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INFLUENCE OF PRECOOLING AND PULSING ON THE GROWTH OF MICROORGANISMS IN THE VASE WATER OF CUT ROSE CV. NOBLESSE

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Mwangi Mariam[1]; P.N. Chowdhry[2] and S.K. Bhattacharjee[1]

ABSTRACT

Microorganisms such as fungi and bacteria in the vase water play an important role in the post-harvest spoilage of cut flowers. In this investigation, the influence of precooling and pulsing on the growth of microorganisms in the vase water of cut rose cv. Noblesse was studied. The vase water of different days after precooling with ice-cold water spray for 45 min and cool storage at 4°C for 24 hours and plusing with two chemicals (i) D-fructose (3 %) + 8-HQC* (150 ppm) for 24 hours and (ii) DMSO** (2%) for 15 min. was utilized for culturing the microbes (fungi) in potato dextrose agar (PDA) and (bacteria) in nutrient agar (NA) medium. The fungal genera that were identified in the vase water of cv. Noblesse during the different days of the vase life were *Alternaria alternata, Alternaria brassicola, Aspergillus flavus, Aureobasidium pullulescens, Acremonium sp., Dreschlera specifera, Cladosporium cladosporoides, Cladosporium oxysporum. Fusarium pallidoroseum and bacteria , Streptomyces griseus, and Streptomyces albus*.

* 8-HQC: Hydroxylequinoline citrate

** DMSO : Dimethyl sulphoxide

Key words: Vase water, Cut flowers, Cut Rose Noblesse, Microorganisms, Spoilage

INTRODUCTION

Microbial contamination is one of the major cause of deterioration in cut flowers which is involved in the plugging of rose stems, resulting in the reduction in water uptake by the stem. When the stem is blocked, continuing transpiration by the leaves results in net loss of water by flower and stem tissues. In roses, this often leads to the 'bent neck' disorder **(Van Doorn and Perik, 1990)**. Precooling is an important postharvest operation which is the removal of heat from the harvested material. Precooling of the flowers to the optimal storage temperature reduced the risk of *Botrytis* infection infection and the amount of ethylene inside the package (**Farnham *et al* 1978**). Several microorganisms such as fungal and bacterial genera have been identified from the vase water which cause the plugging of the xylem vessel elements. In the present investigation, an attempt was

1- Division of Floriculture and Landscaping, Indian Agricultural Research Institute, Pusa. New Delhi-110012, India.

2- Division of Plant Pathology, Indian Agricultural Research Institute, Pusa New Delhi-110012, India

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made to isolate and identify the microorganisms present in the vase water affecting the cut rose cv. Noblesse during its vase life, as affected by precooling and pulsing.

MATERIAL AND METHODS

The investigation was undertaken at the Division of Floriculture and Landscaping, and Indian type culture Laboratory in the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi during 2000-2001. Three year old polyhouse grown rose plants of Hybrid Tea rosa cv. Noblesse of uniform size and vigour, large light pink coloured buds which are borne on erect thorny stems were selected for the experiment Rose buds with stems measuring 30 cm length, with four compound leaves were harvested when the buds were fully mature and the sepals were well spread and unfurled downwards. Two sets of experiments were laid in Completely Randomized designs with three replications. Five flowers were placed in each treatment. In the first experiment, two precooling methods were used (i) spraying the cut flowers for 45 min. at 3 minutes interval with ice-cold water (2±1°C), and (ii) cool storage of cut flowers at 4°C for 24 hours. The cut flowers precooled with ice-cold water spray were then pulsed with two chemicals viz., (a) DMSO (2%) for 15 min. and (b) D-frucose (3%) + 8-HQC (150 ppm) for 24 hours. Cut flowers under the cool storage treatment, were also simultaneously pulsed with the above mentioned chemicals while in cool storage. The control was an extra set of (five) cut roses that were neither precooled nor pulsed. There were altogether seven treatments including control. After the precooling and pulsing treatment were over the flowers were kept in a conical flask with a known amount of tap water and kept at ambient or room temperature in the laboratory.

The second experiment consisted of three parts i.e. isolation, pure culture and identification. The vase water of different days after the precooling and pulsing treatment were given, was utilized for culturing the microbes in potato dextrose agar (PDA) and nutrient agar (NA) medium. For isolating fungi, PDA and for bacteria NA media were used. Individual fungal colonies were isolated from Petriplates containing PDA medium by inoculation needle in the laminar flow chamber. Similarly, bacterial colonies were isolated from Petriplates containing NA medium. Individual fungal colonies were developed in the PDA slants by following single hyphal tip culture and colonies of pure culture were subjected for identification under microscope. The bacterial colonies per ml of vase water for different days of vase life was counted by serial dilution techniques. The morphological informations, especially the different shapes of bacteria were also observed microscopically using Gram staining procedure.

RESULTS AND DISCUSSION

It is evident from Table (1) that the cut rose cv. Noblesse was affected by a number of fungal genera in the vase water during different days in the vase. Maximum activity of the fungal and bacterial pathogens in the precooled and pulsed cut roses were observed on the 4^{th} day of vase life, thus resulting in the end of vase life on the 5^{th} day. In the control treatment where no precooling and no pulsing

Table 1. Influence of precooling and pulsing on the growth of microorganisms in the vase water of cut rose cv. 'Noblesse'

Treatments	1st day	2nd day	3rd day	4th day	5th day
No precooling + No pulsing	*Alternaria alternata* *Dreschlera specifera*	*Cladosporium oxysporum* Alternaria alternata *Dreschlera specifera*	*Cladosporium oxysporum* *Alternaria alternata* *Drechslera specifera*	*Aspergillus flavus* *Alternaria alternata* *Cladosporium oxysporum* *Dreschlera specifera*	*Streptopmyces albus* *Cladosporium oxysporum* *Dreschler specifera* *Alternaria alternata* *Aspergillus flavus*
Precooling – Ice cold water spray for 45 min + No pulsing	*Acremonium* sp. Bacteroa	Bacteria *Acremonium* sp.	Bacteria *Acremonium* sp.	*Alternaria alternata* Bacteria *Acremonium* sp.	*Cladosporium cladosporoides* *Alternaria alternata* *Acremonium* sp., Bacteria
Ice cold water spray for 45 min + 8-HQC + D-fructose (3%) (150 ppm)	*Cladosporium cladospoiroides* Bacteria	*Cladosporium cladospoiroides* Bacteria	*Alternaria alternata* *Streptomyces griseus* *Cladosporium cladosporoides* Bacteria	*Aspergillus flavus* *Alternaria alternata* *Cladosporium cladosporoides* Bacteria *Streptomyces griseus*	-
Ice cold water spray for 45 min. + DMSO (2%)	Bacteria *Aureobasidium pullulescens*	*Alternaria alternata* *Aureobasidium pullulescens* Bacteria	*Alternaria alternata* Bacteria *Aureobasidium pullulescens*	*Alternaria alternata* Bacteria *Aureobasidium pullulescens*	*Alternaria alternata* Bacteria *Aureobasidium pullulescens*

Table 1. Cont.

Treatments	1st day	2nd day	3rd day	4th day	5th day
Cool storage at 4°C + No pulsing	-	-	*Acremonium strictum* Bacteria	*Cladosporium oxysporum* *Alternaria alternata* *Acremonium strictum* Bacteria	-
Cool storage at 4°C + D-fructose (3%) + 8-HQC (150 ppm)	*Alternaria alternata* *Alternaria brassicicola* Bacteria	*Alternaria alternata* *Alternaria brassicicola* Bacteria	*Alternaria alternata* *Alternaria brassicicola* Bacteria	*Alternia brassicicola* Bacteria *Alternaria alternata*	-
Cool storage at 4°C + DMSO (2%)	-	-	*Alternaria alternata*	*Alternaria alternata* *Cladosporium oxysporum*	*Alternaria alternata* *Fusarium pallidoroseum* *Cladosporium oxysporum*

was given, maximum number of fungal genera such as *Alternaria alternata, Dreschlera specifera, Cladosporium cladosporoides, Aspergillus flavus, and Streptomyces albus* was found activated on 5th day resulting in the end of vase life of the cut rose on the same day. In case of precooling with ice-cold water spray for 45 min and no pulsing, three number of fungal genera such as *Acremonium sp, Alternaria alternata,* bacteria and *Cladosporium cladosporoides* were present on the 5th day of vase life. Precooling with ice-cold water spray for 45 min. + D-fructose (3%) + 8-HQC (150 ppm) for 24h pulsing resulted in bacteria and *Cladosporium cladosporoides* presence on 1st and 2nd day. On 3rd day of vase life, two fungal and bacterial genera i.e. *Alternaria alternata* and *Streptomyces griseus* was identified in the vase water. On 4th day, *Aspergillus flavus* was identified along with the other fungal genra, thus resulting in the end of vase life on 4th day itself.

Ice-cold water spray for 45 min. and DMSO (2%) pulsing for 15 min. had bacteria, *Aureobasidium pullulescens,* and *Alternaria alternata* only present in the vase water on the 5th day of vase life. However, in treatments of precooling with cool storage at 4°C and no pulsing and pulsing with DMSO (2%) for 15 min, no activity of microorganisms was observed upto the 2nd day of vase life. This could be due to the low temperature of precooling method at which most of the microbial activity is retarded and also due to the antimicrobial nature of DMSO as reported by **Sankar (2001)**.

The longevity of cut rose cv. Noblesse was significantly affected by the number of bacteria present in he vase water (Table, 2). On day 0, the precooled and not pulsed flower vase solutions differed significantly with a maximum microbial growth observed (3×10^2) whereas precooled and pulsed flower vase solutions were having minimum number of bacterial colonies per ml of vase water (1×10^1). Number of bacterial colonies per ml of vase water significantly varied from first day to senescence day of the total vase life of cut flowers. However, there was no significant difference on the number of bacterial colonies in the vase water of 3rd and 4th day of precooled and not pulsed treatment, and between 4th day and 5th day of the rice-cold water spray for 45 min and pulsed with DMSO (2%) for 15 min. there was a significant increase of bacterial colonies in ice-cold water spray for 45 min without pulsing towards the senescence i.e 7th day. Maximum number of colonies per ml of vase water was recorded on the 7th day i.e on senescence day (16×10^4) with ice-cold water spray for 45 min. and no pulsing whereas minimum (11×10^3) was recorded with ice-cold water spray for 45 min with DMSO (2%) pulsing for 15 minutes. High bacterial counts in the vase water can shorten flower longevity of cut carnation flowers cv. Scania and White Sim, which was related to inhibition of water uptake was also reported by (**Van-Doorn *et al* 1995**). **Van Doorn *et al* (1989)** also reported that whenever the number of bacteria exceeded 106 colony forming units (cfu) per gram fresh weight, the vascular blockage was found, after 3 days in pure water or after a long period in the presence of an antimicrobial compound.

Table (3) reveals the different shapes and structure of bacteria ranging from small rod shaped, cocci, spherical, thick curved like a comma were present in the

Table 2. Bacterial counts in the vase water of cut rose cv. 'Noblesse' on different days in vase

Days	No. of bacterial colonies per ml of water	
	Ice cold water spray for 45 min and no pulsing	Ice cold water spray for 45 min + 2% DMSO for 15 min
0	3.0×10^2	1×10^1
1	10×10^3	4×10^3
2	10×10^4	6×10^4
3	4.0×10^4	15×10^3
4	4.5×10^4	5×10^3
5	1.6×10^4	4×10^3
6	1.1×10^5	5×10^4
7	1.6×10^4	11×10^3
'F' test	**	**
S.Em ±	3824.55	2332.21
CD at 5%	11464.78	6991.22

vase water of cut rose cv. Noblesse during different days of vase life. There was increase in various shapes and number of bacteria from 4th day upto senescence i.e. 7th day. **Van Doorn and Perik (1990)** also reported that a considerable number of bacteria high enough to result in vascular occlusion were present in th stems. A population of bacteria is also present on the outside of the stems. These bacteria may rapidly multiply in the water, the freshly cut stem and the xylem vessels as reported by **Van Doorn and Tijskens (1991)**. In cut roses cv. Norens bacteria which cause vascular blockage were observed in the xylem vessels of stem ends after 10 days in distilled water. These bacteria were identified as *Pseudomonas fluorescens, Klebsiella oxytoca,* and *Aeromonas hydrophila* **(Kim-KiuWeon *et al* 1997)**.

CONCLUSION

A number of fungal genera and bacterial population both Gram positive (+) and Gram negative (-) was found to be affecting the vase water of cv. Noblesse during its vase life. In treatments of precooling with cool storage at 4°C and no pulsing as well as pulsing with DMSO (2%) for 15 min. had no activity on microorganisms upto the 2nd day of vase life inferring that precooling with cool storage at 4°C for 24 h and pulsing with DMSO (2%) for 15 min was beneficial in controlling the growth of microorganisms for 2 days and resulted in a vase life of 5 days.

Table 3. Bacterial isolates (Gram positive or negative) isolated from vase water on different days in vase

Days		Structure	Gram reaction
0	(i)	Small, rod shaped	-
1	(i)	Small, rod shaped	-
	(ii)	Small, thick, curved like comma	+
	(iii)	Small, round cocci / spherical	+
2	(i)	Small, thick, curved like comma	+
	(ii)	Rod shaped in a chain	-
	(iii)	Small, round cocci / spherical	+
3	(i)	Small rod shaped	-
	(ii)	Small, thick, curved like comma	+
	(iii)	Small, round cocci / spherical	+
4	(i)	Small rod shaped	-
	(ii)	Small, thick, curved like comma	-
	(iii)	Small, round cocci / spherical	+
5	(i)	Small, rod shpaed	-
	(ii)	Small, round cocci / spherical	+
	(iii)	Small, thick curved like comma	+
6	(i)	Small, rod shaped	-
	(ii)	Small, round cocci / spherical	+
	(iii)	Small, thick curved like comma	+
	(iv)	Rod shaped in a chain	-
7	(i)	Small, rod shaped	-
	(ii)	Small, round cocci / spherical	+
	(iii)	Small, thick curved like comma	+
	(iv)	Rod shaped in a chain	-

REFERENCES

De, L.C. and S.K. Bhattacharjee (2000). Methods for prolonging vase life of cut flowers A review. ***The Orissa Journal of Horticulture 28(1): 73-87.***

Farnham, D.S.; J.F. Thompson; A.M. Kofranek; P.F. Hasek and R. Rij (1978). Forced air cooling questions and answers. ***Floriculture Review, 162(4188): 33, 79-82.***

Kim, KiuWeon; Kim, Wantae and Kim, Sang Dal (1997). Isolation and identification of bacteria causing bent-neck of cut roses. ***J. Kor. Soc. Hort. Sci. 38(5): 592-596.***

Sankar, V. (2001). ***Postharvest Life and Quality of Cut Roses as Affected by Storage and Packaging***. Ph.D. Thesis, IARI, New Delhi, India.

VanDoorn, W.G. and R.R.J Perik (1990). Hydroxyquinoline citrate and low pH prevent vascular blockage in stems of cut rose flowers by reducing the number of bacteria. ***J. Am. Soc. Hortic. Sci. 115: 979-981.***

Van Doorn, W.G.; K. Schurer and Y. De Witte (1989). Role of endogenous bacteria in vascular blockage of cut rose flowers. ***J. of Plant Physiology, 134: 375-381.***

Van Doorn, W.G. and L.M.M. Tijskens (1991). FLORES : a model on the keeping quality of cut flowers. ***Agric. Syst. 35: 111-127.***

Van Doorn, W.G.; Y. De Witte and H. Harkema (1995). Effect of high numbers of exogenous bacteria on the water relations and longevity of cut carnation flowers. ***Post Harvest Biology and Technology, 6(1-2): 111-1119.***

مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٤٨ ، ع(١)، ٣٤٣-٣٥١ ، ٢٠٠٣

تأثير تبريد أزهار الورد المقطوعة ومعاملتها بالكيماويات على نمو الكائنات الحية الدقيقة فى مياه المزهريات

[٢٥]

موانجى ماريام[١] - ب.ن. شودهرى[٢] - بهاتتاشارجى[١]

١- قسم الزينة - معهد البحوث الزراعية الهندى - بوذا - نيودلهى ١١٠٠١٢ - الهند

٢- قسم أمراض النبات - معهد البحوث الزراعية الهندى - بوذا - نيودلهى ١١٠٠١٢ - الهند

تلعب الكائنات الحية الدقيقة مثل الفطريات والبكتريا فى مياه المزهريات دورا هاما فى تلف بعد الحصاد للزهور المقطوعة .

فى هذه الدراسة ، درس تأثير تبريد أزهار الورد والتغذية السكرية للأزهار المقطوعة على نمو الكائنات الحية الدقيقة فى مزهريات الورد المقطوعة صنف نوبلس.

أخذت مياه المزهريات على فترات يوميه بعد معاملة الأزهار بالتبريد عن طريق الرش بالماء المثلج لمدة ٤٥ ق ثم حفظت على درجة ٤°م لمدة ٢٤ ساعة وغذيت بمادتين ١) د. فركتوز (٣%) + هيدروكس كينولين سترات (١٥٠ جزء / مليون لمدة ٢٤ ساعة و ٢) مادة داى مثيل سلفو واكساىد (DMSO) (٢%) لمدة ١٥ ق وذلك لتنمية الميكروبات (فطريات) على أجار البطاطس والدكستروز، و(البكتريا) على الأجار المغذى .

أمكن التعرف على الفطريات : الترناريا الترناريا ، والترناريا براسيسيكولا، وأسبرجلس فلافوس ، واريوبازيديوم بوليوسيتس ، وأكريمونيوم ، ودرسكلرا سبيسيفيرا ، وكلادوسبوريوم كلادوسبورويدس ، وكلادوسبوريوم اكسيبوروم ، وفيوزايوم بالليدوروسوم ، والبكتريا ستربتوميسيس الباس وذلك فى مياه مزهريات الورد فى أيام مختلفة من الحفظ .

تحكيم: أ.د مصطفى حلمى مصطفى