Journal of Applied Horticulture, 19(1): 29-34, 2017

Effects of bioslurry and plant biostimulant Hicure® on yield, flower quality and vase life of carnation (*Dianthus caryophyllus* L.)

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Abstract

Two greenhouse experiments were conducted in Finlays, Lemotit Flower Farm, Kenya to determine the effect of bioslurry and plant biostimulant Hicure® on yield, quality and vase life of carnation. The experiments were laid in split plot embedded in a randomized complete block design with three replications. Four levels of bioslurry: 0, 0.125, 0.25 and 0.5 L m^{-2} were applied in the main plot while four levels of Hicure®: 0, 2.0, 2.5 and 3.0 L ha^{-1} were used in the sub-plot. Results showed that bioslurry or plant biostimulant did not have a significant effect on carnation's flower yield, weight, flower stem length and flower stem diameter. However, the interaction of bioslurry and plant biostimulant particularly at the rate of 0.5 L m^{-2} and 3 L ha^{-1} significantly improved the flower stem length by 1.11 cm as compared to control. The application of bioslurry significantly improved the flower head size in second trial from 21.09 mm in control to 21.68 mm, 21.81 mm and 21.90 mm for the carnation's flower head diameter and from 40.34 mm in control to 40.96 mm, 40.97 mm and 40.88 mm for the carnation's flower head length, respectively for the rate of 0.125, 0.25 and 0.5 L m^{-2}. The flower head diameter was significantly improved from 22.12 mm in control to 22.32 mm, 22.30 mm and 22.40 mm by respective application of Hicure® at the rate of 2.0, 2.5 and 3.0 L ha^{-1} during first trial. Their interaction also improved the flower head length in second trial. Application of bioslurry had no significant effect on the vase life while plant biostimulant at the rates of 2.0, 2.5 and 3.0 L ha^{-1} significantly reduced the vase life by two days in first trial and one day in second trial. It was concluded that application of bioslurry at the rate of 0.5 L m^{-2} and plant biostimulant Hicure® at the rate 3 L ha^{-1} can therefore, be adopted for improvement of carnation quality parameters such as stem length and flower head size.

Key words: Bioslurry, carnation, plant biostimulant, vase life, flower quality, flower yield

Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the most popular commercial cut flowers globally, ranking second after roses (Jawaharlal *et al.,* 2009). It is preferred by several exporting countries, on account of its excellent keeping quality, wide range of shapes, sizes and colours and ability to withstand long distance transportation (Salunkhe *et al.,* 1990; Jawaharlal *et al.,* 2009; Kanwar and Kumar, 2009). In 2014, it was ranked 14th among the top 25 cut flowers sold in the Dutch auction with a turnover of €25 million (FloraHolland, 2015). It was fourth among the top ten imported cut flower to the Netherlands after roses, St John's wort and gypsophila (FloraHolland, 2015).

Production of carnations is confronted with many problems that affect quality including calyx splitting and shorter stem length for some varieties. Conventionally, growers obtain the quality parameters such as stem length and girth, flowers size and number by heavy application of inorganic fertilizers and synthetic plant growth regulators. Although this results in increased production, it adversely affects soil productivity and the environment.

Currently, increased enforcement of the European codes of practice on good agricultural practices and environmental standards are affecting cut flower trade in the European market (Rikken, 2011). Hence, there is an urgent need to reorient the research priorities to develop alternative systems in crop production (Padaganur *et al.*, 2005). This can be achieved through elaboration of unconventional and non-pollutant solutions and culture techniques focused on testing the action of some fertilizers and biostimulants with non-pollutant properties which may upgrade the classic culture technology that determine plant growth and development, including the increment of the decorative aspect (Violeta *et al.,* 2010). While there are limited alternatives to inorganic fertilizers for meeting the nutritional requirements of crops, organic products for regulating plant growth and development are lacking in some crops including carnations. Among the available alternatives, organic fertilizers and plant biostimulant are being investigated to supplement inorganic fertilizers in integrated crop management. For instance, the use of compost derived from plant and/or animal wastes as soil amendment or fertilizer additive has been reported in the production of several ornamental plants including marigold (Idan *et al.,* 2014), petunia (Moghadam and Shoor, 2013), dahlia (Ahmed *et al.,* 2004), aster (Balladares *et al.,* 2012) and on tuberose (Padaganur *et al.,* 2005). Bioslurry, a digested biogas effluent, is considered to be a quality organic fertilizer (Islam, 2006; Jeptoo *et al*., 2013). Yield increases following bioslurry applications have been reported in different crops such as okra (Shahbaz, 2011), maize, cabbage (Karki, 2001) and carrot (Jeptoo *et al*., 2013).

Similarly, the use of plant biostimulants has been reported in the production of ornamental and other horticultural crops such as *Antirrhinum majus* (Nahed *et al.,*2009), pepper (Paradiković *et al.,* 2011) and *Eustoma grandiflorum* (Mondal *et al.,* 2015). It has been revealed that plant biostimulants have the capacity of enhancing nutrition efficiency and or stress response (du Jardin, 2012). They consist of humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds, beneficial fungi and beneficial bacteria (du Jardin, 2015). Therefore, this study investigated the effects of bioslurry and application of Hicure® (an amino acids based biostimulant) on yield, quality and vase life of carnations.

Materials and methods

Experimental site: This study was conducted at Lemotit flower farm of Finlays Horticulture Kenya Ltd. situated in Kenya at latitude 0°22' South and longitude 35°18' East and an altitude of 2400 m with an average annual rainfall of 1386 mm. The annual mean maximum and minimum temperatures were 24 and 9 °C, respectively, with an average relative humidity of 85% (Situma *et al.*, 2013).

Planting material: Carnation 'Walker' planting material bred by Selecta, propagated and rooted by Finlays Horticulture Kenya Ltd-Lemotit Farm was used in the experiment. An established carnation crop planted in a greenhouse on soil media at a density of 36 plants per m^2 was used. The application of treatments was done after pinching (three weeks after transplanting).

Experimental design and treatment application: Two different trials were conducted. The first trial was established in September 2014 and harvested in February 2015 and the second was planted in February 2015 and harvested in August 2015. The experimental design was a split- plot embedded in a randomized complete block design with three replications. The main plot measured 5.5 x 1 m (5.5 m^2) while the sub-sub plot was 1 x 1 m (1 m^2). Buffer zone of 0.5 m and 1 m separated inter-plots and individual main blocks, respectively. Bioslurry was applied in main plots at the rate of 0.125, 0.25, 0.5 L m^{-2} and control diluted in one litre of water prior to application. Rates of Hicure® used were 2.0, 2.5 and 3.0 L ha^{-1} and control thoroughly mixed with water at the rate of 5000 L ha^{-1} and applied to the sub-plots. Both, bioslurry and plant biostimulant Hicure® were drenched four times at bi-weekly intervals after pinching. Prior to application, bioslurry was analysed for nitrogen content using Kjeldahl method (Watson *et al.,* 2003) and mineral content using atomic absorption spectrophotometer as described by Kovar (2003). The characteristics of the bioslurry are summarized in Table 1.

The plant biostimulant Hicure® used contained a balanced mixture of free amino acids and peptides (hydrolysed protein) of natural origin: amino acids and peptides (62.5%), total nitrogen (10.9%) and organic carbon (29.4%). All treatments received a weekly application of mineral fertilizers through fertigation using per square metre: 3.06 g N, 3.51 g P_20_5, 5.19 g K_20, 1.71 g Ca and 0.74 g Mg, plus trace elements. Routine management practices included irrigation, supporting, weeding, training, disbudding and pest management. Harvesting was done at the paint brush stage when petals started to elongate outside the calyx.

Table 1. Bioslurry characteristics

	Trial 1	Trial 2
pH	7.44	7.46
Nitrogen (%)	0.23	0.16
Phosphorus (ppm)	4.58	6.69
Potassium (ppm)	89.30	68.06
Calcium (ppm)	4.31	3.32
Magnesium (ppm)	19.91	19.91
Density	1.02	1.01

Data collection: Data were collected from 10 tagged sample plants per subplot. Length of flower stem was measured in centimetres from the point just below the bud to the point of origin of branch on the main stem at harvest. Diameter of flower stem was measured in millimetres with vernier callipers at middle of the flower stalk. The flower head length was recorded in millimetres from the point just below the calyx to the upper point of the flower while the flower head diameter was recorded in millimetres at harvesting from each harvested cut flower. The weight of freshly harvested flowers with stalk was determined in grams using electronic balance. Immediately after harvesting, flowers were put in fresh water for two hours to remove the field heat. After that, flowers were kept in bucket containing three litres of water and placed in vase life room at a temperature of 4 °C. Fading of outer row petals was considered as the end of vase life of flowers, which was expressed in days. All data were subjected to analysis of variance (ANOVA) using GENSTAT 14th Edition. Separation of means was performed using the Least Significant Difference at $P \leq 0.05$.

Results

Flower stem length: There was no significant effect of bioslurry and Hicure® on flower stem length in both the trials (Table 2 and 3). The interaction between different levels of bioslurry and levels of Hicure® did not have significant differences in trial 1 while there was a significant difference in second trial ($P \leq 0.05$). The combination of the rate of 0.5 L m^{-2} of bioslurry and 3.0 L ha^{-1} of Hicure® recorded the longest flower stems (Table 4).

Flower stem diameter: The application of different rates of bioslurry and Hicure® did not significantly affect the flower stem diameter (Table 2 and 3). There was no interactive effect of bioslurry and Hicure® on the flower stem diameter (Table 4).

Flower head length: Bioslurry did not significantly increase the flower head length in trial 1 but it had a significant effect in trial 2 ($P \leq 0.05$). Application of bioslurry at the rate of 0.125, 0.25 and 0.5 L m^{-2} significantly improved the flower head length compared to the control and the similar trend was observed in first trial although there was no significant difference (Table 2). Similarly, different rates of Hicure® did not have a significant effect on the flower head length. In both trials, the application of Hicure® at the rate of 2 L ha^{-1} recorded the highest values (Table 3). There was no interactive effect between levels of bioslurry and levels of Hicure® in the first trial while there was a significant effect in second trial (Table 4).

Flower head diameter: There was mixed effect of different rates of bioslurry and of Hicure® on the flower head diameter. In the first trial, the application of different levels of bioslurry did not have a significant effect on flower head diameter although a slight increase was observed (Table 2). Although there was a significant difference between different rates of bioslurry on the flower head diameter in the second trial. Application of bioslurry at the rate of 0.125, 0.25 and 0.5Lm^{-2} increased the flower head

Table 2. Effect of bioslurry on carnation flower quality

Bioslurry levels ($L\ m^{-2}$)	Trial 1				Trial 2			
	Stem Length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)	Stem length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	65.10	5.78	22.15	39.39	69.63	5.30	21.09c	40.34b
0.125	65.92	5.76	22.38	39.52	69.57	5.36	21.68b	40.96a
0.25	65.62	5.77	22.29	39.55	69.54	5.32	21.81ab	40.97a
0.5	65.83	5.77	22.32	39.42	69.46	5.30	21.90a	40.88a

Mean separation within columns by least significant difference test at $P \le 0.05$

Table 3. Effect of plant biostimulant Hicure® on carnation flower quality

Level of Hicure® ($L\ ha^{-1}$)	Trial 1				Trial 2			
	Stem length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)	Stem length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	66.07	5.78	22.12b	39.49	69.61	5.36	21.59	40.70
2.0	65.34	5.75	22.32a	39.57	69.48	5.30	21.55	40.94
2.5	65.43	5.78	22.30a	39.36	69.36	5.31	21.67	40.62
3.0	65.64	5.76	22.40a	39.46	69.76	5.32	21.65	40.89

Mean separation within columns by least significant difference test at $P \le 0.05$

Table 4. Effects of the interaction of bioslurry and plant biostimulant Hicure® on carnation flower quality

Level of Bioslurry ($L\ m^{-2}$)	Level of Hicure ($L\ ha^{-1}$)	Trial 1				Trial 2			
		Stem length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)	Stem length (cm)	Stem diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	0	65.30	5.79	21.63	39.34	69.42ab	5.33	21.22	40.21de
	2	65.20	5.76	22.25	39.44	69.85a	5.30	20.78	40.23cde
	2.5	64.92	5.77	22.28	39.40	69.95a	5.27	21.20	40.54abcde
	3	64.99	5.80	22.46	39.36	69.32abc	5.31	21.15	40.36bcde
0.125	0	66.56	5.79	22.32	39.58	69.99a	5.40	21.65	41.01ab
	2	65.24	5.74	22.38	39.66	68.74cb	5.33	21.61	41.02ab
	2.5	65.28	5.78	22.41	39.49	69.98a	5.36	21.59	40.89abc
	3	66.60	5.73	22.42	39.35	69.57ab	5.33	21.85	40.89abc
0.25	0	66.19	5.79	22.21	39.55	69.77ab	5.43	21.70	40.88abcd
	2	65.63	5.76	22.28	39.60	69.38abc	5.22	21.71	41.15a
	2.5	65.63	5.79	22.39	39.46	69.39abc	5.37	22.00	40.96ab
	3	65.03	5.73	22.27	39.56	69.60ab	5.27	21.81	40.90abc
0.5	0	66.23	5.77	22.32	39.48	69.25abc	5.28	21.78	40.69abcde
	2	65.28	5.74	22.36	39.57	69.94a	5.33	22.10	41.35a
	2.5	65.88	5.79	22.14	39.07	68.12c	5.22	21.91	40.07e
	3	65.92	5.78	22.44	39.57	70.53a	5.36	21.79	41.40a

Mean separation within columns by least significant difference test at $P \le 0.05$

diameter compared to the control (Table 2). On the other hand, the application of various levels of plant biostimulant Hicure® had a significant effect on the flower head diameter in the first trial while there was no significant difference on the effect of different rates of plant biostimulant Hicure® on the flower head diameter in the second trial (Table 3). There was no interactive effect between levels of bioslurry and different levels of Hicure® (Table 4).

Number of flowers per plant: Application of different levels of bioslurry did not result in a significant increase in number of flowers per plant in first and second trial ($P \le 0.05$) (Table 5). Similarly, the application of the biostimulant Hicure® did not increase the flower yield significantly although slight increases were recorded on plant which received this biostimulant at the rate of 3 $L\ ha^{-1}$ in both first and second trial (Table 6). There were

Table 5. Effect of bioslurry on carnation stems yield, weight and vase life

Bioslurry levels ($L\ m^{-2}$)	Trial 1			Trial 2		
	Flowers per plant	Weight (g)	Vase life (in days)	Flowers per plant	Weight (g)	Vase life (in days)
0	5.62	39.25	19.42	6.38	32.45	16.06
0.125	5.63	39.29	18.25	6.54	32.22	15.33
0.25	5.41	39.97	17.75	6.76	32.06	15.72
0.5	5.64	38.55	17.42	6.43	32.42	15.92

Table 6. Effect of plant biostimulant Hicure® on carnation stems yield, weight and vase life

Levels of Hicure® ($L\ ha^{-1}$)	Trial 1			Trial 2		
	Flowers per plant	Weight (g)	Vase life (in days)	Flowers per plant	Weight (g)	Vase life (in days)
0	5.44	38.96	19.83a	6.60	33.02	16.42a
2.0	5.49	39.02	17.58b	6.38	33.28	15.81b
2.5	5.63	39.46	17.67b	6.50	31.03	15.36b
3.0	5.74	39.61	17.75b	6.62	31.82	15.44b

Mean separation within columns by least significant difference test at $P \leq 0.05$

no significant interaction effects on carnation flower yield in all the combination of bioslurry and Hicure® biostimulant.

Flower weight: Application of levels of bioslurry did not have any significant effect on flower weight in both trials (Table 5). A similar trend was also recorded among plants which received different rates of Hicure® biostimulant (Table 6). There was no interactive effect of levels of bioslurry and Hicure® on the weight of flower.

Vase life: Application of bioslurry 0.125, 0.25 and 0.5 L m^{-2} reduced the vase life as compared to the control in both first and second trial (Table 5) although it was not significant ($P \leq 0.05$). On the other hand, the application of Hicure® significantly reduced the vase life ($P \leq 0.05$). The rate of 2, 2.5 and 3 L ha^{-1} did not differ significantly but had a vase life reduced by two days in first trial and one day in second trial compared to the control which recorded a longer vase life (Table 6). Data collected in both trials did not, however, show any significant interactive effect between levels of bioslurry and the plant biostimulant on the vase life of carnations.

Discussion

Application of bioslurry increased the number of flower stems per m^2 compared to control (Table 5). However, this increase affected the stem weight as the increased number of stems reduced the weight per stem particularly in the second experiment (R^2=0.96). This is probably because the application of bioslurry adds more nutrients during early days when shoots are developing and then these compete for the nutrients supplied by the inorganic fertilizers. This justifies why stems in the control had more weight than those where bioslurry was applied.

The nutrients content of bioslurry seems to have played a key role in the effect observed on flower head length and diameter (Table 2). For instance, its application added various nutrients, particularly phosphorus, which contributed in enhancing the quality of carnation. In fact, phosphorus in cow dung slurry and poultry manure slurry is released in higher amount compared to their original state (Haque *et al.,* 2015). Phosphate compounds act as an energy currency in plants, play an important role in photosynthesis and the metabolism of carbohydrates and they are also stored for subsequent growth and reproductive processes (Islam *et al.,* 2010). It was also reported by Zubair and Wazir (2007) that phosphorus significantly improved all floral characters in gladiolus. This is probably one reason for the increase of flower head size. The other probable reason is the increased efficiency in uptake of other nutrients. This efficiency in uptake of nutrients could be attributed to improvement in soil and water conservation, increased microbial population, buffering capacity, exchange capacity of the soil following application of bioslurry as organic amendment along with inorganic fertilizers (Muhmood *et al.,* 2014). These nutrients are directed to reproductive organs as previously revealed on okra (Shahbaz *et al.,* 2014). This increased availability and uptake of nutrients particularly of soil nitrogen as a result of bioslurry application (Shahbaz *et al.,* 2014; Islam *et al.,* 2010) is suspected to be one of the cause of the observed vase life reduction as previously reported on tuberose (Khalaj *et al.,* 2012) and Sandersonia (Clark and Burge, 1999). Alternatively, this reduction of vase life may be simply interpreted as the effect of bigger flower size which is a heavy sink during vase life as they deplete the dry matter quickly. The weight of stems, the flower stem length and the flower head size played a significant role in reducing the vase life as they act as heavy sinks during vase life and deplete stored reserves faster.

The application of plant biostimulant did not show consistent results in both trials. For instance, it recorded the highest number of flowers and heavier flowers in first trial and not in second (Table 6). It also recorded significantly increased flower head diameter in first trial and not in second trial (Table 3). This would imply that the effect of biostimulants is not observed in all growing conditions. Possibly, the regulatory mechanisms of soil and plant as well as the optimum supply of nutrients through fertigation may have lead to the absence of the effect. This was reported in study by Gioseffi *et al.* (2012) on wheat where down-regulation mechanisms on the uptake of organic and inorganic nitrogen were observed. Moreover, ammonium, which was used as a source of nitrogen, has been reported to down-regulate amino acids (Henry and Jefferies, 2003 cited by Gioseffi *et al.,* 2012; Thornton and Robinson, 2005 cited by Gioseffi *et al.,* 2012). Other reasons may be the rate, timing and frequency of application of the plant biostimulant in this study. In a study on roses, amino acids produced an effect when they were applied weekly for five months (Di Benedetto *et al.,* 2006). Also, a study by Mondal *et al.* (2015) demonstrated that some amino acids had an effect on Eustoma flower only when applied during the seedling stage while others needed supply until the anthesis. However, this may be speculative as the fate of amino acids based biostimulants has not been explored under variable soil conditions. The application of plant biostimulant Hicure® significantly reduced the vase life in both trials (Table 6). This may be a result of increased nitrogen uptake as aforementioned.

The interactive effect of bioslurry rates and plant biostimulant rates was observed on flower stem length and on flower head height (Table 4). This is probably due to the improved nutrients uptake by application of amino acids plant biostimulants (Calvo *et al.,* 2014) and also as a result of application of bioslurry (Shahbaz *et al.,* 2014 and Islam *et al.,* 2010). This was observed in a study on *Leucospermum cordifolium* where treatments with greater contribution of nitrogen and amino acids resulted in increased nitrogen, phosphorus, calcium, magnesium, iron and manganese nutrients removal by the harvested flowers (Hernández *et al*., 2014).

Based on these results we can deduce that bioslurry can improve the carnation flower head size. The application of plant biostimulant Hicure® did not improve the yield of carnations. However, it has shown a significant effect on the flower head size. Nevertheless, the application of this biostimulant reduced the vase life. The interaction between bioslurry and plant biostimulant did not affect the yield of carnation; however, it showed some effects on carnation's flower stem length and flower head length. Further studies should focus on using the foliar application of the Hicure® plant biostimulant to explore whether the media did not affect its uptake. It would be necessary to study the application of both products under lower rates of inorganic fertilizers and extend over many production flushes to find out whether both bioslurry and plant biostimulant Hicure® have residual effects.

Acknowledgement

The authors are thankful to James Finlays Company for providing the research materials and allowing us to conduct experiments in their Lemotit farm. Syngenta East Africa Ltd is acknowledged for providing the plant biostimulant Hicure®. We also thank Tatton Agricultural Park of Egerton University for the supply of bioslurry.

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Received: January, 2016; Revised: March, 2016; Accepted: May, 2016