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INFLUENCE OF APPLICATION OF DIFFERENT PROPORTIONS OF SUBSTRATES AND BIOGAS SLURRY-BASED FERTILIZERS ON THE GROWTH AND QUALITY OF PURPLE RAPE – *BRASSICA CAMPESTRIS* L. SSP. *CHINENSIS* (L.) HANELT

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Abstract

Purple rape is a widely used leafy vegetable in china which exhibits an enormous nutritional value. In this study, different proportions and ratios of substrates, including turf as the main ingredient, coconut coir dust and perlite as raw substrate in combination with varying ratios of biogas slurry (BGS), were applied to cultivate purple rape. Several morphological, physiological and yield indexes were studied to provide an experimental background for more efficient growth of purple rape. Results revealed that the morphological attributes of treatment groups were found to be significantly (p<0.05) different from control groups, under different BGS ratios. Notably, T4-10 treatment in combination with 10% BGS, registered the highest total chlorophyll content. The highest plant fresh weight and aboveground fresh weight were recorded in T5-10 treatments. Proportions of compound substrates and slurry among different groups also exhibited marked effects on the biochemical parameters, and substantially improved the quality of purple. Overall results demonstrated that the T4 (turf: coir dust: perlite at a ratio of 2:4:2) in combination with 10% BGS exhibited the most significant positive effects. In conclusion, our findings may offer a new insight to the sustainable and productive organic agriculture.

Key words: Purple rape; Organic substrate; Biogas slurry, Morphological traits, Quality, Biochemical parameters.

Introduction

Scarcity of adequate nutrient supply and poor soil physico-chemical structure are the leading factors resulting in low agriculture productivities. Employment of chemical-based fertilizers are not the justifiable solution to encounter these limitations, since chemical fertilizers are expensive and pose a serious threat to human health and ecosystem (Arisha & Bradisi, 1999; Jeptoo *et al.,* 2012). Moreover, the monetary cost of inorganic fertilizers is increasing enormously, so as a result they are unaffordable to most small-scale, resource marginal farmers and growers. Incorporating organic fertilizers with inorganic fertilizers considerably enhances the physiological attributes and crops yields by providing plentiful growth-regulating ingredients and manipulation of the soil physico-chemical behavior. Thus, it is of worth significance to explore relatively safe and cheaper nutrient sources as potential substitute to chemical fertilizers.

Biogas slurry (BGS) is a by-product from the biogas plant following the digestion of dung or other biomass for generation of methane rich gas. BGS supplies necessary nutrients, improving water-holding capacity and root growth and prevents undesired weed seed germination. It is also important in public hygiene, pollution control as well as environmental protection stand point. Key factors restricting the wide application of BGS include discrepancy of components and imbalanced nutrient elements, which consequently interrupt vegetable development and yields (Parry *et al.*, 2005; Liu *et al.*, 2009). In such scenario, optimizing BGS composition by supplementing some additional nutrients renders it pertinent for commercial application. Yield enhancement by applying bio-slurry has been documented in many crops (Ahmad *et al.*, 2014). In addition, Singla (2013) reported that komatsuna vegetable cultivated using bio-slurry had better quality, yield and N agronomic efficiency than those produced using chemical-based fertilizer.

Purple rape is a type of *Brassica campestris* L. ssp. *Chinensis* (L.) Hanelt, having good antioxidant capacity to eliminate ring harmful radicals in human body and healthcare function of delaying nerve aging. It exhibits fast growing rate, short cultivation cycle, good taste and high ornamental value. Recently, increasing research attention has been devoted in China towards healthcare and interestingly, purple vegetables have appeared to become a most applauded dish. Due to its relatively high requirement of substrates and lack of well-developed cultivating technologies, home-gardening growers tend to give up following first trials. Substrates play a significant role of fixing root system and providing necessary nutrition and living environment for plants. Qiu *et al*., (2016) studied on mixed substrates of purple rape and found out that mixed substrate of turf: coconut coir dust = 2:1 (V: V) is most suitable combination for improving the quality of purple rape. Coconut coir dust has good water retention and water permeability, as well as relatively slow natural decomposition rate and suitable pH value, thus applicable to plug seedling (Manickam & Subramanian, 2006).

At contemporary, there is an intensive research trend of studying BGS application to agricultural production, in particular, focusing on biogas slurry for seed soaking, leaf development, and soil fertilization as well as insecticidal and bactericidal purposes. Previously, a wide range of studies have demonstrated the application of BGS as nutrition source increased the yield and quality of *Brassica chinensis*, and other green leafy vegetables (GLVs) (Liu *et al.*, 2009a, b). However, there is scarcely any report investigating the collective influences of different proportions of substrates and BGS on quality and yield of purple rape. In consideration of present-day environmental and resource problems, the exploration of an environmental-friendly and sustainable cultivation technology is particularly important (Ammer *et al.*, 2016; Khalid *et al.*, 2017a,b). Therefore, the present study was carried out to evaluate the cultivation effects of purple rape in terms of morphology, quality and yield indexes. Different proportions of substrates and biogas slurry were applied to find out the optimal combination in improving yield, quality and ornamental value of purple rape.

Materials and Methods

Plant material and substrate: Purple rape (*Brassica campestris* ssp. *Chinensis*) seeds were purchased from the Italian Franchi Sementi Seeds Company. Coconut coir dust, perlite and BGS were kindly provided by Shanghai Yangke Eco-agricultural Technology Co., Ltd.

Plants cultivation and treatments: Experimental plants were grown in a greenhouse, located in Engineering Centre of Shanghai Jiao Tong University, Shanghai, China. Healthy seeds were sown (one seed per cell) in a 200-cell type tray then transplanted to a 32-cell type tray (model XT32A, cells 4 × 8 (32), size 450 × 280 mm, capacity 190 cc/cell and material PS) with five replicates per treatment. Plants were irrigated with tap water until three true leaves stage in a specified ratio of different substrates (turf: coir dust: perlite) and then treated with specific proportion of BGS according to the designed treatment (Table 1). The chemical and physical properties of different proportions of substrates were determined and listed in Table 2. Biogas slurry was applied in water flush way with fixed quantity per plug (every 10 days; 3 times in total). Plants were also irrigated with tap water once every 3 days under greenhouse conditions with a temperature around 15°/8°C day/night, a relative humidity of 60/70%, 14/10 h light/dark period, and at a photon flux density of about 300–400 μmol/$m^{2}\cdot$ s. Plants were harvested after 60 days of treatment and analyzed for growth and biomass yield.

Measurement of morphological attributes: After harvesting, the morphological attributes including leaf traits (leaf width, leaf length, and leaf area), plant fresh weight, dry weight, and plant height were measured and their average values were calculated. Leaf area per plant was calculated according to the following formula:

$$A = \frac{K}{L \times W}$$

where, A indicates the area, K is the coefficient constant whose value is 0.7501, L and W represent the length and width of leaf per plant.

Table 1. Proportions of selected substrates and biogas slurry used in the present investigation.

Treatment	Slurry ratio (%)	Proportions (volume ratio)		
		Turf	Coir dust	Perlite
T1-S	0	5	1	2
T1-5	5			
T1-10	10			
T1-15	15			
T2-S	0	4	2	2
T2-5	5			
T2-10	10			
T2-15	15			
T3-S	0	3	3	2
T3-5	5			
T3-10	10			
T3-15	15			
T4-S	0	2	4	2
T4-5	5			
T4-10	10			
T4-15	15			
T5-S	0	1	5	2
T5-5	5			
T5-10	10			
T5-15	15			

Table 2. Physio-chemical characteristics of varying proportions of substrates.

Treatment	pH	EC (mS/cm)	Bulk density (g/L)
T1	5.21 ± 0.03b	0.98 ± 4.06d	133.07 ± 0.07a
T2	5.21 ± 0.06b	1.48 ± 2.6b	84.11 ± 0.17b
T3	5.53 ± 0.03a	0.57 ± 3.79e	86.23 ± 0.17b
T4	5.46 ± 0.01a	1.57 ± 2.65a	84.77 ± 0.16b
T5	5.24 ± 0.03b	1.25 ± 4.41c	65.86 ± 0.06c

The leaf water content and chlorophyll content were determined by previously reported method of (Baslam *et al.*, 2011) and (Bruuinsma, 1963), respectively. For biomass measurements, the fresh plants samples were wrapped in aluminium foil for quick-freeze in liquid nitrogen followed by storage in -80°C fridge until further analysis.

Measurement of biochemical parameters: A previously described method was adopetd for the analysis of soluble sugar (Buysse & Merckx, 1993), whereas the total protein content were quantified by Bradford using Bovine serum albumin (BSA) as a calibration standard (Sedmak & Grossberg, 1977). The anthocyanin content was determined by a spectrophotometric method as reported earlier (Mehrtens *et al.*, 2005). Nitrate nitrogen contents were measured with salicylic acid nitration method (Hao *et al.*, 2006). For the determination of vitamin C content, a previously described method was followed with minor modification (Francisco *et al.*, 2010).

Data analysis: The measurements of growth, physiological index, and biochemical parameters were conducted at least five replicates. Data were subjected to analysis of variance (ANOVA) and treatment means were compared by Duncan's New Multiple Range Test at $p<0.05$.

Table 3. Morphological indexes of purple rape under different biogas slurry fertilizer ratios.

Treatments	Leaf number	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Plant height (cm)
T1-S	15.00 ± 0.58cde	5.57 ± 0.35g	3.70 ± 0.25e	15.55 ± 1.83ef	10.23 ± 0.15g
T1-5	15.33 ± 0.33bcde	7.17 ± 0.09f	5.13 ± 0.03d	27.59 ± 0.18d	15.27 ± 0.37e
T1-10	16.67 ± 0.88abc	8.77 ± 0.15cde	7.10 ± 0.15ab	46.71 ± 1.74c	18.40 ± 0.67d
T1-15	17.00 ± 0.00ab	8.90 ± 0.21bcd	7.30 ± 0.06a	48.71 ± 0.78ab	20.83 ± 0.44abc
T2-S	12.00 ± 0.58gh	3.40 ± 0.25h	2.43 ± 0.09fg	6.20 ± 0.48g	7.07 ± 0.35h
T2-5	14.33 ± 0.33ef	7.13 ±0.30f	5.07 ± 0.07d	27.14 ± 1.48d	13.60 ± 0.46f
T2-10	17.00 ± 0.58ab	10.00 ± 0.5ab	7.33 ± 0.17a	55.13 ± 3.94ab	19.10 ± 0.49d
T2-15	17.67 ± 0.33a	10.23 ± 0.43a	7.73 ± 0.15a	59.39 ± 3.05a	21.13 ± 0.7abc
T3-S	13.00 ± 0.00fg	5.03 ± 0.22g	2.90 ± 0.06f	10.93 ± 0.34fg	10.10 ± 0.21g
T3-5	14.00 ± 0.58ef	8.00 ± 0.29def	5.57 ± 0.22cd	33.39 ± 1.64d	16.15 ± 0.38e
T3-10	15.33 ± 0.33bcde	9.00 ± 0.29bcd	7.23 ± 0.15a	48.85 ± 2.12ab	20.67 ± 0.67bc
T3-15	16.67 ± 0.67abc	9.67 ± 0.17abc	7.40 ± 0.40a	53.75 ± 3.88abc	21.70 ± 0.91ab
T4-S	10.67 ± 0.33h	3.13 ± 0.47h	2.00 ± 0.21g	4.84 ± 1.10g	5.27 ± 0.18i
T4-5	14.67 ± 0.33de	7.77 ± 0.43ef	5.57 ± 0.07cd	32.43 ± 1.84d	15.90 ± 0.49e
T4-10	16.00 ± 0.58abcd	9.43 ± 0.57abc	7.17 ± 0.44ab	50.99 ± 5.69ab	21.10 ± 0.10abc
T4-15	17.33 ± 0.67a	9.83 ± 0.17abc	7.47 ± 0.29a	55.13 ± 2.93ab	21.13 ± 0.24abc
T5-S	11.00 ± 0.58h	3.60 ± 0.46h	2.10 ± 0.21g	5.81 ± 1.34g	5.53 ± 0.33i
T5-5	14.00 ± 0.58ef	6.00 ± 0.46g	4.13 ± 0.19e	18.70 ± 2.20e	9.50 ± 0.40g
T5-10	16.33 ± 0.67abc	10.33 ± 0.33a	6.60 ± 0.10b	51.13 ± 1.41bc	22.23 ± 0.18a
T5-15	16.67 ± 0.33abc	7.90 ± 0.31def	5.83 ± 0.17c	34.61 ± 2.11d	19.63 ± 0.75cd

Results

Morphological characteristics of purple rape: The morphological attributes of purple rape such as leaf number, plant height, leaf length, and leaf width and leaf area were determined. The results are shown in Table 3. The morphological indexes of treatment groups were found to be significantly (p<0.05) different from those of control groups, under different BGS ratios. The leaf numbers of groups treated with different slurry ratios are higher than control group. An average highest (17.67) and lowest (10.67) leaf numbers were noted in T2-15 and T4-S group, respectively. The utmost leaf number exhibited in control group S and T1-S, while the lowest one in T4-S. T2-15 displays a prominently improved (64.94%) plant growth by compared with T4-S; whereas, the T1-15 treatment has 13.33% growth over T1-S; T2-15 has a remarkable 47.25% growth over T2-S; T3-15 has a 28.23% growth over T3-S; T4-15 has a 62.41% growth over T4-S; T5-15 has a 51.54% growth over T5-S.

Amongst plant height and leaf characteristics, the plant height, leaf length and leaf width were found to be augmented with BGS ratios than those of respective control groups (Table 3). More BGS results in higher plant height, Treatment groups under 15% BGS ratios (excluding T5) have the highest morphological indexes (Table 3, Fig. 1). There are five treatments possessing 21.10 cm or more in plant height, including T5-10, T3-15, T2-15, T4-15, and T4-10 (in descending order), among which the highest height was observed in T5-10 (22.23 cm), 4 times higher than that of T4-S. Similar trends were also observed in leaf length, leaf width, and leaf area therefore. There are five treatments possessing 9.5cm or more in leaf length, including T5-10, T2-15, T2-10, T4-15, and T3-15. The highest leaf width was observed in treatments plus 15% BGS (except T5-15), 7.73cm, 7.40 cm and 7.47 cm for T2-15, T3-15, T4-15, respectively. Treatments with 50 cm^2 or more in leaf area include T2-15, T4-15, T2-10, T5-10 and T4-10. Comparatively all morphological indexes revealed that treatment T4-S showed the lowest effect and T2-15 showed the best. As shown in Table 4, all treatment groups have higher plant fresh weight as compared to control treatment, and higher BGS is better for accumulating plant biomass. The highest plant fresh weight and aboveground fresh weight were recorded in T5-10. The aboveground dry weight was identical as fresh weight. Except from T5-15, all treatment groups have higher aboveground dry weight than control (Table 4). Root shoot ratios are inversely proportional to aboveground plant biomass. Root shoot ratio of most treatment groups was equal to or less than that of control, except for T3-5 (Table 4).

Biochemical parameters of purple rape: Chlorophyll A contents of all treatment groups were found to be doubled than chlorophyll B contents. The total chlorophyll was found to be significantly different under different proportions of substrates and biogas slurry (Fig. 2). Among treatment groups, T1-S showed the lowest chlorophyll content, while T4-10 has remarkably higher than that of other groups. Chlorophyll content of T4-10 was about 5 times increased than T1-S.

The soluble protein content is positively correlated to slurry ratio in combination with each substrate. Amongst them, T2, T3 and T4 substrates had most influential effect on the protein content. The soluble protein in plants treated with T2-15 exhibited only slight difference from those of T3-15, T4-10 and T4-15, indicating that T1, T2 and T4 compound substrates had limited effects on soluble protein content. The descending order of the soluble protein content of different treatment groups was T2-15>T3-15>T4-10>T4-15 (Fig. 3). T2-15 presented the highest content signifying most suitable proportion of substrates and biogas slurry for the accumulation of soluble protein at this treatment. It should be noted that high ratio of coir dust in compound substrates showed slightly increased soluble protein content in purple rape plant without influencing the flavor (Fig. 2). Proportions of complex substrates and slurry among different groups have no significant effects on soluble sugar content but (T1-5 and T1-10) was found to be higher compared to the controls (Fig. 4).

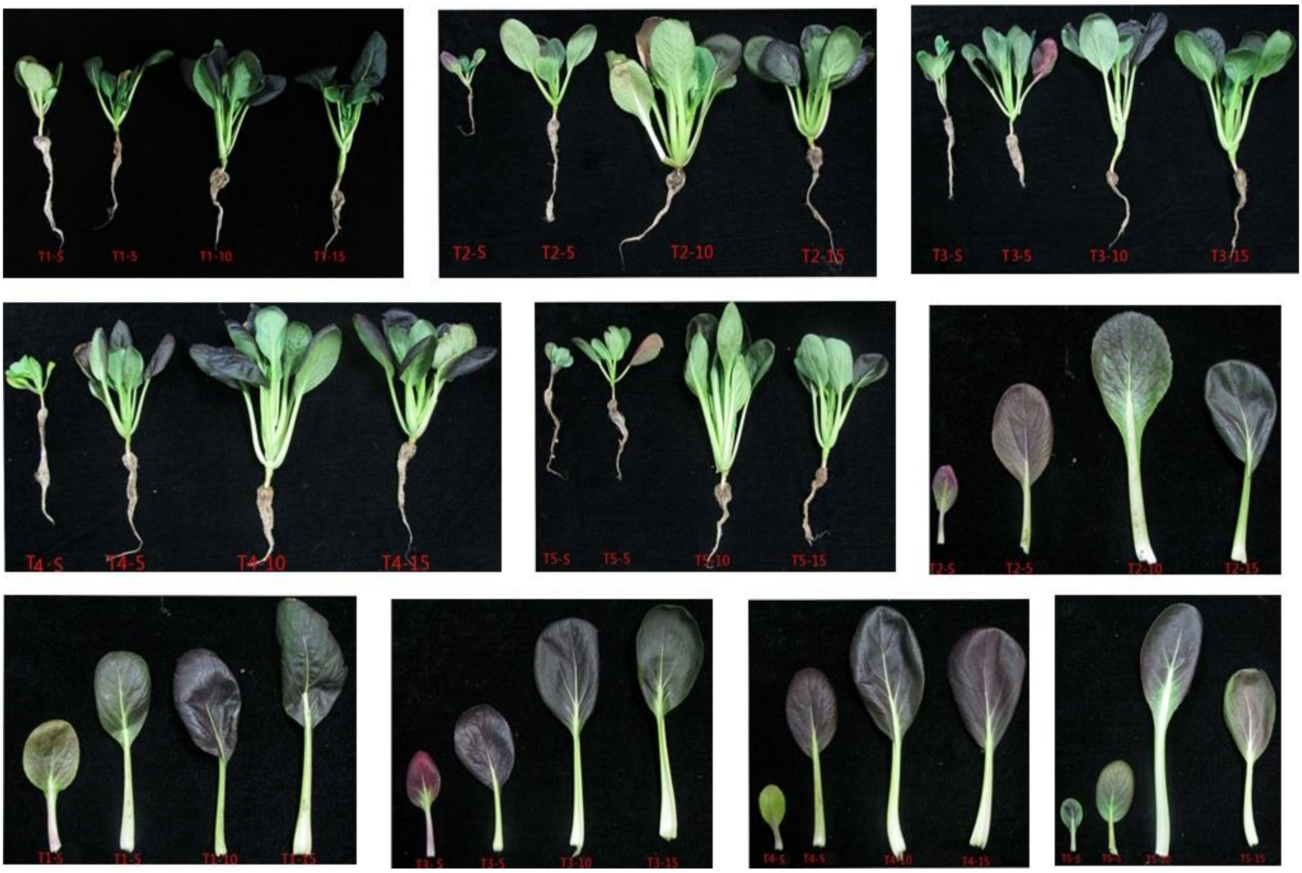

Fig. 1. Plant morphology (left) and leaf morphology (right) of treatment groups.

Table 4. Analysis of fresh weight and dry weight of purple rape.

Treatments	Leaf (FW g/Plant)	Root (FW g/Plant)	Plant (FW g/Plant)	Leaf (DW g/Plant)	Root (DW g/Plant)	Root shoot ratio
T1-S	6.82 ± 0.48h	1.83 ± 0.25cdef	8.65 ± 0.54i	0.59 ± 0.13fgh	0.26 ± 0.05c	1.19 ± 0.04bc
T1-5	5.75 ± 0.28h	1.51 ± 0.13efgh	7.26 ± 0.16i	0.36 ± 0.02hij	0.21 ± 0.03cdef	0.27 ± 0.04bc
T1-10	14.28 ± 0.18fg	1.87 ± 0.14cde	16.16 ± 0.23g	0.72 ± 0.01efg	0.14 ± 0.01fgh	0.13 ± 0.01bcd
T1-15	13.39 ± 0.22g	1.76 ± 0.16defg	15.14 ± 0.06gh	0.80 ± 0.01ef	0.15 ± 0.03efgh	0.13 ± 0.01bcd
T2-S	1.00 ± 0.23j	0.58 ± 0.09j	1.58 ± 0.26k	0.10 ± 0.02j	0.07 ± 0.02h	0.66 ± 0.20a
T2-5	6.73 ± 0.50h	1.16 ± 0.15ghij	7.89 ± 0.35i	0.47 ± 0.01ghi	0.13 ± 0.02fgh	0.18 ± 0.03bcd
T2-10	23.64 ± 0.76d	3.14 ± 0.04a	26.78 ± 0.75c	1.21 ± 0.06bc	0.42 ± 0.02b	0.13 ± 0.01bcd
T2-15	25.98 ± 0.28c	2.58 ± 0.36b	28.57 ± 0.39b	1.28 ± 0.11b	0.24 ± 0.04cd	0.10 ± 0.01bcd
T3-S	3.64 ± 0.26i	0.70 ± 0.02ij	4.34 ± 0.26j	0.22 ± 0.01ij	0.11 ± 0.00gh	0.20 ± 0.02bcd
T3-5	15.49 ± 0.72f	3.37 ± 0.08a	18.86 ± 0.80f	1.16 ± 0.02bcd	0.65 ± 0.04a	0.22 ± 0.00bcd
T3-10	19.70 ± 0.72e	0.79 ± 0.1ij	20.49 ± 0.77e	0.89 ± 0.07de	0.09 ± 0.02gh	0.04 ± 0.00cd
T3-15	27.78 ± 0.19b	1.91 ± 0.41cde	29.69 ± 0.51b	1.41 ± 0.38b	0.29 ± 0.11c	0.07 ± 0.01cd
T4-S	1.42 ± 0.33j	0.88 ± 0.03ij	2.30 ± 0.31k	0.37 ± 0.26hij	0.09 ± 0.01gh	0.69 ± 0.17a
T4-5	12.85 ± 0.27g	1.29 ± 0.07efghi	14.14 ± 0.33h	0.59 ± 0.03fgh	0.16 ± 0.02defg	0.10 ± 0.00bcd
T4-10	18.40 ± 0.67e	1.24 ± 0.02fghi	19.64 ± 0.66ef	0.96 ± 0.05cde	0.16 ± 0.02defg	0.07 ± 0.00cd
T4-15	23.39 ± 0.66d	2.22 ± 0.41bcd	25.61 ± 0.61cd	1.45 ± 0.09b	0.21 ± 0.04cdef	0.10 ± 0.02bcd
T5-S	1.41 ± 0.18j	0.78 ± 0.06ij	2.19 ± 0.22j	0.16 ± 0.00j	0.10 ± 0.01gh	0.56 ± 0.06a
T5-5	3.54 ± 0.32i	1.05 ± 0.13hij	4.59 ± 0.32k	0.26 ± 0.01ij	0.13 ± 0.01fgh	0.30 ± 0.05b
T5-10	29.65 ± 0.86a	2.43 ± 0.16bc	32.08 ± 0.94a	1.73 ± 0.07a	0.24 ± 0.02cde	0.08 ± 0.01cd
T5-15	23.24 ± 0.59d	1.57 ± 0.05efgh	24.80 ± 0.54d	1.4 ± 0.11b	0.23 ± 0.01cde	0.07 ± 0.00cd

The lower nitrate nitrogen content demonstrates a higher dietary value. Among all treatment groups, the nitrate nitrogen content was found in the range of 100-620 mg/kg, which was less than the edible security range of nitrate nitrogen content (1000 mg/kg). Among T1 treatment groups, nitrate nitrogen contents under different slurry ratios were found to be relatively high; nevertheless, nitrate nitrogen contents of other different treatment groups were relatively low, and T5-5 showed the lowest nitrate nitrogen content (Fig. 5). The maximum vitamin C content was observed in treatment T4-10 (2.5 mg/g) (Fig. 6). Elevated anthocyanin content (2.82 mg/g) was presented in treatment T4-15 followed by T2-5, T3-10, T3-5, T3-15 and T4-10 (Fig. 7).

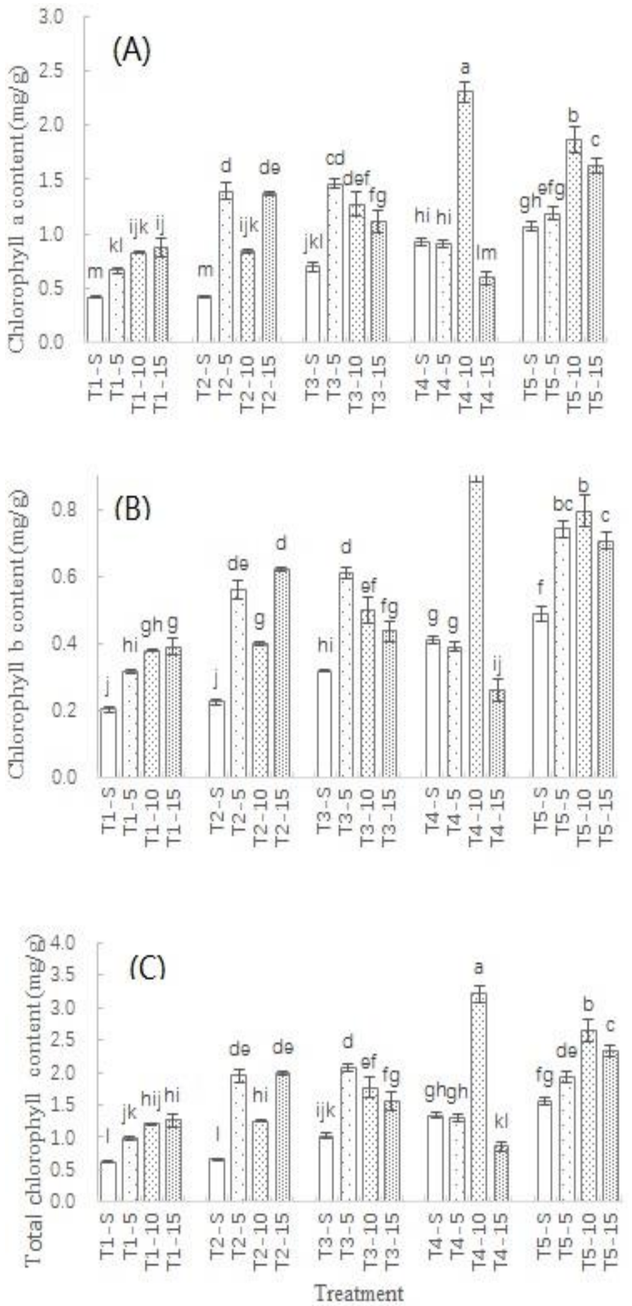

Fig. 2. Effects of different proportions of substrates and biogas slurry on A) chlorophyll A, B) chlorophyll B, and C) total chlorophyll content.

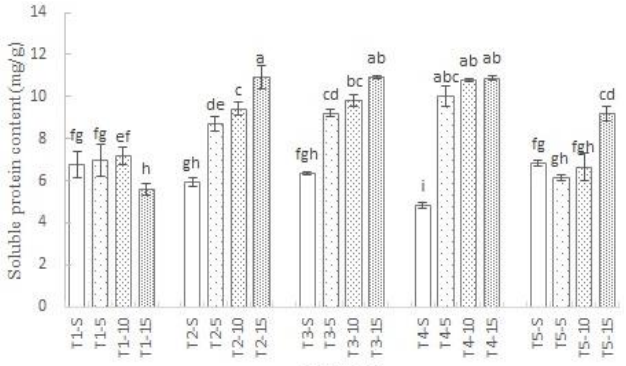

Fig. 3. Effects of different proportions of substrates and slurry on soluble protein.

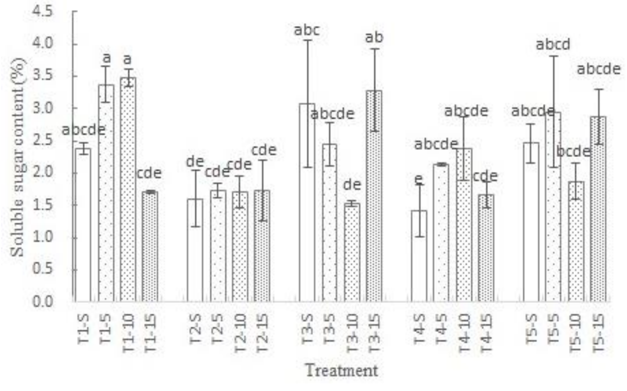

Fig. 4. Effects of different proportions of substrates and slurry on soluble sugar.

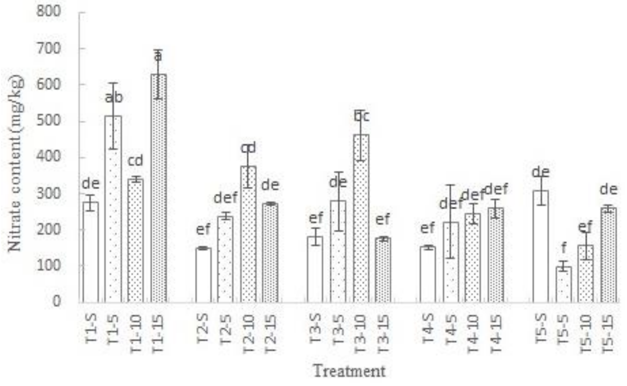

Fig. 5. Effects of different proportions of substrates and slurry on nitrate nitrogen.

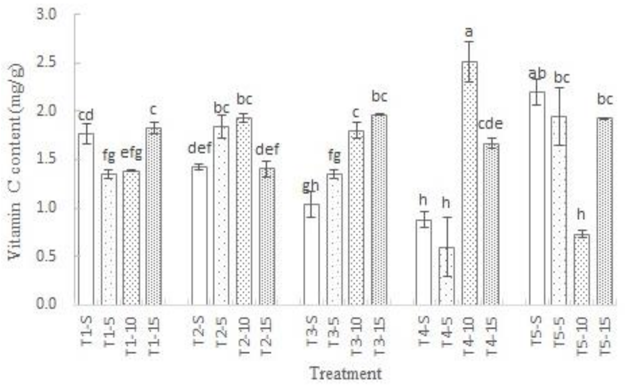

Fig. 6. Effects of different proportions of substrates and slurry on vitamin C.

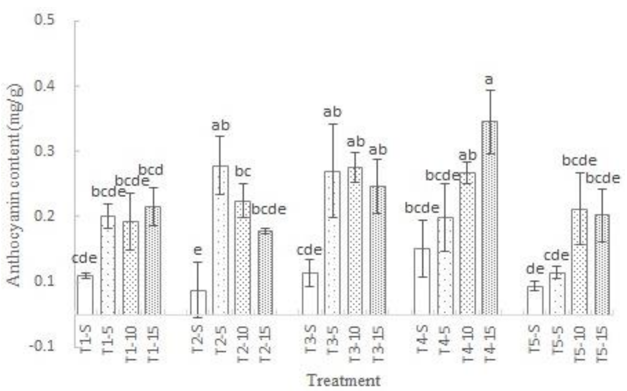

Fig. 7. Effects of different proportions of substrates and slurry on anthocyanin content.

Table 5. Effects of different proportions of substrates and slurry on principal component analysis of growth indexes.

Compositor	Treatment	fac1	fac2	fac3	fac4	Score
1	T4-10	0.92	2.18	-1.42	0.62	0.58
2	T3-15	0.80	0.27	0.64	0.96	0.55
3	T5-10	0.67	1.41	1.12	-1.00	0.52
4	T2-10	1.08	-0.80	0.98	-0.08	0.46
5	T4-15	1.56	-1.01	0.11	-0.88	0.44
6	T2-15	0.58	0.58	0.77	-0.31	0.39
7	T5-15	0.39	1.12	0.01	0.70	0.39
8	T3-5	-0.04	0.50	2.47	0.22	0.38
9	T3-10	1.18	-0.29	-1.80	-0.59	0.17
10	T1-10	0.14	-0.76	0.19	1.07	0.08
11	T1-15	0.97	-1.52	-1.16	-0.12	0.04
12	T4-5	-0.09	-0.36	0.21	-0.50	-0.11
13	T2-5	-0.02	0.48	-1.13	-0.34	-0.12
14	T1-5	-0.32	-1.39	-0.10	1.09	-0.23
15	T5-5	-1.34	1.18	-0.29	1.04	-0.33
16	T1-S	-0.81	-1.10	0.67	0.42	-0.37
17	T3-S	-1.41	-0.38	0.15	0.61	-0.57
18	T5-S	-1.41	0.30	-0.97	0.68	-0.60
19	T2-S	-1.40	-0.71	-0.45	-0.46	-0.79
20	T4-S	-1.46	0.31	-0.01	-3.14	-0.89

While evaluating the effects of substrate and slurry combination on purple rape, solitary quality index cannot fully represent the comprehensive quality of purple rape. Therefore, multiple-object comprehensive evaluation method was used to evaluate the comprehensive effect of purple rape. Different factors including morphological attributes, yield indexes and physiological indexes were considered using SPSS 22 to analyze 20 treatments and calculating their scores as shown in Table 5. Comprehensive evaluation of groups is ranked as (only top 6 is listed): T4-10> T3-15> T5-10> T2-10> T4-15> T2-15, amongst which T4-10 group has 0.58 comprehensive score (the best comprehensive performance), while T4-S has -0.89 (the lowest comprehensive performance).

Discussion

The present study designed twenty treatment groups based on the combination of substrates (including varying ratios of turf, coconut coir dust, perlite and BGS) and investigated their effects on the purple rape. The purple rape plants treated with different ratios of BGS possessed higher leaf numbers, plant height, leaf length, leaf width and leaf area as compared to control groups. Amongst all treatment groups, T5 treatment displayed the best results for all measured parameters, especially under maximum BGS ratio (15%). Notably, the growth attributes were significantly improved by different ratios of BGS which were in agreement with earlier studies indicating that BGS could considerably improve the vegetables growth qualities, as well as biochemical parameters (Waller *et al.*, 2005). Similarly, previous report has shown that coir and perlite mixture was the best combination in ameliorating the quality and yield of gerbera at a ratio of 2:1 (Cho *et al.*, 2006). Moreover, coconut coir dust, vermiculite and perlite have also been exploited as raw materials to incorporate with BGS. Their biochemical characteristics were found to be good for the growth of purple rape, which was in consistent with the findings of previous studies, in which the mixture of these substrates were used in different combination for the cultivation of tomato, *Celosia cristata*, *Dracaena marginata*, peanut and achieved promising results (Arenas *et al.*, 2002; Awang *et al.*, 2009; Stamps & Evans, 1999; Zheng *et al.*, 2016). It was observed that 15% ratio of BGS in combination with other substrates appeared as the most applicable amount which significantly improved the growth of purple rape. The improved purple rape growth might be due to the increased acquisition of beneficial nutrients from substrate and therefore exerting a progressive effect in accumulating health beneficial biochemical compounds. Such a better performance of compound substrate on growth of cucumber together with increasing uptake of phosphorous and potassium has been elucidated in a previous study (Lin, 2006). Mixed combinations of substrates and slurry remarkably increased the total chlorophyll content and chlorophyll A contents of treatment groups were doubled than chlorophyll B. A substantial variation in total chlorophyll content indicated the significant effects of different proportions of substrates and BGS. Several previous studies have reported similar improvement in chlorophyll content following the application of these substrates (Arenas *et al.*, 2002; Ayesha *et al.*, 2011; Gajewska *et al.*, 2006; Wilson *et al.*, 2001). Zhang *et al.*, (2007) reported that vitamin C and reducing sugars in rape plants treated with BGS were higher than those treated with chemical fertilizers, whereas nitrate content decreases. A similar finding was observed in this present study, which corroborated with the previous investigations (Ayesha *et al.*, 2011; Camacho-Cristóbal & González-Fontes, 1999; Qin, 2009). Coir dust in compound substrates increased the soluble protein content slightly in purple rape plants without influencing the flavor which was in corroboration with previous reports (Reghuvaran & Ravindranath, 2010; Wu *et al*., 2014). Notably, the proportion of substrates and BGS in T2-15 treatment was observed to be the optimal to accumulate the soluble protein.

Low ratio BGS might decrease nitrate content in purple rape, while in combination of substrates and BGS, nitrate nitrogen accumulation might be controlled, since mixed combination provided a sufficient nutrition and energy for microbial flora such as denitrifying bacteria and improve its activity. Secondly, denitrifying bacteria in the substrates effectively decrease nitrate nitrogen content in the substrate (control the source of nitrates) to minimize the accumulation. The finding was consistent with previous study (Hao *et al*., 2008).

Conclusions

Taken together, the utilization of substrates in combination with BGS can effectively enhance the growth, yield as well as the quality of purple rape. It also displayed encouraging results in accumulating the soluble protein, sugar, vitamin C, and anthocyanin content in purple rape, accompanying with a decrease in nitrate nitrogen content by using BGS. Moreover, the multiple-object comprehensive evaluation revealed that the quality of purple rape following selected treatments was considerably improved. In conclusion, the present findings might provide relatively safe and less expensive nutrient sources as potential substitute to chemical fertilizers and will be helpful for the sustainable and productive organic agriculture.

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References

Ahmad M., Z. Zahir, M. Jamil, F. Nazli, M.L. Makik and M.F.Z. Akhtar. 2014. Integrated use of plant growth promoting rhizobacteria, biogas slurry and chemical nitrogen for sustainable production of maize under salt-affected conditions. *Pak. J. Bot*., 46(1): 375-382.

Ammer, M.R., S. Zaman, M. Khalid, M. Bilal, S. Erum, D. Huang and S. Che. 2016. Optimization of antibacterial activity of *Eucalyptus tereticornis* leaf extracts against Escherichia coli through response surface methodology. *J. Rad. Res. & Appl. Sci*., 9(4): 376-385.

Arenas, M., C. Vavrina, J. Cornell, E. Hanlon and G. Hochmuth. 2002. Coir as an alternative to peat in media for tomato transplant production. *Hort. Sci., 37*(2): 309-312.

Arisha, H. and A. Bradisi. 1999. Effect of mineral fertilizers and organic fertilizers on growth, yield and quality of potato under sandy soil conditions. *Zagazig J. Agric. Res*, 26: 391-405.

Awang, Y., A.S. Shaharom, R.B. Mohamad and A. Selamat. 2009. Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of Celosia cristata. *Amer. J. Agri.& Biol. Sci., 4*(1): 63-71.

Ayesha, R., N. Fatima, M. Ruqayya, K.M. Qureshi, I.A. Hafiz, K.S. Khan and A. Kamal. 2011. Influence of different growth media on the fruit quality and reproductive growth parameters of strawberry (*Fragaria ananassa*). *J. Med. Plants Res., 5*(26): 6224-6232.

Baslam, M., I. Garmendia and N. Goicoechea. 2011. Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. *J. Agr. and Food Chem.*, 59(10): 5504-5515.

Bruuinsma, J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. *Photoch. & Photobio.*, 2(2): 241-249.

Buysse, J., and R. Merckx. 1993. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.*, 44(10): 1627-1629.

Camacho-Cristóbal, J.J. and A. González-Fontes. 1999. Boron deficiency causes a drastic decrease in nitrate content and nitrate reductase activity, and increases the content of carbohydrates in leaves from tobacco plants. *Planta*, 209(4): 528-536.

Cho, M.S., Y.Y. Park, H.J. Jun and J.B. Chung. 2006. Growth of Gerbera in mixtures of coir dust and perlite. *Hort. Env. and Biot.*, 47(4): 211-216.

Francisco, M., P. Velasco, D.A. Moreno, C. García-Viguera and M.E. Cartea. 2010. Cooking methods of *Brassica rapa* affect the preservation of glucosinolates, phenolics and vitamin C. *Food Res. Int.*, 43(5): 1455-1463.

Gajewska, E., M. Skłodowska, M. Słaba and J. Mazur. 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. *Biol. Plant.*, 50(4): 653-659.

Jeptoo, A., J.N. Aguyoh and M. Saidi. 2012. Improving carrot yield and quality through the use of bio-slurry manure. *Sust. Agr. Res.*, 2(1): 164.

Hao, J.X., J.P. Hong, Y.H. Xie and J. Wen. 2008. Effect of biogas slurry on quality and yield of cdery. *Chinese Agr. Sci. Bul*., 24(7): 408-412.

Hao, J.J., K.L. Zhang and Y. Yu. 2006. Experimental technique of plant physiology. Chemical Industry Press, Beijing, China.

Khalid, M., M. Bilal, D. Hassani, S. Zaman and D. Huang. 2017a. Characterization of ethno-medicinal plant resources of karamar valley Swabi. Pakistan. *J. Rad. Res. & Appl. Sci*.,30: 1-12.

Khalid, M., M. Bilal, D. Hassani, H.M. Iqbal, H. Wang and D. Huang. 2017b. Mitigation of salt stress in white clover (*Trifolium repen*s) by *Azospirillum brasilense* and its inoculation effect. *Bot. Stu.*, 58(1): 5.

Lin, M.H. 2006. Effects of thedifferent substrates on cucumber growth. *J. Anhui Agr. Sci.*, 34(21): 5502-5503.

Liu, W.K., Q.C. Yang and S.Q. Wang. 2009 a. A review on effect of biogas slurry on vegetables and Soil [J]. *Chi. Bio.*, 1: 014.

Liu, W.K., L.F. Du and Q.C. Yang. 2009b. Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. *Acta Agr. Scandinavica*, 59(3): 260-264.

Manickam, I.N. and P. Subramanian. 2006. Study of physical properties of coco peat. *Internat. J. of Green Energy*, 3: 197-144.

Mehrtens, F., H. Kranz, P. Bednarek and B. Weisshaar. 2005. The Arabidopsis transcription factor MYB12 is a flavonol-specific regulator of phenylpropanoid biosynthesis. *Plant Phy.*, 138(2): 1083-1096.

Parry, M., J. Flexas and H. Medrano. 2005. Prospects for crop production under drought: research priorities and future directions. *Ann. Appl. Biol.,* 147(3): 211-226.

Pill, W.G. and K.T. Ridley. 1998. Growth of tomato and coreopsis in response to coir dust in soilless media. *Hort Tech.,* 8(3): 401-406.

Qin Z.. 2009. Effect of biogas slurry application on yield, nutrition quality of purple cabbage and soil quality. *Acta Agr. Jiangxi,* 21(7): 83-86.

Qiu, S.F., L.Q. Yang, D.F. Huang and D.Q. Tang. 2016. Effects of compound substrates with peat and coco peat on the growth characters and the quality of *Brassica campestris* L. ssp. *Chinensis* (L.) 'Ziyoucai'. *J. Shanghai Jiao Tong Univ. (Agr Sci)*., 34(2): 40-46.

Reghuvaran, A. and A.D. Ravindranath. 2010. Efficacy of biodegraded coir pith for cultivation of medicinal plants. *J. Ofentific & Indus. Res.,* 69(7) :554-559

Sedmak, J.J. and S.E. Grossberg. 1977. A rapid, sensitive, and versatile assay for protein using *Coomassie brilliant* blue G250. *Anal. Bioc*., 79(1-2): 544-552.

Singla, A., S.K. Dubey, H. Iwasa and K. Inubushi. 2013. Nitrous oxide flux from komatsuna (*Brassica rapa*) vegetated soil: A comparison between biogas digested liquid and chemical fertilizer. *Bio. and Fer. Soils*, 49(7): 971-976.

Stamps, R.H. and M.R. Evans. 1999. Growth of *Dracaena marginata* and *Spathiphyllum* 'Petite' in sphagnum peat-and coconut coir dust-based growing media. *J. Env. Hort.,* 17: 49-52.

Waller, F., B. Achatz, H. Baltruschat, J. Fodor, K. Becker, M. Fischer and D. von Wettstein. 2005. The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *PNAS,* 102(38): 13386-13391.

Wilson, S., P. Stoffella and D. Graetz. 2001. Compost-amended media for growth and development of Mexican heather. *Comp. Sci. utiliz.,* 9(1): 60-64.

Wu, L.Y., P. Zheng, J.X. Zhao and G.Y. Wang. 2014. The effect of biogas sluryy irrigation on chinese cabbage *Beassica pekinensis* L. and the soil quility. *China Biogas*, 32(3): 90-93

Zhang, Y., J.P. Hong, J.X. Ren and S.T. Shao. 2007. Research on biogas slurry impact on the output and quality of oilseed rape. *J. Shanxi Agr. Sci*., 35(5): 54-57.

Zheng, X., J. Fan, J. Cui, Y. Wang, J. Zhou, M. Ye and M. Sun. 2016. Effects of biogas slurry application on peanut yield, soil nutrients, carbon storage, and microbial activity in an Ultisol soil in southern China. *J. Soils and Sedi,* 16(2): 449-460.

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