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The efficacy of biostimulants in the management of *Agrobacterium tumefasciens* the cause of crown gall disease of roses in Kericho, Kenya

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Abstract

Experiments were conducted at James Finlay Kenya, Tarakwet farm in Kericho county to test the efficacy of various biostimulants, i.e., biozyme 2.5 ml/L, hicure 2.5 ml/L, foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L and control (water sprayed only) in controlling *Agrobacterium tumefasciens* the cause of crown gall disease in roses. Treatments were either sprayed or drenched. Both greenhouse and pot trials were conducted on a susceptible rose variety '*Tropical amazone*'. Plots treated with biozyme 2.5 ml/L, hicure 2.5 ml/L, foltron 2.0 ml codamine radicular 2.0 ml/L, alexin 2.5 ml/L were longer, with a bigger head size and had better yield of marketable rose stems compared to control plots treated with water only. It was therefore concluded the application of various biostimulants on roses affected by *A. tumefasciens* by drenching or spraying improved the yield and quality of marketable rose stems. This was attributed to the fact that biostimulants boosted the immune response of roses to *A. tumefasciens* through improving the nutrient use efficiency of the plant and enhanced tolerance to biotic and abiotic stresses. However, more research is needed to elucidate this.

Keywords: *Agrobacterium tumefasciens, Biostimulants, Roses, Stress, Tumor*

1. Introduction

Kenya's horticultural industry is one of the largest in the world and ranked second in foreign exchange earnings after tea. Floriculture is the most developed sector and accounts for about 38% of all horticultural exports in the European Union. With a global market of 7% share, Kenya is the third largest exporter of cut flowers in the world (Kenya Flower Council, 2019). Floriculture provides employment to 100,000 people directly and two million people indirectly. Roses make up 74% of Kenya's flower export followed by carnations (Kenya Flower Council, 2019). Despite the tremendous contribution to the Kenyan economy, profitable production of roses is constrained by a number of plant diseases such as crown gall caused by *Agrobacterium tumefasciens* (Horst, 1983). Rose plants infected by *A. tumefasciens* are stressed and manifest the following symptoms; slower growth, stunting, yellowing, chlorotic leaves and fail to produce healthy flowers (Kado, 2002).

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Crown gall incidence causes losses of between 75 to 95% on susceptible rose varieties (Maina *et al.*, 2013). A number of measures have been adopted to reduce the impact of *A. tumefasciens* in production of roses. However, no method has been effective in reducing crown gall completely. Current rose management regimes in Kenya lack protocol for this disease hence farmers resort to untested solutions. In addition, there are no registered pesticides in Kenya and in East Africa for controlling the disease (Maina *et al*., 2013). 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 need to develop environmental safe solutions for controlling pests and disease in the flower industry. For this reason, the efficacies of various biostimulants were tested as control agents of *A. tumefasciens* the cause of crown gall disease in roses.

Plant biostimulants are material which contain substance (s) and/or microorganisms when applied to plants stimulate natural processes such as nutrient uptake, nutrient efficiency, tolerance to biotic stress independently of its nutrient content (Rouphael, 2015). Biotic and abiotic stresses which prevent plants in achieving their full potential may be prevented by optimizing plant growth conditions and through provision of water, nutrients and plant growth regulators such as auxins, cytokinins, gibberellins, strigolactones, and brassinosteroids. In addition to these traditional approaches, biostimulants are increasingly being integrated into production systems with the goal of modifying physiological processes in plants to optimize productivity (Du Jardin, 2015).

Biostimulants have two modes of action, i.e., activating a plant immune response, commonly known as Systemic Induced Resistance (SIR) and acting as fertilizers, despite the fact that their constituents differ from typical N: P: K fertilizers (Thomson, 2004; and Percival, 2010) but may be involved in promoting beneficial physiological processes or mycorrhizal associations known to be involved in plant defense (Azcon-Aguilar *et al.,* 2002). Biostimulants may also be less susceptible to fungicidal insensitivity (Tronsmo, 1991) and because of their natural constituents are considered less toxic to the environment and humans. Previous research has shown applications of SIR products alone can result in resistance-induced yield increases of up to 36% (Burr *et al*. 1998), induced rooting and cut fungicide applications to nearly zero (Thompson, 2004). The findings of these research suggest that application of various biostimulants that is; biozyme 2.5 ml/L, foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L and hicure 2.5 ml/L either through spraying or drenching increased the yield and quality of marketable roses despite the presence of *A. tumefasciens*.

2. Material and methods

2.1. Green house trials

The experiment was conducted at James Finlay Kenya, Flowers division Tarakwet farm in Kericho county, located at 0° 27 south/35° 27 east and 2,100 meters above sea level. Average maximum and minimum temperatures range from 19-22 °C, respectively with a total annual rainfall ranging from 2,000 to 2,500 mm. The experiment was conducted in a commercial green house F36 variety '*Tropical amazone*' four years old that is susceptible to crown gall infection. The rose plants were already infected with crown gall disease and were grown hydroponically on pumice media with a pH range of 5.8-6.2.

2.2. Experimental design and treatment application

A modification of the method described by Biondi *et al*. (2009) was used where a green house with susceptible rose variety (*Tropical amazon*) infected with crown gall tumors was used for the experiment. Ten galls were tagged on each treatment replicate and the initial diameter of the galls measured using a Vernier calipers. The galls were then cut using a sterilized roll cut (roll cut was sterilized by dipping into spore kill solution - Didocyldimethyl ammonium chloride before cutting each gall). Treatments shown in Table 1 were drenched or sprayed at the dose rates shown after every two weeks in order to determine the suitable method of application. The treatments were replicated four times with replications arranged in a Randomized Complete Block Design (RCBD) giving a total of 44 plots. Each plot comprised 40 plants measuring 2×1 m^2 with guard rows of 1 m^2 between plots.

The number of fresh galls growing was counted and measured once a month using a Vernier calipers. Tumor diameter of fresh crown gall growths was also measured once a month. Mean tumor weight of the fresh galls was determined after 12 months. The crown gall tumors were cut and weighed using servo weighing balance after twelve months. Yield of marketable stems was counted daily from each treatment replicate for a period of twelve months.

Table 1: Various biostimulant treatments and their composition

Treatments and composition of various biostimulants	Composition / active ingredient
Control (water only - spray)	
Hicure 2.5 ml/L - drench	Inorganic nitrogen, amino acids and ashes
Hicure 2.5 ml/L - spray	
Biozyme 2.5 ml/L - spray	organic matter from plants, Zinc sulphate and sodium octoborate
Biozyme 2.5 ml/L - drench	
Foltron 2.0 ml/L - spray	macro and micro nutrients and Folycystein
Foltron 2.0 ml/L- drench	
Codamine radicular 2.0 ml/L - spray	Nitrogen Phosphorus, potassium and free amino acids
Codamine radicular 2.0 ml/L- drench	
Alexin 2.5 ml/L- spray	Calcium, magnesium, boron, potassium and salicylic acid
Alexin 2.5 ml/L- drench	

The quality of stems harvested was determined by sampling 30 stems once a week from each treatment replicate and the following parameters measured; stem length, weight and head length and width.

The experimental area was separated from the rest of the green house to minimize interference while spraying outside the trial area. Other pests and diseases were controlled using suitable crop protection pesticides when encountered and other agronomic operations such as weeding, removal of suckers were also done. Fertilizers comprising both macro and microelements were administered on a daily basis.

2.3. Pot trials

In order to monitor the crown gall growths closely, a pot experiment was conducted. A modification of the method described by Artur *et al*. (2012) was used for the experiment. Rose seedlings (two months old) grafted on natal briar root stock already infected with crown gall were used for the experiment. The seedlings were grown in plastic containers measuring (30 × 15 × 30 cm) filled with pumice as the growing media. The initial diameter of the galls measured as described in the field experiment and similar treatments used. Treatments comprised of three plants replicated four times with replications arranged in a Complete Randomized Design (CRD). Tumor diameter and weight were measured as described in field experiment.

3. Results

Results on the effect of various biostimulants; biozyme 2.5 ml/L, foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L and hicure 2.5 ml/L either through spraying or drenching are shown in Tables 2, 3, 4 and 5. Significant differences ($p \leq 0.05$) were observed on the yield of marketable roses on plots sprayed with biozyme 2.5 ml/L and control plots sprayed with water only. Plots sprayed with biozyme 2.5 ml/L had a higher yield of marketable roses compared to control plots treated with water only. The yield of roses increased by 14%. No significant differences at 5% level were observed in the yield of roses on plots treated with the various biostimulants; biozyme 2.5 ml/L hicure 2.5 ml/L, codamine radicular 2.0 ml/L, foltron 2.0 ml/L and alexin 2.5 ml/L either sprayed or drenched (Table 2).

No significant differences ($p \leq 0.05$) were observed in the number new galls, gall weight and gall diameter on plots treated with biozyme 2.5 ml/L, hicure 2.5 ml/L, codamine radicular 2.0 ml/L, foltron 2.0 ml/L and alexin 2.5 ml/L either sprayed or drenched and control plots sprayed with water only (Table 3).

Significant differences ($p \leq 0.05$) were also observed in the quality of rose stems produced. Plots treated with biozyme 2.5 ml/L hicure 2.5 ml/L, codamine radicular 2.0 ml/L, foltron 2.0 ml/L and alexin 2.5 ml/L either sprayed or drenched had better quality of roses that is, they were longer by at least 8 cm, heavier by 7 g with a

Table 2: Mean yield of roses and percentage increase on plots treated with hicure 2.5 ml/L, biozyme 2.5 ml/L, Foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml /L sprayed or drenched and control plots sprayed with water only (pooled data for one year)

Treatments	Mean yield	Percentage increase
Control (water only (spray))	441.8 b	0
Hicure 2.5 ml/L (drench)	475.8 ab	7.7
Hicure 2.5 ml/L (spray)	460.3 ab	4.0
Biozyme 2.5 ml/L (spray)	514.8 a	14.0
Biozyme 2.5 ml/L (drench)	456.5 ab	3.2
Foltron 2.0 ml/L (spray)	492.5 ab	10.3
Foltron 2.0 ml/L (Drench)	455.3 ab	2.6
Codamine radicular 2.0 ml/L (spray)	450.3 ab	2.0
Codamine radicular 2.0 ml/L(drench)	470.3 ab	6.5
Alexin 2.5 ml/L (spray)	496.8 ab	12.6
Alexin 2.5 ml/L (drench)	500.3 ab	13.4

Note: *Means within a column followed by the same letter are not significantly different by Turkey's test at 5% level.

Table 3: Mean fresh galls, gall diameter and gall weight on plots treated with hicure 2.5 ml/L, biozyme 2.5 ml/L, Foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L sprayed or drenched and control plots sprayed with water only (pooled data for one year)

Treatments	Mean new galls	Mean gall diameter (cm)	Mean gall weight (g)
Control (water only (spray))	246.0 a	4.2 a	23.4 a
Hicure 2.5 ml/L (drench)	179.8 a	3.8 a	23.0 a
Hicure 2.5 ml/L (spray)	171.3 a	5.1 a	26.1 a
Biozyme 2.5 ml/L (spray)	206.3 a	4.0 a	23.5 a
Biozyme 2.5 ml/L (drench)	245.8 a	4.1 a	28.5 a
Foltron 2.0 ml/L (spray)	166.3 a	3.4 a	26.6 a
Foltron 2.0 ml/L (drench)	170.5 a	4.1 a	25.2 a
Codamine radicular 2.0 ml/L (spray)	222.8 a	5.3 a	29.0 a
Codamine radicular 2.0 ml/L (drench)	216.0 a	3.9 a	25.3 a
Alexin 2.5 ml/L (spray)	219.8 a	3.0 a	27.2 a
Alexin 2.5 ml/L (drench)	223.8 a	3.4 a	26.1 a

Note: * Means within a column followed by the same letter are not significantly different by Turkey's test at 5% level.

bigger head size compared to control plots sprayed with water only. The rose head length and width both increased by 3 mm compared to the control. Results showed that various biostimulants applied either through spraying or drenching improved the quality of roses produced despite the presence of crown gall disease. No differences were observed in the methods of application, i.e., drenching or spraying (Table 4).

Table 4: Mean stem length, head length, head width and stem weight on plots treated with hicure 2.5 ml/L, biozyme 2.5 ml/L, Foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L sprayed or drenched and control plots sprayed with water only (pooled data for one year)

Treatments	Mean stem length (cm)	Mean head length (cm)	Mean head width (cm)	Stem weight (g)
Control (water only (spray))	50.0 b	4.0 a	2.3 a	21.4 b
Hicure 2.5 ml/L (drench)	58.2 a	4.4 a	2.7 a	28.5 a
Hicure 2.5 ml/L (spray)	63.0 b	4.3 a	2.8 a	28.3 a
Biozyme 2.5 ml/L (spray)	60.0 a	4.3 a	2.8 a	28.8 a
Biozyme 2.5 ml/L (drench)	57.1 a	4.3 a	2.6 a	28.7 a
Foltron 2.0 ml/L (spray)	57.4 a	4.3 a	2.6 a	28.5 a
Foltron 2.0 ml/L (Drench)	58.1 a	4.3 a	2.7 a	29.6 a
Codamine radicular 2.0 ml/L (spray)	57.5 a	4.2 a	2.8 a	28.8 a
Codamine radicular 2.0 ml/L(drench)	57.4 a	4.1 a	2.7 a	28.5 a
Alexin 2.5 ml/L (spray)	58.3 a	4.2 a	2.9 a	28.6 a
Alexin 2.5 ml/L (drench)	58.4 a	4.2 a	2.8 a	28.5 a

Note: * Means within a column followed by the same letter are not significantly different by Turkey's test at 5% level.

For the pot experiment, no Significant differences ($p \leq 0.05$) were observed in the number of new galls, gall diameter and gall weight on plots treated with hicure 2.5 ml/L, biozyme 2.5 ml/L, Foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L sprayed or drenched and control plots sprayed with water only (Table 5).

Table 5: Mean new alls gall diameter and gall weight on plots treated with hicure 2.5 ml/L, biozyme 2.5 ml/L, Foltron 2.0 ml/L, codamine radicular 2.0 ml/L, alexin 2.5 ml/L sprayed or drenched and control plots sprayed with water only (pooled data for one year pot experiment)

Treatments	Mean new galls	Mean gall diameter (cm)	Mean gall weight (g)
Control (water only (spray))	6.0 a	2.3 a	33.4 a
Hicure 2.5 ml/L (drench)	5.2 a	4.3 a	33.0 a
Hicure 2.5 ml/L (spray)	9.5 a	4.9 a	36.1 a
Biozyme 2.5 ml/L (spray)	6.8 a	3.1 a	33.5 a
Biozyme 2.5 ml/L (drench)	10.1 a	5.5 a	38.1 a
Foltron 2.0 ml/L (spray)	5.5 a	2.1 a	36.0 a
Foltron 2.0 ml/L (Drench)	10.3 a	4.4 a	32.2 a
Codamine radicular 2.0 ml/L (spray)	8.3 a	3.9 a	39.0 a

Table 5 (Cont.)

Treatments	Mean new galls	Mean gall diameter (cm)	Mean gall weight (g)
Codamine radicular 2.0 ml/L(drench)	8.0 a	3.4 a	35.3 a
Alexin 2.5 ml/L (spray)	8.0 a	3.9 a	37.2 a
Alexin 2.5 ml/L (drench)	7.8 a	2.3 a	33.1 a

Note: * Means within a column followed by the same letter are not significantly different by Turkey's test at 5% level.

4. Discussion

The various biostimulants tested that is biozyme 2.5 ml/L, hicure 2.5 ml/L, codamine radicular 2.0 ml/L, foltron 2.0 ml/L and alexin 2.5 ml/L either sprayed or drenched improved the yield and quality of roses produced despite the presence of *A. tumefasciens.* This might be attributed to the fact that biostimulants improved the nutrient use efficiency of the plant and enhanced tolerance to biotic and abiotic stresses (Du Jardin, 2012). Similar results have been observed in leafy vegetables where biostimulants increased leaf pigments (chlorophyll and carotenoids) and plant growth by stimulating root growth and enhancing the antioxidant potential of plants. In floriculture, biostimulants used in bedding plant production stimulated the growth of plants, which reached the blooming and commercial stages earlier, thus optimizing space in the greenhouse (Rouphael, 2015). Application of biozyme also improved plant growth, fruit set, yield and physiochemical properties of guava (Sayan *et al.,* 2015). Increased total sugar percentage with the application of biozyme in pear was reported by Inomata *et al.,* (1992), Gyul - Aakhmedov and Gasonova (1992) also found similar results in apple with the application of micronutrients. Similarly, the use of biostimulants have been reported in production of ornamental and other horticultural crops such as *Antirrhinum majus* (Nahed *et al*., 2009), pepper (Paradikvoic *et al.,* 2012) and *Eustoma grandiflorum* (Mondal *et al.,* 2015). A combination of bioslurry and hicure was also found to improve the stem length and flower head size of carnations (Niyokuri *et al*., 2017). Biostimulants have two modes of action i.e. activating a plant immune response, commonly known as SIR and acting as fertilizers, despite the fact that their constituents differ from typical N: P: K fertilizers (Thomson, 2004; and Percival, 2010) but may be involved in promoting beneficial physiological processes or mycorrhizal associations known to be involved in plant defense (Azcon-Aguilar *et al.,* 2002).

5. Conclusion

The findings of this study suggest that biostimulants boosted the immune response of roses to *A. tumefasciens*, however more research should be done to confirm this.

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