± 0.19 (a)	0.21 ± 0.04 (a)	7.74± 0.34 (a)	0.18± 0.03 (a)	8.07± 0.10 (a)	0.31± 0.06 (a)
Bare + Si	7.93 ± 0.13 (a)	0.19 ± 0.06 (a)	7.84± 0.16 (a)	0.14± 0.03 (bc)	7.84± 0.05 (a)	0.24± 0.04 (b)
Rice + Si	7.95 ± 0.21 (a)	0.21 ± 0.04 (a)	7.70± 0.30 (a)	0.17± 0.02 (ab)	8.06± 0.11 (a)	0.31± 0.03 (a)

*Diatomite as Si source; **Rice husk biochar as Si source
Mean value having same alphabets in parentheses did not differ significantly at $p \leq 0.05$

Table 4. Effect of diatomite and rice husk biochar on available Si content of post-harvest soil

Treatments	*Summer 2015		*Kharif 2015		**Kharif 2016	
	CCSi	AASi	CCSi	AASi	CCSi	AASi
	(mg kg^{-1})					
Bare	46.2 ± 9.0 (a)	71.9 ± 6.8 (b)	36.6 ± 7.9 (a)	104.1 ± 21.0 (a)	70.2 ± 13.7 (a)	191.0 ± 48.2 (a)
Rice	19.6 ± 3.4 (c)	58.5 ± 15.5 (b)	22.5 ± 2.8 (b)	91.2 ± 13.8 (a)	39.4 ± 5.6 (b)	175.2 ± 16.8 (a)
Bare + Si	35.8 ± 4.7 (b)	96.1 ± 9.8 (a)	32.8 ± 13.8 (ab)	107.5 ± 13.0 (a)	65.8 ± 22.7 (ab)	197.7 ± 19.3 (a)
Rice + Si	17.8 ± 4.8 (c)	57.4 ± 16.6 (b)	32.0 ± 7.9 (ab)	92.3 ± 18.9 (a)	58.8 ± 29.9 (ab)	174.2 ± 23.9 (a)

*Diatomite as Si source; **Rice husk biochar as Si source
Mean value having same alphabets in parenthesis did not differ significantly at $p \leq 0.05$

of Si fertilization, but within each Si treatment and that of control, there was significant variation in Si concentration and its uptake among different cultivars.

pH and EC of post-harvest soil

Soil pH and EC in post-harvest soil was varied significantly among the treatments (Table 3). There was a significant difference in soil pH between absolute control and rest of the treatments. The treatment under the cropping situation recorded higher soil pH (7.95 ± 0.21) along with the DE application. Increase in the EC of post-harvest soil compared to initial soil was noticed in the present investigation. During *kharif* 2015 and 2016, soil pH did not differ significantly among the treatments but there was a significant difference in soil EC. In general, higher EC values were recorded under cropping situation than without crop. Increase in soil pH as a result of submergence is well known (Ponnamperuma 1972) and attributed to the consumption of protons during reduction processes. Increase in the EC of soil due to increase in the solubility of the salts present in the soil and also salts contributed by Si source.

Available Si content of post-harvest soil

In general, plant available Si as extracted by 0.01 *M* $CaCl_2$ and 0.5 *M* acetic acid was found to be lower in presence of crop irrespective of with or without Si application (Table 4). During *summer* 2015, calcium chloride extractable Si (CCSi) content was significantly higher in control where no crop was grown with (35.82 ± 4.71 mg kg^{-1}) or without (46.24 ± 9.03 mg kg^{-1}) added Si. Acetic acid extractable Si (AASi) (96.19 ± 9.87 mg kg^{-1}) increased significantly with application of DE @ 300 kg ha^{-1} where no rice was grown, whereas Si content decreased in the presence of crop even though the DE was applied. In *kharif* 2015, CCSi was significantly affected and found to be highest in control (36.61± 7.95 mg kg^{-1}) and decreased significantly in the presence of crop either with or without DE application. Whereas, AASi content did not differ significantly among the treatments. However, during *kharif* 2016, Si content was found to be higher compared to previous two seasons. The CCSi was found to be highest in control (70.27 ± 13.77mg kg^{-1}) and decreased in the presence of crop. However, AASi content did not differ significantly among the treatments. The processes could be predicted for explaining decrease in Si content in soil may be crop (Keeping 2017) /algal uptake (Opfergelt *et al.* 2011) and/or adsorption on to ferrihydrite (Delstanche *et al.* 2009).

Conclusions

Application of DE and RHB as Si source increased the straw and grain yield consistently in all three seasons. Straw and grain Si content and uptake also increased with application of Si but greatly varied among seasons. Plant available Si content (CCSi and AASi) in post-harvest soil decreased in presence of crop with or without Si application indicate higher requirement of Si for rice crop. In spite of continuous application of Si sources, there was increase in the straw and grain yield, which indicated requirement of Si supplementation in wetland rice. Both DE and rice husk biochar can be effectively used as Si sources for sustainable rice farming.

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