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SHORT COMMUNICATION

Impact of co-application of biomethanated spent wash with bio-char on soil micro-nutrients and crop growth

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Abstract

Biomethanated spentwash (BMS) and biochar are novel soil conditioners considered as value addition to the bi-products from agro-industry. The high nutrient content and organic matter of BMS and high retention of nutrient and organic carbon on biochar has potential of benefits for co-application. We studied the effect of BMS, biochar and their co-application on soybean, which is a common rotation crop in Sugarcane. A field experiment was conducted in randomized block design in vertisols. BMS was produced at Ugar sugar works ltd. and biochar from agriculture waste. Treatments comprised of BMS application@ 60, 66 and 72 m3 ha-1, as sole amendment and in combination with biochar @ 2.5 t ha-1. Post-harvest soil analysis demonstrated a non-significant effect of treatments on soil pH, electrical conductivity and micro-nutrients. However, available Zn, Fe and Mn were numerically increased for all BMS treatments. BMS and biochar or their co-application had no significant effect on germination and plant height of soybean. However the number of soybean pods and grain yield was significantly improved. Grain yield of soybean in BMS 60 m3 ha-1 plus biochar co-applied treatment was significantly higher than all other treatments. Grain yield of BMS 66 m3 ha-1 and other BMS plus BC treatments were at par with each other and significantly higher than sole biochar 2.5 t ha-1, RDF and control.

Keywords: Biomethanated spentwash; Biochar; Vertisol; Sugarcane-soybean rotation

Sugarcane is an important cash crop cultivated over 4.2 million hectare (Mha) land in India with a production of 348 million tons (MT). The 532 operational sugar factories are important component of agro-industries in India. Likewise, distilleries in India process as much as 13.8 MT molasses, generating 40 billion liters of spent-wash annually (Biswas et al. 2017; ISMA, 2020). In recent years, wide row planting is being adopted to increase sustainable production of sugarcane. The row spacing and initial slow growth period of 100 – 120 days (Chogatapur et al. 2018) provides opportunity for a short duration crop in sugarcane. Soybean is a promising rotation crop because of its ability of biological nitrogen fixation (Wang et al. 2020). Incorporating soybean as a rotation crop in sugarcane has significant increase in the net land productivity (Chagas et al. 2016; Fituma and Argaw 2019). Soybean is also an important oilseed crop, with annual cultivation over 10.3 Mha, production of 10.9 MT and in addition, soybean oil import of 3.15 MT (DAC&FW, 2019). However, harnessing the benefit of sugarcane-soybean cropping system is restricted by low soybean productivity of 1.05 tha-in India. On the contrary, the rainfed yield potential of soybean is much higher at 2.1 t/ha. Declining soil fertility is one of the major reasons for low crop productivity (Agarwal et al. 2013).

Disposal of spent-wash is an important concern of sugarcane agro-industry. It has high nutrient content and organic matter and therefore is applied as fertigation either directly or after bio-methanation. Application of biomethanated spentwash (BMS) to soil results in improvement of soil nutrients, organic matter and physical properties (Kamble et al. 2017). Studies have reported positive effects of BMS application on mustard yield and attributed its high N, P, K and S content to improved plant physiology, seed yield and quality (Kumari et al. 2015). Application of BMS mainly results in incorporation of readily soluble nutrients and biodegradable organic matter, which is gradually subjected to leaching and microbial degradation.

Biochar is a novel material that has received widespread attention in the past two decades. It is produced from pyrolysis of biomass including wastes from agriculture and agro-industries. It has a highly recalcitrant structure, high porosity and a small fraction of labile carbon and nutrients. Studies have reported significant crop yield improvement with application of biochar (Xu et al. 2015) and attributed to high retention of nutrients, organic carbon and moisture (Lehmann et al. 2007).Therefore, the effect of biochar in combination with various organic fertilizers has been studied and reported in the published literature (Lashari et al. 2014; Mekuria et al. 2014; Xie et al. 2013). However, studies on combined application of biomethanated spent-wash and biochar in crop are scarce, especially in context of soil micro-nutrients. With this background the effect of BMS, biochar and their co-application on soybean yield was studied. Both sugarcane and soybean are widely cultivated in vertisols. Productivity of these soils is restricted by poor physical properties under moist conditions, low organic carbon (Virmani et al. 1982), low nutrient status and declining fertility (Behera et al 2007). In context of vertisols, divergent results of biochar application were reported, with significant yield improvement (Bayyinah et al. 2017), non-significant effect (Macdonald et al. 2014) as well as negative effect on crop yield (Nguyen et al. 2016).

The evidence from the published literature suggests beneficial effects of co-applying biomethanatedspentwash BMS and biochar. Soybean is a short duration crop in sugarcane – soybean cropping system. Studying the effect of BMS - biochar application on soybean crop would serve as an indicator for its potential effects in subsequent crop. In this present study, effect of biomethanatedspentwash (BMS) produced at Ugar Sugar works and agri-waste biochar (BC) and was studied on soybean. The effect of BMS and BC on soil carbon and macro-nutrients are well reported. Our study emphasized on the effect on soil micro-nutrients, crop yield.

Materials and Methods

Biochar production

Biochar was produced by using a mixture of crop residue and waste wood as a feedstock. Pyrolysis of the feedstock was performed as slow pyrolysis, in a temperature range of 450º C to 600º C. A vertical kiln type reactor with a brick lining was used for the production. The feedstock was shredded into pieces for use in the production process. Cooled biochar lumps were crushed and sieved through 4 mm sieve prior to storage for use. (Information obtained from manufacturer - BIOSAT Anulekh Agrotech Pvt. Ltd.).

Description of experimental site and agro-climate

The experimental plot was located 16°38’34” N latitude and 74°49’57” E longitude, at Ugar sugar works farm, in Belagavi district, Karnataka, India. A semi-arid, hot and dry climate over the region with a mean annual precipitation of 630 mm. As per NARP-ICAR agro-climatic zoning, this region is classified as scarcity zone. Experimental plot had a clayey soil, classified as Vertisol (FAO, 1974), with a pH of 7.87, deep profile depth and referred as black soil as per local nomenclature. The experimental plot had a long history of sugar-cane cultivation, along with soybean as intercropping in recent years. The field received annual application of 100 kg/ha di-ammonium phosphate, 75 kg/ha muriate of potash and 5 tons compost every 3 years. Detail chemical analysis of the soil at experimental site before start of the experiment is provided in Table 1. The present experiment was conducted during 2017.

Soil and biochar analysis

Soil analysis was performed as per the procedures recommended in "soil testing manual" of government of India. It involved measurement of pH and electrical conductivity using PVC sleeved glass electrode (EQ-610 model, Equiptronics, India). Available zinc, manganese, iron and copper were determined by DTPA extraction (Lindsay and Norvell 1978). Hot water extraction was used for determination of available boron and 1 M ammonium acetate extraction for available molybdenum. Inductively coupled plasma was used to measure concentrations of Cu, Fe, Mn, Mo and B in the soil extracts. Soil texture was analyzed by hydrometer (DA&C - GOI 2011).

The biochar analysis for total elemental carbon, hydrogen, nitrogen, sulphur and oxygen fraction was determined with a dry combustion elemental analyser (FLASH EA 1112 series, Thermo finnigan, Italy), at Sophisticated Analytical Instrument Facility, of Indian Institute of Technology Bombay. Methods recommended by International Biochar Inititative were adopted for chemical analysis of biochar. Electrical conductivity and pH was determined in 1:20 (w:v) biochar to distilled water (Rajkovich et al. 2011), 1 M hydrochloric acid digestion was adopted for determining available micronutrients in biochar (Camps-Arbestain et al. 2015). Biochar to water ratio 1:10 (w:v) was adopted for extraction of water soluble carbon fraction and measured by using TC/ TOC flow analyzer (Lin et al. 2012). Specific Surface Area was determined by nitrogen adsorption-desorption isotherm [Brunauer-Emmett-Teller (BET) method (ASTM D6556)].

Table 1. Chemical properties of soil and biochar used for the experiment

Parameters	Unit	Biochar	Soil	Nutrients	Unit	Biochar	Unit	Soil
pH		7.57	7.87	P	g kg^{-1}	3.00 (1.4)	kg/ha^{-1}	22.3
EC	$mScm^{-1}$	1.9	0.29	K		17		295.6
CEC	$molckg^{-1}$	21.21	55.2	Ca		2.43 (1.4)		nd
TC	%	65.04	nd	Mg		1.63 (0.43)		nd
TN	%	10.6	nd	Fe	mg kg^{-1}	540	ppm	5.98
TH	%	1.90	nd	Mn		374		14.10
BET SSA	$cm2g^{-1}$	59.68	nd	Zn		72		1.62
Water soluble C	ppm	125	nd	Cu		35		5.02
SOC	%	nd	1.02	B		35		0.51

Note: For Biochar, figure in parenthesis is total nutrient content and outside is available nutrient content; $ - kg/ha^{-1}; nd: Not determined

Experiment design

The field experiment was conducted in a randomized block design (RBD) with three replications. The experiment comprised of nine treatments namely T1: unfertilized control (UC), T2: recommended dose of chemical fertilizers only (RDF), T3: only biochar @ 2.5 t/ha-I (BC 2.5), T4: only biomethanated spent wash (BMS) @ 60 m3 ha^{-1}, T5: only biomethanated spent wash @ 66 m3 ha^{-1}, T6: only biomethanated spent wash @ 72 m3 ha^{-1}, T7: biomethanated spent wash @ 60 m3 ha^{-1} plus biochar @ 2.5 t/ha^{-1}, T8: biomethanated spent wash @ 66 m3 ha^{-1} plus biochar @ 2.5 t/ha^{-1}, T9: biomethanated spent wash @ 72 m3 ha^{-1} plus biochar @ 2.5 t/ha^{-1}. Individual treatment plots were of the size of 3.05 m x 3 m, which were separated with 0.6 m buffer spacing within a replication and 1.05 m between the replications. RDF for soybean was applied in combined form of urea and di-ammonium phosphate at 25 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} (Deosarkar et al. 2002). Biochar application rates upto 5 t ha-1 adopted in this study are in consideration with the practical feasibility for implementation and affordability of small and marginal farmers. Biochar was broadcasted on soil surface and mixed in top soil manually. Biochar was moistened prior to application to restrict losses due to wind blowing.

Common agronomic practices

Experiment was conducted during kharif season of 2017 with Soybean variety KDS 344. Treatment plots were prepared in the fourth week of June and irrigated prior to sowing operation. Fully mature plants were harvested after 105 DAS in the second week of October 2017. Seed treatment was performed @ 10 g kg-1 seeds with rhizobium and phosphate solubilizing micro-organisms and @ 5 g kg-1 seeds with *Trichoderma* (obtained from the laboratory of plant pathology, Agricultural College, Kolhapur, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. Sowing was performed by dibbling at a crop spacing of 52.5 x 21.5 cm. Uniform plant population was maintained by thinning at 10 days after sowing (DAS). Total 3 irrigations were given to the crop to overcome the rainfall deficit. Preventive pest control was adopted by chemical pesticides namely, Chloropyriphos 48 EC at 15 DAS (2 ml/L-1), Triazophos 40 EC at 25 DAS (2 ml/L-1), Thiamethoxam at 45 DAS (1 g/L-1), Benzisothiazolin-3-1 (0.019%) at 60 DAS (0.5 ml/L-1) and Propiconazole 25 EC at 75 DAS (3ml/L-1) for controlling sucking and chewing pest, and disease causing pathogens.

Soil and plant sampling

Initial soil properties were analyzed from a composite soil sample collected from the experimental plot and sampling was performed in each plot for post-harvest soil analysis. Physiological performance of the crop was studied by intermediate sampling of plant height and number of pods. Ten random representative plants were sampled at 40 and 55 DAS for plant height and 85 DAS for number of pods. Each plot was harvested manually at full maturity of 105 DAS to obtain total grain yield and yield attributes of 10 randomly selected plants in each plot.

Statistical analysis

Statistical significance of the treatment effect on growth, grain yield and soil properties were tested with analysis of variance (RBD-ANOVA) using Microsoft excel™. Significance was tested at 95% confidence interval using F-test.

Results and Discussion

Effect of biochar on soil properties

Results of post-harvest soil properties are presented in Table 2. Biochar 2.5 t/ha-1 and BMS at any application rate had no significant effect on soil pH compared to control. This was observed because both biochar and BMS had near neutral pH that was similar to pH of the study soil. Similar results are reported in neutral or alkaline soil (Liu and Zhang, 2012). Similarly, application of biochar @ 2.5 t/ha^{-1} or BMS at any application rate had no significant effect on electrical conductivity of soil. Biochar has low concentration of soluble salts (El-Naggar et al. 2019) and has limited effect on soil electrical conductivity. BMS has high concentration of soluble salts, however it is subjected to leaching during the growth period. Similar results are reported by Naorem et al. 2017. Biochar @ 2.5 t/ha^{-1} and BMS at any application had no significant effect on exchangeable zinc, copper, ferrous, manganese or boron in the soil. Non-significant effect of biochar on soil nutrients has also been previously reported, depending on the inherent soil nutrients (Guerena et al. 2013) and biochar properties (Namgay et al. 2010). In the present study, biochar was applied at 2.5 t/ha^{-1}. Lack of significant effect of biochar on soil nutrients, at application rates similar to the present study, has also been previously reported (Barbosa de souse et./ al. / 2014). In case of BMS application, although the effect on soil micronutrients was non-significant, an increasing trend was with application rate was observed with and without Biochar. This was particularly observed with available zinc, ferrous and manganese. The positive effect on soil micronutrients is attributed to the micro-nutrient content of the BMS. Significant increase in soil micronutrients on BMS application has been previously reported, however at higher application rates greater than 100 m^3 ha^{-1} (Deshpande et al. 2017). BMS application rate in the present study is in range of 60 to 72 m^3 ha^{-1}, BMS at all three application rates, recorded numerical increase in soil micronutrients, however the effect was non-significant.

Effect of biochar on soybean

Results of the effect of BMS @ 60, 66 and 72 m^3 ha^{-1} and biochar @ 2.5 t/ha^{-1} on soybean yield and

Table 2. Effect of Co-application BMS and Biochar on soil properties

Treatment	pH	EC (dS m^{-1})	Zn (ppm)	Cu (ppm)	Fe (ppm)	B (ppm)	Mn (ppm)
Control	7.88	0.329	1.36	4.41	5.67	0.49	13.78
RDF	7.88	0.282	1.37	4.59	5.48	0.50	13.63
Sole BC 2.5	7.72	0.379	1.33	4.58	5.52	0.46	14.56
BMS 60	7.87	0.343	1.45	3.63	6.04	0.52	17.73
BMS 66	7.97	0.360	1.48	4.11	6.28	0.59	17.23
BMS 72	7.92	0.263	1.55	4.18	6.57	0.59	18.39
BMS 60 + BC 2.5	7.79	0.281	1.73	4.21	6.60	0.59	18.21
BMS 66 + BC 2.5	7.94	0.354	1.72	4.53	6.12	0.58	18.05
BMS 72 + BC 2.5	7.84	0.373	1.69	4.41	6.00	0.55	18.10
S. Em.	0.14	0.06	0.16	0.42	0.40	0.05	1.99
CD	ns	ns	ns	ns	ns	ns	ns

Significance calculated at $P < 0.05$; CD provided for significant treatments; n.s.: non-significant

RDF: Recommended dose of chemical fertilizers; BC 2.5: Biochar @ 2.5 t ha^{-1};
BMS 60: Biomethanated spentwash @ 60 m^3 ha^{-1}; BMS 66: Biomethanated spentwash @ 66 m^3 ha^{-1};
BMS 72: Biomethanated spentwash @ 72 m^3 ha^{-1}

physiological parameters are presented in Table 3. No significant effect of BMS @ any application rate and BC @ 2.5 t/ha^{-1} was recorded on soybean germination at 10 DAS. The mean plant population in the plots was maintained at 88,650 per hectare and the germination ranged between 76.8 to 86.5 percent. Similar results have also been previously reported in the published literature for four different type of biochar treatments (Solaiman et./al./2012; Shamim et al. 2018). No significant effect of BMS @ any application rate and BC @ 2.5 t/ha^{-1} treatments was recorded on plant height measured at 40 and 50 DAS. Mean plant height on both sampling events was at par for sole biochar and various rates of BMS compared with each other or RDF (25 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) and control. Lack of significant effect on vegetative growth of soybean is attributed to the non-significant effect of treatments on soil properties, explained by lower application rates of the treatments adopted in the present study. Similar observation has also been previously reported (Yooyen et al. 2015). Significant effect of biochar @ 2.5 t/ha^{-1} and BMS @ 60, 66 and 72 m^3 ha-1 was recorded on number of pods at 85 DAS. Highest number of pods was recorded for BC @ 2.5 t/ha^{-1} plus BMS at 60 m3 ha-1, which was significantly higher compared to all other treatments. BMS 66 m^3 ha^{-1} was the second best treatment, which was significantly higher than RDF (25 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}), sole BC @ 2.5 t/ha^{-1} and control, and was at par with BMS 72 m^3 ha^{-1} plus biochar 2.5 t/ha^{-1}, sole BMS 60 m^3 ha^{-1}, sole BMS 66 m^3 ha^{-1} and sole BMS 72 m^3 ha^{-1}. All BMS and biochar @ 2.5 t/ha^{-1} plus BMS treatments also recorded a numerically higher soil zinc, ferrous and manganese as compared to RDF, control or biochar treatments. Therefore, positive

Table 3. Effect of biochar on soybean yield and yield attributing parameters

Treatment	Germination	Plant height 40 DAS	Plant height 55 DAS	No. of Pods	Pod wt	Fresh Biomass yield	Grain yield
Unit	percentage	cm	cm	Nos.	gm	gm PL^{-1}	Kg ha^{-1}
Control	82.05	55.1	71.300	94.600	2.38	58.6	734.242
RDF	78.52	56.8	75.167	96.633	3.00	67.0	1074.650
Sole BC 2.5	84.5	57.2	73.567	96.267	2.78	61.7	858.292
BMS 60	86.54	61.2	74.233	112.700	2.86	77.0	1423.725
BMS 66	82.47	57.9	75.100	117.067	2.70	71.2	1434.042
BMS 72	79.70	56.8	75.533	105.733	2.75	68.6	1202.325
BMS 60 + BC 2.5	76.82	59.9	76.267	141.100	2.45	82.2	1770.042
BMS 66 + BC 2.5	80.13	55.4	70.700	108.167	2.69	68.3	1243.325
BMS 72 + BC 2.5	83.44	57.4	72.533	110.767	2.90	72.1	1334.758
S. Em.	20.29	2.34	2.5	9.43	0.57	0.09	92.6
CD	ns	ns	ns	20.19	ns	ns	198.2

Significance calculated at $P < 0.05$; CD provided for significant treatments; n.s.: non-significant

RDF: Recommended dose of chemical fertilizers; BC 2.5: Biochar @ 2.5 t ha^{-1};
BMS 60: Biomethanated spentwash @ 60 m^3 ha^{-1}; BMS 66: Biomethanated spentwash @ 66 m^3 ha^{-1};
BMS 72: Biomethanated spentwash @ 72 m^3 ha^{-1}

effect on number of pods is attributed to increase in micro-nutrient availability. However it could also be attributed to increased macro-nutrient availability as commonly reported in the published literature (Kizito et al. 2019). Biochar @ 2.5 t/ha^{-1} and BMS @ 60, 66 and 72 m^3 ha^{-1} treatments had a non-significant effect on 10 plant biomass weight and 10 plant pod weight at harvest. The lack of effect could be explained by non-significant effect of treatments on vegetative growth of soybean as recorded in plant height measurement. The overall trend of effect with respect to various treatments was in alignment with the trend of number of pods. Significant effect of BC @ 2.5 t/ha^{-1} and BMS @ 60, 66 and 72 m^3 ha^{-1} treatments was recorded on grain yield in the present experiment. All treatments except BC at 2.5 t/ha^{-1}, recorded significantly higher grain yield over control. Sole biochar application at 2.5 t/ha^{-1} also recorded non-significant effect on soil micro-nutrients, which were at par with control. Lack of significant effect of sole biochar treatment on grain yield is attributed to non-significant effect on soil nutrients. BMS has high nutrient content as compared to biochar, so much that biochar is reported to serve as a medium to adsorb nutrients from liquid fertilizers like BMS (Kizito et. al., 2019). This explains the marginal effect of biochar on soil micro-nutrients as compared to BMS treatments. RDF (25 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) application significantly increased the grain yield as compared to control, which was 46 percent higher. Highest grain yield was recorded with BMS 60 m3 ha^{-1} plus BC @ 2.5 t/ha^{-1}, which was significantly superior to all other treatments and 140 percent higher than control. Significant increase in soybean yield with BMS application has also been previously reported (Hati et al. 2007; Chandel et al. 2011) and is attributed to high nutrient content (Deshpande et al. 2017). Combined application of BC @ 2.5 t/ha^{-1} with BMS @ 66 m^3 ha^{-1} and BC @ 2.5 t/ha^{-1} plus BMS @ 72 m^3 ha^{-1} recorded significantly higher grain yield compared to control, sole BC @ 2.5 t/ha^{-1}, RDF (25 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1}) and sole BMS @ 72 m^3 ha^{-1}, however was at par with all other BMS plus BC 2.5 t/ha^{-1} treatments. Among sole BMS treatments, BMS 66 m^3 ha^{-1} was the optimum treatment. BMS 72 m^3 ha^{-1} significantly reduced the grain yield compared to BMS 60 m^3 ha^{-1} and BMS 66 m^3 ha^{-1} Inhibitory effect of BMS at excess application rate and higher yield at optimum application rate has also been previously reported in the published literature (Jain and Srivastava 2011). Based on soil analysis results, there is limited evidence to attribute the improvement in crop yield to increase in soil micronutrients. However, based on the non-significant increase in zinc, ferrous and manganese with BMS application, demonstrate a positive involvement in enhancing the crop yield. In the present study, sole biochar application had no significant effect on grain yield. However combined application of BMS 60 m^3 ha^{-1} and biochar 2.5 t/ha^{-1} and recorded significantly higher grain yield as compared to control and all other treatments. While BMS is a source of available nutrients and bio-available organic carbon, biochar application is reported to improve nutrient retention (El-Naggar et al. 2015) as well as physical properties of soil (Zong et al. 2018). The particular biochar BMS combination had a beneficial effect on crop growth. However, co-application of BC and BMS at very high rates, would have undesirable effect on plant growth by nutrient immobilization due to high organic loading (Hankins et al. 2017).

It is concluded that application of biochar at low rates of 2.5 t/ha^{-1} without co-application of chemical or organic fertilizer has limited scope for soybean yield improvement, due to high nutrient requirement of soybean. In case of BMS, application in excess to an optimum rate can result in crop yield decline. Combined application of BMS at 60 m^3 ha^{-1} and biochar at 2.5 t/ha^{-1}, demonstrated a significant effect on crop yield. There is limited evidence to attribute the gains in grain yield to micro-nutrient added with the treatments.

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