# Repurposing distillation waste biomass and low-value mineral resources through biochar-mineral-complex for sustainable production of high-value medicinal plants and soil quality improvement

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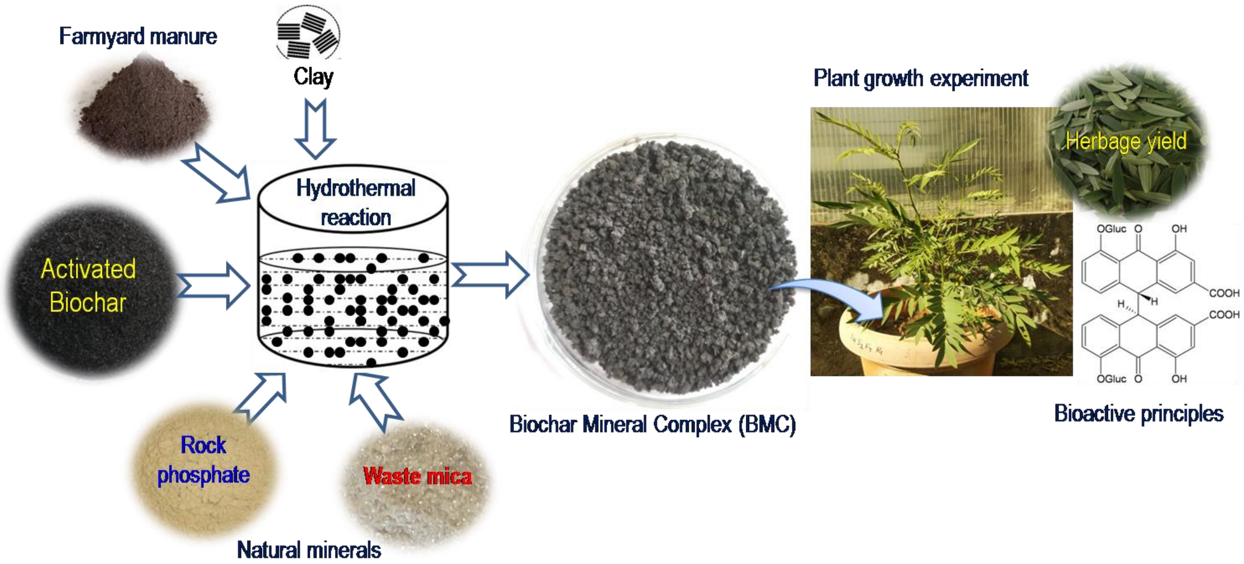
## Highlights

- Novel biochar-mineral-complex (BMC) was prepared as an effective soil amendment.
- BMC had better surface properties and nutrient contents than pristine biochar.
- BMC performed better than conventional organic and chemical fertilizers.
- BMC improved herbage and bioactive compound yields of senna plants.
- BMC improved soil OC, available nutrients and biological properties.

## Graphical abstract



## Abstract

High cost of synthetic fertilizers and their hazardous effects catapult the exploration of alternative nutrient formulations and soil amendments. This study aimed to synthesize a novel biochar-mineral-complex (BMC), and evaluate its nutrient supplying and soil improvement performances. In a hydrothermal reaction, the BMC was prepared using a biochar derived from distillation waste of Lemongrass (*Cymbopogon flexuosus*) and farmyard manure, for the first time via fortification with low-grade rock phosphate and waste mica. The BMC showed improved physico-chemical properties and nutrient availability than the pristine biochar. When applied to a deeply weathered acidic soil, the BMC significantly ($p<0.05$) improved the herbage and bioactive compound (sennoside) yields of a medicinal plant (senna; *Cassia angustifolia* Vahl.) compared to the pristine biochar, farmyard manure, vermicompost, and chemical fertilizers. The BMC also improved the soil quality by increasing nutrient and carbon contents, and microbial activities. Soil quality improvement facilitated greater nutrient uptake in senna plants under BMC compared to the pristine biochar, and conventional organic and chemical fertilizer treatments. This study thus encourages the development of BMC formulations not only to overcome the limitation of sole biochar application to soils, but also to phaseout chemical fertilizers in agriculture. Moreover, BMC could bestow resilience and sustainability to crop production via value-added recycling of waste biomass and low-grade mineral resources.

***Key words:*** Alternative fertilizers; Distillation waste biomass; Low-grade minerals; Medicinal plants; Resource recycling; Soil quality improvement

## 1. Introduction

A sharp increase in the price and demand of chemical fertilizers in global agriculture and the huge carbon footprint to produce them emphasized the need to invent efficient nutrient management strategies based on cost-effective and environment-friendly nutrient sources. Recycling of waste biomass, crop residues, animal manures, and also naturally occurring low-grade minerals has been regarded as an avenue to reduce the need of costly synthetic fertilizers (Basak, 2017). Farmyard manure (FYM) and compost are frequently used as soil amendments and important nutrient sources in organic agriculture (Basak et al., 2020). Recently, biochar is also considered as a promising soil amendment to conserve carbon and nutrients present in organic materials and biomass, and thereby address the environmental issues concerning with sustainable agricultural nutrient management (Mandal et al., 2016; El-Naggar et al., 2019; Purakayastha et al., 2019).

Extraction of essential oil through distillation of aromatic plants contributes 3 Mt of solid biomass every year in India (Saha and Basak, 2019). The waste biomass generated after the extraction of essential oil (0.5 – 1.0% of total fresh biomass) may contribute to emission of greenhouse gases if the biomass is dumped openly or burnt (Saha et al., 2018). Recycling of the aromatic plant waste biomass (APWB) into effective soil amendments (e.g., compost and biochar) might not only mitigate the greenhouse gas issues but also increase the farmers' benefits by supplying and retaining nutrients, at least as a partial replacement of chemical fertilizers. Application of biochar and compost is also known to improve the physical, chemical, and biological qualities of agricultural soils (Sanchez-Monedero, 2018; Saha et al., 2019; Basak et al., 2020). However, nutrient contents in the biochar prepared from APWB is not sufficient to support crop demand; particularly major nutrients such as N, P and K are a concern (Jha et al., 2010). Fortification of biochar with rock phosphate (RP) and waste mica via biochar-mineral-complex (BMC) preparation could be a promising approach to increase the nutrient contents in biochar-based products because these low-grade mineral materials are an inexpensive source of P (Basak et al., 2020) and K (Basak, 2018). The P and K release from these minerals could be accelerated by thermal alteration (dos Santos Teixeira et al., 2015; Tumbure et al., 2020) which is an integral practice for biochar preparation. No report is currently available for the production of BMC from APWB and RP/waste mica, and their nutrient release pattern during crop cultivation. P and K are non-renewable nutrient sources, and many developing countries including India are dependent on the import of commercial fertilizers (100% K fertilizers, and >60% P fertilizers) from other countries (Basak, 2019). An effective recycling of the locally available minerals via BMC thus offers enormous potentials in crop production by reducing the dependency on costly chemical fertilizers.

High specific surface area (SSA) and a range of reactive surface functional groups (e.g., -COOH, -OH, and $-NH_2$) enable biochar to exhibit various chemical properties (e.g., hydrophilic vs hydrophobic, acidic vs basic properties) (Wallace et al., 2019; Zhang et al., 2020). The SSA of biochar can significantly contribute to adsorption-desorption, acid-base, and redox reactions with the soil matrix (Ding et al., 2016; Matin et al., 2020). Recent findings revealed that biochar could form organo-mineral complexes by interacting with surrounding organic matter, ash, clay, and other minerals in the soil (Farrar et al., 2019; Zhao et al., 2019; Lu et al., 2020).

BMC having properties similar to natural soil organo-mineral aggregates could be produced by mixing organic matter, ash, clay, and other minerals, and subsequently heating at a moderate temperature (up to 240°C) (Chia et al., 2014). The organo-mineral reaction during torrefaction at 220-240°C could form a nutrient-rich BMC (Lin et al., 2013). Li et al. (2014) reported an increase of surface reactivity and cation exchange capacity (CEC) in BMC due to the formation of oxygenated functional groups, and Lewis acid and base sites. The production of BMC could conserve nutrient elements by incorporating them into the heterocyclic carbon structures, which would provide a comparatively stable nutrient rich formulation with slow-release property. Through improved physicochemical and biological activities, BMC application could facilitate soil nutrient cycling and mobilization, and ultimately plant uptake (Ye et al., 2016). However, nutrient availability from BMC and its role in plant growth and nutrition have been investigated only sparsely.

Poor nutrient use efficiency from chemical fertilizers in highly weathered acidic tropical soils covering nearly one-third of the continental surface is a significant global issue (Jien and Wang, 2013; Anda et al., 2015). Owing to high SSA, CEC and pH ameliorating ability, BMC could improve nutrient retention in such deeply weathered soil, and support the production of crops maximizing benefits from the degraded soils while simultaneously recycling biowaste and mineral resources. It was hypothesized that BMC would be more effective than chemical fertilizers and conventional organic nutrient sources (e.g., FYM and vermicompost) in sustaining plant growth by improving the nutrient use efficiency in highly weathered soils. The specific aims of this study are to (1) prepare BMC from APWB, RP and waste mica as an amendment of a highly weathered (degraded) soil, (2) explore the physico-chemical characteristics of the BMC, (3) examine BMC-induced soil property changes in comparison to chemical fertilizer, FYM and vermicompost application, and (4) investigate the yield and quality of a medicinal plant (*Cassia angustifolia* Vahl.) following soil application of the BMC.

## 2. Materials and methods

### *2.1. Preparation of biochar-mineral-complex (BMC)*

Residual biomass obtained after the distillation of Lemongrass (*Cymbopogon flexuosus*) in a hydro-distillation unit of a local farmer in Anand, Gujarat, India, was collected for the preparation of biochar. The solid residual biomass was air-dried, and processed into powder (≤ 2 mm). The powdered biomass was pyrolyzed at 350°C (heating rate: 5°C $min^{-1}$) for 2 h in a muffle furnace equipped with $N_2$ purge (flow rate: 2 mL $min^{-1}$) to make a limited oxygen environment (Saha et al., 2019). The BMC was prepared following a reported method (Lin et al., 2013; Chia et al., 2014; Ye et al., 2016) with some modification. The BMC was synthesized with a proportionate mixture of the above biochar (32 g), minerals (i.e., 30 g kaolinite clay, 12.5 g RP, and 5.5 g waste mica) and 20 g organic matter (FYM) (Table S1; Supplementary Information). The RP used in this study contained 8.6% and 0.003% total and water-soluble P (Basak, 2009a). The waste mica contained 9.72% total K ($K_2O$), and 0.011% water soluble K (Basak, 2019b). The biochar was pre-treated with 10% phosphoric acid (Chia et al., 2014) before mixing it with other raw materials. The phosphoric acid treatment was employed to increase the porous structure, and abundance and stability of surface functional groups such as carbonyl, phenolic, alcoholic, hydroxyl, and ether groups in the biochar (Zhao et al., 2017; Chu et al., 2018). This treatment also would lead to loss of hydrogen from the surface, and increase the aromaticity of the biochar by increasing the proportional content of C (Lin et al., 2012). The biochar was washed repeatedly with distilled water to remove the excess phosphoric acid until the solution pH became neutral. Then, the proportionate amounts of other raw materials (Table S1) were mixed thoroughly with the acid treated-biochar. Boiling double distilled water was poured into the mixture, and stirred at 80°C for 2 h to let the materials coagulate, and finally a homogeneous mixture was obtained. The mixture was transferred to a batch hydrothermal reactor (20 cm × 20 cm × 30 cm), and raised to 220°C within 1 h, and maintained at this temperature for 3 h to get the BMC (Fig. S1). The moderate torrefaction temperature and duration were adopted to enhance the dissolved organic C content in the BMC (Lin et al., 2013). The final product (BMC) was cooled under atmospheric condition, and stored in an airtight container until further use.

### *2.2. Physico-chemical properties of BMC*

The biochar and BMC were finely ground, and passed through ≤ 2-mm sieve for further analysis. The pH and electrical conductivity (EC) were measured by using a digital pH and conductivity meter (Aquamax KF, GR Scientific, UK), at a solid: deionized water ratio of 1:5 (w/v). The CEC was measured by extracting the samples with 1N sodium acetate solution (pH = 8.2) (Sumner and Miller, 1996). Acid neutralizing capacity (ANC) was determined by using the potentiometric titration method (Johnson, 1990). The SSA was measured by the Brunauer-Emmett-Teller (BET) adsorption method. Total C and N were determined by a CHN analyzer (PE-2400, Perkin Elmer, USA). Other nutrient contents (e.g., P, K, Ca, Mg, Zn, Cu, Fe, and Mn) in the biochar and BMC were analyzed by Inductively Coupled Plasma Optical Emission Spectrometer (ICP – OES) (Optima 3300 RL, Perkin Elmer, USA) after digestion with $HNO_3$-HCl mixture [3:1(v/v)] in a microwave digestion system (Discover@ SPD, CEM Corporation, USA). The crystal structure, morphology, and surface chemistry of BMC were studied through X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) spectroscopy(Supplementary Information).

### *2.3. Plant growth experiment*

A pot culture study was conducted under natural conditions in a net house. A deeply weathered acidic soil was selected for the experiment, and the bulk sample was collected from an experimental field (0-15 cm depth) of the Agriculture Research Station at Kadapa, Andhra Pradesh, India. The soil sample was air-dried in the laboratory, grinded, and sieved through a 2-mm sieve for the analysis of initial properties. The experimental soil was light in texture (sandy loam) with pH=6.9, EC=0.18 dS $m^{-1}$, and organic C (OC) = 3.89 g $kg^{-1}$. The soil belongs to the order Alfisol, and subgroup Typic Haplustalf (Soil Survey Staff, 2010). Fertility status of the soil was low with available N, P and K contents of 62.8, 12.7 and 72.4 mg $kg^{-1}$, respectively.

A medicinal herb, senna (*C. angustifolia*), was grown in the pot experiment. Senna is widely used in traditional and modern medicines. India is the main producer of senna leaves and pods (economic parts of the plant for medicinal use), and holds a monopoly in the international market with an annual turnover of ₹300 million (Basak and Gajbhiye, 2018). Cultivation of aromatic and medicinal plants in degraded marginal soils, as chosen in this study, could fetch both economic and environmental benefits, and would be easily adopted by farmers (Basak and Gajbhiye, 2018). Medicinal and aromatic plants need less but a steady supply of nutrients for providing the best yield of high-quality products (Basak and Gajbhiye, 2018), which is likely to be met by BMC. FYM and vermicompost were obtained from the Anand Taluka Cooperative, and Livestock Research Station, Anand Agricultural University, respectively. The chemical fertilizers (CF) were applied according to the recommended dose of nutrients for senna crop: 60 kg $ha^{-1}$ N (urea), 40 kg $ha^{-1}$ P (single super phosphate), and 20 kg $ha^{-1}$ K (muriate of potash). The six selected treatments comprised of: $T_1$- Control, $T_2$ – FYM (5 t $ha^{-1}$); $T_3$- Vermicompost (5 t $ha^{-1}$); $T_4$- Biochar (5 t $ha^{-1}$); $T_5$ - BMC (5 t $ha^{-1}$), and $T_6$ – CF.

Sieved (< 2-mm) 10 kg of soil was taken into earthen pots (32 cm inner diameter and 26 cm height), and mixed thoroughly with different treatment combinations. Senna seeds were sown in four replicated pots of each treatment, and placed in a randomized block design. A single plant per pot was maintained to grow for the period of 120 days. The pots were irrigated at regular intervals to maintain the soil moisture content at field capacity.

### *2.4. Growth and yield parameters of senna plant*

The above-ground senna plant biomass was harvested after 120 days of sowing (DAS) which coincided with the flowering (pod formation) stage of the plant. The plant height, number of branches, and fresh and dry herbage (leaf + pod) yields were recorded at harvest. The fresh pods and leaves yield per plant was recorded immediately after harvesting. The dry weight of pods and leaves were recorded when the samples reached a constant weight under shade drying. The shed-dried plant samples were stored for further analysis.

### *2.5. Analysis of plant and soil*

Senna leaves and pods are known for their laxative properties due to the presence of sennoside (Reddy et al., 2015). The sennoside content in pulverized (<0.5 mm) leaves and pods was analyzed on a High-Performance Liquid Chromatography (HPLC) equipment (LC-20AD, Shimadzu Corporation, Kyoto, Japan) following extraction in 70% methanol (Supplementary Information).

The major nutrient (N, P and K) contents in the dried (65$^{o}$C) plant biomass were analyzed by standard methods (Supplementary Information). Soil sample (0-15 cm) from each pot was collected after harvesting of senna plants. Immediately after collection, a set of soil samples was stored separately in a refrigerator (4°C) for the analysis of soil biological parameters. Another set of samples was air dried, grinded, and passed through a 2-mm sieve, and then analyzed for pH, EC, OC content, and available nutrients (e.g., N, P and K) (Supplementary Information). Samples were allowed to reach the room temperature after taking out from refrigerator, and used for determination of inorganic N, microbial biomass carbon (MBC), soil respiration, and enzyme activities (e.g., dehydrogenase and alkaline phosphatase) (Supplementary Information). The moisture content of original soil samples was determined, and all results were expressed on oven dry-basis.

### *2.6. Data analysis*

The data generated from the laboratory and pot experiments were expressed as the mean of four replicates. An analysis of variance (one-way ANOVA) was conducted according to the experimental design followed (Completely Randomized Design). Statistical significance among treatments was worked out by estimating the critical difference ($P < 0.05$) in SPSS-20 (SPSS Inc., Chicago, USA) software package. Microsoft Excel (Microsoft Corporation, USA) was also used for data calculation, tabulation and graphical representation.

## 3. Results and discussion

### *3.1. Physico-chemical properties of amendments*

Physico-chemical properties of BMC in comparison with FYM, vermicompost and the pristine biochar are given in Table 1, and Table S2. Among the amendments, the lowest total C was recorded in BMC, but it contained the highest amount of water soluble, and total P and K (Table 1). BMC was found slightly acidic (pH=6.85±0.05) in nature as opposed to the alkaline pristine biochar (pH=8.12 ± 0.06), and it contained the highest CEC among all the amendments (Table 1). Low pH value in BMC was resulted likely from the release of organic acids (e.g., humic acid) from FYM during BMC preparation (Sekhar et al., 2001). The CEC value [66.1 ± 2.3 cmol($p^+$) $kg^{-1}$] exhibited by BMC (Table 1) was greater than an enriched biochar [40.67 cmol($p^+$) $kg^{-1}$] reported earlier (Chia et al., 2014). Compared to the pristine biochar, total C content in the BMC was low (Table S2) because the mineral addition created a dilution effect for the C content. In addition, reaction of the biochar with phosphoric acid might have reduced the C content too by an individual or combined action of (i) biochar surface oxidation, (ii) carbonate C elimination, and (iii) inorganic P precipitation (dilution effect) (Chia et al., 2014). The hydrothermal reaction of biochar with RP and waste mica most likely increased the available, and total P and K contents in BMC (dos Santos Teixeira et al., 2015; Dissanayake et al., 2018). The SSA of the BMC was 26.7 ($m^2$ $g^{-1}$) with a total pore volume of 2.407 (mL $g^{-1}$) (Table S2). The SSA of BMC was comparatively lower than a bamboo-based biochar-montmorillonite composite (156 $m^2$ $g^{-1}$) (Viglasova et al., 2018), but higher than a municipal solid waste biochar-montmorillonite composite (6.51 $m^2$ $g^{-1}$) (Ashiq et al., 2019). Riddle et al. (2019) reported that the reduction of SSA in biochar-mineral composite compared to pristine biochar could be due to the coating of biochar with metal hydr(oxide)s (e.g., magnesium hydroxide).

## 3.2. Characterization of BMC

### *3.2.1. Scanning electron microscopy*

RP, waste mica, and kaolinite were mixed with the biochar, and thus the minerals were incorporated into the biochar structure in BMC. Surface morphology of the pristine biochar was random, disordered and porous in nature (Fig. 1a). The SEM image of BMC at 1000X magnification showed a thin film-like structure covering the BMC surface (Fig. 1b). At 5000X magnification, the thin film-like structure appeared to be a further prominent layered coating on the BMC surface (Fig. 1c). This type of layered coating is common for the surface morphology of clays, as reported in the literature (Ashiq et al., 2019a; 2019b). SEM images of BMC (Fig. 1b and c) also showed partial filling of biochar pores with mineral particles that entered into the biochar pores (Premarathna et al., 2019). The course mineral particulates coated the biochar surface giving it a rough texture with irregularly shaped agglomerates and flaky structures, which is again a confirmation of clays being incorporated in the BMC (Fig. 1b and 1c). Nevertheless, the entire biochar surface was not covered by the mineral particles, and some pores were still accessible. Pores originated from the raw biomass are important as they provide habitats for beneficial microorganisms, and also retain and recycle nutrients to improve the soil fertility (Viglasova et al., 2018). Partial covering of biochar pores in BMC was rather advantageous because an excessive clay coating could have made the pores inaccessible to microorganisms (Premarathna et al., 2019), hampering the nutrient cycling. The partial coating obtained in this study thus could be ideal for the purpose of soil fertility improvement.

### *3.2.2. Fourier transform infrared spectroscopy*

Considerable difference was observed between the FTIR spectra of pristine biochar and BMC (Fig. 2). Compared to the pristine biochar, the presence of a sharp band at around 1034 $cm^{-1}$ in the BMC spectrum indicated the incorporation of Si-O functional groups onto the BMC, possibly coming from the silicate minerals. Bands around 1030-1040 $cm^{-1}$ confirmed an out-of-plane deformation of the -CH groups, symmetric stretching vibration of C-O groups of cellulose, hemicellulose and lignin, and O-P-O bending vibration due to the incorporation of RP. A small band at around 915 cm$^{-1}$ represented the Al-OH deformation of kaolinite clay mineral (Saikia et al., 2016), which confirmed the incorporation of the mineral components on the BMC surface (Darby et al., 2016; Premarathna et al., 2019). Sharp and intense bands for BMC at 3693 and 3619 cm$^{-1}$, which were absent in the pristine biochar, could also be attributed to the inclusion of kaolinitic clay onto the BMC surface (Darby et al., 2016), and a broader band at around 3422 cm$^{-1}$ indicated the stretching vibration of O-H groups of bonded water (Darby et al., 2016). Bands at 794 cm$^{-1}$ indicated the presence of silica (Darby et al., 2016). Representative bands for aliphatic carbon, a shoulder like peak at about 1627 cm$^{-1}$ might be associated with the C=O stretching of ketone and carboxylate derivatives (Joseph et al., 2013). As compared to the pristine biochar, aliphatic C-H stretching bands were missing at around 2925 cm$^{-1}$ in the spectrum of BMC, which was a characteristic feature of BMC. Li et al. (2018) reported that the incorporation of major minerals (e.g., kaolinite, metakaolin, and quartz) could hinder the expression of some functional groups from carbon fractions in the spectra of biochar-composite materials. The intense bands at 600 cm$^{-1}$ to 400 cm$^{-1}$ might be associated with the stretching vibrations of metal-O, halogen stretching, and phosphate bands (Li et al., 2017), which possibly originated from the mineral materials added into the BMC.

### *3.2.3. X-ray diffraction*

The XRD pattern of the pristine biochar revealed a reflection at 2θ = 28°, which was the only observable reflection of a crystallographic structural phase. Compared to the pristine biochar, several new and intense reflections were observed in the XRD pattern of BMC (Fig. 3), indicating that biochar formed a complex with minerals. The presence of kaolinite in the BMC was confirmed by the sharp reflection at 2θ = 12° and 25° (Fig. 3), which was in line with the

SEM and FTIR results establishing the complex formation (Liu et el., 2019). Additionally, the reflections at 2θ = 27° indicated the presence of quartz ($SiO_2$), and reflection at 2θ = 32-34° corresponded to calcite ($CaCO_3$) (Zhao and Nartey, 2014). Thus, a number of reflections in the XRD pattern re-confirmed the formation of BMC. The presence of diffuse and broad reflections at around 2θ = 5 to 70° were indicative of short-range order carbon structures in the BMC. Moderate temperature pyrolysis (350°C) in the present study would have resulted in biochar with a limited extent of order in the resultant carbon structure (Khan et al., 2015).

### 3.3. Plant growth and senna yield

Senna plant height and number of branches were significantly ($P<0.05$) increased by conventional organic treatments (FYM and vermicompost) over the control (Table 2). However, the highest plant height (74.6 cm), and number of branches (16.7) were observed with the application of BMC followed by chemical fertilizers (CF). Application of biochar and vermicompost significantly improved the plant growth over the control, and FYM. However, plant growth parameters recorded in the case of biochar and vermicompost treatments were significantly lower than BMC. Treatment receiving CF was found effective in improving plant growth parameters better than the control and FYM, but at par with biochar and vermicompost. The growth and vigor shown by the treatments, particularly biochar, vermicompost and BMC, were more effective in improving the total biological yield, which was also reflected in the herbage yield (leaf + pod) of senna. Application of FYM, vermicompost, biochar and BMC significantly increased the fresh as well as dry herbage yields over the control (Table 2). Treatment receiving BMC (5 t $ha^{-1}$) recorded the highest fresh (176.1 g $plant^{-1}$), and dry (57 g $plant^{-1}$) herbage yields followed by CF. Application of biochar and vermicompost were found equally effective to CF in improving the herbage yield. However, the BMC treatment recorded 18.1% higher total herbage yield than same rate of biochar application (5 t $ha^{-1}$). Herbage (leaf +pod) is the main economic part of senna, and BMC had a more profound influence on plant growth and herbage yield than biochar and vermicompost. The enriched nutrient composition of BMC prepared from natural minerals (Table 1; Table S2) might have immediate effect on plant growth and herbage yield. During BMC preparation, some physico-chemical properties of biochar were improved (Table 1) due to the formation of organo-mineral complexes, which likely contributed to high CEC and available nutrients (Chia et al., 2014). Due to the improvement of CEC, BMC would retain plant nutrients more efficiently in soil than biochar (Lin et al., 2013; Archanjo et al., 2017). Moreover, it could have a significant positive influence on soil biological activity (Ye et al., 2016). So, the contribution of BMC in terms soil quality improvement might have attributed to the highest plant growth and yield. The CF treatment could not perform well (statistically at par with BMC) due to low use efficiency of nutrient from chemical fertilizers in the highly weathered tropical soil (Basak, 2019b). The BMC in this case would have behaved as a slow-release nutrient source as opposed to chemical fertilizers (Farrar et al., 2019; Tumbure et al. 2020).

### 3.4. Bioactive compound production

Different treatments significantly influenced the leaf and pod sennoside contents (Fig. 4). However, biochar and BMC were found more effective in improving the sennoside content in leaf and pod than FYM and vermicompost. The treatment receiving BMC recorded the highest sennoside content, both in leaf (2.73%) and pod (3.15%), which were significantly ($P < 0.05$) higher than CF treatment. Treatments comprised of control, FYM, vermicompost, and CF did not show any difference in sennoside contents. However, significant variation in sennoside yield was found particularly in leaf, and total sennoside yield in individual treatment. Application of vermicompost, biochar, BMC and CF significantly improved the sennoside yield over the control. The sennoside yield (1.58 g plant$^{-1}$) in the case of BMC was 15.9 and 12.8% higher than CF and pristine biochar applications, respectively.

The sennoside yield results indicated that nutrient availability was indispensable for the production of sennoside by senna plants irrespective of the nutrient sources (biochar, fertilizer, or other organic amendments). The synergistic effects of nutrient availability and soil environment improvement due to BMC application probably stimulated the plant growth and physiological processes, which might have attributed to the highest sennoside content with BMC. BMC likely improved the transformation, retention, and use efficiency of nutrients (Lin et al., 2013; Chia et al., 2014), and also played a synergistic role in soil microbial colonization (Ye et al., 2016), which most possibly influenced the primary plant metabolism, and consequently diversion to secondary metabolites. Results of the present investigation were also supported by previous reports, where sennoside content in senna (Basak and Gajbhiye, 2018), withanolide content in ashwagandha (*Withania somnifera*) (Pratibha et al., 2013), and andrographolide content in kalmegh (*Andrographis paniculata*) (Saha et al., 2019) were enhanced respectively by enriched compost, bio-augmented vermicompost, and biochar applications.

## 3.5. Nutrient contents in plant tissues

Application of vermicompost, biochar, BMC, and CF significantly improved the plant nutrition (N, P and K contents) over the control (Fig. 5). The highest N (1.23%), P (0.196%), and K (0.691%) contents were recorded with the treatment receiving BMC. However, no significant (P

$< 0.05$) difference in N content was observed due to application of vermicompost, biochar, BMC, and CF. Similar trend was also observed as in the case of plant nutrient uptake (Fig. 5). Application of BMC recorded much higher P and K uptake over the control, FYM, and biochar, but at par with CF treatment.

Results of the present study thus suggested that BMC was a superior nutrient source than FYM, vermicompost, and biochar. Significantly higher nutrient contents in senna plant was recorded in the treatment receiving BMC, which might be due to better availability of the nutrients in the soil under this treatment, and also due to the effective supply of nutrients on plant demands. This might be explained by the higher nutrient contents (Table 1; Table S2) in BMC than other organic treatments. These results agree with a previous study (Ye et al., 2016) where a BMC (made of wood biochar, chicken manure, and kaolinitic clay) performed better than chicken manure compost in improving nutrient uptake by pakchoi (*Brassica rapa* L., ssp. *chinensis*). Similarly, Basak and Gajbhiye (2018) reported enhanced senna plant growth and P uptake under RP-enriched compost application. Therefore, BMC might have triggered the senna plant growth and nutrition, which ultimately reflected in the total plant nutrient uptake.

### 3.6. Soil pH, EC and organic C

Notable changes in soil pH, EC, and OC were found due to the application of BMC, organic amendments and CF after the harvest of senna (Table 3). Application of FYM, vermicompost and biochar significantly improved soil pH, EC, and OC than the control and CF. However, treatment receiving biochar and BMC showed distinctly higher soil pH, EC and OC values as compared to other treatments. The highest soil pH (7.2), EC (0.34 dS $m^{-1}$), and OC (4.15 g $kg^{-1}$) was recorded with the treatment receiving biochar, which was statistically at par with BMC.

Increase in EC and OC of soil was expected due to biochar and BMC because the materials themselves had substantially higher EC and total C content (Table 1; Table S2) than that of the experimental soil. The alkaline nature and acid neutralizing capacity of biochar and BMC might have a role in increasing the soil pH (Lin et al., 2013; Saha et al., 2019). Previous studies too indicated that biochar and BMC application increased soil EC and OC (Li et al., 2014). During pyrolysis of biomass residues (high temperature), the biochar products would be composed of oxides of Ca, Mg and K, which might have increased the EC of highly weathered soil (Jien and Wang, 2013). The soil OC (SOC) content significantly ($P < 0.05$) improved with organic amendments, biochar and BMC application than the control and CF. SOC contributes significantly in improving soil physical and chemical properties which help in maintaining the soil fertility (Rao et al., 2017). Improvement of SOC after biochar and BMC application indicated their determining roles in increasing SOC which likely boosted the soil properties and plant growth in this study (Ye et al., 2016; Arif et al., 2017).

### 3.7. Inorganic N, and available P and K in soil

Similar to SOC, available nutrient status was also significantly improved with the application of vermicompost, biochar and BMC as compared to the control (Table 3). The highest inorganic N (47.83 mg kg$^{-1}$) was recorded with BMC application, which was 9.7 and 14.2% higher than biochar and CF applications. Available P content in soil was found distinctly higher in BMC application, which was 46.2 and 17.1% higher than biochar and CF. The highest available K (84.91 mg kg$^{-1}$) was recorded in the treatment receiving BMC, which was significantly ($P < 0.05$) higher than the biochar (80.23 mg kg$^{-1}$), and CF (79.37 mg kg$^{-1}$). As expected, available nutrients would improve significantly in soil with the addition of different organic amendments and chemical fertilizer treatments. An additional increase in nutrient content was observed due to the application of BMC. This phenomenon could be explained by the nutrient composition of BMC (Table 1) which was prepared from natural sources of P and K.  However, application of biochar also improved the soil nutrient availability, which indicated that apart from nutrient composition, the physico-chemical properties of biochar and BMC also played an important role. This hypothesis was supported by previous studies (Jien and Wang, 2013) where biochar notably improved the physico-chemical properties of highly weathered soils, maintained the SOC level, and increased nutrient use efficiency. Here, the increase in nutrient efficiency was expected from biochar and BMC applications due to higher nutrient retention and availability in the soil. Simultaneously, nutrient supply might have improved due to the prevention of nutrient loss occurring through leaching and volatilization in highly weathered soils of tropical regions (Major et al., 2010). The BMC was a nutrient rich formulation with improved physico-chemical properties derived from the combinational effects of biochar and minerals through organo-mineral complex formation (Lin et al., 2013). Therefore, BMC had some additional advantages over biochar in terms of nutrient composition, which was reflected in the present study. The outcomes of this study also corroborated with the findings a previous study (Ye et al., 2016) where BMC was found a superior nutrient source than other organic amendments. In the present study, BMC was produced from natural minerals, such as RP and waste mica, which were found effective in improving P (Basak, 2019a) and K (Basak,2019b) use efficiency, respectively, in highly weathered soil. These minerals might have contributed to the overall performance of BMC as a nutrient rich soil amendment.

### 3.8. Soil biological properties

Application of organic amendments, biochar, BMC, and CF had significant impact on soil biological properties (Fig. 6). A significant boost of MBC was observed in the soils treated with FYM, vermicompost, biochar, BMC, and CF. However, the highest MBC (153.1 mg kg$^{-1}$) was observed in the treatment receiving BMC, which was significantly ($P < 0.05$) higher than vermicompost, biochar, and CF (Fig. 6a). Generally, FYM and biochar are known for rich source of C, but having low nutrient content. On the other hand, chemical fertilizers are devoid of C source, but rich in mineral nutrients. Here, BMC acted as a balanced source of C and nutrients for soil microbes. The same was reflected in the results showing higher MBC in the soil treated with BMC due to a faster growth and proliferation of soil microbes. Soil respiration (SR) rate also followed a similar trend as MBC, where the treatment receiving vermicompost, biochar and BMC showed significantly ($P < 0.05$) higher SR than the rest of the treatments (Fig. 6b). The highest SR (2.74 mg $CO_2$-C kg$^{-1}$ day$^{-1}$) was recorded in the treatment receiving BMC, however, it was at par with vermicompost and biochar treatments (Fig. 6b). The SR represents the metabolically active microbial population in the soil, which was stimulated by the supply of substrates from vermicompost, biochar, and BMC. This was reflected in this study by higher SR in the treatments receiving vermicompost, biochar, and BMC. In case of soil enzyme activities, both DHA and ALP significantly ($P < 0.05$) increased in the treatment receiving vermicompost, biochar, and BMC as compared to FYM and CF. The application of BMC recorded the maximum DHA (22.4 µg TPF g$^{-1}$ h$^{-1}$) and ALP (49.2 µg PNP g$^{-1}$h$^{-1}$) in the soil, which was significantly ($P < 0.05$) higher than vermicompost and biochar (Fig. 6c and 6d). DHA is a metabolic enzyme which might have improved due to the balanced supply of substrates and mineral nutrients from BMC. ALP plays a vital role in the cycling of soil P, and is governed by the external P sources. Here, the application of P through RP in BMC had a much higher effect in improving ALP activity, which was accounted for 38.9% higher than soluble P fertilizer application (Fig. 6d).

The results of soil biological activities indicated that BMC was much more effective nutrient source than other organic amendments and chemical fertilizers in the highly weathered soil. MBC is considered as the most sensitive part of SOC, and is significantly influenced by the input supply (Nielsen et al., 2014). Here, application of FYM, vermicompost, and biochar acted as source substrates (C) for soil microbes, which was reflected in high soil MBC (Saha et al., 2019). The high soil MBC was found in the treatments receiving biochar and BMC that stimulated the microbial proliferation due to the supply of substrates, soluble nutrients, and congenial habitat. This result corroborated the finding of other workers where application of enriched compost (Basak, 2017), and enhanced biochar (Nielsen et al., 2014) improved soil MBC. The SR and DHA represent the microbial population, specifically the physiologically active microbes thriving in the soil (Nannipieri et al., 1990). The high SR and DHA with the addition of biochar and BMC could be explained by relatively high substrate availability, which could stimulate soil microbial population. Alkaline phosphatase is a predominant enzyme in soil, and is mainly governed by the soil microbial activities with low P availability (Spohn and Kuzyakov, 2013). In the present study, soluble chemical fertilizers showed a negative impact on ALP because of the abundance of readily available P. On the contrary, the supply of OC as well as insoluble P source (RP) from BMC stimulated the microbes for ALP activity. It was earlier observed that APL was not only influenced by the P source but also by the nature of C source (Spohn et al., 2015). Results of the current work had close conformity with earlier findings, where soil treated with P enriched compost significantly improved ALP both in soil incubation (Basak, 2017), and pot culture (Basak and Gajbhiye, 2018) experiments.

## 4. Conclusions

This study demonstrates successful utilization of waste biomass and low-grade mineral powders for the production of an inexpensive BMC which can be a precursor for environmental sustainability with the potential of phasing out chemical fertilizers in crop production. Blending of low-grade rock phosphate and waste mica minerals with biochar significantly improved the surface properties and nutrient contents of the BMC product. The enriched BMC had a remarkable positive impact on senna plant growth and nutrition, and was even more effective than chemical fertilizers in a highly weathered soil. The BMC also improved the soil quality by enhancing available nutrients and biological activities. Thus, BMC served as an excellent starter fertilizer, and could be a potential alternative of chemical fertilizers in medicinal plant cultivation. However, intensive field experiment is needed to evaluate the full potential of BMC for phasing out chemical fertilizers in order to sustain agricultural production. Nevertheless, this study provides a new advent of BMC as a sustainable and alternative nutrient source for boosting up crop production in highly weathered soil through effective recycling of biomass waste and mineral resources.

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## Declaration of interest

The authors declare no competing financial interests for this study.

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## Title of tables

Table 1. Comparative physico-chemical properties of farmyard manure (FYM), vermicompost (VC), biochar (BC), and biochar-mineral-complex (BMC).

Table 2. Comparative effects of biochar mineral complex, organic amendments, and chemical fertilizers on the growth and herbage yield of senna (*Cassia angustifolia*).

Table 3. Soil physio-chemical properties as influenced by the application of biochar-mineral-complex, organic amendments, and chemical fertilizers.

## Figure legends

Fig. 1. SEM images of (a) pristine biochar, and biochar-mineral-complex at (b) 1000X magnification, and (c) 5000X magnification.

Fig. 2. FTIR spectra of pristine biochar, and biochar-mineral-complex.

Fig. 3. XRD patterns of pristine biochar, and biochar-mineral-complex.

Fig. 4. Comparative effects of biochar-mineral-complex, organic amendments, and chemical fertilizers on bioactive molecule sennoside in *Cassia angustifolia* plant parts (Bars indicate standard error of mean, n=3).

Fig. 5. Primary nutrient contents in *Cassia angustifolia* plant tissues as influenced by biochar-mineral-complex, organic amendments, and chemical fertilizer applications (Bars indicate standard error of mean, n=3).

Fig. 6. Comparative effect of biochar-mineral-complex, organic amendments, and chemical fertilizers on soil biological properties under *Cassia angustifolia* growth (Bars indicate standard error of mean, n=3).

## Tables

Table 1. Comparative physico-chemical properties of farmyard manure (FYM), vermicompost (VC), biochar (BC), and biochar-mineral-complex (BMC).

| | pH | CEC [cmol($p^+$) $kg^{-1}$] | Total C (%) | Mineral N (mg $kg^{-1}$) | TKN (%) | WSP (mg $kg^{-1}$) | TP (%) | WSK (mg $kg^{-1}$) | TK (%) |
|---|---|---|---|---|---|---|---|---|---|
| FYM | 6.87 ± 0.09 | 43.9 ± 1.3 | 31.9 ± 0.4 | 57.4 ± 2.7 | 0.53± 0.07 | 93.7± 2.3 | 0.21± 0.05 | 89.6 ± 1.1 | 0.47 ± 0.01 |
| VC | 7.51 ± 0.10 | 47.1 ± 2.1 | 35.8 ± 0.6 | 143.2 ± 4.8 | 1.12 ± 0.13 | 118.1± 2.6 | 0.66 ± 0.05 | 128.2 ± 1.8 | 0.72 ± 0.04 |
| BC | 8.12± 0.06 | 49.7 ± 1.7 | 47.6 ± 1.1 | 5.2 ± 0.3 | 0.72 ± 0.08 | 103.7± 1.9 | 0.19 ± 0.03 | 92.3 ± 1.4 | 0.68 ± 0.03 |
| BMC | 6.85± 0.05 | 66.1± 2.3 | 8.91 ± 0.8 | 47.5 ± 2.3 | 0.45 ± 0.07 | 209.4 ± 2.7 | 1.38 ± 0.07 | 143.5± 2.1 | 2.74 ± 0.06 |

[Mineral N: ($NH_4^+$ + $NO_3^-$); TKN: Total Kjeldhal nitrogen; WSP: water soluble P; TP: Total P; WSK: water soluble potassium; Total K]

Table 2. Comparative effects of biochar mineral complex, organic amendments, and chemical fertilizers on the growth and herbage yield of senna (*Cassia angustifolia*).

| Treatments | Plant height (cm) | Number of branches plant$^{-1}$ | Fresh herbage yield (g plant$^{-1}$) | | Dry herbage yield (g plant$^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | Leaf | Pod | Leaf | Pod |
| $T_1$: Control | 56.4 | 12.8 | 119.1 | 18.4 | 40.5 | 4.54 |
| $T_2$: FYM | 63.7 | 13.7 | 129.4 | 20.5 | 42.7 | 5.09 |
| $T_3$: VC | 71.7 | 14.6 | 140.5 | 22.8 | 45.3 | 5.83 |
| $T_4$: BC | 71.9 | 14.9 | 142.8 | 24.3 | 46.3 | 6.15 |
| $T_5$: BMC | 74.6 | 16.7 | 150.2 | 25.9 | 50.7 | 6.29 |
| $T_6$: RFD | 72.9 | 15.1 | 143.4 | 23.8 | 47.3 | 6.02 |
| *CD ($p = 0.005$)* | *2.86* | *1.72* | *6.14* | *1.94* | *3.12* | *0.21* |

[FYM: Farmyard manure; VC: Vermicompost; BC: Biochar; BMC: Biochar mineral complex; RFD: Recommended dose of fertilizers; CD: Critical difference]

Table 3. Soil physio-chemical properties as influenced by the application of biochar-mineral-complex, organic amendments, and chemical fertilizers.

| Treatments | pH | EC ($dS\ m^{-1}$) | SOC ($g\ kg^{-1}$) | Mineral N ($mg\ kg^{-1}$) | Available P ($mg\ kg^{-1}$) | Available K ($mg\ kg^{-1}$) |
|---|---|---|---|---|---|---|
| $T_1$: Control | 6.89 | 0.21 | 3.91 | 34.50 | 13.40 | 72.03 |
| $T_2$: FYM | 7.02 | 0.25 | 3.97 | 37.43 | 14.37 | 75.53 |
| $T_3$: VC | 7.06 | 0.26 | 4.00 | 42.57 | 17.20 | 76.00 |
| $T_4$: BC | 7.20 | 0.34 | 4.15 | 43.61 | 14.63 | 80.23 |
| $T_5$: BMC | 7.17 | 0.31 | 4.10 | 47.83 | 21.40 | 84.91 |
| $T_6$: RFD | 6.86 | 0.23 | 3.85 | 41.89 | 18.28 | 79.37 |
| *CD (p = 0.005)* | *0.17* | *0.09* | *0.18* | *6.09* | *2.87* | *4.21* |

[FYM: Farmyard manure; VC: Vermicompost; BC: Biochar; BMC: Biochar mineral complex; RFD: Recommended dose of fertilizers; CD: Critical difference]

# Figures

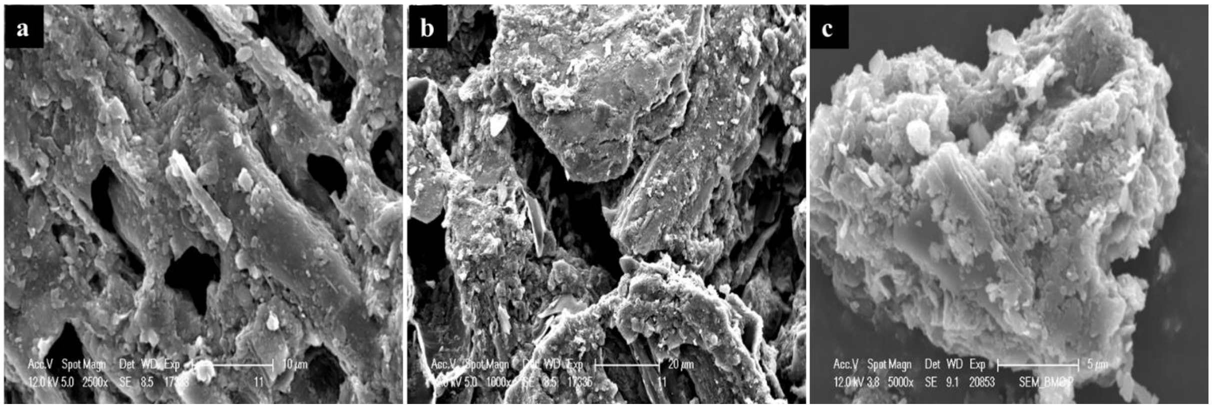


Fig. 1.

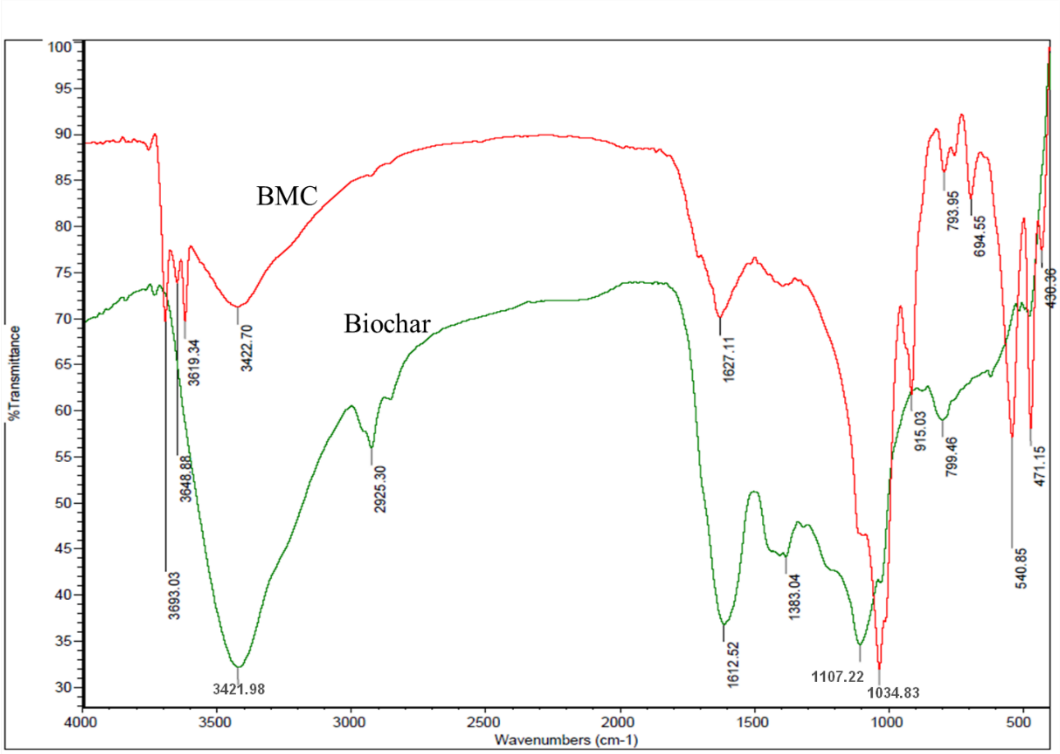
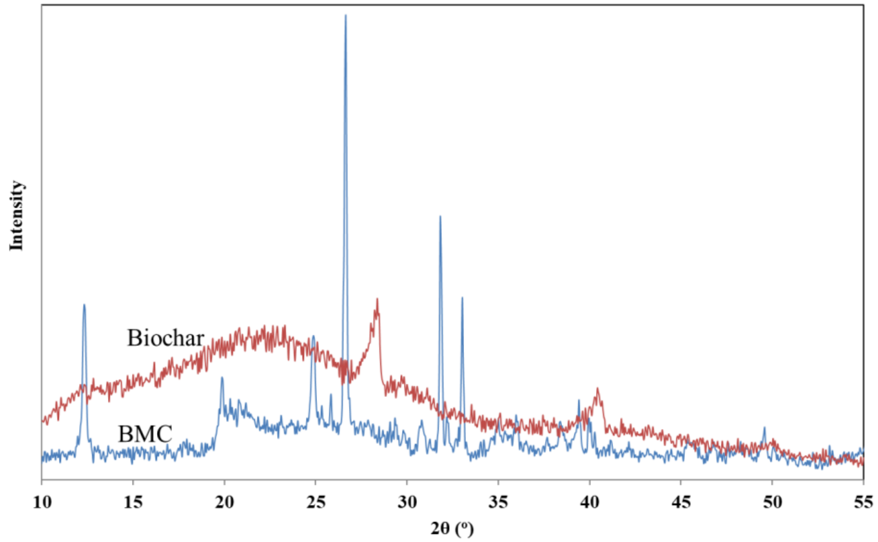
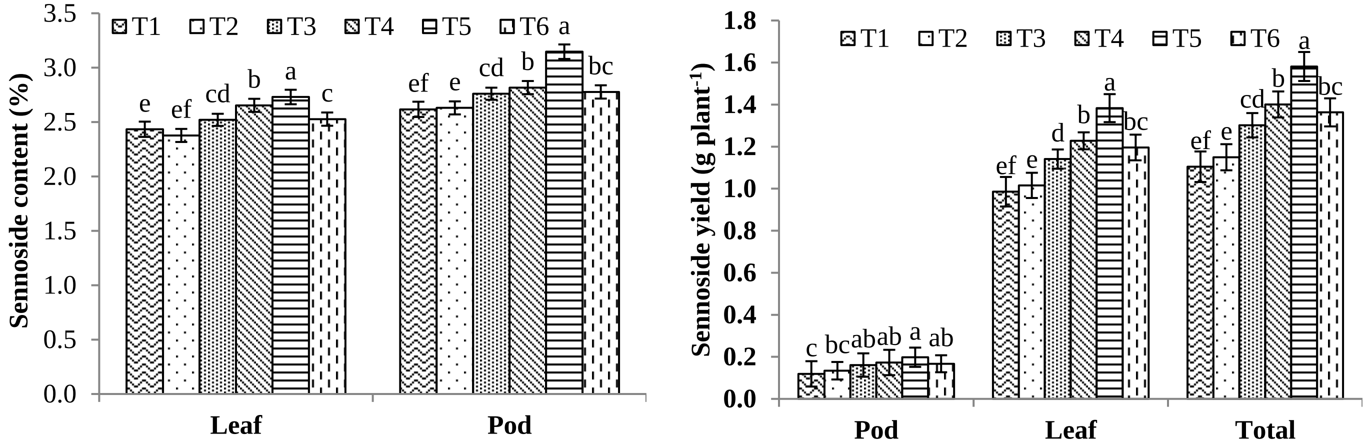


Fig. 2.



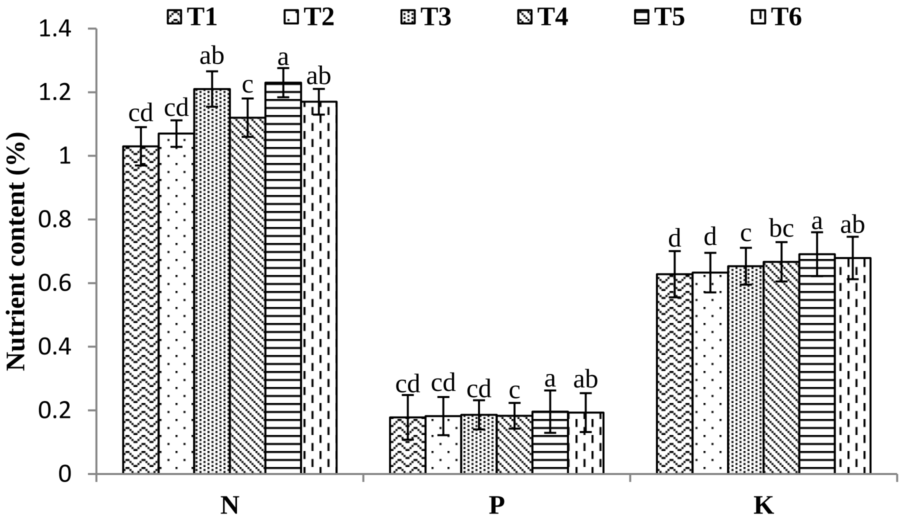
Intensity
Biochar
BMC
10
15
20
25
30
35
40
45
50
55
2θ (º)

Fig. 3.



T1
T2
T3
T4
T5
T6
Sennoside content (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
e
ef
cd
b
a
c
ef
e
cd
b
a
bc
Leaf
Pod
Sennoside yield (g plant-1)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
c
bc
ab
ab
a
ab
ef
e
d
b
a
bc
ef
e
cd
b
a
bc
Pod
Leaf
Total

Fig. 4.



737

738 Fig. 5.

739

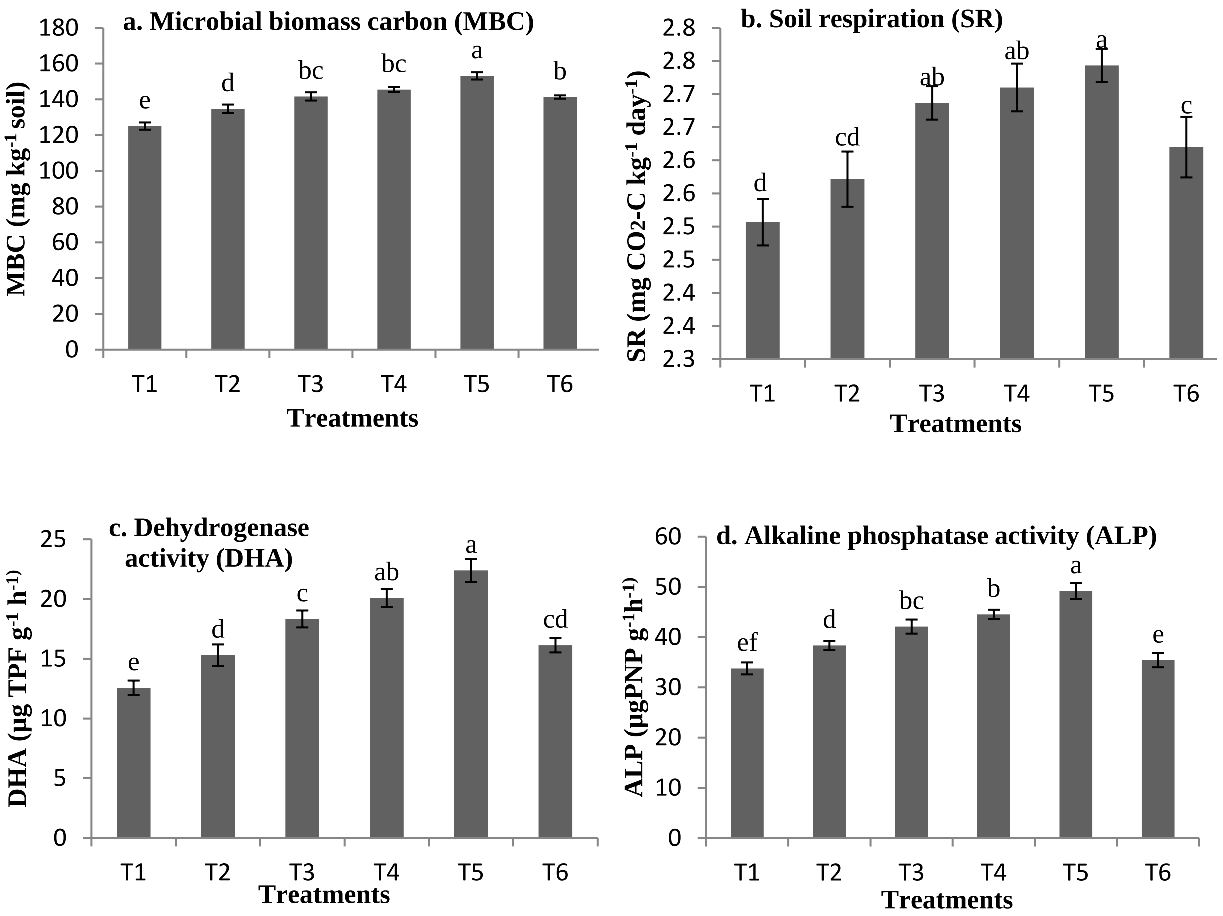

a. Microbial biomass carbon (MBC)
MBC (mg kg$^{-1}$ soil)
0
20
40
60
80
100
120
140
160
180
e
d
bc
bc
a
b
T1
T2
T3
T4
T5
T6
Treatments
b. Soil respiration (SR)
SR (mg CO$_2$-C kg$^{-1}$ day$^{-1}$)
2.3
2.4
2.4
2.5
2.5
2.6
2.6
2.7
2.7
2.8
2.8
d
cd
ab
ab
a
c
T1
T2
T3
T4
T5
T6
Treatments
c. Dehydrogenase activity (DHA)
DHA (µg TPF g$^{-1}$ h$^{-1}$)
0
5
10
15
20
25
e
d
c
ab
a
cd
T1
T2
T3
T4
T5
T6
Treatments
d. Alkaline phosphatase activity (ALP)
ALP (µgPNP g$^{-1}$h$^{-1}$)
0
10
20
30
40
50
60
ef
d
bc
b
a
e
T1
T2
T3
T4
T5
T6
Treatments

Fig. 6.

Supplementary Information:

# Repurposing distillation waste biomass and low-value mineral resources through biochar-mineral-complex for sustainable production of high-value medicinal plants and soil quality improvement

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### *Structural characterization and surface morphology of BMC*

Fourier transform infrared (FTIR) spectra of the pristine biochar and BMC were taken on a Shimadzu IR-Prestige-21® spectrometer (Shimadzu, Japan). Potassium bromide (KBr) pellets were prepared by mixing powdered samples, and spectra were recorded in 4000-400 $cm^{-1}$ scan range.

Morphology of the biochar and BMC surface was imaged on a scanning electron microscope (SEM) (Philips XL-30®, SEMTech Solutions, MA, USA) at different magnifications in order to confirm the complex formation. For this purpose, powdered sample was smeared on specimen stubs, and then gold coated (10 nm) under vacuum before being observed under SEM.

The powdered biochar and BMC were also studied by X-ray diffraction (XRD) technique. XRD pattern was acquired on a Philips PW 1710 diffractometer (Royal Philips, The Netherlands) with monochromatic CuKα radiation ($\lambda$=1.54 Å) at generator voltage 40 mA, and tube current 40 kV, with $2\theta$ value ranging from 5 to $80^o$, where $\theta$ is the diffraction angle.

### *Analysis of bioactive compound in plant tissues*

Dried leaf and pod samples were pulverized to make powder (<0.5 mm). Pulverized samples (100 mg) were extracted with aqueous methanol (70%, 20 mL) by sonication (10 min) (Srivastava et al., 1983). Extracts were centrifuged and filtered by syringe filter (0.45 µm), and analyzed on a High-Performance Liquid Chromatography (HPLC) equipment (LC-20AD, Shimadzu Corporation, Kyoto, Japan) configured with SPD-20A UV-Vis detector at 270 nm, an auto sampler (SIL-20AC HT), and GraceAlltima (100 × 4.6 mm, 3 µm) column with mobile phase consisting of methanol and 1.25% acetic acid in water.

### *Analysis of plant nutrients*

For the analysis of N, P and K contents, plant biomass samples were dried at 65°C in an oven, and crushed to powder in a Wiley mill (5-mm size). Samples were digested on an electric hot plate with a di-acid mixture ($HNO_3$:$HClO_4$: 9:4) (Piper, 1967). The K content in the digest solution was measured on a flame photometer (Model 128, Systronics, India). The P content in the digest solution was measured on a spectrophotometer (Model 117 Systronics, India) after developing a yellow color complex (vanadomolybdo-phosphate) (Jackson, 1973). For the determination of total N, samples were digested using $H_2SO_4$ and a catalyst mixture ($K_2SO_4$: $CuSO_4$: Se in the ratio of 200:10:1) at 400 ± 5°C for one hour in a micro-Kjeldahl digestion system (Model KES20L, Pelican Equipment, India). The digested samples were distilled in the micro-Kjeldahl system to measure the N content.

### *Analysis of soil samples*

Soil pH and EC were analyzed on a digital pH and EC meter (Aquamax KF, GR Scientific, UK), respectively, by suspending the soil in double distilled water (1:2.5(w/v) soil: water ratio) (Jackson, 1973). Soil OC was measured by rapid titration following the Walkley-Black method (Nelson and Sommers, 1996). Inorganic N ($NH_4^+$ + $NO_3^-$) was estimated by extracting the soil sample with 2 M KCl solution (Keeney and Nelson, 1982) followed by micro-Kjeldahl distillation. Available P (AP) was analyzed after extracting the soil with Mehlich I extractant solution (Mehlich, 1953), and the P concentration in the extract was measured by the spectrophotometer after developing blue color with ammonium molybdate (Watanabe and Olsen, 1965). Available K (AK) content in soil was analyzed by extracting with neutral 1N ammonium acetate ($NH_4OAc$) solution (Hanway and Heidel, 1952) followed by estimation of K on the Flame Photometer.

Chloroform fumigation-extraction method (Jenkinson and Powlson, 1976) was used for the MBC assay. Soil respiration (SR) was estimated by measuring $CO_2$-C released during incubation by alkali trap method (Anderson, 1982). Dehydrogenase activity (DHA) was assayed by measuring pink color intensity on the spectrophotometer due to the production of triphenyl formazan from triphenyl tetrazolium chloride (Klein et al., 1971). Alkaline phosphatase activity (ALP) in soil was estimated by measuring the yellow color intensity on the spectrophotometer due to the release of $\rho$-nitrophenol from $\rho$-nitrophenol phosphate substrate (Tabatabai and Bremner, 1969).

***Supplementary tables***

Table S1. Raw materials used for the preparation of enriched biochar mineral complex.

| Raw material | Description | Content by weight (g) |
|---|---|---|
| Biochar | Lemon grass (*Cymbopogon flexuosus*) biochar, produced at 350°C for 2 h, and treated with phosphoric acid | 32 |
| Clay | Air dried kaolinite (Kaolin) procured from Molychem, Mumbai | 30 |
| Organic matter | Farm yard manure (FYM) obtained from local cattle shed at Anand | 20 |
| Mineral powder | Low-grade minerals such as rock phosphate and waste mica. | |
| | - Rock phosphate from Rajasthan State Mines and Minerals Ltd., Udaipur, India | 12.5 |

| | | |
|---|---|---|
| | - Waste mica from mica mine of Koderma, Jharkhand, India | 5.5 |
| Water | Double distilled water | 30 |

Table S2. Selected characteristics of biochar mineral complex (BMC) prepared from distillation waste biomass of lemon grass and low-grade rock phosphate and waste mica.

| Properties (dry weight basis) | BMC$^*$ |
|---|---|
| BET Surface area (m$^2$ g$^{-1}$) | 26.7 (0.78) |
| Total pore volume (mL g$^{-1}$) | 2.41 (0.17) |
| pH | 6.85 (0.03) |
| Electrical conductivity (d Sm$^{-1}$) | 0.62 (0.06) |
| Cation exchange capacity (c mol (p$^+$) kg$^{-1}$) | 66.1 (1.21) |
| ANC (% CaCO$_3$ equivalent) | 10.3 (0.21) |
| Total Carbon (%) | 8.91 (0.3) |
| Total Nitrogen (g kg$^{-1}$) | 4.51 (0.08) |
| Total P (g kg$^{-1}$) | 13.8 (0.43) |
| Total K (g kg$^{-1}$) | 27.4 (0.47) |
| Ca (g kg$^{-1}$) | 30.8 (1.37) |
| Mg (g kg$^{-1}$) | 3.54 (0.83) |
| Zn (mg kg$^{-1}$) | 11.4 (0.23) |
| Cu (mg kg$^{-1}$) | 13.1 (0.41) |
| Fe (mg kg$^{-1}$) | 12212 (4.21) |

| | |
|---|---|
| Mn (mg $kg^{-1}$) | 186.8 (2.56) |

$^*$Value in the parenthesis indicates (± SE).

## *Supplementary figure*

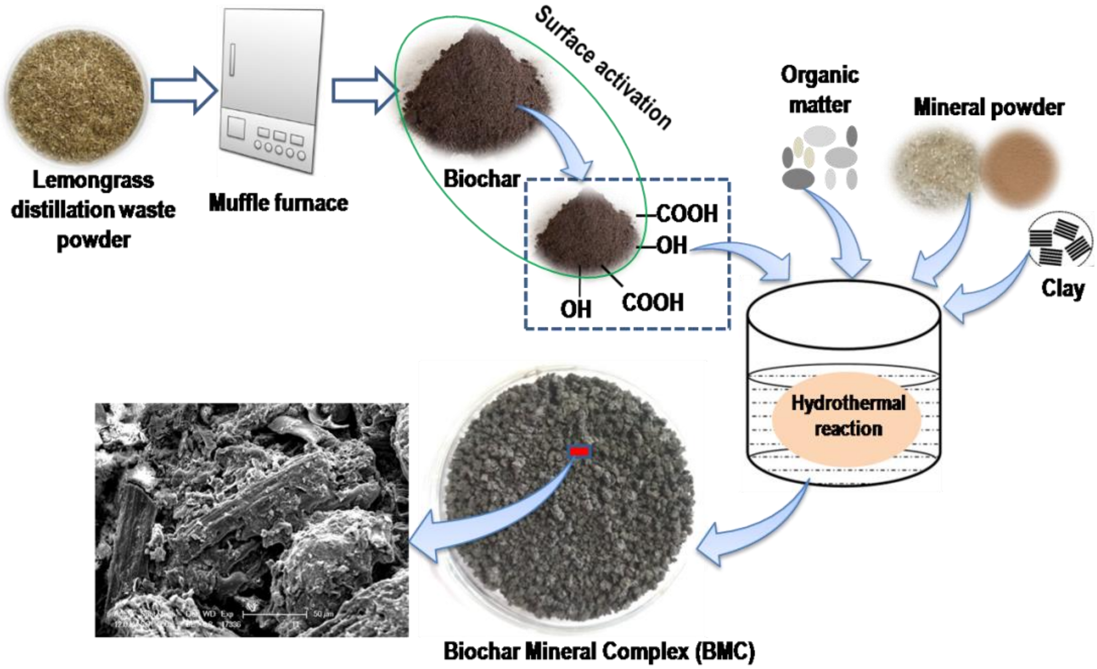

Fig. S1. Schematic diagram showing the preparation steps of biochar mineral complex.

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