Pak. J. Bot., 50(1): 123-129, 2018.

IMPACT OF BIOGAS SLURRY FERTILIZER ON GROWTH, QUALITY AND BIOCHEMICAL CHARACTERISTICS OF ORNAMENTAL LETTUCE'BISCIA ROSSA'

JUAN JIAXIANG[1], MUHAMMAD KHALID[1], ZHAO HONG[1], YANG LEQI[1],MUHAMMAD BILAL[2], GAO ZHAOLIANG[1], TANG DONGQIN[1*] AND HUANG DANFENG[1]

[1]*School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, 200240, P.R. China*
[2]*State KeyLaboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China*
[*]*Corresponding author's email: dqtang@sjtu.edu.cn*

Abstract

Biogas slurry is a good source of plant nutrients and substituting chemical fertilizers with biogas slurry not only achieve resource utilization of slurry, but also reduce the amount of fertilizer. The present study investigated the influence of different ratios of biogas slurry fertilizer (BSF) on the growth, yield and quality characteristics of ornamental lettuce 'Biscia Rossa'. Results revealed that BSF significantly (p<0.05) improved the growth of ornamental lettuce on treatment with Z1 fertilizer; the plant height was increased by 12.66% and 16.75%, while leaf area was improved by 19.97% and 42.03%, in comparison with control group. The improvement in yield of ornamental lettuce was noted in varying degrees by BSF. The highest dry and fresh weights were observed in case of Z1 treated lettuce plants. The fresh weight was 33.52%, 44.77%, 43.96% and 49.04% higher than that of other 2 fertilizers and 2 control groups, respectively. Whereas the dry weight was 2.7 and 3.17-times greater than that of two control groups. The biogas slurry irrigation also considerably improved the quality of ornamental lettuce. Moreover, it was observed that vitamin C, anthocyanin, soluble sugar and protein were higher than that of control groups. Contrarily, the BSF led to decrease in nitrate-nitrogen content in ornamental lettuce.

Keywords: Ornamental lettuce; biogas slurry; yield; quality, biochemical attributes

Introduction

Biogas slurry is the residual liquid when organic matters such as livestock manure undergo mixed anaerobic fermentation (Amon *et al*., 2006). It contains a large quantity of organic and inorganic salt, and high concentration of essential nutrients such as ammonium salt, sylvine, carbon and phosphate (Zhao *et al*., 2015). With high nutrition value and without viable bacteria or eggs, it is valuable and quick-acting compound fertilizer (Clemens *et al*., 2006). Development and utilization of biogas slurry as bio-organic fertilizer will not only improves the plants quality and yield, provides good methods of treating and recycling livestock manure, but also have environmental and economic values (Ahmad *et al.*, 2014). Reports indicate that "as a kind of bio-organic fertilizer" biogas slurry significantly improved the growth, yield and quality of different plants (Berenguer *et al*., 2008).

The degraded odorous compounds after fermentation, leave high amount of carbon/nitrogen and also reduce the dry matter content (Goldstein, 2000; Wentzel and Joergensen, 2016).Most of carbon is lost in the form of methane and carbon dioxide, while 80% to 90% of the raw nutrients like nitrogen, phosphorous and potassium retain in biogas slurry (Zhang *et al*., 2009). Moreover, biogas slurry is rich in hydrolytic enzymes, vitamins B, amino acids, plant hormones, and compounds with pests inhibiting efficiency along with a variety of other macro- and micronutrients (Zha*et al*., 2008; Wentzel & Joergensen, 2015). Thus, biogas slurry is a good source of nutrients and its application increases the nitrogen content of the soil and improves organic matter (Sorensen & Amato 2002; Chantigny *et al*., 2004; Berenguer *et al*., 2008). Moreover, it also improves the physical properties of soil by increasing enzymatic activities, retaining water as well as regulating soil fertility, soil environment gas and heat (Guojun *et al*., 2008).

An improvement in whole plant yield, fruit set rate, vitamin C content and sugar content has been observed as compared to unfermented substrates and control (Banik & Nandi 2004, Garg *et al*., 2005). It is estimated that China produce more than 200 million tons biogas slurry per year (Huang & Liao, 2005). Due to large pollution load, its discharge may lead to huge environmental pollution. Resource utilization can be achieved by processing biogas slurry which seems to be the potential alternative than chemical fertilizers. Such a resource utilization is of profound importance from the public hygiene point of view, environment protection and pollution control (Terhoeven-Urselmans *et al*., 2009). In literature, very scarce studies have been reported investigating the influence of biogas slurry on ornamental lettuce quality and yield under different fertilizer ratios and methods. Therefore, in the present study, different ratios of biogas slurry were applied to replace chemical fertilizer and their influence was analysed on the quality characteristics of lettuce plant. The current study also aimed to find the most suitable ratio of biogas, its optimum application to get the desired quality of ornamental lettuce plant and to provide a reference to the proper usage of resource biogas slurry in the agriculture field.

Materials and Methods

Experimental materials: The ornamental lettuce 'Bisciarossa' seeds were procured form Italian Franchi Sementi Seeds Company. The Zhongnuo organic substrate ($N + P_2O_5 + K_2O \geq 2.0\%$, organic matter ≥ 40%) was purchased from Huaian Zhongnuo Agricultural Technology Development Co., Ltd, whereas, the organic compound fertilizer ($N + P_2O5 + K_2O \geq 6.0\%$, organic matter ≥ 45%) was obtained from Shanghai Runhua Eco-Agricultural Technology Co., Ltd. Biogas slurry, used in the present experiment was provided by Shanghai Yangke Ecological

Agriculture Technology Co., Ltd.The basic components of biogas slurry are listed in Table 1. All the chemical or reagents used were of high purity grade and mainly obtained from Sigma-Aldrich (USA) and Keyi Chemical Reagent Co., Beijing, China.

Table 1.The basic components of biogas slurry used in this study

Parameter	Biogas slurry
pH	8.13+0.34
EC (μS/cm)	58.5+1.28
Total nitrogen (mg/L)	5083.09+4.32
Total phosphorus (mg/L)	463.5+3.81
Total potassium (mg/L)	99.1+2.76
Available nitrogen (mg/L)	1450.8+4.65
Available phosphorus (mg/L)	2157.75+4.91
Available potassium (mg/L)	435.6+2.89

Experimental design: Studies were carried out in glass greenhouse located in Engineering Centre of School of Agriculture and Biology, Shanghai Jiao Tong University (SJTU), Shanghai China. Equal-size seeds were sown on 3 October, 2014 to grow seedlings, which were then grown in Zhongnuo organic substrate supplemented with 15% organic compound fertilizer. The seedlings were shifted to pots (4 seedlings/ pot) when they grow to three leaves stage. Experimental setup consists of 3 treatments and 2 control groups as portrayed in Table 2; where each treatment group contained 5 pots (5 repetitions) with 4 plants per pot. The first and second fertilizations were carried out at the 10^{th} and 20^{th} day, respectively following transplantation. The fertilizers were provided in the whole growth period. After stipulated growth period, the plants were harvested and their morphological, yield and physiological indexes were observed and measured.

Morphology and yield indexes: The morphological characteristics such as plant height (from stem foot to tip of the longest leaf), leaf length (cm), leaf width (cm), leaf number, plant type, leaf colour, leaf shape and leaf margins were observed and measured during harvesting. The plant height, leaf length and leaf width were measured with ruler, whereas the leaf numbers were counted manually using five replicates from each treatment. Qualitative indexes such as, leaf colour, leaf shape and leaf margin were recorded through observational methods. After harvesting, five plants per treatment were chosen to determine their fresh and dry weights (g). The plant samples were dried in an oven to constant weight for measuring dry weight of each sample. Leaf water content was measured using the following formula (FW-DM)/ DMb × 100) and the results were calculated in percentages (Baslam *et al*., 2011). Chlorophyll contents were calculated using the method described previously(Bruuinsma, 1963).

Anthocyanin measurement: Anthocyanins were determined following the method reported earlier (Cevahir *et al*. 2004). For this, fresh leaves samples were thoroughly homogenized with 1.0 mL acidified methanol followed by overnight dark incubation at 4°C in a temperature-controlled incubator. After the designated incubation time, the solution mixture was centrifuged and the resulting supernatants were filtered through passing four-layer cheese cloth before measuring optical density at A_{530} and A_{657} in a double beam UV-vis spectrophotometer (HITACHI U-2900 spectrophotometer). The relative amount of total anthocyanin content was calculated per plant or per gram of FW as previously described (Mancinelli, 1984).

Total sugar and protein analysis: For soluble sugar analysis, a previously described method of Buysse & Merckx, (1993) was used. Fresh leaf sample (0.2 g) was taken in a test tube with 5.0 mL of distilled water and placed in a boiling water bath (100 °C) for 30 min. The sample mixture was filtered into volumetric flask and 5.0 mL anthrone solution was added to 1.0 mL of filtrate followed by incubation in water bath for 10 min. After 10 min, the solution was taken out, cooled to room temperature and absorbance was measured at 620 nm. A rapid and versatile method of Bradford using Bovine serum albumin (BSA) as standard was used for the quantification of total protein contents (Sedmak & Grossberg 1977).Results were calculated as milligrams per gram of fresh weight. Nitrate nitrogen contents were measured with salicylic acid nitration methodology (Cataldo *et al*., 1975).

Vitamin C content: Ascorbic (AA) acid content was determined following the method of Francisco *et al*., (2010). In an Erlenmeyer flask containing 2.0 g of fresh grounded leaf powder, 1.0 mL of $ZnSO_4$ (30%) and potassium ferrocyanide (15%) were thoroughly mixed and then 1.0% oxalic acid was added to the final volume of 100 mL. The resulting homogenate was filtered and 2.0 mL of 2, 6-dichlorphenol indophenol and 5.0 mL of xylene solutions were added to the 4.0 mL of filtrate, vortex mixed and optical density was recorded at 500 nm.

Quantification of ornamental characteristics: Quantification of ornamental characteristics of lettuce was carried out based on periodic observations and the indexes were recorded, with reference to assessment and evaluation standards for leaf ornamental vegetables (Piao & Liu, 2008).

Table 2. The different ratios of biogas slurry fertilizer and fertilizing methods

Treatment	Base fertilizer	Additional fertilizer
Z1	None	5.0% biogas slurry
Z2	None	10% biogas slurry
Z3	None	15% biogas slurry
CK1	None	None
Ck2	15% organic compound fertilizer	None

Statistical analysis

Experimental measurement of growth, physiological index, and biochemical parameters were relied on means of at least three replicates with standard deviations. The means and standard errors were computed for each treatment, and the SE values were displayed as Y-error bars in the Figures. Data were subjected to analysis of variance (ANOVA)and treatment means were compared by Duncan's New Multiple Range Test (DMRT) at $p<0.05$ using a software package (IBM SPSS Statistics 21).

Results

Morphological characteristics of lettuce: The morphological attributes of lettuce such as leaf number, plant height, leaf length, leaf width and leaf area were determined at different control and treatment groups, and responses thus recorded, are summarized in Table 3. It was observed that morphological indexes of treatment groups were found to be significantly ($p<0.05$) different from those of control groups under different BSF ratios. The leaf numbers of treatment groups were greater than that of control groups under different biogas slurry fertilizer ratio. Notably, the highest average leaf numbers (up to 8.60±0.24) were recorded in Z2 fertilizer-treated lettuce plants followed by 8.4±0.47 and 7.2±0.21 in Z1 and Z3, respectively. The maximum leaf numbers in Z2 were 23.26% and 18.60% higher than those treated with CK1 and CK2, respectively.However, no significant difference ($p>0.05$) in leaf numbers was found between two control groups. Similarly, in case of plant height, leaf length, leaf width and leaf area indexes, the treatment groups were also observed to be better as compared to control groups. The treatment group Z1 displayed superior growth attributes followed by Z2 and Z3. In ornamental lettuce, the highest plant height (20.3±0.56 cm) was recorded in Z1 treatment, which was 12.66% and 16.75% higher than that of control groups, respectively. Whereas, the least plant height (18.40±0.26) was noted in Z3 treatment which was only 3.64% and 8.15% higher in contrast with CK1 and CK2, respectively. The leaf area of Z1 treated plants was 19.97% and 42.03% greater in comparison to CK1 and CK2, respectively. Among two control groups, CK2 (treated with organic compound fertilizer) possess lower growth indexes than CK1 (no fertilizer). The quality and ornamental characteristics of lettuce were also assessed and results are displayed in Table 4.

Table 3. Morphological indexes of ornamental lettuce under different biogas slurry fertilizer ratios

Treatment	Leaf number	Plant height	Leaf length	Leaf width	Leaf area
Z1	8.4 ± 0.47a	20.30 ± 0.56a	18.40 ± 0.17a	7.40 ± 0.43a	59.10a
Z2	8.6 ± 0.24a	19.97 ± 0.32a	17.83 ± 0.45a	6.40 ± 0.56bc	47.3b
Z3	7.2 ± 0.21b	18.40 ± 0.26b	15.27 ± 0.51b	6.37 ± 0.38bc	43.32b
CK1	6.6 ± 0.53b	17.73 ± 0.27bc	15.03 ± 0.49b	6.90 ± 0.44ab	47.30a
CK2	7.0 ± 0.58b	16.90 ± 0.6c	13.57 ± 0.15c	5.67 ± 0.35c	34.26c

Table 4.Assessment of quality and ornamental characteristics of lettuce

Indexes	Level 1 (5 points)	Level 2 (4 points)	Level 3 (3 points)	Level 4 (2 points)	Level 5 (1 points)
leaf color	Stem leaf is bright purple	Purple	Leaf has red or purple spots	Evergreen stem leaf has bright color	Evergreen stem leaf has dim color
Leaf shape	Big, wavy or pinnate	long strip or deep cracks	Dentate or shallow cracks	Silence	No characteristics
Texture	Plain, bright no pubescence no powder	Plain, bright no pubescence no powder	Plain, bright with pubescence with powder	Rough surface no pubescence	Rough surface with pubescence
Plant shape	Compact Rosette	Compact Loose	Compact Upright stem	Upright stem Loose	Upright stem Loose

Yield of lettuce: Fig. 1 shows the lettuce yield under different BSF ratios. Results revealed that significant differences ($p<0.05$) were found among all the treatment groups. Amongst all treatment groups, the ornamental lettuce treated with Z1 displayed the highest fresh weight (9.05 g) and dry weight (0.36 g).The recorded improvements were 68.09% and 66.10% than that of CK1 and CK2, respectively, for fresh weight (Fig. 1A). Evidently, the fresh weights of Z2 and Z3 treatment groups were also greater than that of control groups. Likewise, the dry weight the Z1treated lettuce showed the highest dry weight of 0.48 g which was 2.7 and 3.17 times higher than that of CK1 and CK2 groups, respectively. The percentage improvements were noted to be 43.96% and 49.04% with respect to CK1 and CK2, respectively (Fig. 1B). It was worth mentioning that by increasing the BSF ratio, the fresh weight and dry weight were reduced. On the other hand, the fresh weight and dry weight of two control groups did not have any significant difference ($p>0.05$); nevertheless, in CK2 treatment, the lettuce has relatively higher fresh weight than CK1, while the dry weight of CK2 was found to relatively lower than that of CK1.

Leaf watercontent: Fig. 2 illustrates the differences of leaf water content in ornamental lettuce under different BSF ratios. By increasing the biogas slurry fertilizer ratios, the leaf water content of ornamental lettuce was increased and therefore Z3 treatment revealed the highest water content of 97.39%, while CK2 exhibited the lowest water content of 94.30%. The water content of Z3 was recorded to be 2.79% and 2.36% higher than that of Z1 and Z2, and 2.23% and 3.17% higher than that of CK1and CK2 groups, respectively. Apart from Z3 treatment, there was no significant difference (p>0.05) in water content among all other treatment groups. The water content of CK1 is 0.92% higher than that of CK2; however, no significant difference of water content exists between two control groups.

Biochemical parameters of lettuce

Chlorophyll and anthocyanin: As it is evident in Fig. 3A, that chlorophyll content has no significant difference (p>0.05) between treatment and control groups. The highest chlorophyll content (0.88 mg/g) was noted in Z1-treated ornamental lettucewhich was 15.65% and 15.03% higher than that of CK1 and CK2 groups, respectively. Anthocyanin content showed significant difference (p<0.05) among different treatment groups (Fig. 3B). Noticeably, the anthocyanin content was found to be increased by gradually increasing the BSF ratios andZ3 has the utmost anthocyanin content which was significantly greater than that of control groups.

Soluble protein and sugar content: The soluble protein content under different BSF ratio was higher than that of control groups (Fig. 4A). Z2 exhibited the 26.64% and 20.25% higher soluble protein content than CK1 and CK2 groups, respectively. It should be noted that elevated BSF helps to improve the accumulation of soluble protein in ornamental lettuce. On the other hand, Z2 has the highest soluble sugar content of 1.44% while CK1 has the lowest content of 1.05% (Fig. 4B). Soluble sugar content of Z2 is 18.17%, 6.06%, 26.94% and 20.75% higher respectively than that of other control groups. Soluble sugar content under different BSF ratio ranks as Z2＞Z3＞Z1, which indicates that too high or too low BSF ratio will have negative effects on the accumulation of soluble sugar in ornamental lettuce.

Vitamin C and nitrate nitrogen content: Under different BSF ratios, no significant difference (p>0.05) was observed in vitamin C content among fertilized treatments and control groups (Fig. 5A). T3 possesses the highest Vitamin C content which was 8.94% and 19.82% higher than that of CK1 and CK2 groups, respectively. Nitrate-nitrogen content of ornamental lettuce has significant differences (p<0.05) between treatment and control groups (Fig. 5B). The higher BSF ratio led to lower nitrate nitrogen content. Z1 and Z3 exhibited the highest and lowest nitrate nitrogen content, respectively. Nitrate nitrogen content of Z1 is 23.52%, 43.55%, 30.49% and 6.66% respectively higher than that of other control groups. The nitrate nitrogen content of CK1 was 25.54% lower than that of CK2.

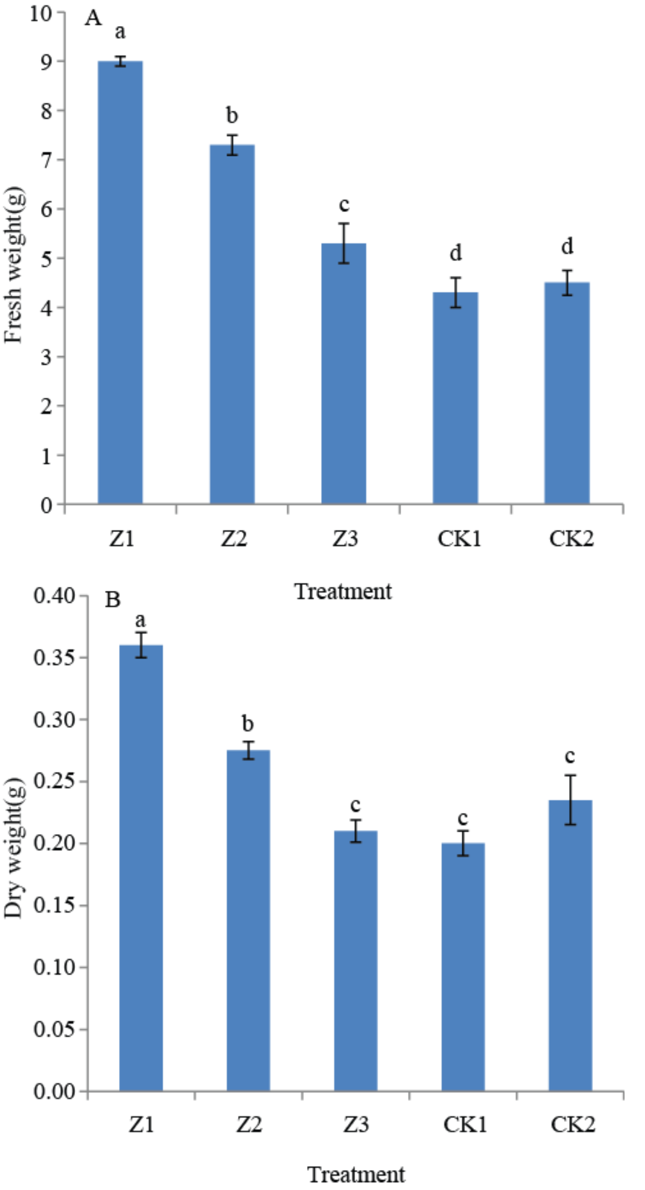

Fig. 1. Effects of different biogas slurry fertilizer ratios on the A) fresh and B) dry weight of lettuce

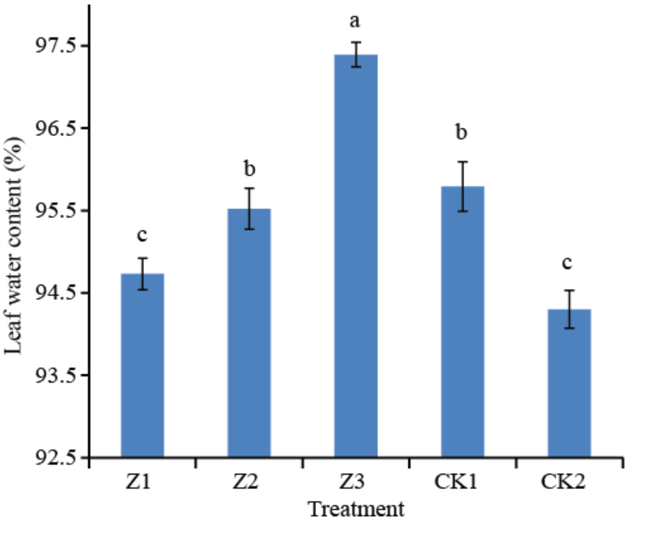

Fig. 2. Effects of different biogas slurry fertilizer ratioson the leaf water content of lettuce.

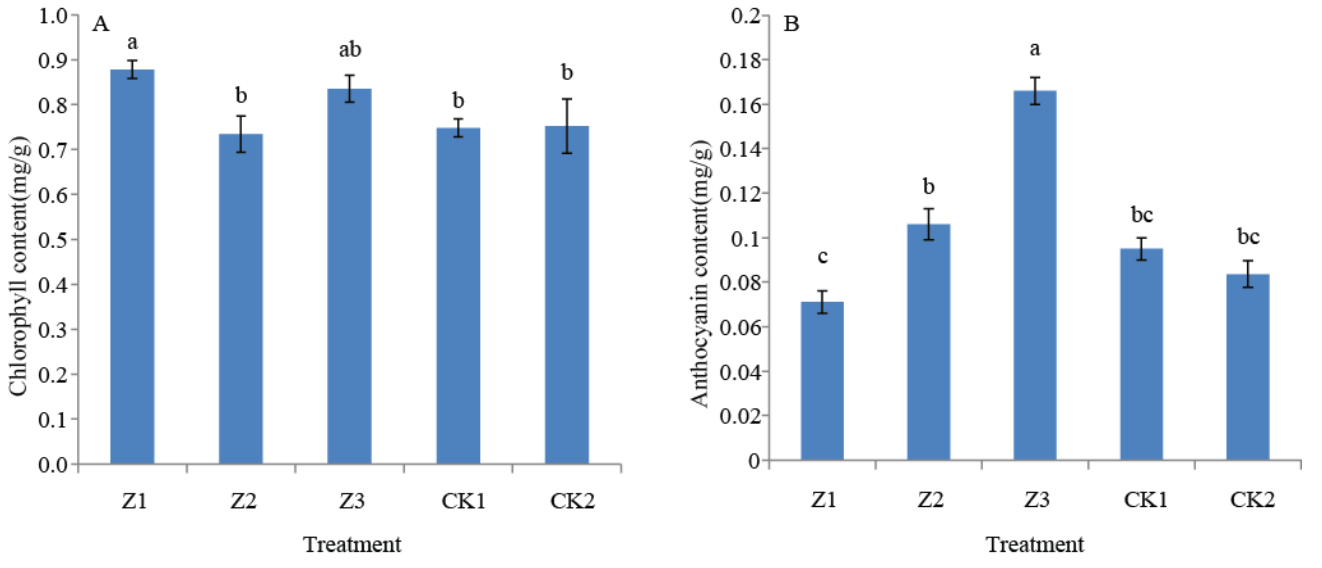

Fig. 3. A) Chlorophyll and B) anthocyanin content of lettuce under different biogas slurry fertilizer ratios.

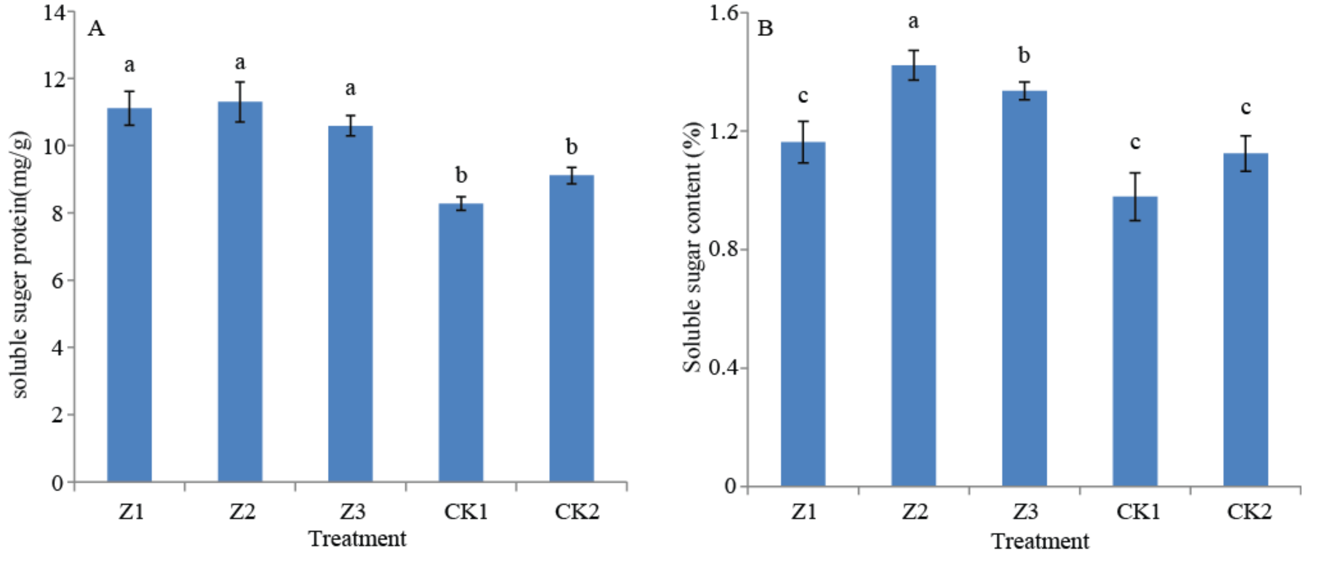

Fig. 4. A) Soluble protein and B) sugar content of lettuce under different biogas slurry fertilizer ratios.

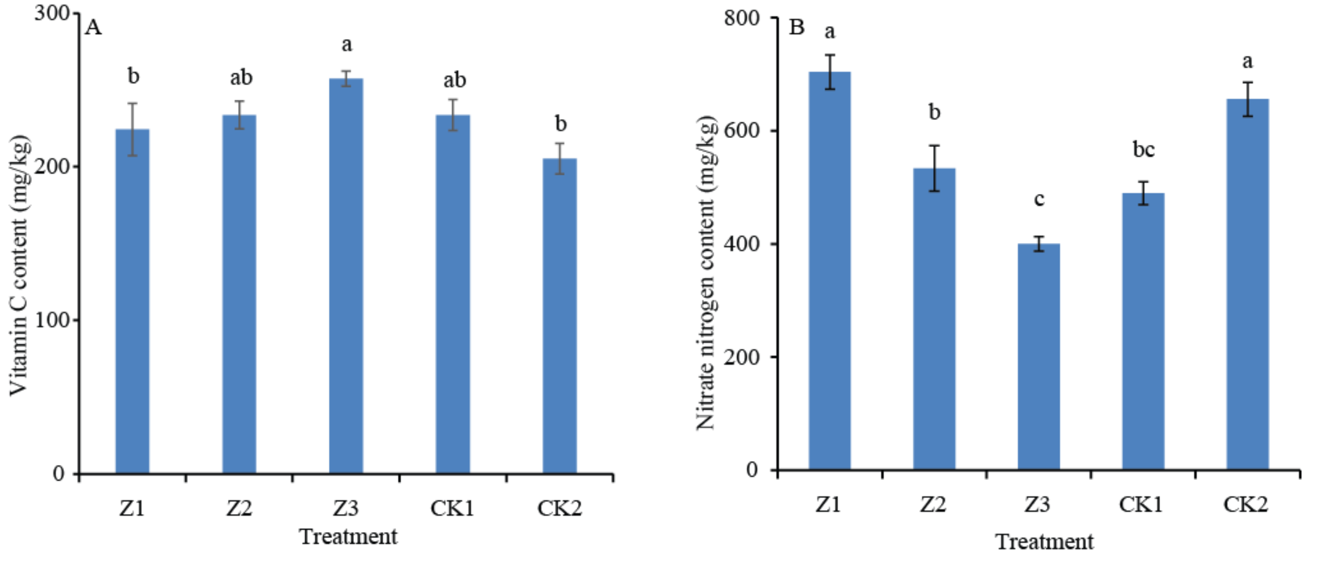

Fig. 5. A) Vitamin C and B) nitrate-nitrogencontent under different biogas slurry fertilizer ratios.

Discussion

It is well known that optimized level of BSF is a good source of plant nutrients and can significantly improves soil properties and crop productivities (Ahmad *et al.*, 2014). Apart from Z3 treatment (15% BSF ratio), other treatments (different BSF ratios) also displayed a significant effect (p<0.05) on leaf numbers, plant height, leaf length, leaf width and leaf area of lettuce as compared to control groups. Such an increase in biological yields of oil-seed rape using optimized level of biogas slurry has been recorded by Wu *et al*. (2013) while, the yield was reduced with the excess use of biogas slurry. Similarly, in order to obtain the maximum growth of maize fodder, an optimal level of biogas slurry (70 kg of slurry N ha^{-1})was suggested to be applied, while excessive use beyond this optimal level might induce adverse effects on plant growth (Islam *et al*., 2010). It has been demonstrated that BSF might increases the availability of macro- and micronutrients particularly nitrogen which in turn promotes the meristematic growth leading to higher bioactive compounds like anthocyanin, vitamin C etc. BSF is the rich source of nitrogen which holds the prime importance since it is the key element in all proteins including enzymes which regulate all the metabolic processes in plants (Islam *et al*., 2010). Nitrogen also has a direct correlation with the important metabolic pathways such as growth, photosynthesis and consequently affect the light energy efficiency and yield because it is the main element of leaf chlorophyll content which is an important physiological/ biological attribute directly related to photosynthesis rate in plants (Shiratsuchi *et al*., 2006; Khalid *et al*., 2017). Plant photosynthetic rate may be linear in correlation to leaf nitrogen content but the continuous lack of nitrogen reduces photosynthesis rate, soluble protein, enzymes and chlorophyll (Boussadia *et al*., 2010).

Elevated amount of phosphorus in biogas slurry may possess positive influences in yielding higher biomass and other beneficial compounds because it play an important role in photosynthesis, metabolism of carbohydrates and reproductive processes. In current study, the soluble protein, soluble sugar, anthocyanin and chlorophyll content were increased with the suitable supply of biogas slurry, while antagonistic results were observed with high enough concentration of biogas slurry. The data showed that an appropriate concentration of biogas slurry application is important to be considered. In our pre-experiment, 20% biogas slurry application causes the burning of ornamental lettuce seedlings, therefore suitable ratio should be determined in order to save cost and improve crops yield. Net photosynthesis rate may increase with optimum supply of biogas while adverse effects can be observed with the higher usage of biogas slurry presumably due to accumulation of different ions, which destroy chloroplast structure and decrease photosynthesis (Xu *et al*., 2013, Yan *et al*., 2016). Oxidative stress caused by reactive oxygen species (ROS) decrease the chlorophyll content (Figueroa *et al*., 2010).

Results also showed that biogas slurry reduces the nitrate nitrogen accumulation efficiently in Z3 treatment. Biogas slurrycan provide nitrogen supply for plant in both inorganic and organic forms that may consist of more than 10 kinds of amino acids. Several studies have reported that amino acid supply either multiple or single amino acid treatments decrease nitrate accumulation in vegetables (Liu, 1993; Güneş *et al*., 1996; Chen *et al*., 2007; Liu *et al*., 2007). Due to complex constituents of biogas slurry such as mixed amino acid and other bioactive substances of low molecular weight may cause up regulation of nitrate reductase which need to be further investigated.It can be extrapolated that biogas slurry is a good source of nutrient solution and could feasibly be used for growing good quality of ornamental lettuce with low nitrate concentration.

Conclusions

The results indicated that biogas slurry fertilizer can effectively improvegrowth, development as well as the yield of ornamental lettuce. Moreover, it has advantages in accumulation of vitamin C, soluble sugar, soluble protein and anthocyanin in lettuce and decreases nitrate-nitrogen content. Compared to organic compound fertilizer and non-fertilizer treatment, BSF remarkably improved the quality of ornamental lettuce; nevertheless, it is worth mentioning that BSF ratio should not be too high or too low because it directly influences the crop’s yield.

Acknowledgments

This work was supported by the National High Technology Research and Development Programme (863) of China under grant 2013AA103006 from the Ministry of science and technology of China.

References

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

Amon, B., V. Kryvoruchko, T. Amon and S. Zechmeister-Boltenstern. 2006. Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agri. Ecosys. & Environ*., 112: 153-162.

Banik, S. and R. Nandi. 2004. Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. *Indus. Crops & Prod*., 20: 311-319.

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

Berenguer, P., F. Santiveri, J. Boixadera and J. Lloveras. 2008. Fertilisation of irrigated maize with pig slurry combined with mineral nitrogen. *European J. Agron*., 28: 635-645.

Boussadia, O., K. Steppe, H. Zgallai, S.B. El Hadj, M. Braham, R. Lemeur and M.C. Van Labeke. 2010. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars ‘Meski’and ‘Koroneiki’. *Scientia Horticulturae*, 123: 336-342.

Bruuinsma, J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. *Photochem. & Photobiol*., 2: 241-249.

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

Cataldo, D.A., M. Maroon, L.E. Schrader and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Comun. Soil Sci. and Plant Analy.*, 6(1): 71-80.

Cevahir, G., S. Yentur, M. Yazgan, M. Unal and N. Yilmazer. 2004. Peroxidase activity in relation to anthocyanin and chlorophyll content in juvenile and adult leaves of" mini-star" Gazania splendens. *Pak. J. Bot*., 36: 603-610.

Chantigny, M.H., D.A. Angers, T. Morvan and C. Pomar. 2004. Dynamics of pig slurry nitrogen in soil and plant as determined with N. *Soil Sci. Soc. of Amer. J*., 68:637-643.

Chen, Y., S. Sun, J. Wang, X. Gao, Z.Q. Yang, Q.H. Zhai and S.P. Li. 2007. Study on nutritional effects of organic-N on solution cultured lettuce. *J. of Henan Agri. Uni*., 41(5): 506-510.

Clemens, J., M. Trimborn, P. Weiland and B. Amon. 2006. Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agri. Ecosys. & Environ*., 112: 171-177.

Figueroa, F., A. Israel, A. Neori, B. Martínez, E.-J. Malta, A. Put, S. Inken, R. Marquardt, R. Abdala and N. Korbee. 2010. Effect of nutrient supply on photosynthesis and pigmentation to short-term stress (UV radiation) in Gracilaria conferta (Rhodophyta). *Marine Pollution Bulletin*, 60:1768-1778.

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: 1455-1463.

Garg, R., H. Pathak, D. Das and R. Tomar. 2005. Use of flyash and biogas slurry for improving wheat yield and physical properties of soil. *Environ. Monitoring & Assess*., 107: 1-9.

Goldstein, J. 2000. Anaerobic digestion advances. *Biocycle*, 41: 30-32.

Güneş, A., A. İnal and M. Aktaş. 1996. Reducing nitrate content of NFT grown winter onion plants (*Allium cepa* L.) by partial replacement of NO 3 with amino acid in nutrient solution. *Scientia Horticulturae*, 65: 203-208.

Huang, S.W. and X.Y. Liao. 2005. Progress and prospect of biogas fermentation residues (MFR) application on rice planting [J]. *China Biogas*, 2: 006.

Islam, M.R., S.M.E. Rahman, M.M. Rahman, D.H. Oh and C.S. Ra. 2010. The effects of biogas slurry on the production and quality of maize fodder. *Turk. J. Agri. & Forest*., 34: 91-99.

Khalid, M., M. Bilal, D. Hassani, H. M. Iqbal, H. Wang and D. Huang. 2017. Mitigation of salt stress in white clover (*Trifolium repens*) by *Azospirillum brasilense* and its inoculation effect. *Botanical Studies*, 58: 5.

Liu, G. and S. Li. 1993. Seasonal fluctuation of amide-nitrogen effects on yield and nitrate accumulation of nonheading Chinese cabbage in liquid culture. *J. Nanjing Agri. Uni*., 16(2): 111-113.

Liu, X.Q., K.Y. Ko, S.H. Kim and K.S. Lee. 2007. Enhancement of nitrate uptake and reduction by treatment with mixed amino acids in red pepper (*Capsicum annuum* L.). *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 57: 167-172.

Mancinelli, A.L. 1984. Photoregulation of anthocyanin synthesis VIII. Effect of light pretreatments. *Plant Physiol*., 75: 447-453.

Piao, Y.J. and R.Y. Liu. 2008. The basic research on the application of ornamental vegetables in landscape architecture through questionnaire-based survey [J]. *Chinese Landscape Architecture*, 2: 90-94.

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

Shiratsuchi, H., T. Yamagishi and R. Ishii. 2006. Leaf nitrogen distribution to maximize the canopy photosynthesis in rice. *Field Crops Res.*, 95: 291-304.

Sørensen, P. and M. Amato. 2002. Remineralisation and residual effects of N after application of pig slurry to soil. *European J. Agron*., 16: 81-95.

Terhoeven-Urselmans, T., E. Scheller, M. Raubuch, B. Ludwig, and R.G. Joergensen. 2009. CO_2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley. *App. Soil Ecol*., 42: 297-302.

Wentzel, S. and R.G. Joergensen. 2015. Quantitative microbial indices in biogas and raw cattle slurries. Engineering in Life Sciences.16(3): 231-237.

Wentzel, S. and R.G. Joergensen. 2016. Effects of biogas and raw slurries on grass growth and soil microbial indices. *J. Plant Nutr. & Soil Sci.*, 179(2): 215-222.

Wu, J., Q. Yang, G. Yang, F. Shen, X.-H. Zhang and Y.-Z. Zhang. 2013. Effects of biogas slurry on yield and quality of oil-seed rape. *J. Plant Nutr*., 36: 2084-2098.

Xu, C., Y. Tian, Y. Sun and L. Dong. 2013. Effects of biogas slurry irrigation on growth, photosynthesis, and nutrient status of *Perilla frutescens* seedlings. *Comm. in Soil Sci. & Plant Analysis*, 44: 3381-3390.

Yan, C., R. Muñoz, L. Zhu and Y. Wang. 2016. The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae *Chlorella* sp. *Energy*, 106: 554-561.

Zha, G., W. Zhang, F. Ying, S. Liu and R. Xu. 2008. Seperating solid and liquid of Dianchi′ s Eichhorniacrassipes for Biogas Generation [J]. *Chinese Wild Plant Resources*, 27(1): 36-38.

Zhang, J., M.X. Zhang, S.D. Shan, L.P. Luo and M.Y. Wang. 2009. Growth status, grain yield and heavy metals content of rice (*Oryza sativa* L.) as affected by biogas slurry application. *J. Agro-Environ. Sci*., 10: 004.

Zhao, Y., S. Sun, C. Hu, H. Zhang, J. Xu and L. Ping. 2015. Performance of three microalgal strains in biogas slurry purification and biogas upgrade in response to various mixed light-emitting diode light wavelengths. *Biores. Technol*., 187: 338-345.

(Received for publication 2 January 2017)