Biorational Preservation of Rose (*Rosa hybrida* L.) Cut-Flower Using Stevia (*Stevia rebaudiana* B.) and Thyme (*Thymus vulgaris* L.) Extracts

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Abstract. Rose cut flower is one of the widely grown cut flowers in Kenya. However, most roses have a challenge of short vase life. This study aimed at determining the efficacy of plant extracts from thyme and stevia in preservation of rose cut-flowers. Two rose cut-flower cultivars; ‘radiance and ‘high & sparkling’ were subjected to stevia and thyme extracts each at three levels (0.2, 0.4, and 0.6gL⁻¹). Thyme extracts at a concentration of 0.2 gL⁻¹ significantly (p≤001) extended the vase life of rose cut flower by 3.5 days and floral absorption rates by 10.4% compared to the commercial preservative (chrysal) at the same concentration rates. Application of higher doses (0.4gL⁻¹ and 0.6gL⁻¹) of plant extracts led to shorter vase life (6 days) of rose cut flower and maximum bent neck records at day 8. The response of rose cut flower to the treatments did not vary between cultivars. The results from this study indicate that thyme extracts offer an attractive alternative to the use of chemical floral preservatives for prolonging the vase life and enhancing quality of rose cut flower. The efficacy of extracts is however depended on the concentration level with 0.2gL⁻¹ dosage recording the best results.

Introduction

Postharvest life is a major concern in rose cut flowers especially due to their ethylene production property. Chemical floral preservatives mainly containing sugars and germicides are used to improve postharvest life of rose flowers. However, most of the chemical floral preservatives contain either aluminium sulphate, silver nitrate, silver thiosulphate, calcium chloride which have negative effects on the environment [1]. This study used extracts from stevia and thyme to preserve rose cut flowers. *Stevia rebaudiana* is an herbaceous plant grown for its leaves. The plant has high levels of sugar relating to stevioside and rebaudioside concentrations [2]. The extracts from stevia have been tested for their ability to preserve cut flowers especially in improving fresh weight and extending diameter [3]; in preventing chlorophyll degradation during postharvest handling [4] and in preventing bent neck development [5]. There is however, limited research on applicability of stevia extracts as flower food. There is a need therefore to explore its potential since stevia extracts have been reported to contain natural stevioside [2] which could substitute chemical preservatives.

Thyme herb (*Thymus vulgaris*) is an evergreen herb with culinary, medicinal and ornamental value. Essential oil from thyme contains thymol and carvacrol which exhibit anti-microbial properties [6-9]; hence improving relative fresh weight and solution uptake of cut flower. Thyme monoterpenes have been found to inhibit ethylene production [10] hence improve floral quality [3] and vase life. Whereas many studies have been done on potential use of thyme extracts in preservation of cut flowers, differing results have been reported on the best application rate of thyme extracts for improving floral quality. Furthermore, most of the studies on thyme extracts have been done on carnations and gerbera flowers and very few studies have focused on roses especially radiance and high and sparkling cultivars. This study therefore aimed at determining the best application rates of thyme and stevia extracts and their efficacy on improving the postharvest life and quality of two rose cultivars.

Materials and Methods

Production of Stevia and Thyme Plants

A field experiment was established at Karatina University agricultural farm. The region lies at an altitude of 1980m above sea level with annual rainfall of 1200mm. The soil is mainly red volcanic soil. The average temperature ranges from 15⁰C -18⁰C. The field experiments were set up following a randomized complete block design. Beds measuring 1.35m width on top and 1.55 m at the base and 25m length were prepared. Each bed had 4 rows at a spacing of 35cm. Plant to plant spacing was 16 cm. A total of 20 beds were prepared. The plants were planted and all agronomic practices carried out.

Harvesting of Stevia and Thyme Leaves

Initial harvesting was done at four months after transplanting. Leaves were harvested by plucking. The leaves were harvested during the vegetative stage prior to flowering and the plants were trimmed completely leaving 4 inches from the ground. New plants were harvested again one month after a new flush of leaves sprouted.

Postharvest handling and storage of stevia and thyme Leaves

After harvesting, the leaves were shade dried under a net to prevent volatilization of plant chemical compounds due to strong sun light effect. The dried leaves were then crushed using a grinding machine to form powder. The powder obtained was stored in polythene bags at room temperature.

Preparation of stevia and thyme Extracts

Shade dried stevia powder was mixed with 0.5L of 100% pure acetone and 0.5L pure water. A mechanical shaker was used to shake the mixture for seventy two hours. The mixture was then sieved using filter paper and then transferred to an oven at 70°C temperature for seventy two hours to obtain uniform powders [11]. Thyme extracts was prepared by mixing dried leaves with 1L of 85% ethanol and shaken for seventy two hours. The resulting mixture was then sieved with a filter paper. The extract was transferred into a vacuum distillation unit at 80°C temperature. The solvent was evaporated and condensed. The extract was concentrated in the oven at 70 °C for seventy two hours [11].

Phytochemical analysis of thyme and stevia extracts

Dried parts of stevia and thyme plants (about 100g) were cut into small pieces and subjected to hydrodistillation (HD) for 3hrs using a Clevenger type apparatus. Oils obtained from the dried plant parts were dried using anhydrous sodium sulphate. The volatile compounds isolated by hydrodistillation were analysed by GC/MS, using an Agilent Technologies 6890N GC. The fused HP-5MS capillary column was coupled to an Agilent Technologies 5973B MS (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 250°C while that of the detector was 280°C (split ratio: 1/100). Helium gas (99.995% purity) was used as a carrier gas at a flow rate of 1.2 ml/min. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 to 550 m/z. The percent phytochemical composition of thyme and stevia are shown in Table 1 and 2.

Table 1. Percent (%) phytochemical composition of thyme extracts

Components	Concentration (%)
Thymol	46.21
Carvacrol	2.44

Table 2. Percent (%) phytochemical composition of stevia extracts

Compound	Concentration (%)
Stevioside	10
Rebaudioside	2
Pulcoside	0.7

Production and management of rose cut-flower cultivars

Two rose flower cultivars (radiance and high and sparkling) were hydroponically raised in a greenhouse at Zena roses, Thika. Temperature and relative humidity were controlled at 17-18⁰c and 65-80% respectively. The flowers were fertigated twice per week. Pest and disease control was done based on daily scouting reports. The flowers (1st bloom) were harvested at bud stage 12 weeks after planting by cutting slightly above the second five leaflet leaf using a secateur.

Post-harvest Handling of Rose Cut Flowers

The flowers were immediately placed in buckets half filled with water [12] after harvesting, then transported to a pack house. Flowers were pre-cooled for two hours to remove field heat in a pack house, graded in a grading room and then trimmed to a length of 35cm using a sterilized scalpel and immediately taken to a post-harvest laboratory.

Determination of the efficacy of thyme and stevia extracts on vase-life and quality of rose cut-flower

Experimental design

The experiment followed a randomized complete block design in a factorial arrangement with three replications. The two rose cultivars were the main treatments while the various levels of thyme and stevia extracts formed the sub-treatments.

Preparation of stock solutions

Thyme and stevia extracts were prepared separately by adding one gram of dried plant material to five litres of sterile distilled water to make a 5litre stock solution of 0.2g/L⁻¹ concentration. Higher concentrations of stevia and thyme (0.4 and 0.6g/L) were prepared by adding 2grams and 3grams respectively to 5 litres of sterile distilled water.

The cut flowers were cut to a length of 35cm. The one litre volume vases were cleaned and the prepared solutions added. A total of thirty six bunches of 6 rose stems each were separately placed in one litre of each stock solution with the respective amount of each of the three treatments each at three concentration levels (0.2, 0.4 and 0.6g/L⁻¹).

Floral arrangement

Six rose flower stems were placed in each flower vase (three stems for each cultivar). The cultivars were placed in one vase since most of rose flowers are sold in mixed bouquets. The vases were sealed in the neck using water proof polythene seal to prevent evaporation of vase solution. Observations were done on daily basis. The temperature of the testing room was maintained at 22⁰c with 12 hours of photoperiod.

Data collection

Data was collected on the following parameters:

Vase Life (day(s)): This is a parameter used to show post-harvest longevity of cut flower [13]. It is defined as the post-harvest period a cut flower retains its aesthetic value until the end of consumer utility [14]. Severe petal discolouration, tips blackening, petal browning and bent neck of flowers was deemed as the end of cut flower life.

Solution absorption rate (%): This was assessed based on the formula described below.

$$\text{Solution absorption rate (\%)} = \frac{\text{Initial solution level} - \text{Final solution level}}{\text{Initial solution level}} * 100$$

Physical Quality; Bent Neck

The physical quality of harvested flowers is among the major factors in the market value of cut flowers. In this study, flower physical qualities were determined using a modified physical quality and vase life termination rating scale as described by [15] & [16; 0: bending 0 - 15°, 1: bending between 16°- 25°, 2: bending between 26°- 65°, 3: bending between 66°- 90°, 4: bending more than 90°.

Statistical Analysis

All collected data was subjected to analysis of variance (ANOVA) using GenStat 12.1 version. Means were separated using the Least Significant Difference at 99% level of confidence.

Results and Discussion

Effects of Stevia and Thyme Extracts on vase life

The results indicated that the efficacy of stevia and thyme extracts on the vase-life and quality of the selected rose cut flower cultivars (high and sparkling and Radiance cultivars) was depended ($p=0.001$) on the concentration level of the extract but independent ($p=0.080$) of the cultivar used.

Treatment of radiance cultivar with stevia and thyme extracts enhanced vase life by 0.6% and 16% respectively compared to the control. The vase life of high and sparkling cultivar was enhanced by 11.1% and 3.2% when treated with thyme and stevia extracts respectively Treatment of compared to the non-treated control. The highest vase life extension was observed on cut flower treated with the commercial floral preservative (Chrysal) enhancing the vaselife of radiance and high and sparkling cultivars by 18.3% and 17.1% respectively compared to the control.

Table 3. Effect of thyme extracts, stevia extracts and chrysal on vase of radiance & high and sparkling rose cultivars

Treatment	Cultivar	Mean vase life (days)
Control	Radiance	8.033a
Control	High & sparkling	8.147a
Stevia extracts	Radiance	8.083a
Stevia extracts	High & sparkling	8.417a
Thyme extracts	High & sparkling	9.167b
Thyme extracts	Radiance	9.583bc
Chrysal	Radiance	9.833c
Chrysal	High & sparkling	9.833c

Means followed by the same letter are not significantly different at 1% LSD

Plant extract type and treatment level significantly ($p \leq 0.001$) affected the vaselife of rose cut-flower cultivars. Thyme extracts, chrysal and stevia extracts at applied to rose cut flower at the rate of 0.2g L^{-1} enhanced vase life by 44.1%, 30.7% and 8.4% respectively compared to the control.

Thyme extracts, stevia extracts and chrysal applied at c had varying effects on extending the vase life of rose cut flower compared to the control. The highest vase life enhancement (16.3%) was observed on chrysal treated flowers followed by stevia (0.9%). Thyme at 0.4gL^{-1} had no effect on vase life enhancement (0 % extension) compared to the control. Thyme and stevia extracts applied at 0.6gL^{-1} decreased the vase life of rose cut flowers by -15.6% and -1.1% compared to the control while treatment with chrysal at 0.6gL^{-1} increased vase life of rose cutflower by 19.1% compared to control. (Fig. 1).

Figure 1. Effect of chrysal, stevia extracts and thyme extracts application dosages on vase life of radiance and high and sparkling rose cultivar. Bars followed by the same letter are not significantly different at $\text{LSD}=0.01$

This indicates that the efficacy of plant extracts (thyme and stevia) decreases with increasing concentration level. Thyme extracts at 0.2gL^{-1} concentration were most efficacious extending vase life of rose cut-flower by 2.833 and 5 days compared to chrysal at 0.2gL^{-1} concentration and stevia at 0.2gL^{-1} concentration respectively. This result agrees with [17] who found that application of 200ppm of thyme essential oil significantly lengthened vase life in carnation, 'sensi cultivar' by 2 days. Similar findings were obtained by [4] indicating that flower senescence of cut roses 'dolce vita' decreased when treated with thyme extracts at 0.2ppm along with pulse treatment of sucrose and calcium chloride. The result is also in agreement with [6] who proved that 100mgL^{-1} thymol and 50mgL^{-1} carvacrol compared to the control was most effective in prolonging the vase life of cut gerbera. This result however differs with [18] who reported that thyme extracts at 900mgL^{-1} concentration was most effective in prolonging the vase life of *lilium santander* cut flower as compared to control. The reduced vase life as concentration of thyme extracts increased could be attributed to scorching of vascular tissues of rose cut flower stems dipped in the vase solution as was observed on the fourth day (Plate 1). This appeared to have rendered the cut flower stems damaged at those particular sections.

Plate 1. Scorching effects of thyme extract at 0.6 gL^{-1} on vascular tissue of rose cut flower

The low efficacy levels observed with increasing concentration levels of thyme, stevia and chrysal treatments could be attributed to increased sugar levels which may have led to increased microbial activity, vascular blockage and phytotoxicity which were not measured in this study. These factors have however been reported as possible causes of reduced vase life. For example, vascular blockage of cut flower stems occurred on the sixth day [6] in stevia treated cut gerbera.

Effects of Stevia and Thyme Extracts on Absorption Rate

Thyme extracts at 0.2 gL^{-1} concentration had the highest mean absorption rate (99%) (Fig. 2). This may be attributed to the anti-microbial properties exhibited by thymol and carvacrol in thyme extracts [8] [7] which prevented vascular blockage [6] of the cut flower stems hence continued solution uptake. These two compounds (thymol and carvacrol) were found to be present in thyme extracts initial sample characterization at 46.21% and 2.44% concentrations respectively. The result concurs with [6] who reported that 100 mgL^{-1} carvacrol essential oil improved the relative fresh weight and vase solution absorption rate of gerbera flower.

Chrysal at 0.2 gL^{-1} concentration had mean absorption rate of 88.6%. Chrysal has a strong germicide; aluminium sulphate that helps to prevent microbial infection of flower stems [19] ensuring continued vase solution uptake. [20] investigated the effect of aluminium sulfate at 50, 100 and 150 mgL^{-1} concentrations on vase solution uptake of cut *Eustoma grandiflora* and found that 150 mgL^{-1} concentration of aluminium sulfate enhanced water uptake and fresh weight.

Thyme extracts at 0.6 gL^{-1} concentration exhibited the lowest mean absorption rate of 5.7%. This was attributed to the phytotoxic effect of flower part immersed in the solution on the fourth day after treatment. Initial characterization of thyme plants in our study showed high content of polyphenols (51.35%) which could be attributed to the low efficacy of thyme extracts at higher concentrations.

Stevia extracts at 0.2 gL^{-1} did not differ significantly ($p \leq 0.01$) with control and the rest of treatment concentrations in as far as absorption rate was concerned. It was found that the high sugar content present in stevia extracts [2] accelerated microbial growth leading to vascular blockage [6] of the flower stems hence low solution uptake.

Figure 2. Effect of chrysal, stevia extracts and thyme extracts application dosages on vase solution absorption rate in rose flower

Effect of Treatments on bent neck of Rose Flowers

The effect of applied treatments significantly varied ($p \leq 0.01$) with type and concentration level (Table 4). Non-treatment (control) rose flower and treatment with thyme extracts at 0.4gL^{-1} and 0.6gL^{-1} recorded maximum bent neck score (4) at day 8 while treatment with stevia extracts (0.2gL^{-1} , 0.4gL^{-1} , 0.6gL^{-1}) and chrysal at 0.4gL^{-1} and 0.6gL^{-1} recorded maximum bent neck on the 10th day. Rose flowers treated with chrysal at 0.2gL^{-1} showed the maximum bent neck of 4 on the day 12. The vase life of the rose flowers was terminated at the maximum bent neck level. Apart from the control, stevia extracts 0.2gL^{-1} concentration exhibited the highest level of bent neck compared to thyme and chrysal at the same concentration. The high level of sugar component (10% in our stevia extract) and in 0.4gL^{-1} and 0.6gL^{-1} thyme and chrysal application levels may explain the early (at day 6) and maximum rose cut flower bent neck recorded on day 8. High sugar concentrations in vase solutions has been reported to cause vascular blockage and flower infection and the cut end in gerbera cut flower [2]; [6].

The best treatment in preventing bent neck was observed on rose flower treated with thyme extracts at 0.2gL^{-1} concentration. This treatment showed little or no signs of bent neck up to the twelfth day (12) of the vase life (Plate 2). The result may be attributed to the high levels of thymol (46.21%) and carvacrol (2.44%) oils observed in thyme plant extracts. The oils have been reported to exhibit antimicrobial properties which prevent vascular embolism and preserve the integrity of cut-flower. [8] reported anti-microbial property of thyme extracts as having deterred infection of the cut flower stems hence ensuring continued solution uptake and hence cell turgidity is maintained. The result conforms to that of [17] who found that bent neck in thyme extracts treatment at a concentration of 0.1 mgL^{-1} remained low up to day 12 in cut gerbera in comparison with the control. [5], comparing stevia and thyme extracts reported lower stem bending in gerbera cut flower treated with thyme extracts.

Plate 2. Effects of thyme, stevia and chrysal treatments on rose cut flower bent neck prevention on day 12. a) 0.2g/l thyme extracts b) 0.2g/l stevia extracts c) 0.2g/l stevia extracts

Conclusion

This study showed that natural plant extracts can be used to enhance rose flower vase life and floral quality. In particular, thyme extracts offer the best alternative to commercial chemical floral preservatives in extending the vase life and enhancing the quality of rose flower. The efficacy of the plant extracts however is not depended on the rose flower cultivar. The two cultivars (radiance and high and sparkling cultivars) used in our study had statistically the same response to the application of stevia, thyme extract treatments. This study also established that the efficacy of plant extracts is depended on the phytochemical composition, concentration level of the phytochemicals and the extract dosage applied. Application of low dosages (low concentration levels) of extract had best results in extending vase life and enhancing quality of rose cut flower as evidenced with the thyme extracts at 0.2gL^{-1} concentration level. Application of higher dosages could have undesirable effects on rose flower life and qualities as was observed with the application of 0.4gL^{-1} and 0.6gL^{-1} dosages which led to shorter vase life (6 days) and maximum bent neck records at day 8. Therefore this study unraveled the efficacy of extracts from stevia and thyme plants grown in Kenya on the rose flower vase life and quality and the results highlight the potential of the plants in offering environmentally sound alternatives to the use of chemical preservatives in cut flower preservation. Further studies should be undertaken to establish the economic viability in the use plant extracts as alternatives to chemical floral preservatives.

Conflict of Interest

The authors declare no conflict of interest.

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