Pak. J. Agri. Sci., Vol. 53(3), 593-598; 2016
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/16.3769
http://www.pakjas.com.pk

BIO-CONTROL ACTIVITY OF BACTERIAL STRAINS ON POSTHARVEST PERFORMANCE OF *Gladiolus* L. HYBRIDS 'MAMMOTH'

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A study was carried out to elucidate the efficacy of different bacterial strains on controlling detrimental bacteria and on vase life extension of *Gladiolus* L. hybrids 'Mammoth'. In a preliminary study, three bacterial strains (*Bacillus pumilus*, *Delftia acidovorans*, and *Herbasperillum* sp.) were isolated from the vase solutions of cut *Gladiolus*, identified and cultured to obtain the bacteria to be used in the study. These isolated strains were compared with two strains of *Pseudomonas fluorescens* (PF-279 and PF-417). All tested strains produced similar vase life of cut gladiolus stems, which was also similar to that of stems placed in tap water. However, stems placed in solutions with PF-279 *Pseudomonas fluorecsens* resulted in highest water uptake, while the stems placed in solutions with higher concentrations of *Herbasperillum* sp. (10 or 20 mL L^{-1}) had lowest water uptake. Cut stems require low pH for sustaining water uptake and vase life extension, but use of nutrient broth to culture bacteria increased initial solution pH, which resulted in early senescence of cut stems due to rapid bacterial growth, vascular occlusion due to embolisms and reduced stem hydraulic conductance compared to the stems placed in tap water (control). In summary, tested bacterial strains had no effect on controlling detrimental bacteria present in the vase solutions or vase life extension of cut gladiolus and cannot be used in organic floral preservatives for holding cut stems after harvest.
Keywords: *Pseudomonas fluorescens*, *Bacillus pumilus*, *Herbasperillum* sp., *Delftia acidovorans*, beneficial microbes, vase life.

INTRODUCTION

Gladiolus (*Gladiolus* L. hybrids), one of the most popular bulbous cut flowers of the world, has the problem of shorter vase life due to vascular occlusion by bacteria. Stem blockage in cut flowers is generally caused by bacterial proliferation along with their decay products (Teixeira-da-Silva, 2003). Bio-control through bacteria represent a potential alternative management approach (Jetiyanon and Kloepper, 2002) and may help in developing an organic method for effectively controlling detrimental microbes in the vase solutions (Carlson *et al.,* 2015). The bio-control agents are used for biological management of pests to control a specific microbe (Shanmugam *et al.,* 2011; Sajjad *et al*., 2014). Among bacterial antagonists, *Pseudomonas fluorescens* is most effective against a wide range of plant pathogens infecting different plants such as carnation, bean, radish, cucumber, tomato, and tobacco (van Loon *et al.,* 1998), while *P. fulva* has extended vase life of cut zinnia stems (Carlson *et al.,* 2015). Moreover, *Burkholderia cepacia* and *Bacillus* spp. (spore forming Gram-positive bacteria) have effectively been used to control plant diseases (Kloepper *et al.,* 2004). Use of compatible and multiple bio-control agents in various groups also helps to control plant diseases, such as combinations of bacteria (Raupach and Kloepper, 1998; Shanmugam *et al.,* 2002), fungi (Paulitz *et al.,* 1990), bacteria and fungi (Duffy *et al.,* 1996), yeasts (Janisiewicz, 1996), and bacteria and yeast (Janisiewicz and Bors, 1995).

An experiment was conducted to compare different beneficial bacterial strains for their efficacy to control detrimental bacteria in vase solutions with gladiolus stems. There is dire need to develop organic preservatives for keeping flower organic grown without chemicals until end of vase life. However, there are currently no effective organic preservatives available in the market and those available are not effective (Ahmad *et al.,* 2014). Organic carbohydrate source and acidifier are available but organic biocides are not available. Therefore, this study was conducted to elucidate the effect of bio-control bacterial strains, some of which have been proved effective for various agronomic crops, in controlling detrimental bacteria in vase solutions and effect on the postharvest water relations and quality characteristics of cut gladiolus. Moreover, the findings of the study would help develop an organic floral preservative to be used for handling organically grown cut flowers.

MATERIALS AND METHODS

The study was conducted at the Postharvest Laboratory, Department of Horticultural Science, NC State University, Raleigh, NC, during 2012-13. Prior to the study, a preliminary trial was conducted by placing fifteen stems in three vases [containing 500 mL deionized (DI) water] with five stems per vase. Stems were observed daily for differences in appearance due to senescence. Aliquots of the vase solution were taken on day 7 of vase life (which was when the stems started showing differences in appearance) from each vase, diluted, and cultured on nutrient agar plates to isolate the bacterial strains. On the basis of size and color, three types of colonies were isolated and re-cultured until pure cultures were obtained and submitted to the NCSU Plant Disease and Insect Clinic for identification of strains. Two other strains, *Pseudomonas fluorescens* (PF-279) and *Pseudomonas fluorescens* (PF-417), were obtained from Washington State University, WA, USA, and University of North Carolina, Chappel Hill, NC, USA, respectively, and compared along with strains isolated from the vase solutions.

***Plant material*:** Cut stems of gladiolus (*Gladiolus* L. hybrids 'Mammoth') were received from a commercial grower, Glad-A-Way Farms, California, USA, within four days of harvest. On arrival, stems were sorted into 19 similar groups on the basis of stem caliper. Initially stems were sorted into groups with similar diameters followed by distribution uniformly among the treatments. Thus, each treatment would contain the same number of thick, intermediate and thin stems. Stems were dipped in soapy solution for 5 sec., rinsed in tap water, surface sterilized by spraying 70% alcohol, recut with sterilized secateurs to final uniform stem length of 80 cm, labeled, and placed in vases (three stems per vase) in a vase-life evaluation room set at 21±1°C temperature, 40-60% relative humidity (RH) and a 12 h light period provided by cool-white fluorescent tubes, which provided a photosynthetic photon flux of ~20 µmol m^{-2} s^{-1} measured with a 1078 QMSW Quantum meter (Apogee Instruments, Inc., Logan, UT, USA).

***Treatments*:** Inoculum of each strain was prepared by inoculating single colony from isolated pure cultures and grown for 48 hrs. with continuous shaking. Vases containing 700 mL of tap water were sterilized and covered with parafilm to reduce the chances of air contamination. Initial bacterial population varied from 1 x10^4 to 25 x10^4 cfu mL^{-1} for different strains. There were 19 treatments with all five strains

Table 1. Effect of different concentrations of various bacterial strains on vase life and weight changes of 'Mammoth' gladiolus. Stems were placed in either tap water, pure nutrient broth, or in nutrient broth plus *Pseudomonas fluorescens* (PF-279), *Pseudomonas fluorescens* (PF-417), *Bacillus pumilus*, *Herbasperillum* sp., or *Delftia acidovorans*, at 5, 10, or 20 mL L^{-1}.

Treatments		Vase life	Initial fresh	Termination fresh	Dry weight (g)	Fresh weight
Strain	Conc. (mL L^{-1})	(d)	weight (g)	weight (g)		change (g)
Tap water (-ve control)	-	9.5[z]	77.1	73.2	8.2	-3.9
Nutrient Broth (+ve control)	5	9.5	76.8	73.5	8.3	-3.4
	10	8.8	79.4	77.6	8.4	-1.8
	20	9.3	82.5	79.6	8.7	-2.9
Pseudomonas fluorescens (PF-279)	5	8.9	84.4	78.5	8.3	-5.9
	10	9.4	80.2	77.3	8.5	-2.9
	20	9.1	82.4	76.4	8.2	-6.0
Pseudomonas fluorescens (PF-417)	5	9.1	81.4	78.1	8.7	-3.3
	10	9.4	79.4	71.0	8.0	-6.2
	20	9.0	77.2	75.2	8.4	-4.2
Bacillus pumilus	5	9.3	83.6	80.9	8.6	-2.6
	10	9.3	71.1	66.6	7.7	-4.5
	20	9.1	78.2	76.9	8.4	-1.3
Herbasperillum sp.	5	9.6	71.8	64.4	7.8	-7.4
	10	9.2	85.8	77.8	8.8	-8.0
	20	9.4	77.5	72.1	8.3	-5.4
Delftia acidovorans	5	9.4	75.0	74.6	7.9	-0.3
	10	9.1	74.6	73.6	7.9	-1.0
	20	9.0	80.1	75.6	8.3	-4.5
Significance[y]						
Overall		NS	NS	NS	NS	0.012
Strain (S)		NS	NS	NS	NS	NS
Conc. (C)		NS	NS	NS	NS	NS
S x C		NS	NS	NS	NS	NS

Data represent means of five vases of three stems each or 15 individual stems; [z] Mean separation within columns by LSD at $P \leq 0.05$; [y]*P* values were obtained using General Linear Models (GLM) procedures (version 9.3; SAS Inst. Inc., Cary, NC) for significant effects of different strains and their concentrations; [NS] Nonsignificant at $P > 0.05$.

and nutrient broth at 5, 10, or 20 mL L^{-1} along with tap water (control). Each treatment was applied to five vases of three stems each.

***Measurements*:** Data were recorded for vase life [time period (days) from placing stems in vases to the time of stem termination], initial and final fresh weight (one designated stem from each vase), dry weight (measured at termination after drying in oven at 70°C for 72 h), fresh weight change, water uptake (measured in milliliters from all vases when first stem was terminated in entire experiment), percentage of florets opened during vase period, initial and final pH, pH change, initial and final EC, EC change, and symptoms of termination, which included bent stem or petal wilting (Saleem *et al.,* 2013). Cut stems were observed every day during vase period and every stem was terminated if it had developed one or more of the above mentioned symptoms on more than half of the flowers/petals, foliage, or stem (Ahmad *et al.,* 2013a).

***Statistical analysis*:** The experimental layout was completely randomized design with factorial arranged treatments and five replicate vases of three stems each. Data were analyzed using analysis of variance (ANOVA) and General Linear Models procedures of SAS (version 9.3, SAS Inst., Inc., Cary, NC) and Fisher's LSD at $P \leq 0.05$ was used to separate means (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Use of bio-control strains did not extend vase life of gladiolus stems and resulted in vase life similar to the stems placed in tap water (Table 1). Moreover, all stems had similar fresh weight changes during vase period and dry weight of a stem. However, stems placed in vase solutions with *Pseudomonas florescens* (PF-279) at 5 mL L^{-1} maintained water uptake and had a higher uptake than stems placed in solutions with *Herbasperillum* sp. or *Pseudomonas florescens* (PF-417) at 10 or 20 mL L^{-1} (Table 2). Use of higher concentrations of all bacterial strains tested lowered the water uptake, but had no effect on opening of the florets during vase period. In another study, bacterial strains viz. *P. fulva* and *Escherichia* coli K12 extended vase life compare to other tested bacterial strains, nutrient broth and control (DI), but all bacterial strains had no effect on water uptake of cut stems of zinnia (Carlson *et al.,* 2015).

Table 2. Effect of different concentrations of various bacterial strains on water uptake, number of opened florets, and pH changes of 'Mammoth' gladiolus. Stems were placed in either tap water, pure nutrient broth, or in nutrient broth plus *Pseudomonas fluorescens* (PF-279), *Pseudomonas fluorescens* (PF-417), *Bacillus pumilus*, *Herbasperillum* sp., or *Delftia acidovorans*, at 5, 10, or 20 mL L^{-1}.

Treatments		Water uptake	Number of opened	Initial pH	Final pH	pH change
Strains	Conc. (mL L^{-1})	(mL)	florets (%)			
Tap water (-ve control)	-	309.0 ab	70.8 abc	5.3 h	4.9 e	-0.31 a
Nutrient Broth (+ve control)	5	299.0 abc	76.8 a	8.0 g	6.2 abc	-1.76 bc
	10	292.0 abc	69.3 bcde	8.1 f	6.1 abc	-1.99 cd
	20	272.0 bcd	64.1 e	8.1 f	6.2 abc	-1.91 bcd
Pseudomonas fluorescens (PF-279)	5	327.0 a	70.6 abc	8.4 c	5.8 d	-2.6 h
	10	293.0 abc	64.3 de	8.4 c	6.1 abc	-2.30 efg
	20	282.0 abcd	73.3 ab	8.1 g	6.3 a	-1.71 b
Pseudomonas fluorescens (PF-417)	5	286.0 abcd	72.0 abc	8.6 a	6.1 abc	-2.53 gh
	10	257.0 cde	68.3 bcde	8.4 c	6.1 abc	-2.33 efg
	20	243.0 de	66.8 cde	8.4 c	6.2 abc	-2.24 ef
Bacillus pumilus	5	277.0 bcd	69.3 bcde	8.0 g	6.1 abc	-1.92 bcd
	10	277.0 bcd	70.4 bcd	8.3 d	6.1 abc	-2.23 de
	20	289.0 abcd	71.5 abc	8.2 e	6.2 abc	-1.96 cd
Herbasperillum sp.	5	293.0 abc	70.2 bcde	8.5 b	6.0 cd	-2.48 fgh
	10	217.0 e	71.5 abc	8.5 b	6.1 abc	-2.37 efgh
	20	261.0 cde	68.0 bcde	8.4 c	6.3 ab	-2.14 de
Delftia acidovorans	5	282.0 abcd	70.0 bcde	8.5 b	6.1 abc	-2.37 efgh
	10	269.0 bcd	73.0 abc	8.4 c	6.0 bc	-2.35 efg
	20	281.0 abcd	70.2 bcde	8.2 e	6.2 abc	-1.99 cd
Significance[y]						
Overall		NS	NS	<0.0001	<0.0001	<0.0001
Strain (S)		0.0189	NS	<0.0001	NS	<0.0001
Conc. (C)		0.0138	NS	<0.0001	<0.0001	<0.0001
S x C		NS	0.004	<0.0001	0.006	<0.0001

Data represent means of five vases of three stems each or 15 individual stems; [z] Mean separation within columns by LSD at $P \leq 0.05$; [y]*P* values were obtained using General Linear Models (GLM) procedures (version 9.3; SAS Inst. Inc., Cary, NC) for significant effects of different strains and their concentrations; [NS] Nonsignificant at $P > 0.05$.

Table 3. Bacterial populations of various bacterial strains, *Pseudomonas fluorescens* (PF-279), *Pseudomonas fluorescens* (PF-417), *Bacillus pumilus*, *Herbasperillum* sp., or *Delftia acidovorans*, sampled on day 0, 3, 6 or 9. Inoculum of each strain was added at 5, 10, or 20 mL L^{-1}.

Strain	Volume of inoculant added (mL L^{-1})	Bacterial counts (cfu 0.1 mL^{-1})			
		Day 0	Day 3	Day 6	Day 9
Water (-ve control)			$11x10^3$ $1x10^6$ -	$28x10^3$ $1x10^6$ -	$51x10^3$ - -
Nutrient broth (+ve control)	5		$288x10^4$ - -	$235x10^4$ $7x10^6$ -	$235x10^4$ $4x10^6$ -
	10		$366x10^4$ $6x10^6$ -	$112x10^4$ $10x10^6$ -	$187x10^4$ $2x10^6$ -
	20		$298x10^4$ $53x10^6$ -	$225x10^4$ $32x10^6$ -	$133x10^4$ $1x10^6$ -
Pseudomonas fluorescens (PF-279)	5	$156x10^2$ $8x10^3$ $1x10^4$	$287x10^4$ $5x10^6$ $1x10^8$	$170x10^4$ $4x10^6$ -	$210x10^4$ $3x10^6$ -
	10		$463x10^4$ $2x10^6$ $1x10^8$	$184x10^4$ $6x10^6$ -	$256x10^4$ $8x10^6$ -
	20		$612x10^4$ $2x10^6$ -	$186x10^4$ $5x10^6$ -	$120x10^4$ $2x10^6$ -
Pseudomonas fluorescens (PF-417)	5	$356x10^2$ $200x10^3$ $53x10^4$	$201x10^4$ $92x10^6$ -	$105x10^4$ $2x10^6$ -	$31x10^4$ $8x10^6$ -
	10		$290x10^4$ $5x10^6$ $1x10^8$	$131x10^4$ $1x10^6$ -	$107x10^4$ $4x10^6$ -
	20		$415x10^4$ $6x10^6$ $1x10^8$	$152x10^4$ $2x10^6$ -	$203x10^4$ $4x10^6$ -
Bacillus pumilus	5	$413x10^2$ $60x10^3$ 8x104	$136x10^4$ $11x10^6$ $1x10^8$	$180x10^4$ $2x10^6$ -	$216x10^4$ $3x10^6$ -
	10		$313x10^4$ $15x10^6$ -	$224x10^4$ $18x10^6$ -	$400x10^4$ $19x10^6$ $1x10^8$
	20		$246x10^4$ $10x10^6$ $1x10^8$	$120x10^4$ $7x10^6$ -	$109x10^4$ $11x10^6$ -
Herbasperillum sp.	5	$456x10^2$ $90x10^3$ $22x10^4$	$280x10^4$ $42x10^6$ $2x10^8$	$35x10^4$ $1x10^6$ -	$285x10^4$ $7x10^6$ -
	10		$495x10^4$ $43x10^6$ $4x10^8$	$186x10^4$ $4x10^6$ -	$149x10^4$ $73x10^6$ -
	20		$510x10^4$ $50x10^6$ $8x10^8$	$81x10^4$ $1x10^6$ -	$35x10^4$ $1x10^6$ -
Delftia acidovorans	5	$296x10^2$ $47x10^3$ $11x10^4$	$184x10^4$ $2x10^6$ -	$290x10^4$ $8x10^6$ -	$288x10^4$ $18x10^6$ -
	10		$416x10^4$ $13x10^6$ -	$556x10^4$ $43x10^6$ -	$607x10^4$ $29x10^6$ -
	20		$640x10^4$ $23x10^6$ $2x10^8$	$240x10^4$ $5x10^6$ -	$210x10^4$ $11x10^6$ -

Values represent means of samples from two or three vases.

During vase period, pH of the solutions containing bacterial strains became much more acidic with the greatest decrease (2.6 units) for PF-279 at 5 mL L^{-1}, which might be the reason for continued water uptake by the stems (Table 2). Higher water uptake due to low pH has also been reported in other studies with different ornamental species (Ahmad *et al.*, 2013b; Carlson and Dole, 2013). However, the solution EC

changes for all treatments were statistically similar (data not presented).

The bacterial population in different vase solutions greatly varied when sampled on 3 day intervals during vase life (Table 3). Although no significant extension in vase life of cut gladiolus stems occurred, the PF-279 bacteria appeared to have countered the negative effects of detrimental bacteria in the vase solutions to produce similar vase life as with tap water. It has been reported that avoiding bacterial proliferation in vase solutions is more important as compared to their populations in the solutions (Carlson *et al.,* 2015). Certain bacterial strains may have little or no effect but several species may shorten the vase life of various cut flower crops (van Doorn *et al.,* 1991; Jacob and Kim, 2010) by blocking the vascular system (Put, 1990), by producing enzymes which kill plant tissues (Membre and Burlot, 1994), or by producing senescence causing hormones, such as ethylene (van Doorn *et al.,* 1991). Therefore, more comprehensive studies need to be conducted by using different strains, concentrations and populations of the bacteria for evaluation of their effects on extending postharvest longevity of cut flowers.

In summary, *Pseudomonas florescens* (PF-279) yielded better results among all tested strains by maintaining water uptake by the stems (Gast, 2000) due to low solution pH, which is beneficial in extending vase life of many cut flower species by slowing bacterial growth in vase solution (van Doorn and Perik, 1990), avoiding stem vasculature blockage and embolisms (Durkin, 1979), maintaining higher stem hydraulic conductance (Marousky, 1971), reducing water stress symptoms and numerically lengthening the vase life of cut gladiolus stems.

***Acknowledgements*:** The authors would like to acknowledge the Glad-A-Way Farms, CA, USA, for providing cut gladiolus stems, and Washington State University, WA, USA, for providing bacterial strain, *Pseudomonas fluorescens* (PF-279), for the study.

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