Adv. Hort. Sci., 2018 32(1): 137-142
DOI: 10.13128/ahs-21148

Short note

AHS
Advances in Horticultural Science

Residual effects of bioslurry and amino acids plant biostimulant on carnation (*Dianthus caryophyllus* L.) flower quality

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Key words: bioslurry, carnation, flower quality, plant biostimulant, residual effect.

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Citation:
NIYOKURI A.N., NYALALA S., MWANGI M., 2018 - *Residual effects of bioslurry and amino acids plant biostimulant on carnation (*Dianthus caryophyllus *L.) flower quality.* - Adv. Hort. Sci., 32(1): 137-142

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 7 August 2017
Accepted for publication 23 October 2017

Abstract: A greenhouse experiment was conducted in Finlays, Lemotit Flower Farm in Kenya to determine the residual effect of bioslurry and an amino acids plant biostimulant on carnation flower quality. A second flush of carnation plants growing in previous experimental plots was used. This experiment was laid out in a split plot design with three replications. Four levels of bioslurry: 0, 0.125, 0.25 and 0.5 L m^{-2} were applied in the main plots while four levels of plant biostimulant: 0, 2.0, 2.5 and 3.0 L ha^{-1} were used in the sub-plots. Results showed that there was no significant residual effect of bioslurry on studied parameters. Residual effects of plant biostimulant applied at 2.0, 2.5 and 3.0 L ha^{-1} resulted in a significant increase in carnation flower stalk length by 1.08 to 1.72 cm compared to control. However, there was negligible reduction of the flower stalk diameter (0.1 mm) and no significant residual effect of plant biostimulant on flower head size. Moreover, there were no residual interactive effects of bioslurry and plant biostimulant on studied parameters. These results suggest that plant biostimulant can be used to improve the flower stem length in the subsequent flush of carnation plants supplied with a full dose of inorganic fertilizers.

1. Introduction

Carnation *(Dianthus caryophyllus* L.) is a popular cut-flower throughout the world (Roychowdhury and Tah, 2011). It is preferred to other cut flowers in several exporting countries as it lasts longer after being cut, has a wide range of attractive forms and colours, has the ability to withstand long distance transportation and significant ability to rehydrate after continuous shipping (Salunkhe *et al.,* 1990; Kanwar and Kumar, 2009;

Renukaradya *et al.,* 2011).

Although it is indigenous to the Mediterranean region, carnation can be grown in almost every climate in glasshouses, plastic houses, and shade nets as well as in open field (Aydinsakir *et al.,* 2011). It was fourth among the top ten imported cut flower to the Netherlands after roses, St John's wort (*Hypericum* spp.) and gypsophila with a turnover of €18 million (FloraHolland, 2017). It was also one of the main cut flowers exported by Kenya in 2012 (CBI, 2013) and among the leading cut flowers locally used in flower arrangements and in value addition of flowers, in form of bouquets. In 2014, carnations contributed 4% of the domestic value of floriculture in Kenya (HCD, 2015).

The quality of carnations is currently affected by many problems such as calyx splitting and short stem length for some varieties. To meet the required quality parameters such as stem length and girth, flowers size and number, farmers resort to heavy application of inorganic fertilizers and synthetic plant growth regulators. Although this results in increased production and quality, it adversely affects soil productivity and the environment. This is because pesticides, phosphorus and nitrate used in carnation production represent the major agricultural pollutants that threaten the environment (Nardi *et al.,* 2016).

With the increasing relevance of social and environmental standards, current research is focusing on developing alternative systems in crop production through development of unconventional and non-pollutant solutions. Currently, there are limited organic alternatives to meet plant nutrients. Moreover, organic alternatives for plant growth regulators are lacking in many crops and limited in other crops such as carnations.

Use of compost derived from plants and/or animal wastes as soil amendment or fertilizer additive has been reported as an alternative in the production of several ornamental plants. Moreover, bioslurry, the residual manure generated through anaerobic decomposition of various organic materials is considered a quality organic fertilizer (Islam, 2006). Similarly, the use of biostimulants in sustainable agriculture has been growing particularly for their capacity of enhancing nutrition efficiency and stress response (du Jardin, 2012). Biostimulants can be obtained from different organic materials and include complex organic materials, humic substances, beneficial chemical elements, peptides and amino acids (protein hydrolysates), seaweed extracts, inorganic salts, chitin and chitosan derivatives, antitranspirants, amino acids and other N-containing substances (du Jardin, 2015; Nardi *et al.,* 2016).

Many studies (Karki, 2001; Islam, 2006; Shahbaz, 2011; Jeptoo *et al*., 2013; Shahariar *et al.,* 2013) reported yield increases and quality improvement on many crops such as Okra (*Hibiscus esculentus* L.), maize (*Zea mays*), cabbage (*Brassica oleracea* var. *capitata)* and carrot (*Daucus carota*) following bioslurry application. Studies by Nahed *et al.* (2009 a, b), Paradiković *et al*. (2011) and Mondal *et al.* (2015) reported that plant biostimulants could be successfully used in the production of ornamental and other horticultural crops such as *Antirrhinum majus*, Gladiolus (*Gladiolus grandflorum* L.) sweet yellow pepper (*Capsicum annuum* L.) and *Eustoma grandiflorum*.

However, many studies only evaluated the bioslurry and biostimulant direct effects, whereas there are only a few studies which focused on their residual effects on the quality and yield of many crops including carnations. This study was therefore aiming at evaluating residual effects of bioslurry and plant biostimulant on flower quality in carnation plants which previously received applications of bioslurry and plant biostimulant.

2. Materials and Methods

Experiment location

The study was conducted in a greenhouse at Lemotit flower farm of Finlays Horticulture Kenya Ltd. situated in Kenya at latitude 0^o 22' South and longitude 35^o 18' East, from March to September 2015.

Experimental design and treatments application

Carnation 'Walker' plants planted on soil media at a density of 36 plants per m^2 were used. These plants had received drench applications of bioslurry and plant biostimulant, four times at bi-weekly intervals after pinching (three weeks after transplanting) during the period September 2014 to February 2015. The experimental design was a split-plot design with three replications. The main plot measured 5.5 x 1 m (5.5 m^2) while the 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. Cow dung 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 plant biostimulant 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 they were applied to the sub-plots during the period of September 2014 to February 2015.

Bioslurry used had at wet basis a pH of 7.44, 0.23% of N, 4.58 ppm of P, 89.3 ppm of K, 4.31 ppm of Ca, 19.91 ppm of Mg and density of 1.0195 kg L^{-1}. The plant biostimulant used was Hicure®, an amino acids based plant biostimulant. This plant biostimulant contains a balanced mixture of free amino acids (with higher proline and glycine contents) and peptides (hydrolysed protein) of natural origin. It is composed of amino acids and peptides (62.5%), total nitrogen (10.9%) and organic carbon (29.4%). After harvest in February 2015, carnation plants were allowed to grow for subsequent season in order to study the residual effects of bioslurry and plant biostimulant.

Maintenance practices

All treatments benefited from a weekly application of mineral fertilizers through fertigation using: 3.06 g N, 3.51 g P_2O_5, 5.19 g K_2O, 1.71 g Ca and 0.74 g Mg, plus trace elements per square metre. Routine crop 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.

Data collection and analysis

Data were collected from 10 tagged sample plants per sub-plot on three flower quality parameters namely the length of flower stalk, diameter of flower stalk and flower head size (diameter and head length). The length of flower stalk 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 using digital vernier callipers. 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 at paint brush stage using digital vernier calliper. All data were subjected to analysis of variance (ANOVA) using GENSTAT 14th Edition. Separation of means was performed using the Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

3. Results

There was no significant residual effect of bioslurry on measured parameters (Table 1). However, a significant residual effect of plant biostimulant on plants which had received any level of plant biostimulant was observed on flower stalk length and flower stalk diameter (Table 2). Carnations plants that had received 2.0, 2.5 and 3.0 L of plant biostimulant had significantly longer flower stalks and significantly thinner flower stalks compared to the control (Table 2).

The application of different rates of plant biostimulant did not show a significant residual effect on flower head size (diameter and head height) as presented in table 2. The interaction between different levels of bioslurry and those levels of plant biostimulant had no significant residual effects on measured parameters (Table 3).

Table 1 - Residual effect of bioslurry on carnation flower quality

Bioslurry levels (L m^{-2})	Flower stalk length (cm)	Flower stalk diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	81.01	5.52	21.77	40.58
0.125	81.18	5.56	22.04	40.44
0.25	81.02	5.55	21.86	40.56
0.5	81.15	5.55	21.81	40.48

Table 2 - Residual effect of plant biostimulant on carnation flower quality

Levels of plant biostimulant (L ha^{-1})	Flower stalk length (cm)	Flower stalk diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	80.12 b*	5.628 a	21.97	40.53
2	81.20 a	5.512 b	21.83	40.47
2.5	81.20 a	5.515 b	21.74	40.58
3	81.84 a	5.528 b	21.93	40.48

* Means in the same column with the same letter are not significantly different at $P \leq 0.05$ using Tukey's HSD test.

4. Discussion and Conclusions

Results of this study showed a significant increase of flower stalk length as a result of the residual effect of plant biostimulant. This increase of flower stalk length may be as a result of enhancement of macro nutrient uptake by plant biostimulant (Calvo *et al.,* 2014; Rose *et al.,* 2014) which rapidly improves the growth compared to treatments without plant biostimulant. The other probable reason would be the direct uptake of amino acids which are immediately used by carnation plants for their growth and devel-

Table 3 - Residual effects of the interaction of bioslurry and plant biostimulant on carnation flower quality

Bioslurry levels (L m^{-2})	Level of plant bio-stimulant (L ha^{-1})	Flower stalk length (cm)	Flower stalk diameter (mm)	Flower head diameter (mm)	Flower head length (mm)
0	0	79.94	5.57	21.81	40.61
	2	81.07	5.48	21.74	40.57
	2.5	80.83	5.51	21.71	40.45
	3	82.21	5.51	21.8	40.70
0.125	0	79.75	5.65	22.13	40.55
	2	81.30	5.53	21.81	40.46
	2.5	81.68	5.51	21.79	40.67
	3	81.97	5.56	22.44	40.08
0.25	0	80.28	5.67	22.06	40.49
	2	81.14	5.52	21.95	40.50
	2.5	80.85	5.50	21.76	40.60
	3	81.82	5.51	21.69	40.64
0.5 L	0	80.52	5.61	21.88	40.47
	2	81.29	5.52	21.84	40.35
	2.5	81.44	5.55	21.7	40.58
	3	81.35	5.53	21.81	40.41

opment (Calvo *et al.,* 2014). There are strong evidences that the increase in flower stalk length may be attributed to residual auxins and gibberellins like activities of the plant biostimulant (Brown and Saa, 2015). In fact, both auxins and gibberellins are important in plant cell division and elongation (Ertani *et al.*, 2009; Calvo *et al.,* 2014 and Colla *et al.,* 2014). Results of this study are in agreement with previous findings by Ertani *et al.* (2009) and Colla *et al.* (2014). Colla *et al.* (2014) reported an increase in coleoptile elongation rate when compared to the control, in a dose-dependent fashion, comparable with the effects of indole-3-acetic acid following the treatment of maize with the protein hydrolysate. The same study provided additional evidences of a gibberellin-like activity of protein hydrolysate when application of plant-derived protein hydrolysate "Trainer" at all doses significantly increased the shoot length of the gibberellins deficient dwarf pea plants by an average value of 33% in comparison with the control treatment. Similar results previously reported by Ertani *et al.* (2009) showed that the treatment of lettuce with both protein hydrolysate based fertilizers resulted in an increase in the epicotyl length comparable with the effects of exogenous gibberellic acid. The occurrence of this residual effect was perhaps the result of previous down-regulation mechanisms which affected the effect of plant biostimulant in the previous season. 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).

The observed reduction in flower stalk diameter is suspected to be a gibberellin-like activity which promoted the flower stalk length (Ertani *et al.*, 2009; Colla *et al.*, 2014) at the expense of flower stalk diameter.

The absence of residual effect of plant biostimulant on flower head size was possibly due to constant supply of nutrients through fertigation. The flower head size is usually a result of carbohydrates stored for subsequent growth and reproductive processes (Islam *et al.,* 2010). Although there are evidences that biostimulants can enhance macro nutrient uptake (Calvo *et al.,* 2014; Rose *et al.,* 2014), it is possible that the residual effect of plant biostimulant was limited to stimulating the elongation of flower stalk. Hence, the residual effect may have contributed much during early stages when carnation plants started re-growing after the harvest by improving growth and nutrients assimilation as previously revealed by Colla *et al.* (2014).

Bioslurry did not show any significant residual effect on the flower stalk length, flower stalk diameter and the flower head size. Generally, nutrients in cow dung slurry and poultry manure slurry are released in higher amounts compared to their original state (Haque *et al.,* 2015). This is because bioslurry, with its narrower C:N than farmyard manure, shows better results on soil nutrient availability at early stages of its application while, farm yard manure affects the nutrient uptake to the plant in more consistent manner because its mineralization occurs at later stages (Muhmood *et al*., 2014). However, results of our study are not in agreement with Shahzad *et al.* (2015) who reported that the application of bioslurry and composted poultry manure as a bio-fertilizer improves soil organic matter contents and availability of soil nutrients (N, P and K) to the subsequent crop, resulting in increased crop productivity and reducing the cost of fertilizer to subsequent crop. In fact, the residual effect of bioslurry may depend on its initial content, characteristics and the quantity applied and for these reasons, the residual effect of bioslurry on carnation grown in the following season was very limited.

Based on results of this study, we can conclude that the application of plant biostimulant on carnations plants supplied with a full dose of inorganic fertilizers may have residual effect on the subsequent production flush. However, the residual effect of bioslurry on carnations grown in the same conditions may depend on its nutrient content and physico-chemical characteristics. Further works would be necessary to study the application of both products under lower rates of inorganic fertilizers and extend over many productions flushes to find out the extent of their residual effects.

Acknowledgements

The authors are thankful to James Finlays Company for providing the research plant materials and allowing us to conduct experiments in their Lemotit farm.

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