



Paddy straw-based biodegradable horticultural pots: An integrated greener approach to reduce plastic waste, valorize paddy straw and improve plant health

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ABSTRACT

In the present study, we investigated the possible use of Paddy straw (PS) to develop biodegradable pots for the cultivation of transplantable horticulture crops, as alternative to plastic pots. To prepare biodegradable pots, six different Biocomposites (BCs) were developed using PS (untreated and treated) as filler, corn starch (native and cross-linked) as matrix and glycerol as a plasticizer. All the six BCs were characterized on the basis of various parameters (water uptake, mechanical strength, porosity, density, etc.). Biodegradability of all the six BCs was established under lab (*in vitro* CO₂ emission assay) and field condition (weight loss assay), which was further validated through SEM and FTIR analysis. Under *in vitro* conditions, none of the BCs was found antimicrobial. Among the six BCs, pots prepared from BC3, BC4, BC5, and BC6 were found physically stable for up to 28–30 days under greenhouse conditions. The plant growth variables of cucumber plant grown in these pots were equivalent to those grown in plastic pots. However, considering the ease of preparation, only BC3 and BC4 were selected for field studies. When BC3 and BC4 pots along with 30-day old cucumber plants were transplanted to fields, both the pots have shown disintegration and degradation within 10–20 days after transplantation, facilitating the penetration of roots out of the pots. Further, plants were found to be healthy and morphologically equivalent to those grown in plastic pots. In the background of rising concern about plastic waste, agro residue burning and their effect on the environment and human health in the long run, these biodegradable pots open a new path to reduce, reuse and recycle responsibly. Fuss-free transplantation is another resounding benefit associated with these pots. In future, these biodegradable pots may provide better opportunity to manage PS ecofriendly.

1. Introduction

Historically, heavy clay, wooden, metal and stone containers were used for raising horticulture plants under nursery conditions. However, these containers were heavyweight, rotted and broke easily, difficult to sanitize, ship, and expensive. Attempts to replace these materials with user-friendly and low-cost materials lead to experimentation with old food cans, peat pots, recycled paper pots, etc. It is only in the 1960s; plastic pots were introduced, and by the 1970s and 1980s, they had almost wholly replaced the clay pots and other potting materials (Orzolek, 2017). Since then, plastic has been used in various horticultural applications, and its usage is significantly increasing along with the

demand for horticulture produce.

Plastic pots are light in weight, low cost, less fragile and easy to ship (Kruger et al., 2018). On the other hand, they are made up of non-biodegradable polystyrene, polyethene, and polypropylene (Schettini et al., 2013), which makes them non-biodegradable a severe threat to the environment (Evans and Hensley, 2004). Another unnoticed problem with plastic pots is restricted aeration to the young growing roots, which is balanced by using a potting mix with high aeration. In addition to this, during transplantation, these plants are removed from the containers either by pulling or by cutting the container wall with sharp blades. During this process, young roots and root hairs may get damaged, reducing water and nutrient uptake efficiency and acting as

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infection sites for most of the soil-borne pathogens (Schrader, 2000). These undesirable effects of plastic pot usage on the plants and environment lead to explore new eco-friendly and sustainable materials for horticulture applications.

Paddy is a major crop grown in India, with 105.48 million tonnes of grain production in 2014–15 (Government of India, 2017). In the same year (2014–15), the total agro-residue generated by paddy was around 122.6 million tonnes (Devi et al., 2017). It is estimated that about 500 million tonnes of crop residues are generated annually in India, among which 34% was contributed by rice alone. (NPMCR, 2014). These crop residues are of low value and recalcitrant for rapid biodegradation. Further, this problem has intensified manifold because of the shortage of labour and the high cost of residual biomass transportation. In several paddy/wheat-growing regions of northern India, the recent trend is to burn the crop residue in the field to facilitate the agricultural activities for the next crop. This consequence in reduced soil microflora, organic C, nutrient recycling, etc. Further, enhanced air pollution due to the emission of particulate matter and greenhouse gases posing a threat to the local population (Singh, 2018). To valorize paddy straw (PS), several value-added products such as biofuels, composts, nano-silica, medium for mushroom cultivation, paper and cardboard, mulching material, etc., have been developed. However, possible use of PS to develop biodegradable biocomposites (BCs) is least explored and further their application in developing biodegradable horticulture pots was not attempted. Previously, several biodegradable transplantable pots such as Jiffy pots (madeup of peat and wood pulp) (Kruger et al., 2018; Evans and Hensley, 2004), Cowpots (composted cow manure and natural fibre), Coirpot (coir fibres), Fertilpots (wood fibre and peat) (Nambuthiri et al., 2015), and Netpot (rice hull) (Koeser et al., 2013), have been developed as alternative to plastic pots. However their large scale commercial application is still awaited. By considering the above facts, in the present study we aim to (i) use PS to develop biodegradable BCs, (ii) characterize BCs for their physico-chemical characteristics and biodegradation properties, and (iii) compatibility analysis as transplantable pots under greenhouse and field conditions for horticulture applications.

2. Materials and methods

Corn-starch, Glycerol (99%), n-Hexane (99%) AR, Chloroform, Bromothymol Blue (BTB), Curcumin, Sodium hydroxide (NaOH), Potassium hydroxide (KOH), Sulphuric acid (H_2SO_4), Boric acid (BA), Hydrochloric acid (HCl), Acetone and Acetic acid were procured from Sisco Research Laboratory (Mumbai, India). Nutrient Broth (NB), Potato Dextrose Broth (PDB) and Agar powder were of Himedia (Mumbai, India). 2 Ethyl 1, 3 hexanediol and Fluorescein Diacetate (FDA) were procured from Sigma (Darmstadt, Germany).

Paddy straw (dry biomass left after harvesting paddy grains) was purchased from farmers of Agra, Uttar Pradesh (U.P.), India. Microorganisms (*Trichoderma* spp., *Fusarium verticilloides*, *Aspergillus flavus*, *Bacillus marisflavi* and *Escherichia coli*) were procured from the culture collection of Environmental Biotechnology Lab, Centre for Rural Development and Technology, Indian Institute of Technology Delhi (IITD), India. Seeds of cucumber (*Cucumis sativus* L. variety 'Sultan') and Perlite were purchased from the local horticulture shop. The potting mix (organic seed starter) of Casa De Amor (Ghaziabad, India) was used for the greenhouse study. Muslin cloth was purchased from a local vendor.

2.1. Pretreatment of paddy straw

Paddy straw was chopped into 2–3 cm long bits using a sterile chopper knife and dried under sunlight. The dried PS was coarse powdered (1–2 mm size) using a mechanical grinder (750 W, Prestige, India) and used for pretreatment.

Alkali treatment (T1): Paddy straw powder was soaked in 1% NaOH solution (1:20, w/v, g/mL) at 33 ± 2 °C for 2 h. The treated PS powder

was filtered through four layers of muslin cloth and washed with distilled water until the pH of the washout water becomes neutral. The treated PS was dried under sunlight till it reached the constant weight.

Alkali-autoclave treatment (T2): Alkali treatment was performed, as explained above (T1). After the incubation period of 2 h, the PS powder and NaOH solution were autoclaved at 15 psi and 121 °C for 15 min. Afterwards, the PS was washed and dried, as explained in T1.

The treated (T1 and T2) PS were subjected to Scanning electron microscopy (SEM) [(ZEISS EVO 50, Germany) with an acceleration voltage of 20 kV at Central Research Facility (CRF), IIT Delhi, India] and Fourier transform infrared spectroscopy (FTIR) [(Spectrum 1, PerkinElmer, UK) located at Nano Research Facility (NRF), IIT Delhi, (Nanoscale research facility, IIT Delhi)] analysis following standard procedures.

2.2. Crosslinking of starch

Corn starch was crosslinked by using BA as a crosslinker by following the procedure given by Tanetrungroj and Prachayawarakorn (2018) and crosslinking was confirmed by starch solubility assay (Koo et al., 2010).

2.3. Preparation of biocomposite

Biocomposite 1 (BC1): Native starch and raw PS (size = 1–2 mm) were mixed in 2:3 (w/w) ratio. To 5 g of starch and PS mixture, 12.5 mL of distilled water and 1.5 mL of glycerol was added. This mixture was incubated at 90 °C for 45 min with frequent manual stirring. Biocomposite sheets were moulded by hand pressing between two ceramic plates (10 × 10 cm) coated with a nonstick silicon surface with spacers of 4 mm thickness. The obtained sheets were dried in a vacuum oven at 70 °C for 12 h.

Biocomposite 2 (BC2): Native starch and alkali-treated PS (T1) were used for preparing BC2. All the other process was the same as explained above for BC1.

Biocomposite 3 (BC3): Native starch and alkali-autoclaved PS (T2) was used in preparing BC3. All the other process was the same as explained above for BC1.

Biocomposite 4 (BC4): Crosslinked starch and alkali-autoclaved PS (T2) were used to prepare BC4. All the other process was the same as explained above for BC1.

Biocomposite 5 (BC5): Native starch and alkali-autoclaved PS (T2) were mixed in a 2:3 (w/w) ratio. To 5 g of starch and PS mixture, 70 mL of distilled water was added, and 400 mg of BA was used as a cross-linking agent. The pH of the mixture was adjusted to 12, with 0.1 M NaOH. The slurry was kept on a magnetic stirrer (700 rpm) at 50 °C for 12 h. The reaction was stopped by reducing pH to 7 with 0.1 M HCl. The obtained slurry was washed with distilled water thrice by centrifuging at 7500 rpm for 10 min. The pellets were dried in the oven at 50 °C for 24 h and powdered using a mechanical blender. The dried sample (5 g) and 12.5 mL water and 1.5 mL glycerol were used for preparing BC sheets (BC5), as explained above.

Biocomposite 6 (BC6): BC6 sheet was developed similar to BC5 without adding BA.

2.4. Characterization of biocomposites (BCs)

Water uptake, disintegration in the aqueous medium, porosity, density, quantification of macro and micronutrients, BA leachate study and antimicrobial behaviour of BCs were tested following standard procedures. Proximate analysis (Moisture, volatile matter, fixed carbon and ash content), Cellulose, hemicellulose and lignin content of raw and treated PS, and different BCs were done following standard procedures.

2.4.1. Mechanical testing

The mechanical tests were performed following a standard test method (ASTM D638, 2014) with some modifications in a universal test

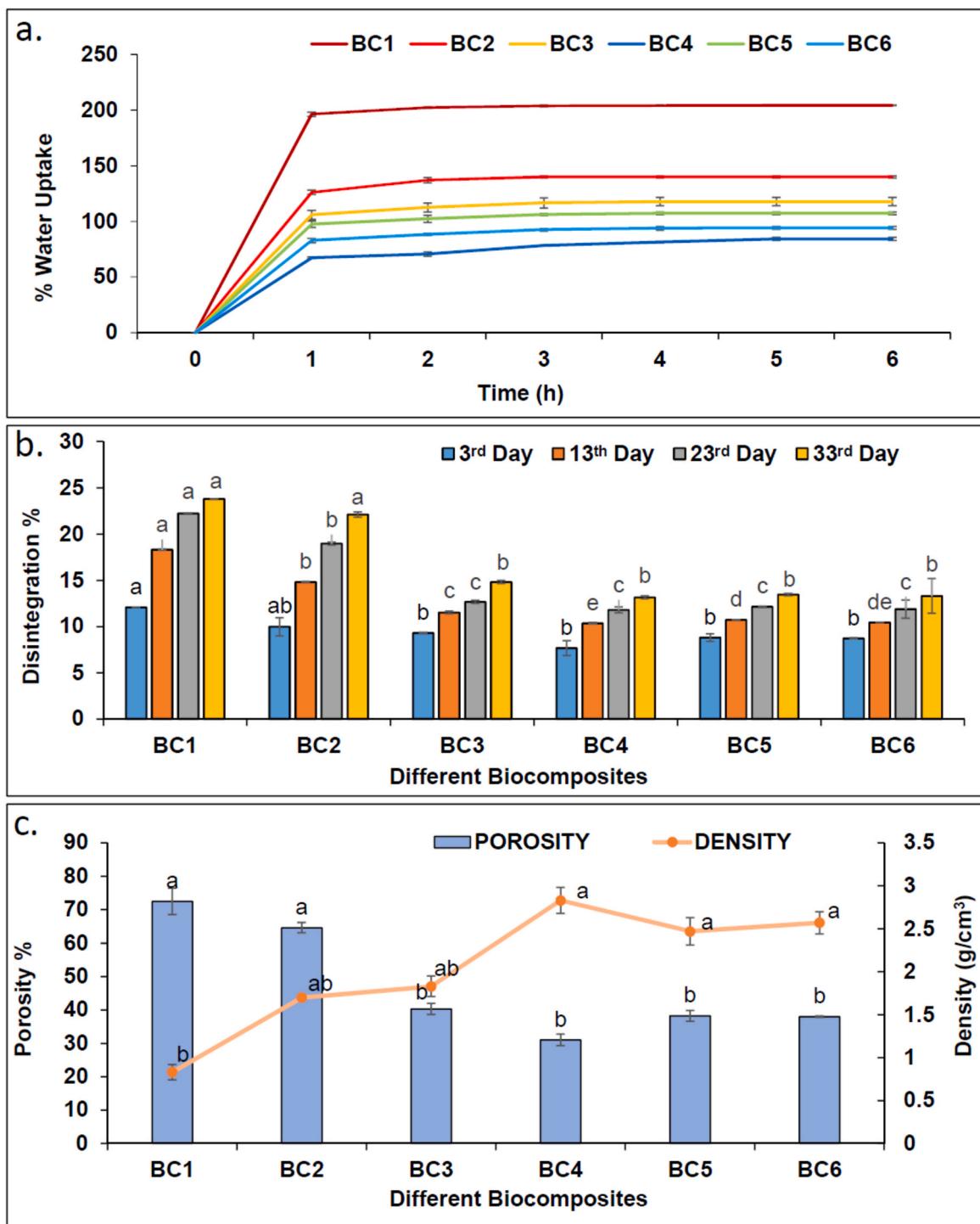


Fig. 1. (a) Water uptake percentage of different BCs with increasing incubation period; (b) Disintegration of various BCs in aqueous medium at different time intervals (days); (c) Porosity percentage and density of various BCs. Values are the means \pm SE of data from three independent experiments. Values followed by same letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

machine (UTM) (INSTRON 3365, United States) ([Figs. S1 and S2](#)) located at Physical Testing Laboratory, Department of Textile Technology, IITD, India.

The specimens of all BCs were dried in a vacuum oven at 70 °C for 12 h before testing. All the BC samples were subjected to mechanical testing under 5 kN load at a displacement rate of 2 mm/min in which the top grip of the instrument moved upward and the bottom grip was kept stationary. The ultimate failure of the sample was visualized by a sharp drop in stress on the load-extension curve. The fractured surface of the

failed specimens was subjected to SEM analysis.

2.4.2. Biodegradation

CO₂ emission during the degradation of BCs was measured under laboratory conditions following the standard method ([ASTM D5988, 2014](#)) with minor modifications ([Fig. S3](#)). In order to test the degradation of BCs under field conditions, the BC samples of 4.5 × 4.5 cm (l × b) were dried at 70 °C for 12 h in a vacuum oven and weighed (W1). These sheets were placed in a plastic frame (4.5 × 4.5 × 0.1 cm; l × b × w) and

buried in pits of 1 feet length, 1 feet width and $\frac{1}{2}$ feet depth, excavated at Mahatma Gandhi Gramodaya Parisar (MGGP), IITD, India (Fig. S4).

During the whole experimental period (March to July 2019), temperature, humidity and rainfall were noted down (for Longitude 77.19 °E and Latitude 28.54 °N) from an online source (<http://www.soda-pro.com, n.d.>) (Fig. S5).

All the BCs were carefully removed from the soil after every 5 days. The recovered samples were cleaned with a brush to remove any soil debris and then dried at 70 °C for 12 h in a vacuum oven and weighed (W2). The weight loss percentage of BC was calculated by employing the formula:

$$\text{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{Eq. (1)}$$

The morphology of BCs before and after the soil burial test were examined under SEM. Further, FTIR was employed for monitoring any change in functional groups of the BCs after degradation.

Cultivable microbial diversity associated with degradation of BCs was analyzed both for bacteria and fungi by following standard procedures. Total and morphologically different fungi and bacteria in each BC were tabulated. Total active microbes associated with degradation of BCs were analyzed relatively following FDA hydrolysis assay using native soil as control (Green et al., 2006).

2.5. Greenhouse and field studies

All the BCs were used to prepare the pots using a hand-pressed moulding machine designed at Environmental Biotechnology Lab, IITD and fabricated at JPC Lab Solution, Delhi, India (Fig. S6a). The pots were dried in a hot-air oven at 70 °C for 12 h and stored in an airtight zip lock cover. The pot dimensions were height - 5.5 cm, end base diameter - 5.5 cm, top base diameter - 6.0 cm, a wall thickness - 2.0 ± 0.1 mm, volume ≈ 130 mL and a weight of 25 ± 1 g (Fig. S6b). Further, 4 holes of diameter 3 mm were made as the bottom of the pot to allow passage of excess water.

A greenhouse study had been performed by growing cucumber seedlings for 28 days at MGGP, IITD. For each BC and plastic pot, four replicates, each with 8 pots, were maintained and arranged in a completely randomized design. There were eight blocks, each containing 28 pots, constituted by four randomly picked pots from each BC. Within each block, the pots were arranged randomly. Seeds were washed thoroughly with distilled water and sown in each BC pot containing a commercial potting mixture. For control, commercial plastic trays were used in place of BC pots. The seedlings were grown without any fertilizer input and irrigated equally for all the pots during the growth period. After 28 days, cucumber seedlings were carefully removed from the pots and root length, shoot length, fresh weight, dry weight and Seedling Vigour Index (SVI) were measured.

In another set of experiments, 28 days old cucumber seedlings along with the BC pots were transplanted in the research field (3×3 m plots) at MGGP, IITD. While in the case of control, cucumber seedlings were removed from the plastic plug tray, then transplanted in the field. The plant growth and degradation of pots and root penetration were visually observed up to 40 days after transplantation. During the whole experimental period (March to June 2020), temperature, humidity, and rainfall (for Longitude 77.19 °E and Latitude 28.54 °N) were noted down from online sources (<http://www.soda-pro.com, n.d.>) (Fig. S7).

2.6. Water loss through the wall of BC3 and BC4 pots

Plastic, BC3 and BC4 pots were filled with standard potting mix. The weight of potting mix saturated with water (W) and the pot and the saturated potting mix (W1) were recorded. The pots were topped with the covers (aluminium foil) to ensure that water loss happens only through the walls of the pots and kept at 28 ± 2 °C. The pots were weighed (W2) after each 24 h. Water loss % was estimated:

$$\text{Water Loss(\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{Eq. (2)}$$

2.7. Statistical analysis

Values obtained in experiments were expressed as means with standard error. The results were statistically analyzed using the one way Analysis of Variance (ANOVA) with SPSS (version 25.0). The significant difference between the means was compared using the highest significant difference (HSD) as obtained using Tukey's test at the $p < 0.5$ level. Data generated by analyzing the different characters of all BCs was subjected to 2D principle component analysis using OriginLab (version 2021b).

3. Results and discussion

Under SEM, PS shows the removal of lignin, wax and hemicellulose and fibrillation with T1 and T2 treatment (Fig. S8). These results were also confirmed through FTIR analysis (Fig. S9). The crosslinked starch has an overall tendency to have less solubility (33%) in comparison to native starch (51%) as confirmed by starch solubility assay (Fig. S10).

Moulding and drying different BCs yielded sheets of thickness 2.5 ± 0.3 mm. These BC sheets were subjected to various physicochemical and biological analyses to study their fitness to develop biodegradable BC pots.

3.1. Water uptake

Water absorption of any BC is one of the essential properties, which play a vital role in determining its end application. In our studies, the water uptake process was very rapid for an initial 1 h for all the BCs tested; after that, it slowed down and reached saturation (Fig. 1a). This behaviour of water uptake is typical of Fickian diffusion (Azwa et al., 2013; Reddy et al., 2018).

Water uptake ranges from 84.19 to 204.28% (Fig. 1a) for various BCs. BC4 recorded the least water uptake of 84.19% compared to other BCs and the highest uptake was by BC1 (204.28%). The values are comparable to those ($\approx 92\text{--}280\%$), reported by Edhirej et al. (2017) for cassava bagasse/sugar palm fibre reinforced cassava starch hybrid composites. However, it was significantly lower than Peat Moss (476%), Wood Fibre (316%) and Cow Manure (391%) based seedling trays (Zhang et al., 2019), indicating better integrity of the BCs developed in the present study.

3.2. Disintegration/solubility

BC with the least solubility/disintegration in an aqueous medium to maintain structural integrity is preferred by considering the end application. Fig. 1b shows the disintegration % of various BCs in the water at different time intervals. BC4 has shown the least disintegration of 13.16%, while BC1 showed the highest disintegration of 23.80%. The pattern of the disintegration of the BCs is the same as observed for water uptake, i.e. it is progressively decreasing from BC1 to BC4. Disintegration % of BC5 and BC6 were not significantly different from BC4.

3.3. Porosity and density

The porosity of BC is primarily due to the presence of air-filled cavities within BC. In the case of BCs, the porosity is inversely proportional to the density. In our experiments, BC1 recorded the highest porosity (72.50%) in comparison to other BCs and the least porosity was recorded by BC4 (31.03%). However, the porosity of BC5 (38.2%) and BC6 (37.97%) was higher than BC4 (31.03%) but not significantly different from each other. The density of the BCs followed an inverse trend, where BC4 (2.83 g/cm^3) and BC1 (0.83 g/cm^3) recorded the

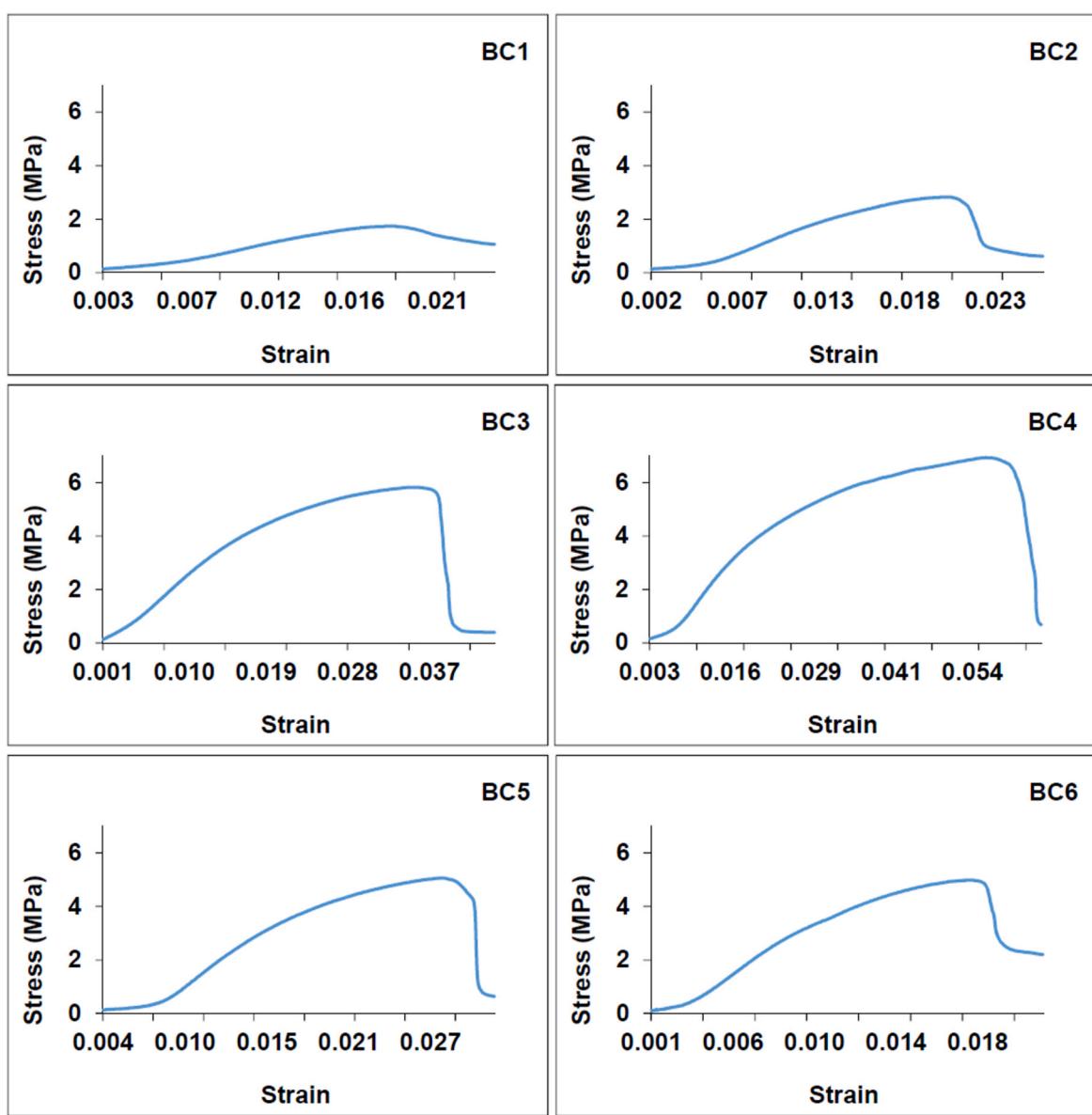


Fig. 2. Typical stress-strain curves for various BCs.

highest and least density, respectively (Fig. 1c). The porosity and density values recorded by various BCs also correlated well with their water uptake ability. Where, BC1 with high porosity and least density recorded highest water uptake and BC4 with low porosity and high density recorded least water uptake.

3.4. Mechanical test

The mechanical strength of pots is an important indicator of their capability to withstand the rigours of biotic and abiotic stress under greenhouse conditions such as transportation, handling, transplantation, semi-mechanized filling, watering, plants and microbes. However, there are no standard values available with reference to agro residue based BC pots. When BCs were subjected to mechanical tests, stress increased with the strain in a non-linear fashion, reaching a peak where the material failed. Typical stress-strain curves for different BCs are shown in Fig. 2. The failed specimens of all the 6 BCs exhibited corrugated edges (Fig. S11) implies that the failure process was a tearing process characteristic of a ductile material.

Different parameters indicating mechanical strength is shown in

Fig. 3a – e. Maximum extension (NM), Maximum tensile strain and Tensile strain (extension) of different BCs were not significantly different. However, BC4 recorded significantly higher Maximum load (N) and Maximum tensile stress (MPa). The tensile strength of all the BCs varied from 1.96 to 6.82 MPa (Fig. 3), which was higher than those reported by Zhang et al. (2019) for Peat Moss (\approx 1.2 MPa), Cow manure (\approx 2 MPa) and Wood fibre-based (\approx 1 MPa) seedling trays. The values of the tensile strength of BCs in this study are comparable to those composite, prepared by using protein hydrolysate, PEG and wood/sawdust (4.0–12.5 MPa) by Sartore et al. (2018) which was used to develop nursery containers. On the other hand, the tensile strength of the developed BCs was lower than those reported by Castronuovo et al. (2015) for polyester/plant fibres based biodegradable pots (\approx 20–22 MPa). The higher tensile strength observed in this case might be because of the synthetic matrix (polyester). Among all of the BCs, BC1 had shown the least tensile strength (1.96 MPa) and the highest was recorded with BC4 (6.82 MPa) (Fig. 3).

The results obtained from the above four parameters indicated BC1 and BC4 as the poorest and strongest material, respectively for producing horticultural pots in terms of their structural integrity and

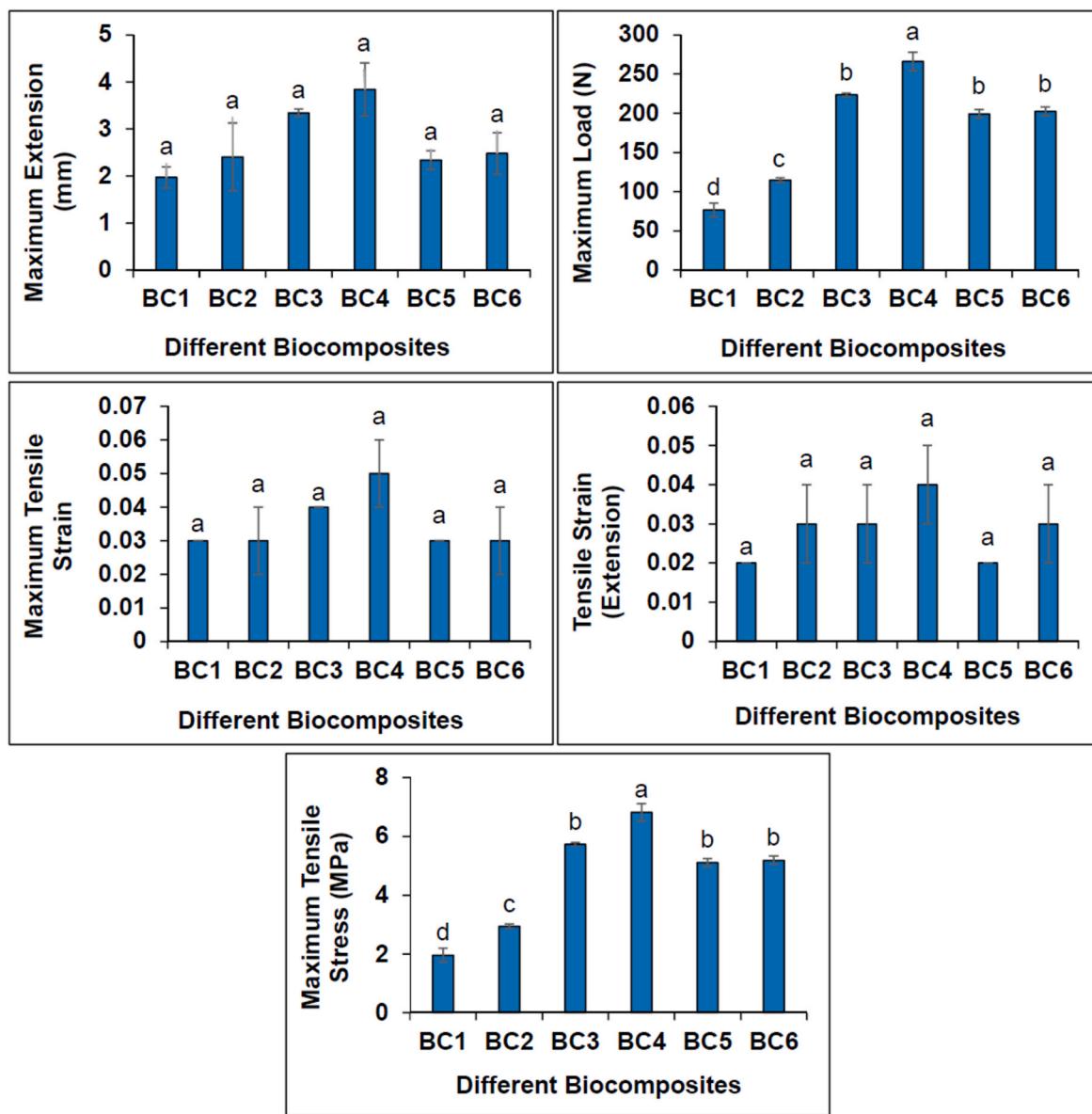


Fig. 3. Tensile properties for different BC (a) maximum extension (b) maximum tensile strain, (c) maximum load, (d) maximum tensile stress and (e) tensile stress at maximum load. Values are the means \pm SE of data from three independent experiments. Values follow by same letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

Table 1

Macro and micronutrients present in different Biocomposites.

	N (mg/g)	P (µg/g)	K (µg/g)	Ca (µg/g)	Mg (µg/g)	Fe (µg/g)	Zn (µg/g)	Mn (µg/g)	Mo (µg/g)	B (µg/g)
BC1	3.36 ± 0.04^a	706.51 ± 18.46^a	4323.54 ± 127.25^a	393.31 ± 8.03^a	1769.51 ± 26.24^b	592.67 ± 8.87^b	393.78 ± 3.53^c	227.92 ± 2.28^a	1.08 ± 0.01^a	20.14 ± 0.07^c
BC2	1.76 ± 0.04^d	407.56 ± 7.07^b	150.5 ± 1.33^b	380.31 ± 4.39^a	1202.86 ± 14.41^c	397.57 ± 3.53^d	328.03 ± 1.11^d	107.67 ± 1.29^f	0.07 ± 0.00^e	8.82 ± 0.13^c
BC3	2.22 ± 0.03^b	431.47 ± 8.06^b	175.00 ± 2.73^b	394.13 ± 5.60^a	1703.12 ± 24.66^b	424.36 ± 8.46^d	300.92 ± 6.33^e	197.24 ± 5.01^c	0.11 ± 0.00^d	9.56 ± 0.22^c
BC4	1.57 ± 0.04^e	419.05 ± 9.77^b	148.91 ± 3.04^b	322.39 ± 4.75^b	1914.06 ± 34.94^a	473.55 ± 2.68^c	509.53 ± 5.15^b	212.28 ± 1.18^b	0.11 ± 0.00^d	99.73 ± 2.73^b
BC5	1.97 ± 0.02^c	302.96 ± 5.72^c	325.74 ± 6.12^b	232.14 ± 1.29^c	1208.66 ± 26.25^c	652.96 ± 11.20^a	402.02 ± 8.12^c	141.70 ± 2.62^e	0.14 ± 0.00^c	610.45 ± 10.80^a
BC6	1.31 ± 0.01^f	333.17 ± 8.06^c	207.47 ± 5.95^b	226.79 ± 1.30^c	1092.87 ± 10.00^d	617.36 ± 16.25^{ab}	534.42 ± 9.65^a	159.54 ± 3.70^d	0.18 ± 0.00^b	20.27 ± 0.53^c

Values are the means \pm SE of data from three independent experiments. Values follow by same superscripted letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

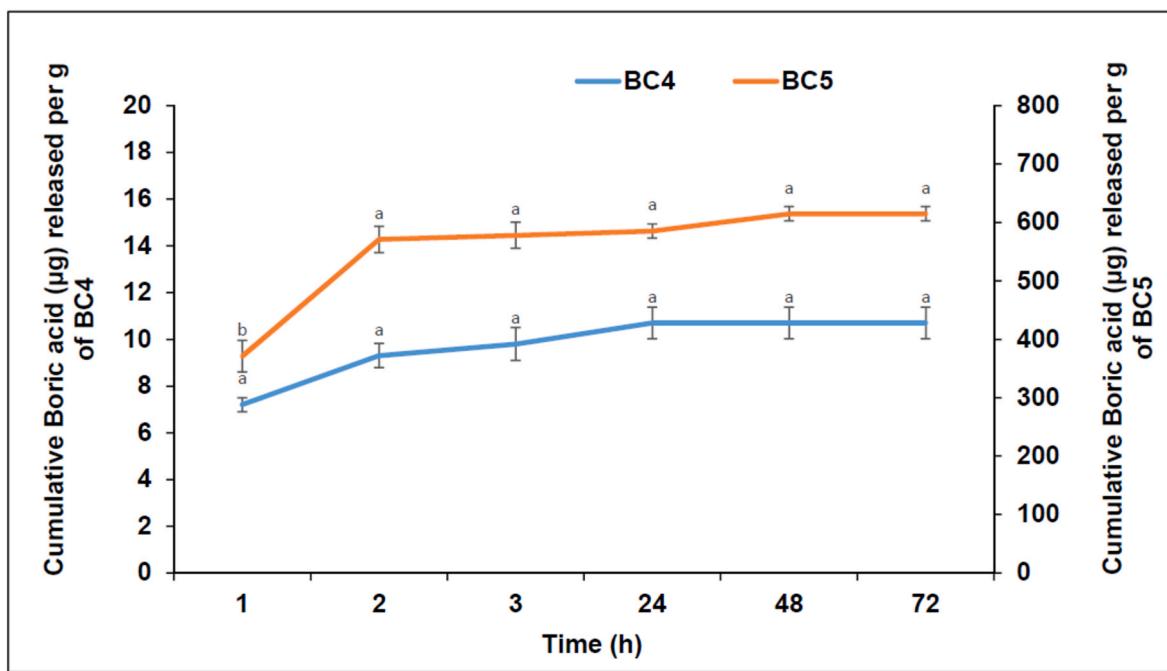


Fig. 4. Boric acid leachate pattern from BC4 and BC5 at different time intervals. Values are the means \pm SE of data from three independent experiments. Values followe by same ltters are not significantly different according to Tukey's test at the $p < 0.5$ level.

mechanical strength. In BC1, lignin, wax, and oil on the surface of raw PS fibre (Fig. S8a) interfere in fibre and matrix interaction, leading to reduced interfacial adhesion between the two. Whereas in the case of BC2, alkali treatment of PS removes the lignin, oil, and wax from the fibres surface (Fig. S8b), leading to fibrillation, i.e., breakdown of the fibre bundles into elementary fibrils (Negawo et al., 2019), resulting in increased surface area for mechanical interlocking between fibre and matrix. Further, removal of lignin exposed the cellulose on the surface and hydroxyl groups of cellulose were free to interact with the matrix that improves mechanical interlocking between fibre and matrix (Chand and Fahim, 2008). Combined alkali and autoclave treatment of PS (BC3, 4, 5 and 6) were more effective in removing non-cellulosic materials (lignin, hemicellulose and pectin) (Fig. S8c and Sup Table 1) from the inter-fibrillar region and creating roughest surface compared to alkali treatment alone (Obeng et al., 2019). Proximate analysis and, cellulose, hemicellulose and lignin quantification supported the above observations. Cellulose content of BC3, BC4, BC5 and BC6 were found higher than BC1 and BC2 which also explains the improved characteristics from BC1 to BC4 (Sup Table 1). These observations evidenced an improved quality in terms of water uptake, disintegration, porosity, density and mechanical strength from BC1 to BC3.

Whereas in BC4, the parameters mentioned above were further improved, using BA-cross linked starch as a matrix. Since BA crosslinker replaces the $-OH$ group on starch and restricts the molecular motion, resulting in more hydrophobicity and stability (Awada et al., 2014; Tanetrungroj and Prachayawarakorn, 2018). In BC5 and BC6 an attempt was made to crosslink the starch and cellulose fibres with or without BA. BC5 and BC6 were not significantly different from BC4 in disintegration, porosity and density. However, their water uptake percentage and tensile strength were significantly inferior in comparison to BC4. Further, more physico-chemical analyses are required to explain this behaviour of BC5 and BC6.

3.4.1. SEM analysis of fracture surface of BC

The structural integrity and mechanical behaviour of all the BC can be explained by considering the SEM micrographs of the fracture surface obtained after the failure of specimens in the mechanical test. Poor mechanical strength of BC1 and BC2 was confirmed by the failure mode

of both the BCs, which show easy fibre pullout from the matrix [Fig. S12 (a, b) and Fig. S13 (a, b)]. The fibre surface was poorly bonded to the matrix, as observed in Fig. S12 (c, d) and Fig. S13e. Further, the presence of large voids was observed on the fracture surface [Fig. S12 (e) and Fig. S13 (c, d)] indicated the ineffective stress transfer from the fibre to the matrix.

However, in other BCs, (BC3, BC4, BC5 and BC6), the presence of a large number of microfibrils increases the effective interfacial area of adhesion between fibre and matrix (Figs. S14, 15, 16 and 17). Further bonding of the matrix with fibre is evident by the coating of fibre with matrix (Figs. S14e, 15e, 16e and 17e). All these properties correspond to the improved mechanical performance of these BCs in comparison to BC1 and BC2. Because of crosslinked starch, BC4 had shown marginal improvement in tensile strength over other BCs.

3.5. Macro, micronutrients, boric acid leachate and antimicrobial study

Macro and micro-nutrient composition of BCs such as N, P, K, Ca, Mg, Mn, Zn, Mo, Fe and B are represented in Table 1. In soil, these nutrients may be released along with the disintegration and degradation of BCs, enhancing the nutrient status. Higher content of N recorded in BC1 indicated that alkali and autoclave treatment removes some of the N containing molecules from PS as evident from the low N content in other BCs. Similar observations were also made for P and K.

Boron (B) is one of the essential elements for the optimum growth and yield of the crop (Shireen et al., 2018). On the other hand, a higher concentration of BA may be toxic to plants ($IC_{50} = 175 \mu\text{g/g}$ of soil for *Brassica napus*) and microbes ($IC_{50} > 2400 \mu\text{g/g}$ of soil) (Becker et al., 2011). In our studies, BA was used as crosslinker in BC4 and BC5. The higher level of BA ($613 \mu\text{g/g}$) was recorded in BC5 followed by BC4 ($100 \mu\text{g/g}$), which was still lesser than IC_{50} values for microbes ($> 2400 \mu\text{g/g}$). However, in BC5, it was relatively higher than the IC_{50} value for plants ($175 \mu\text{g/g}$) (Becker et al., 2011). The effect of BA on plants and microbes depends on the rate at which it releases from BCs. In BC5, $371 \mu\text{g BA/g BC}$ was released in the surrounding environment within 1 h of incubation. Whereas in BC4, BA release was only $7.20 \mu\text{g/g}$ in the first 1 h. During 24 h, cumulative release of BA was $10.7 \mu\text{g/g}$ in BC4; after that there was no release of BA was recorded up to 72 h (Fig. 4). However,

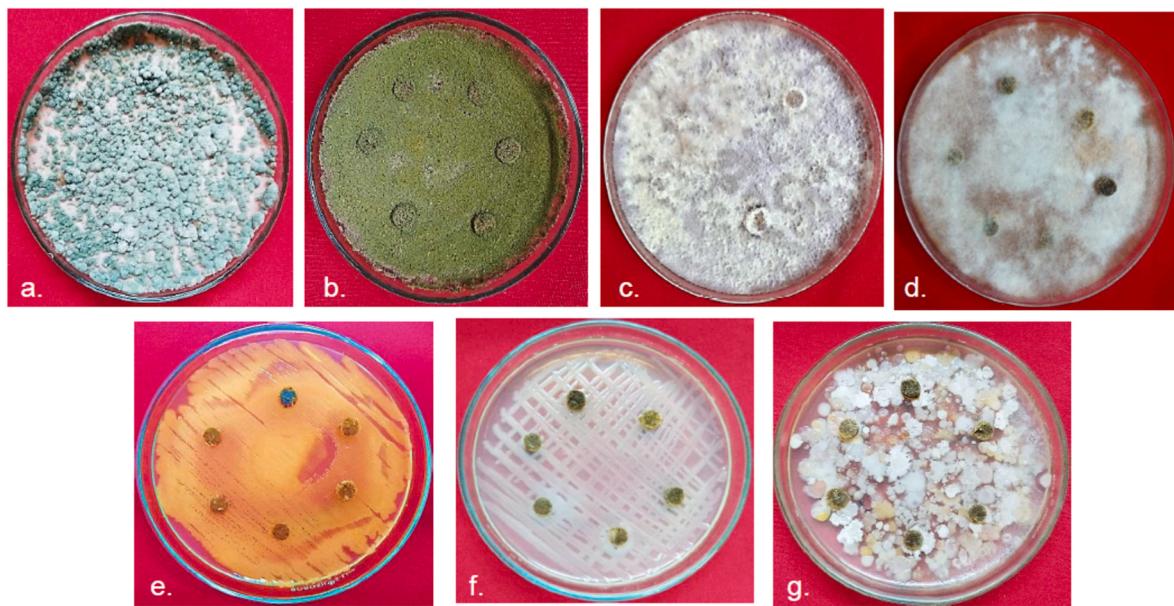


Fig. 5. Antimicrobial activity of different BCs against (a) *Trichoderma* spp.; (b) *Aspergillus* flavus; (c) *Fusarium* verticilloides; (d) Total soil fungi; (e) *Bacillus* marisflavi CRDT-EB-1; (f) *Escherichia* coli and (g) Total soil bacteria.

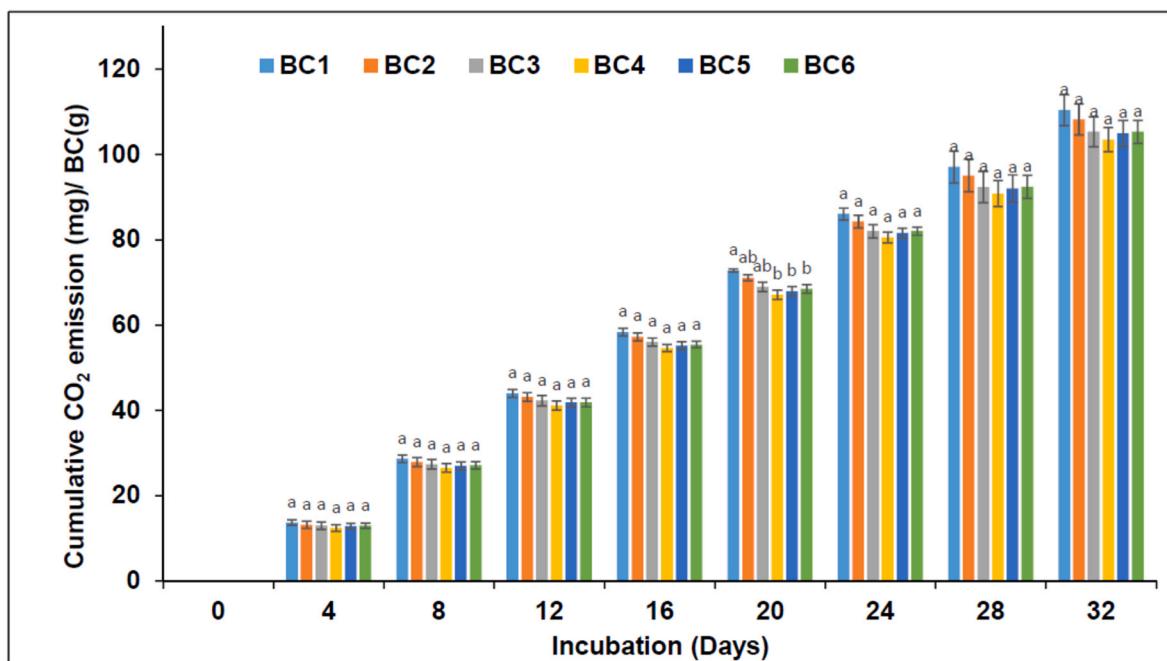


Fig. 6. CO₂ emission for different BCs under simulated environmental conditions. Values are the means \pm SE of data from three independent experiments. Values followe by same ltters are not significantly different according to Tukey's test at the $p < 0.5$ level.

most of the BA present in the BC5 was leached within 24 and 48 h of the leaching experiment (Fig. 4).

Since the BC pot should ultimately degrade in soil, it is desirable that it should not suppress soil microbial growth. All the 6 BCs tested were found not inhibitory to test bacteria, fungi, total cultivable soil bacteria and fungi, as shown in Fig. 5. However, earlier, a pine needle/bark meal reinforced BC and was reported to have antimicrobial properties that delayed its soil degradation (Treinyte et al., 2018).

3.6. Biodegradation

Fig. 6 shows mg CO₂ released per g of BC during the process of

microbial degradation. Cumulative CO₂ emission was highest (110.39 mg/g BC) for BC1 compared to other BCs. Least CO₂ emission was recorded by BC4 (103.45 mg/g BC) followed by BC5 (104.92 mg/g BC), BC6 (105.28 mg/g BC), BC3(105.31 mg/g BC) and BC2 (108.19 mg/g BC). The higher biodegradation of BC1 mainly because of its composition and physicochemical characters, which were discussed earlier.

By considering the application of the developed BC, i.e., transplantable pot, it is imperative that developed BC pot should degrade as soon as possible after transplantation so that BC pot would not cause any obstacle for the establishment of roots in the soil. Weight loss is considered an important tool to evaluate the effect of soil environment on the degradation behaviour of BC. Weight loss % for all the BCs for 20

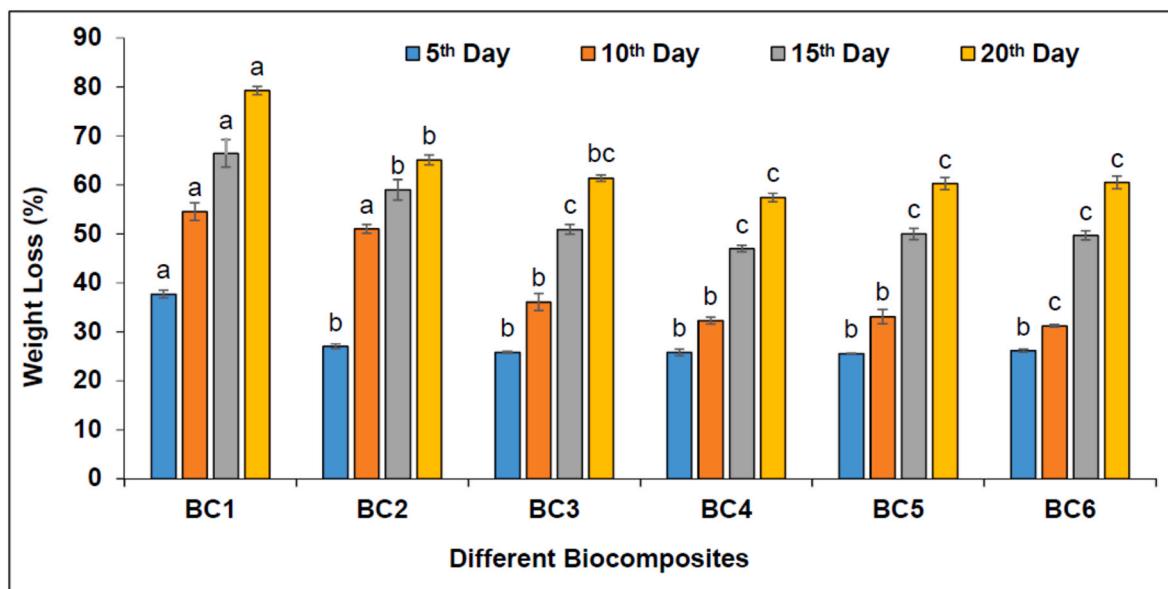


Fig. 7. Weight loss percentage of different BCs at different time intervals (days) of incubation under field condition. Values are the means \pm SE of data from three independent experiments. Values followed by same letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

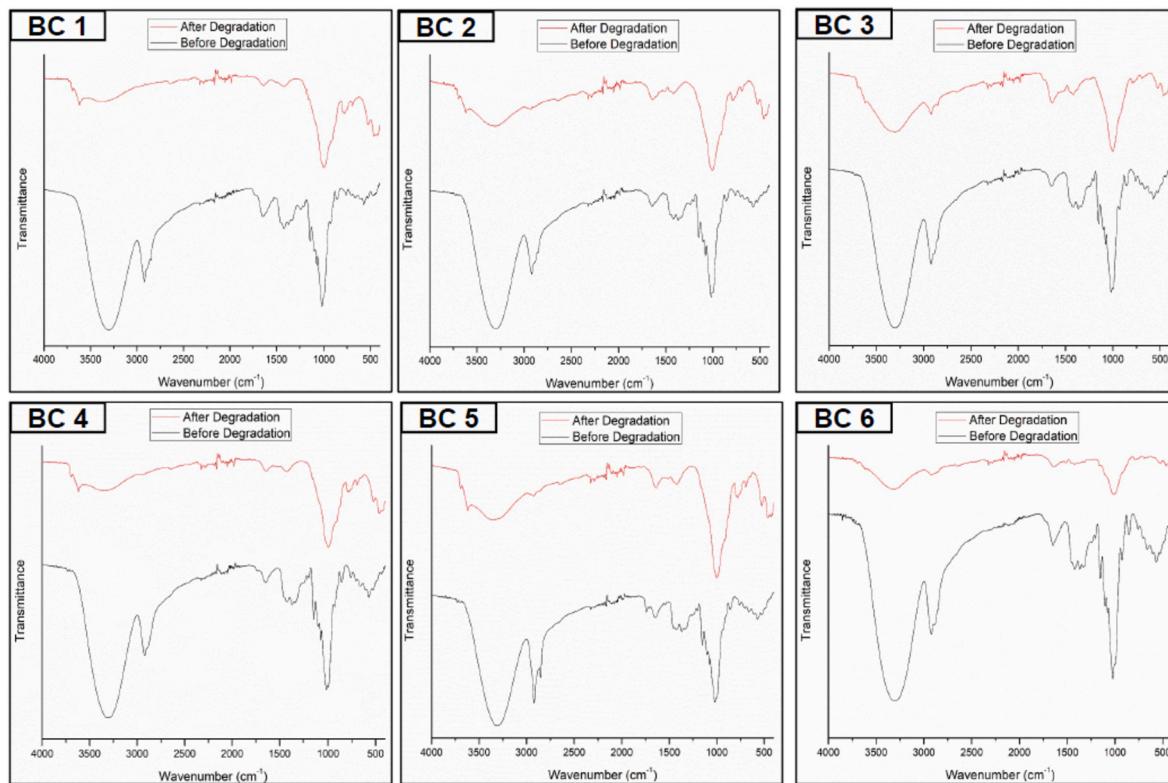


Fig. 8. FTIR spectra of various BCs at $t = 0$ and after 15 days of incubation in field, in wavenumber region $3965\text{--}400\text{ cm}^{-1}$ (a) BC1, (b) BC2, (c) BC3, (d) BC4, (e) BC5 and (f) BC6.

days in the soil is presented in Fig. 7. It was found that % weight loss in all of the BCs was linear with the incubation period of the soil burial test.

The weight loss was 79.32% for BC1 observed at 20 days of soil burial, which was highest compared with other BCs. In the case of BC4, weight loss was the least (57.43%). While BC2, BC3, BC5 and BC6 recorded 65.08%, 61.39%, 60.30% and 60.48% weight loss in the same incubation period. The higher biodegradation observed in BC1 may be because of poor interfacial adhesion between fibre and matrix,

facilitating water penetration and providing more sites for subsequent microbial colonization and enzymatic hydrolysis of starch and fibre (Lee and Wang, 2006; Rajesh et al., 2015). In the case of BC4, crosslinking has imparted a hydrophobic character to starch. Hence less moisture absorption by the composite from the soil may have resulted in a reduced degradation rate.

Changes in surface morphology of all the BCs within 10 days of burial evidenced a gradual degradation. After 15 days in soil, the uniformity of

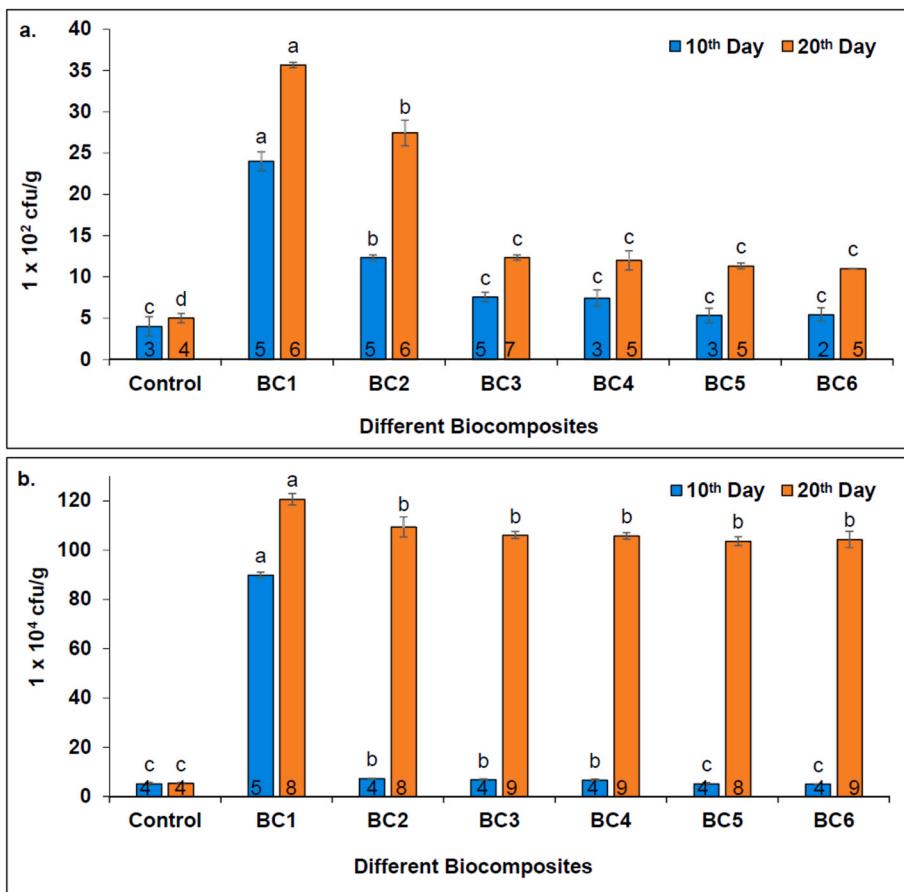


Fig. 9. Population distribution and morphological diversity of bacteria and fungi associated with degradation of BCs (a) after 10 days of soil burial and (b) after 20 days of soil burial. *number inside the bar represents morphologically different bacteria and fungi. *statistical analysis had been carried out among 10 and 20 days samples separately. Values are the means \pm SE of data from three independent experiments. Values follow by same letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

the matrix was disappeared; the surface of the BC becomes heterogeneous along with pores and agglomeration. Due to erosion of fibre and matrix junction, fibres have come out of the matrix at certain places and were broken as well, indicating the attack of microbes at the interface between matrix and fibres (Fig. S18). These results confirm the biodegradability of BCs and agree with the degradation pattern observed by Kaith et al. (2010) for corn-starch and *Saccharum spontaneum* fibres-based BC. In all BCs, the matrix degraded first, exposing the fibres. It was more evident in BC1 due to its fast degradation, as matrix had almost completely disappeared and fibres became more prominent structures with lengthening soil burial time. Due to its amorphous nature, starch was more susceptible to degradation, while the crystalline portion of cellulose fibres was difficult to hydrolysis (Lv et al., 2017).

Since the main mechanism of biodegradation is enzymatic hydrolysis (Zhao et al., 2008), the availability of water and the presence of hydrophilic functional groups in the material are the two most crucial factors involved in this process. The FTIR spectra of all the BC before degradation have a major broad peak between 3200 and 3400 cm⁻¹, which corresponds to the stretching vibration of -OH groups (Edhirej et al., 2017; Kaith et al., 2010) of starch and fibre (Fig. 8). The intensity of this peak was reduced in all the BCs after degradation. Vasile et al. (2018) have reported bands at 3395 cm⁻¹ disappeared after the initial 50 days of soil burial for PLA/chitosan BCs. Similarly, Rivero et al. (2013) also demonstrated that after 150 days of exposition, loss of peaks corresponding to the stretching vibration of -OH group in chitosan matrix could be used to confirm the biodegradation process of the same. The observations indicated that the microbes preferentially attack OH group of the BCs. In addition, the involvement of physical and chemical degradation cannot be ruled out. The bands at 998 cm⁻¹, 1073 cm⁻¹ and 1150 cm⁻¹ corresponding to glycosidic linkages of starch (Tai et al., 2019) were broken down during degradation as these peaks were absent

in spectra of degraded BCs.

Further, the biodegradation of BCs was supported by microbial analysis associated with degrading BCs. Bacteria were found to be more prominent than fungi (Fig. 9 and Fig. S19). The same trend was reported by Sun et al. (2019) for the degradation of composite flower pots made from straw fibre/soy protein. The authors had reported 10⁸ cfu/g bacteria and 10³ cfu/g fungi associated with the degradation of BC.

After the initial 10 days of soil burial, the highest bacterial load was recorded for BC1 (8.99×10^5 cfu/g). The reason may be poor interaction between matrix and fibre in BC1, which provided more sites for decomposer invasion. In contrast, in other BCs, such sites were less abundant due to good adhesion between fibre and matrix. However, with the lengthening of soil burial time, the number of bacteria for BC1, BC2, BC3, BC4, BC5 and BC6 were not significantly different (Fig. 9a). The reason may be because the 3D network structure of these BCs becomes loose and porous by microbial action, which accelerated further colonization of microbes at the surface of the BC. Further, glucose released due to hydrolysis of the starch in the initial stage of soil degradation act as biological fuel for the growth of microbes and their proliferation (Lv et al., 2017).

A similar pattern of the microbial load was recorded for fungi. After 10 days of incubation, the fungal count was maximum in BC1 compared to other BCs. However, the fungal count increased for all the BCs with soil burial time (Fig. 9b). It had been observed that microbes (bacteria and fungi) associated with the degradation of various BCs, were abundant and morphologically diverse than those found in the native soil. Also, the microbial load pattern associated with the degradation revealed that BC with crosslinked starch (BC4) and BA (BC5) was not different from their controls with native starch (BC3) and without BA (BC6).

Hardly 1% of soil microorganisms can be cultivated with classical

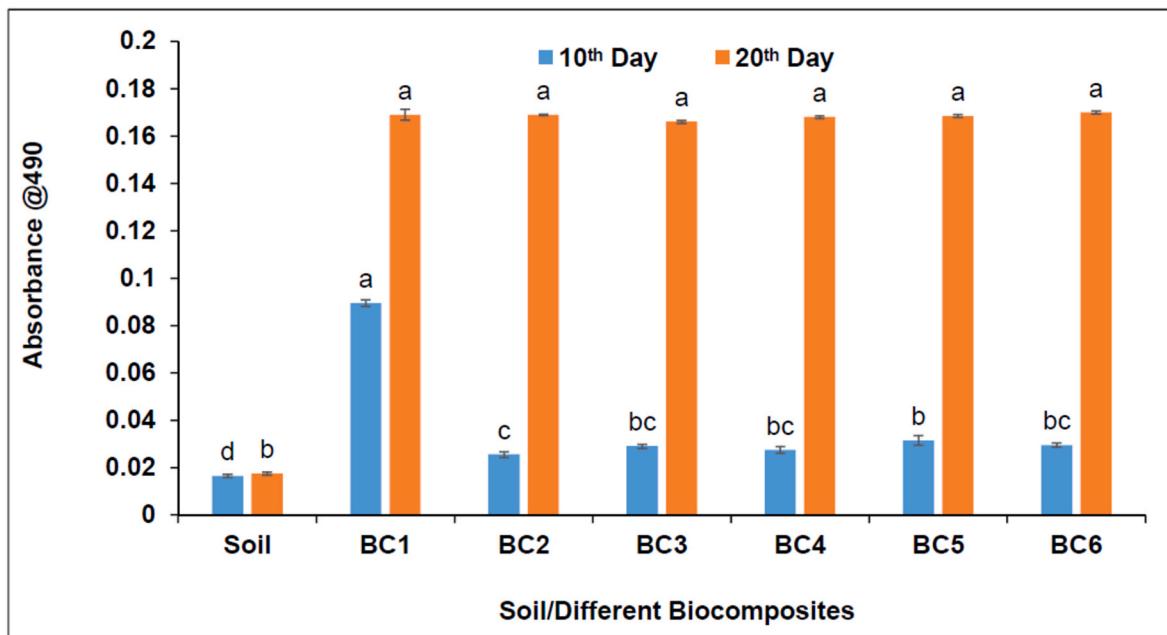


Fig. 10. FDA hydrolyzing enzyme activity for various BCs tested at two different time intervals (10 and 20 days). * Statistical analysis had been carried out among 10 and 20 days samples separately. Values are the means \pm SE of data from three independent experiments. Values follow by same letters are not significantly different according to Tukey's test at the $p < 0.5$ level.

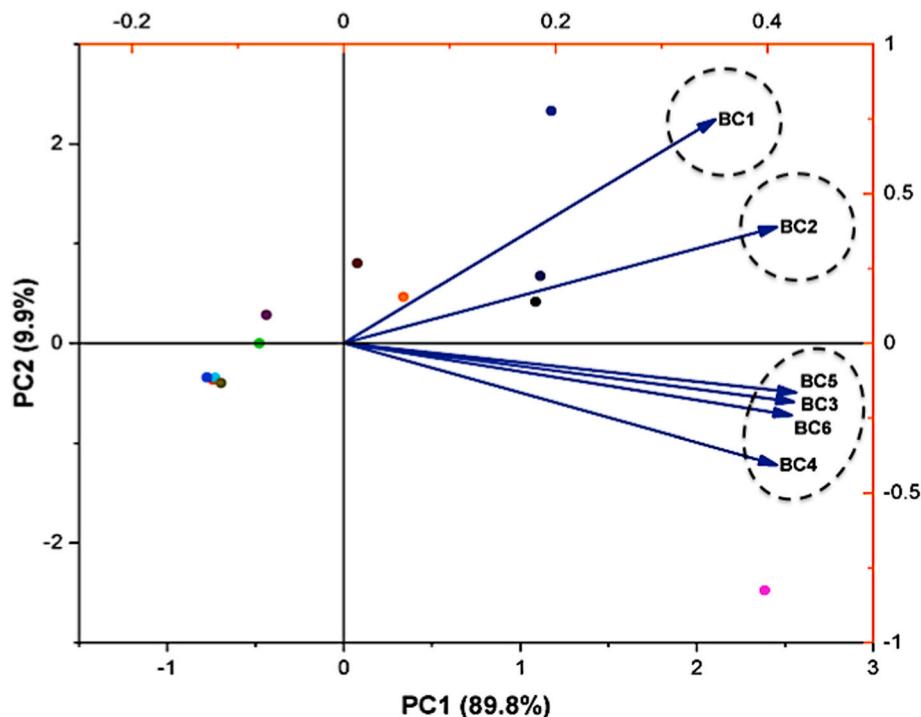


Fig. 11. 2D-Principal component analysis of physico-chemical and biodegradable properties of different BCs. The dot points with different color represents the different characteristics of BCs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

laboratory techniques (Yun et al., 2005). The cultivable method to study microbial diversity gives partial information about the microbial community associated with BC degradation. In this regard, the FDA hydrolysis assay measures both cultivable and uncultivable microbial populations by quantifying proteases, lipases, and esterase activity (Green et al., 2006). In this study, after the initial 10 days of the soil burial period, higher absorbance was recorded in the case of BC1 (Fig. 10), indicating higher enzymatic activity corresponding to higher

microbial load in the BC1 in comparison to other BCs. However, after 20 days, the absorbance of BC1, BC2, BC3, BC4, BC5 and BC6 were not significantly ($p < 0.05$) different from each other. Higher absorbances recorded for degrading BC samples compared to control (native soil), clearly indicated the higher microbial load and activity in BCs.

When all the above obtained data of BCs subjected to 2D-PCA, three distinct clusters were formed which includes, Cluster I: BC1, Cluster II: BC2 and Cluster III: BC3, BC4, BC5 and BC6. The separation these

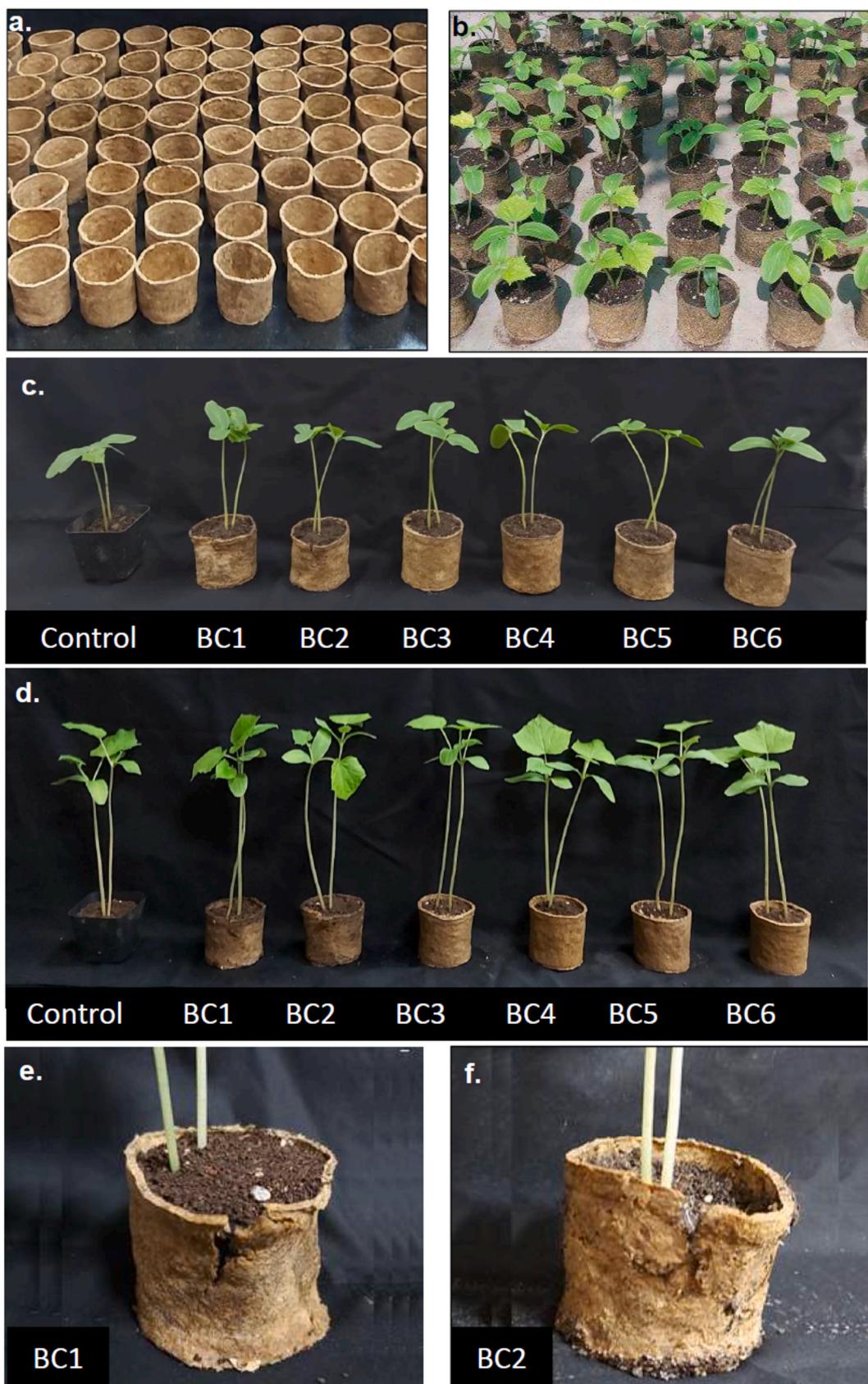


Fig. 12. Greenhouse evaluation of BC pots (a) moulded pots; (b) cucumber (*Cucumis sativus* L.) seedlings in BC pots; (c) BC pots along with 10 days old cucumber seedling; (d) BC pots along with 28 days old cucumber seedling; (e) deformed BC1 and (f) deformed BC 2 pots.

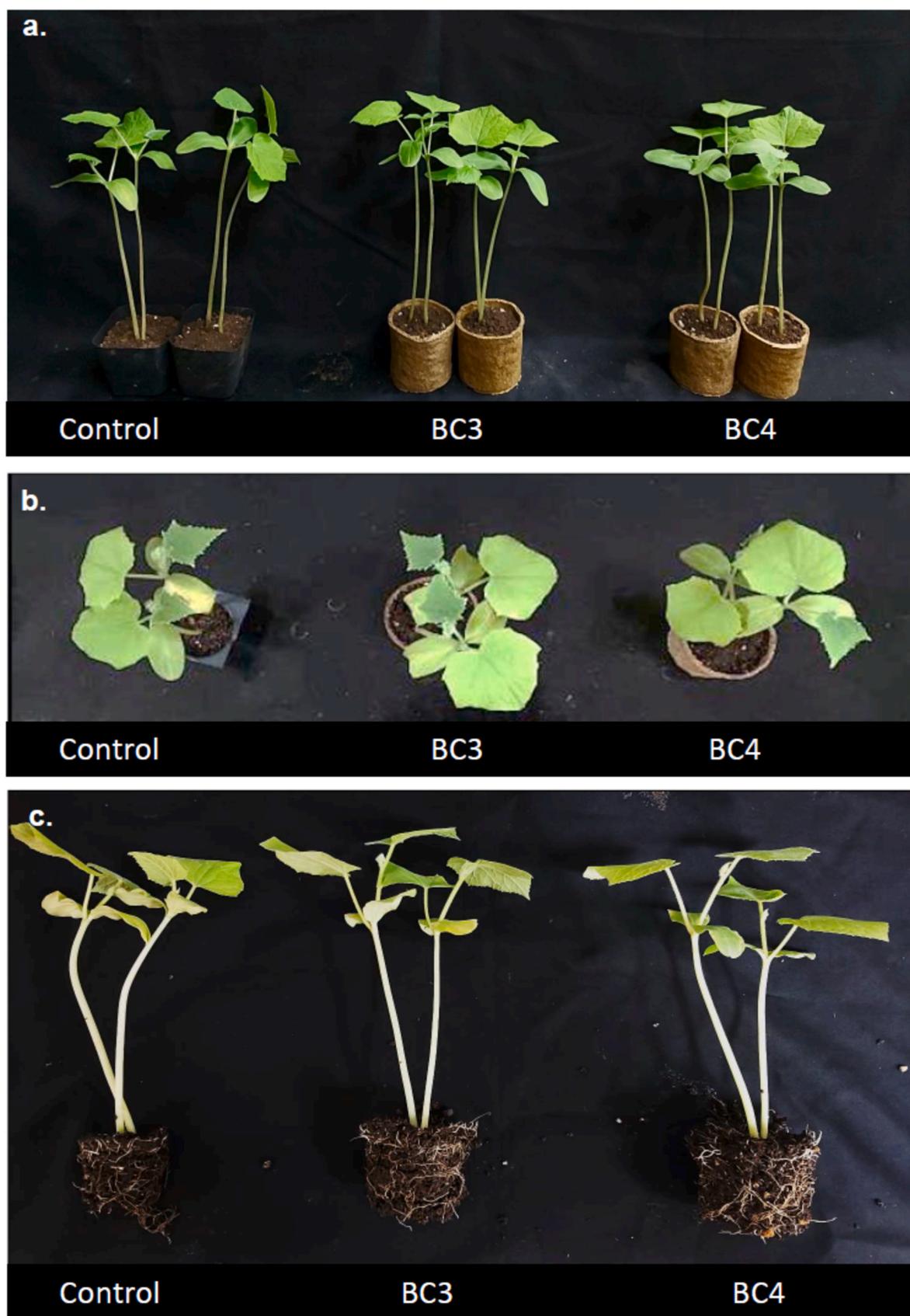


Fig. 13. Cucumber seedlings in BC3, BC4 pots and plastic tray (control) after 28 days of seeding (a) front view; (b) top view and (c) root system of plants.

Table 2

Growth parameters of cucumber (*Cucumis sativus* L.) seedlings grown in different Biocomposite (BC) pots and control.

Pot Type	SL (cm) ^a	RL (cm) ^b	FW (g) ^c	DW (g) ^d	SVI ^e
Control	19.41 ± 0.05 ^a	11.74 ± 0.37 ^a	2.08 ± 0.01 ^a	0.22 ± 0.00 ^a	3115 ± 32 ^a
BC3	19.29 ± 0.21 ^a	11.91 ± 0.22 ^a	2.06 ± 0.07 ^a	0.25 ± 0.01 ^a	3121 ± 40 ^a
BC4	19.56 ± 0.31 ^a	12.26 ± 0.10 ^a	1.99 ± 0.03 ^a	0.24 ± 0.01 ^a	3182 ± 40 ^a
BC5	19.10 ± 0.21 ^a	12.60 ± 0.20 ^a	2.10 ± 0.04 ^a	0.24 ± 0.00 ^a	3169 ± 29 ^a
BC6	18.82 ± 0.35 ^a	12.72 ± 0.04 ^a	2.00 ± 0.02 ^a	0.23 ± 0.00 ^a	3154 ± 38 ^a

Values are the means ± SE of data from three independent experiments. Values followe by same superscripted ltters are not significantly different according to Tukey's test at the p < 0.5 level.

^a Shoot Length/seedling.

^b Root Length/seedling.

^c Fresh Weight/seedling.

^d Dry Weight/seedling.

^e Seedling Vigour Index = Germination % × Seedling Length (Abdul-Baki and Anderson, 1973).

clusters primarily attributed to the use of untreated and treated PS (cluster I: raw PS, cluster II: alkali treated PS and cluster III: alkali and autoclaved PS). Further, within cluster III, BC4 was found separated from BC3, BC5 and BC6, indicating possible role of cross linked starch in differentiating the characteristics of BC4 over other BCs (Fig. 11).

3.7. Greenhouse studies result

During the first stage of the greenhouse evaluation of pots developed from 6 different BCs (Fig. 12a – 12f), BC1 and BC2 pots showed deformation in the structural integrity (Fig. 12e and f)) in the form of cracks in the pot walls just after 10–12 days of seeding. With the experimental period, these cracks enlarged and ultimately, both BC pots (BC1 and BC2) collapsed, indicating inadequate strength to develop pots for nursery cultivation. However, all the other BC pots (BC3, BC4, BC5 and BC6) showed sufficient mechanical resistance to guarantee material functionality. All these BC pots remained intact throughout the entire period of 28 days from seeding to transplantation of cucumber seedlings

(Fig. 12d).

After 28 days of the growth period, seedlings were found normal and healthy without any abnormalities in all tested BC pots and control (Figs. 12d and 13). Mean shoot length, mean root length, fresh weight, dry weight and SVI of cucumber seedling grown in BCs and plastic tray were not significantly (p < 0.05) different (Table 2).

By considering the ease of preparation, BC3 and BC4 pots were selected for field studies. Though BC5 and BC6 were found equivalent in all the physicochemical, biodegradation and greenhouse performance parameters, they were not selected for field studies because of the complexities involved in the preparation process as explained in the materials and methods section. One of the drawbacks we found in these BC pots was their low water use efficiency. The amount of water loss from the walls of BC3 and BC4 pots was 47 and 44%, which were significantly higher than plastic pots (Fig. 14). The variations between BC3 and BC4 can be explained by a relatively high density of BC4 (2.83 g/cm3) in comparison to BC3 (1.83 g/cm3). Further, the BC4 was characterized by low water uptake ability (84.19%) than BC3 (117.68%). Similar observations were reported by Treininte et al. (2018) as peat pots with less density were characterized by more water loss than composite pots (pine needle/bark reinforced PVA composite) with high density.

3.8. Field studies

Plantable pots are suitable for transplantable crops that are transferred in the soil after a short period of growth in nurseries. These pots are able to withstand the rigour of greenhouse production (watering, handling and shipping) and, once planted in the soil, disintegrate and degrade within a short time without restricting root penetration through the wall of the pot (Kuehny et al., 2011). These pots eliminate the need for separate disposal, so these pots help solve the disposal problem associated with plastic pots and reduce the transplantation shock (Khan et al., 2000). Plantable pots must disintegrate and degrade within a short time. However, the type of container material and soil factors such as moisture, temperature, pH, microbial load etc., play a decisive role in the degradation of the pot. Regional differences in degradation rate due to varied climatic and soil conditions cannot be ruled out (Evans et al., 2010).

In the present study, after 10, 20 and 30 days of transplantation, plant growth, degradation of pots, and root penetration were visually

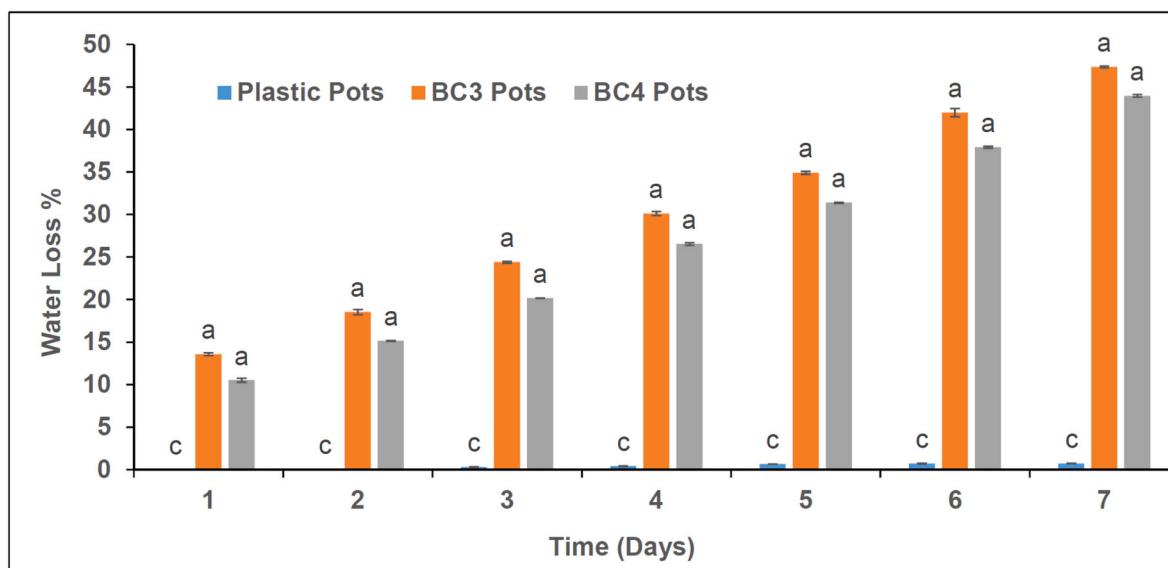


Fig. 14. Water Loss through the walls of pots versus time. Values are the means ± SE of data from three independent experiments. Values followe by same ltters are not significantly different according to Tukey's test at the p < 0.5 level.



Fig. 15. Field studies: (a) Transplantation of cucumber seedlings along with BC pots; cucumber plants after (b) 10 days; (c) 20 days and (d) 30–40 days of transplantation.

monitored (Figs. 15 and 16). It had been found that plant roots started penetrating from the bottom of the pots after 10 days of transplantation (Fig. 16b). After 20 days of transplantation, pots were almost degraded, and plants' roots overgrown the walls of pots (Fig. 16d). After 30 days from the transplantation, pots were degraded entirely without affecting the normal growth of root and aerial plant parts (Figs. 15d and 16e and f).

Even if a biodegradable pot has the best parameters (sustainability and biodegradability), it would not be accepted if plant growth is lesser than the plastic pot. There are both positive and negative results reported in the literature for various biodegradable pots regarding plant growth performance. Lopez and Camberato (2011) evaluated the growth of 'Eckespoint Classic Red' poinsettia (*Euphorbia pulcherrima*), a long-term greenhouse crop in biodegradable pots (Coirpot, cowpot, peat pot, rice hull, moulded fibre, and wheat starch derived resin pot) incomparison with those growing in plastic pots, and reported an improved growth parameters. In contrary, Evans and Hensley (2004) observed that in comparison to the plastic pot, growth of marigold, vinca, impatiens, geranium, and tomato were compromised in feather pot, and the lowest growth of the plants was observed in peat pots. Here, the reduced growth in biodegradable containers was attributed to the water loss through the wall of the peat pot. Earlier BC pots prepared by Treintye et al. (2018) hindered the penetration of roots after

transplantation because of their slow degradation rate in soil. Similarly, Degradation coirpot in soil was slow, so these pots have to be removed or manually broken apart before incorporating into the soil to facilitate root penetration (Nambuthiri et al., 2015). In the present study, both under greenhouse and field conditions, the shortlisted BCs were not inhibiting the plant growth and root penetration.

4. Conclusions

In horticulture, after use plastic pots are primarily disposed of in landfills. On the other hand, burning is considered a quick and straightforward method of disposal of PS in several regions of India. Both the method of disposal have severe implications in terms of human and environmental health. Paddy straw bases biodegradable horticultural pots developed in this study have the potential to address these problems. The product is novel in the sense that performance-wise it is equivalent to plastic pots, but its constituents are derived from natural resources. Apart from this, avoidance of transplantation shock is another benefit associated with these pots. Additionally, BC pots can disintegrate and degrade within 10–20 days of transplantation under normal soil conditions. Plants grown in the BC pots were equivalent to those grown in the plastic pots. Also the principal constituent of BC pots is PS, which is an abundant and renewable resource. During the degradation process,

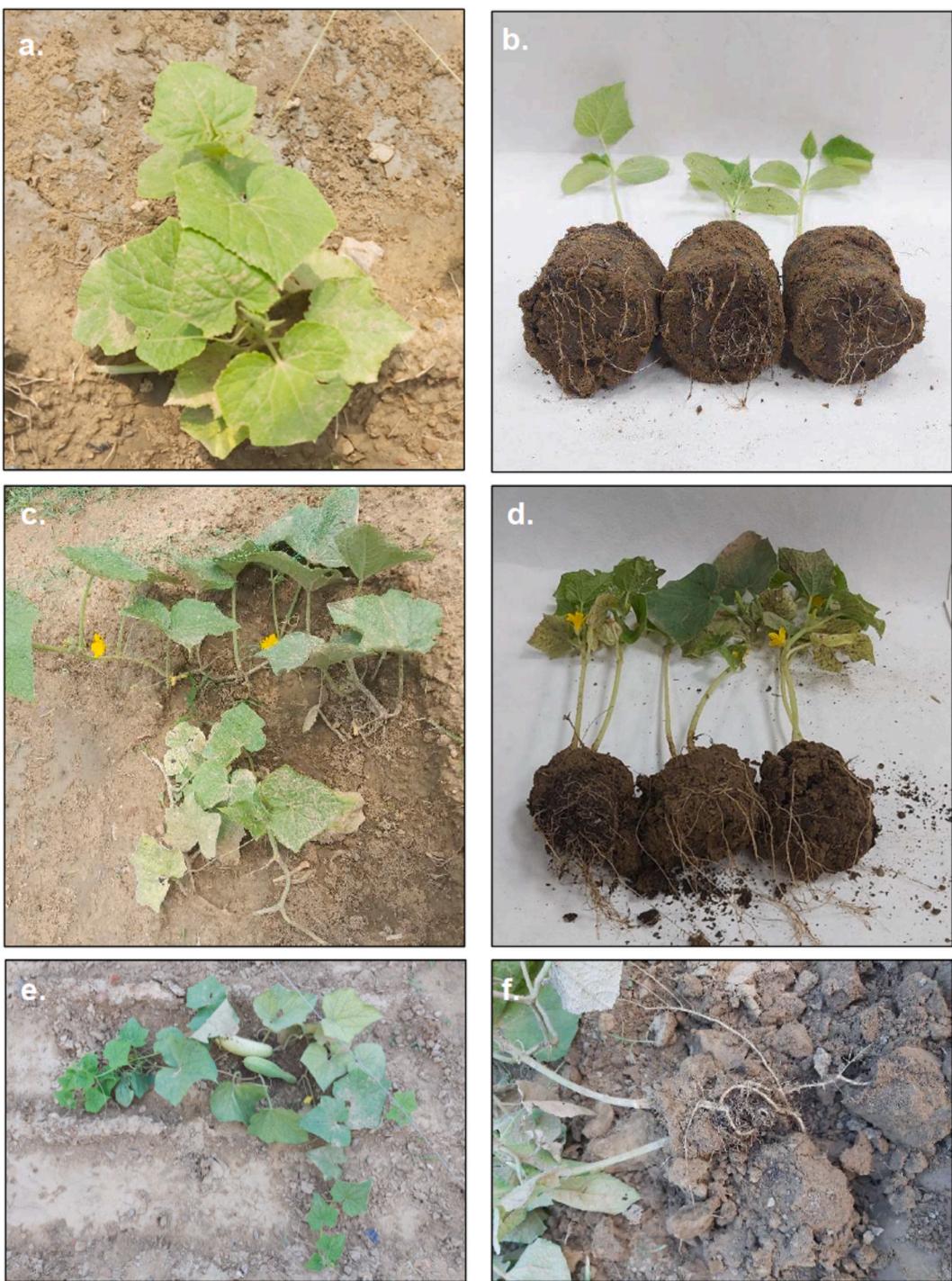


Fig. 16. Field studies: cucumber plants a, b-after 10 days; c, d – after 20 days and e, f - after 30–40 days of transplantation.

microbial enzymes - amylase, cellulose, laccase, etc. would act on the organic matter of the BCs, releasing macro and micronutrients and enhancing the organic C content of the soil.

In future, these BCs can be further evaluated for a wide range of horticulture and floriculture plant cultivation which may require different growth periods under greenhouse conditions. The properties of the pots can be tuned according to the requirements by varying the composition and preparation process. In this regard, our study provides

the platform to conduct more research and develop wide varieties of PS-based BCs considering their broad applications.

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CRediT authorship contribution statement

Pratibha: Investigation, Conceptualization, Methodology, Writing – original draft, Data curation. **Sampa Saha:** Writing – review & editing, Supervision. **Hariprasad P.:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2022.130588>.

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