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Bacterial Population in Holding Solutions Used for Postharvest Handling of Cut Roses and Gerbera at Retail Sites

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ABSTRACT

Bacteria proliferating in cut flower holding solutions at retail sites can block the xylem vessels. This could shorten the vase life of cut flowers at consumer sites. No study has been conducted in Sri Lanka to assess the level of bacterial contamination in holding solutions used for postharvest handling. Therefore, this study was conducted to examine the bacterial counts in holding solutions used for cut *Rosa hybrid* and *Gerbera jamesonii*, in a random sample of ten retail sellers from Western and North Western Provinces of Sri Lanka. Samples were drawn aseptically, serially diluted with distilled water and cultured on Nutrient Agar (plate count technique). Bacterial counts were expressed as colony-forming units per milliliter (cfu ml^{-1}). These counts were considered in relation to frequency of changing the keeping solution and the use of chemicals in water. Results revealed that the number of bacteria at the retail level was within the range from 10^3-10^5cfu ml^{-1}.

KEYWORDS: Bacterial population, Cut Gerbera, Cut Rose, Holding solutions, Postharvest

INTRODUCTION

Floriculture is now recognized as a profitable industry in Sri Lanka. Because of its potential to generate higher income per unit area. Currently, Sri Lanka earns 28 % of annual export income by exporting cut flowers to Japan, Netherlands, Canada and the United Kingdom (Hiriyadeniya *et al.*, 2011). Cut flower production for the local market is carried out in the up country region of Sri Lanka . Postharvest problems are clearly seen at the florists' level at hot humid destinations in the island such as Colombo, Kandy, Negombo, Chilaw and Kurunegale (Weeraratne *et al.*, 2012).

As flowers are viable and actively metabolizing plant parts, they are subjected to ageing processes (Hiriyadeniya *et al.*, 2011). A major cause of early deterioration in cut flowers is the blockage of xylem vessels by microorganisms which accumulate in holding solution from the vessel or vessel conductors (De Silva *et al.*, 2013). Cut flowers are sensitive to microbial contamination at the stem base or in the vase solution. Consequently, will shorten their vase life. When the vessels of stems are blocked, water loss via transpiration cannot be replaced by water uptake, thus, causing a negative water balance in cut stems. A negative correlation has been reported between the number of bacteria in solution of stem and water conductivity in the stem of cut flower (Kazemi and Ameri, 2012). Hydraulic conductance measured in 5 cm stem segments of cut rose was reduced whenever the number of bacteria in the segment exceeded 10^6 to 10^7 cfu g^{-1} fresh weight (Hoogerwerf and van Doorn, 1992).

Bacteria in vase water can block vessels on the cut surface and some bacteria from vase water produced ethylene (Kazemi and Ameri, 2012). Further, more some bacteria produce pectic enzymes which can digest cell walls (De Witte and Van Doorn, 1988).

High bacterial counts in the stems often occur when flowers are kept in water by consumers. However, it could also occur at earlier stages of cut flower handling, because most flower species are kept in water by the growers, wholesalers and retailers (Hoogerwerf and Van Doorn, 1992). Flowers may, therefore, develop resistance to water uptake prior to their vase life at the consumers.

As the day temperature in the Up country ranges from 12-20 ^{0}C and the relative humidity from 75-95 %, harvested flowers could be kept without special postharvest treatments until they are loaded for transportation. As a result, cut flower producers rarely practice correct postharvest techniques during handling and transport which leads to postharvest losses (Weeraratne *et al.*, 2012). Studies done overseas have shown that the bacterial concentration found in vase water at consumer sites was about 10^7 - 10^8 cfu ml^{-1} a few days after placing the stems in water (De Witte and Van Doorn, 1988; Hoogerwerf and Van Doorn, 1992). A study conducted in The Netherlands, involving keeping solutions used for eight flower species, revealed that the number of bacteria, especially at the retail level, was often high enough to reduce flower longevity. In water samples from flower shops the bacterial count was higher. No clear differences were found between the distribution of bacterial counts in the samples

from the various temperate countries (West Germany, France, UK, Italy, Holland, the United States and Sweden).

Bacterial counts of 10^5 and 10^6 cfu ml^{-1} were most frequently found for gerbera at retail sites in temperate countries (Hoogerwerf and van Doorn, 1992).

To date, no study has been conducted in Sri Lanka to measure the level of bacterial contamination in holding solutions used for cut flowers at the retail sites. Therefore, this study was carried out to identify the level of bacterial proliferation in cut rose and gerbera holding solutions prior to purchase by the consumer. We hypothesized that the number of bacteria found before the consumer level will be below 10^7 cfu ml^{-1} because of the relatively rapid transportation of the flowers from one site to the next in the handling chain. To investigate this hypothesis, the numbers of bacteria in holding solutions were measured, in relation to frequency of water renewal, and the use of chemicals in the water.

METHODOLOGY

The study was conducted at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura (NWP), during the period from January to April, 2013.

Method of Sampling

A sample of ten retail dealers was randomly selected from the Western and North Western provinces of Sri Lanka. The consent of the management was obtained beforehand. However, the random collection of holding solution samples was done without prior notice.

The post harvest handling practices of two cut flower species *Rosa hybrida* and *Gerbera jamesonii* were observed at the retail sites. Holding solution samples were collected on the very first day of flowers at retail shops and on third day from the same buckets. Samples were transported and stored under cool conditions, (≤ 5 ^{0}C) for less than a day until culturing.

Determination of Bacterial Numbers

Sample of 5 ml from holding solution of cut rose and gerbera were collected from the retail sites into sterile containers using sterile disposable pipette tips and a pipette after stirring the water. Under aseptic conditions, water samples were serially diluted up to the dilution factor 10^{-5} with sterile distilled water, and 0.1 ml was drawn in triplicate, spread onto Nutrient Agar (NA) plates with a sterile glass rod. Plates were incubated at 30 ^{0}C for 48 hr, after which bacterial growth was enumerated as cfu ml^{-1}.

The number of days the water was in use for holding the flowers, and the chemical products included in the water was noted.

Bacterial Identification

Bacterial colonies grown on NA at 30 ^{0}C for 48 hr were selected and subjected to Gram Staining Test. The colonies were also distinguished under light microscope on the basis of their morphological characteristics.

Statistical Analysis

The experiment was arranged as a Nested Design. The data on bacterial counts were analyzed by Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

According to the observed bacterial counts in keeping solutions. Retail dealers could be categorized in to groups.

The grouping was based on Hoogerwerf and Van Doorn, 1992. The mean bacterial counts are given in Table 1 and Table 2 presents the categories of retailers based on holding solution bacterial populations of low ($\leq 10^4$ cfu ml^{-1}) and intermediate (10^4-10^6 cfu ml^{-1}), and high ($\geq 10^6$ cfu ml^{-1}).

Water was used as the holding solution without an antibacterial compound therefore, bacterial count depended on the frequency of water change and was high ($\geq 10^6$ cfu ml^{-1}) in water that had not been changed within one day. There were no retail shops having high number of bacteria in holding solutions of cut rose and gerbera on day 1 and day 3. If the holding solution is tap water with chlorine or with an antibacterial compound, 10^4 cfu ml^{-1} is a lower bacterial count (Hoogerwerf and van Doorn, 1992). However, number of retailers having high number bacterial count (10^4-10^6 cfu ml^{-1}) was higher on day 3.

This investigation revealed that the numbers of bacteria in the water used for postharvest handling of cut rose and cut gerbera were generally low and intermediate.

Table 1. Number of retailers with a low, intermediate, or high number of bacteria in holding solutions of cut rose and gerbera on day 1

Flower species	Level of bacterial population (n = 10)			
	Low ($\leq 10^4$ cfu ml^{-1})	Intermediate (10^4-10^6 cfu ml^{-1})	High ($\geq 10^6$ cfu ml^{-1})	Total number of measurements
Gerbera	4	6	0	10
Rose	5	5	0	10

Table 2. Number of retailers with a low, intermediate, or high number of bacteria in holding solutions of cut rose and gerbera on day 3

Flower species	Level of bacterial population (n = 10)			
	Low ($\leq 10^4$ cfu ml^{-1})	Intermediate (10^4-10^6 cfu ml^{-1})	High ($\geq 10^6$ cfu ml^{-1})	Total number of measurements
Gerbera	2	8	0	10
Rose	2	8	0	10

It indicates that if an attempt is made to reduce the number of bacteria in the holding solutions that could be achieved effectively with the retailers belong to the group with less than 10^4 cfu ml^{-1}.

Recutting of the stem end of flower stems by 2-3 cm and changing water in the holding buckets / vessels were observed as a daily practice by retailers. This could be a reason for the relatively low bacterial populations observed in holding solutions. Accordingly, all the retailers had holding solutions with relatively low or intermediate bacterial counts (Tables 1 and 2). When compared to previous studies in temperate countries (Hoogerwerf and Van Doorn, 1992) the values on bacterial population were lowered in holding solutions used in Western and Northwestern provinces in Sri Lanka.

Hoogerwerf and Van Doorn (1992) reported that the number of bacteria in water sampled at retailers was very high, both in specialized flower shops and supermarkets. It was because the stems were not re cut and the water in holding solutions remained unchanged for more than a day. In the buckets used in the streets or at open markets the numbers of bacteria were lower than in retail shops or supermarkets. Because of daily water changing and re cutting were routinely practiced.

Low numbers of bacteria were found in holding solutions when chlorine compounds were used. These compounds effectively controlled the number of bacteria in laboratory studies and in practices (Hoogerwerf and Van Doorn, 1992).

In Western and North western Provinces of Sri Lanka most of retailers used tap water as their holding solution. Chlorine compounds used for purification of tap water may have an effect to prevent high bacterial count at retail sites.

During this study, quality parameters of the water used for holding solutions were not measured. However, none of the studied retail sites used antimicrobial compounds in postharvest handing solutions of roses and gerbera. Most of the retailers stored roses under low temperature (4-5 ^{0}C) in bottle coolers while gerbera remained in room temperature or in air conditioned show rooms.

In Sri Lanka, florists are applying only few postharvest practices at retail premises i.e: recutting of stem end and changing of water daily in holding solutions. This water contained relatively low numbers of bacteria even though it generally did not contain an antibacterial compound.

The differences in holding solution bacterial counts among ten retailers were statistically significant ($P < 0.005$) (Figure 1). It could be due to because different practices adopted during handling chains. Few of retailers had their own cultivations that produced high quality flowers, which were transported under cool conditions to the retail premises. Most of retailers purchased flowers from wholesalers who transported by train to the retail sites. Few retailers used cool rooms to store cut flowers in room temperature (especially cut gerbera). The difference between two flower species (rose and gerbera) were statistically significant ($P < 0.005$) both for Day 1 and Day 3.

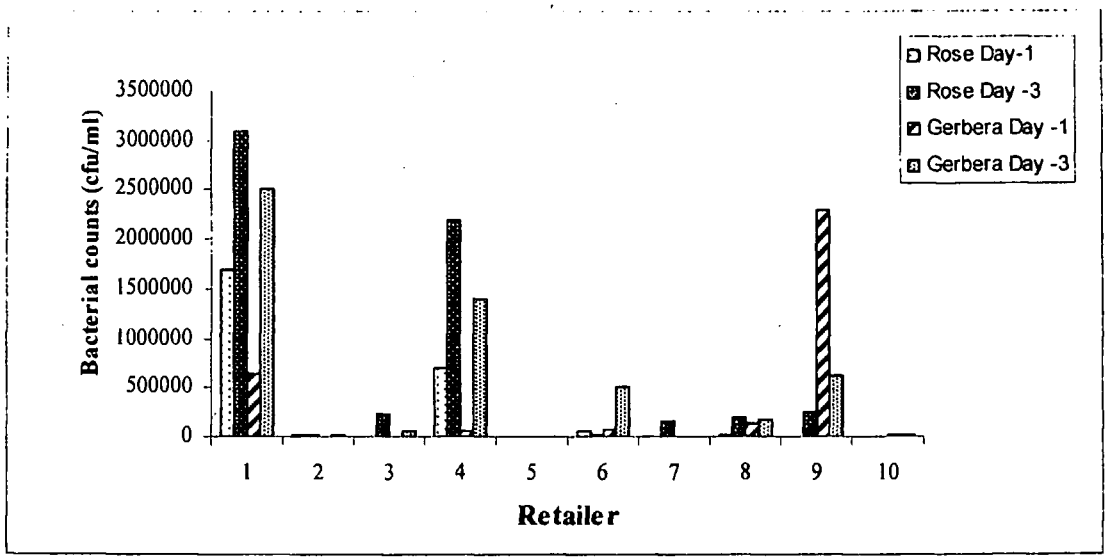

Figure 1. Average bacterial counts (cfu ml^{-1}) of cut rose and gerbera with different retailers and the days in solution

Woody stems of cut roses were more prone to bacterial plugging than fleshy stems of cut gerbera by third day in holding solution (Figure 2). Day 3 was not mentioned on the graph, because in most places, flowers were sold within 3-5 days. According to Hoogerwerf and Van Doorn (1992), bacterial counts of 10^5 and 10^6 cfu ml^{-1}that were most frequently found for gerbera at retail sites in the current study are similar to temperate countries .These numbers sufficient enough to reduce flower longevity. According to bacterial population enumerated from the vase water of cut rose, found no reduction of water uptake at 10^5 cfu ml^{-1} and a strong reduction at 10^7 cfu $ml^{-1}$with all the bacterial strains (Hoogerwerf and Van Doorn, 1992).

Average bacterial population found in rose holding solutions were 2.5×10^5 cfu ml^{-1} and 6.2×10^5 cfu ml^{-1} in day 1 and day 3 respectively. The total bacterial population found in keeping solutions of retail shops was not a sufficient amount to reduce flower longevity at retail sites under current postharvest practices. However, bacterial population found in gerbera holding solutions were 3.2×10^5 cfu ml^{-1} and 6.2×10^5 cfu ml^{-1} in day 1 and day 3 respectively.

Bacterial Identification

The bacterial colonies different in their morphological characteristics. The most frequently isolated were of 3 types: Type A, white, coccus and Gram positive. Type B orange, bacillus, and Gram negative, Type C, pink, bacillus and Gram positive.

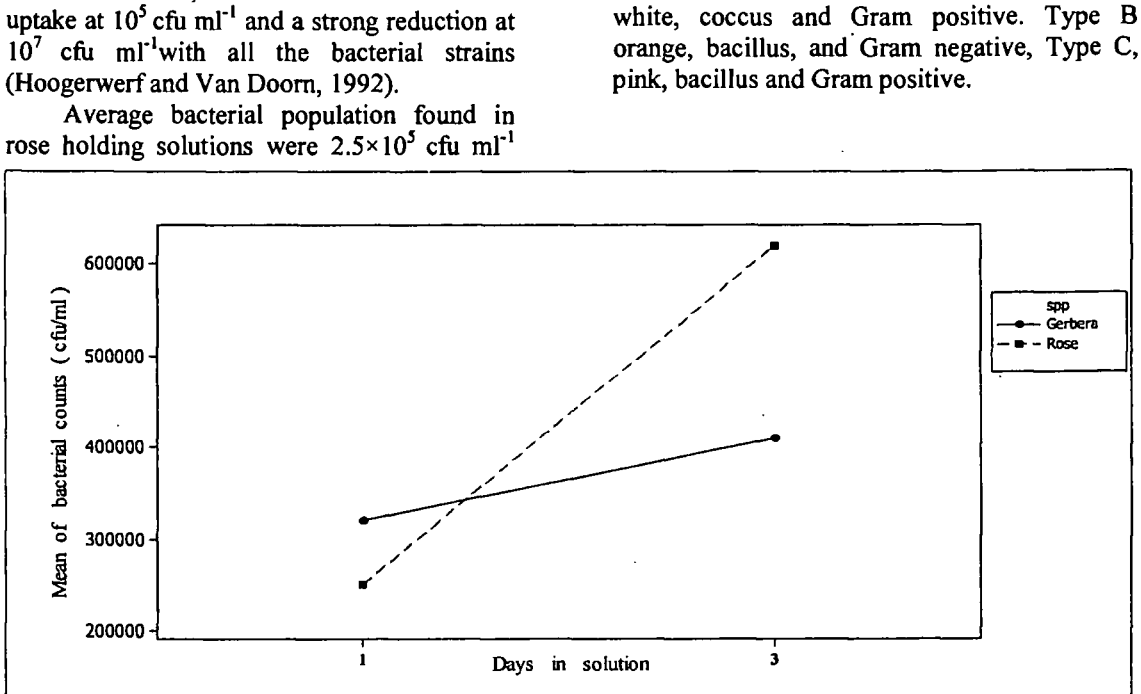

Figure 2. Relationship of the bacterial counts cfu ml^{-1}(values given are the means of 10 sellers) and days in solution for cut rose and cut gerbera

CONCLUSIONS

The level of bacterial population found in holding solutions at retail sites prior to purchase by consumer was within the range from 10^3-10^5 cfu ml^{-1} ($\leq 10^7$ cfu ml^{-1}). Whenever water was used without an antibacterial compound, the number of bacteria depended on the frequency of water renewal and was invariably high (more than 10^6 cfu ml^{-1}) in water that had not been renewed within one day. Future research will focus on ascertaining the effect of holding solution bacterial population on longevity of cut flowers at consumer sites.

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REFERENCES

De Silva, V.M.R., Santos, C.M.G., De Souza, A .V., De Alencar, P.R. and De Silva, L.F., (2013). Physiological blockage in plants in response to postharvest stress, *African Journal of Biotechnology*, **12** (11), 1168-1170.

De Witte, Y., and Van Doorn, W.G., (1988). Identification of bacteria in the vase water of roses and the effect of the isolated strains on water uptake, *Scientia Horticulturae*, **35**, 285-291.

Hiriyadeniya, H.D.P., Somarathna, T., and Wellala, C.K.D., (2011). The impact of low cost treatments in extending the vase life of cut Anthurium and Orchid, Available from: http://digital.lib.ou.ac.lk/docs/handle/701300122/373?mode=ful . (Accessed 27 January 2013).

Hoogerwerf, A. and Van Doorn, W.G., (1992). Number of bacteria in aqueous solutions used for postharvest handling of cut flowers, *Postharvest Biology and Technology*, **1**, 295-304.

Kazemi, M. and Ameri, A., (2012). Postharvest life of cut gerbera flowers as affected by Nano – Silver and Acetylsalicylic Acid, *Asian Journal of Bio Chemisty*, 7 (2) , 106 - 111.

Weeraratne, T.P., Daundasekera, W.A.M., and Wijesundara, D.S.A. (2012). Field survey of postharvest handling of cut flowers produced in the up country of Sri Lanka for the local market, *Ceylon Journal of Science*, **41** (1) , 67-70.