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Evaluation Activity of Some Antimicrobial Agents in Reduction Microbial Load and Their Impact on Vase Life of *Asparagus aethiopicus* L.

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The short vase life is a major problem in the *Asparagus aethiopicus* leafy cut stem industry. The present study showed that several bacterial and fungal species were found to be associated with *A. aethiopicus* cut wilted stems and their vase life. This included six bacterial species, *i.e., Bacillus subtilis, Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Streptomyces* sp. *Streptobacillus* sp. and also five fungal species, *i.e., Alternaria alternata, Fusarium oxysporum, F. solani, Aspergillus flavus and A. niger.* However, *Pseudomonas* sp. and *F. oxysporum* were the most frequent bacterial and fungal microorganisms. Three tested antimicrobial agents, *i.e.,* 8-hydroxy quinoline citrate (8-HQC), sodium hypochlorite and citric acid significantly reduced number of bacterial and fungal proliferation in the different vase treatments where stems basal ends were dipped in them and this effect was increased with increasing their concentrations. However, 8-HQC at 200 ppm + oxytetracycline hydrochloride (1000 ppm) showed reduction percentages of 84.29 and 51.72 % of bacterial and fungal numbers, respectively. Also, sodium hypochlorite at 10 ppm + oxytetracycline hydrochloride (1000 ppm) recorded bacterial and fungal reductions that reached 85.25 and 42.44%, respectively. This was followed by citric acid (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) which showed 83.31and 28.32 %, reductions of bacterial and fungal numbers while antimicrobial agents' treatments without oxytetracycline hydrochloride showed lower reduction effect. All antimicrobial agents' treatments succeeded to decrease weight loss and enhanced water uptake and vase life of the treated *A. aethiopicus* cut stems and this effect was increased with increasing their concentrations. Meanwhile, treatment with the mixture of 8-HQC (200ppm) + oxytetracycline (1000 ppm) was the most effective treatment in decreasing cut stem weight loss to be low as 4.05 % compared to 44.37 % for the untreated control. Also, this treatment enhanced water uptake and vase life to be as high of 44.90 g and 14.50 days compared to 16.81 g and 5.44 days of the control treatment in the previous parameters, respectively. This was followed by the mixture of sodium hypochlorite (10 ppm) + oxytetracycline hydrochloride (1000 ppm) and citric acid at (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) treatments.

Keywords: *Asparagus aethiopicus*, oxytetracycline hydrochloride, *Fusarium oxysporum, Bacillus subtilis, microbial load, vase life*

Asparagus aethiopicus L. is belonging to the Asparagaceae family (Gandipilli and Geddada 2018). It is extensively utilized by florists in flowers ornamentation. Its ornamental foliage is a perfect complementary background to most other flowers designs. It serves as an amazing greenery in delicate flower bouquets, and it can be applied as cascading central points in great designs (Safeena *et al*., 2019).

Its leafy cut stems have been popular in recent years in Egypt as a filling material in vases and rose bouquets. However, they only have a vase life of 4-6 days at room temperature which is much shorter than many other plant filling materials Safeena *et al.*, 2014. Therefore, there is a commercial demand for protracting and elongating their foliage vase life (Shabanian *et al.*, 2018). It has been frequently reported that several bacterial and fungal proliferations are associated with such reduction in vase life (Ichimura *et al*., 2016, Kampowski *et al*., 2018 and Gandipilli and Geddada, 2018). Bacterial and fungal species that reproduce in the vase solution and cut stems prevent water uptake and thus lowering vase life (Balestra *et al.*, 2005 and Ferrante *et al*., 2007). Pathogens (fungal and/or bacteria ones) growth in the vase solution causes a physical objection of xylem then reduces the uptake of water ended to senescence. Also, Coutinho and Wingfield (2017), Younis *et al*. (2018) and Zeiss *et al*. (2019) mentioned that the presence of pathogenic agents often leads to vascular siege in cut stems, damaging of the important cellular processes and accelerates the death of the infected stems. Symptoms caused by the pathogenic agent included hindered wood texture of cut stems that lowering their lifetime and causing insufficient water uptake due to physical blockage of xylem vessels.

So, several antifungal and antibacterial compounds that indirectly enhance water flowing in the xylem in preservative solutions like 8-hydroxyquinoline citrate, citric acid and sodium hypochlorite were proposed and they have been used to attain the best postharvest quality and vase life in several cut flowers and foliage Hashemabadi *et al*., 2015; Rida *et al*., 2016; Balieiro *et al*.; 2018, He *et al.*, 2018; Kantharaj *et al*., 2018; Sarhan *et al*., 2018 and Safeena *et al*., 2019.

The present study, therefore, focused on potential of microbial load proliferation on the cut stems vase life of *A. aethiopicus* and the efficacy of some safe materials to control the associated pathogens and to attain the quality and vase life of *A. aethiopicus.*

Materials and Methods

The present study was conducted at Research Branch, Plant Pathology Research Institute, Ornamental, Medicinal and Aromatic Plant Diseases Research Department, El-Sabihia Agricultural Research Station Alexandria. Samples of *A. aethiopicus* showing symptoms of wilt were collected from different vases in different places in Alexandria during 2018 for isolation and identification of the associated fungal and bacterial species.

1. Isolation and identification of the associated bacteria and fungi:

One cm long segments were cut from the leafy cut stem ends in each flask for isolation of the associated fungal and bacterial species as follows:

1.1. Isolation and identification of the associated fungi:

Samples segment of the were thoroughly washed for several times under running water, cut into small pieces, rinsed in sterilized distilled water, dried between two folds of sterilized filter paper and cultured on PDA in petri plates. Inoculated plates were kept at 26°C±2 for five days. The developed fungal colonies were purified using single spore or hyphal tip techniques and maintained on slant PDA (Al-Jaradi *et al.,* 2018). The isolated fungi were identified using the morphological characteristics of mycelia and spores according to Barnett and Hunter (1986) and Sneh *et al.* (1991). Identification of the isolated fungi was confirmed by the staff members of Department of Mycology, Plant Disease Survey' and Plant Pathology Research Institute, ARC, Giza, Egypt. Percentage of frequencies (%) of the recovered fungal species were then calculated according to, Braithwaite *et al.* (2006) as follows:

$$\text{Frequency (\%)} = \frac{\text{Number of detected fungal colonies}}{\text{Total fungal colonies}} \times 100$$

1.2. Isolation and identification of the associated bacteria:

According to Harrigan and McCance (1996), segments were washed several times with distilled water, then ground in a mortar with 10 ml sterile normal saline solution, strained in cheese cloth, and 0.1 ml of the filtrate was spread on nutrient agar in plates, incubated at 31±1°C for 24 h. Developed bacterial colonies were counted according to the morphological characteristics (Jowkar *et al*., 2012) and streaked onto new nutrient agar plates until separated colonies were obtained. Pure cultures of the bacterial species were maintained in nutrient agar slants and were identified according to Harrigan and McCance (1996), Benson (2002) and Naing *et al*. (2017) by the Agricultural Laboratory (Agro Lab), Sadat City, Egypt. Frequency of the associated bacterial species were calculated as mean CFU (Colony Forming Unit/ milliliter) Bacteria / ml, estimated by the serial dilution method Reynolds (2015).

2. Pathogenicity tests for the recovered fungal and bacterial species:

According to Younis *et al*. (2014), matured outwardly healthy cut stems of *A. aethiopicus* (freshly cut stems with 60-70 cm long stems) were obtained from a commercial well-known nursery in Alexandria. Cut stems were warped in tissue paper and taken to the laboratory, then slanting cuts (2-cm) were made for cut stems under the running tap water for better absorption of vase solution, and lower leaflets were stripped off, sterilized by 1% sodium hypochlorite for three minutes and washed several times with sterile distilled water. Six *A. aethiopicus* cut stems were prepared for each treatment (in three glass vases, 2 in each) and each vase

contained 500ml of sterile distilled water with 10g sucrose where the basal 5-cm of the cut stems were dipped. All vases were sterilized by immersing in 3% sodium hypochlorite for 20 min washed several times with distilled water then inverted and left to dry. All treated cut stems were kept at room temperature of 21±2°C, relative humidity of 63±3%, and 12h/day cool white fluorescent light lamps. All vases were covered with cotton plug to block water evaporation and to reduce the entry of bacteria and fungi from the surrounding air.

2.1. Pathogenicity tests of fungal species:

The tested isolates were cultured on PDA at 26°C±2 for ten days. The spores were scraped from the surface of the culture and introduced into 10 ml of sterile distilled water. Concentration was determined by using Haemocytometer slide. The suspension was adjusted to the concentration of 10^6 spores/ml and 5 ml of the spore suspension were added to each vase which contained 500ml sterile distilled with 10g sucrose. Control vases were inoculated with 5ml sterile distilled with 10g sucrose. After 24 hours of inoculation, six *A. aethiopicus* cut stems (2 in each vase), prepared as previously mentioned, were dipped in vase solution where the basal 5-cm of the cut stems were covered. All vases were kept at room temperature as previously mentioned. Plants in vases were monitored daily and when wilt symptoms were developed (three days after dipping basal leafy stem cuts in inoculated vase solution), disease severity for each fungal species was calculated. To fulfill Koch's postulates, re-isolations were conducted and identification of the recovered fungi was performed as previously mentioned where confirmed the associated fungal species.

2.2. Pathogenicity tests of bacterial species:

According to Li *et al*. (2012) and Rafi and Ramezanian (2013), pathogenicity tests were performed by using bacterial cultures grown at 31±1°C for 24 h. on nutrient agar (NA). Each bacterial species was suspended in sterile distilled water and bacterial concentration was adjust to 10^8 CFU/ml, using the serial dilution method pour plate technique according to Jowkar *et al*. (2012) Reynolds (2015). Pathogenicity tests were performed in the glass vases containing 500 ml sterile distilled water with 10g sucrose. All vase solutions were inoculated with 5ml bacterial suspension (10^8 CFU/ml) and control vases were inoculated with 5ml sterile distilled water with 10g sucrose. After 24 hours of inoculation, six *A. aethiopicus* cut stems (2 in each vase) were dipped in vase solution where the basal 5 cm of the cut stems were covered. All vases were kept at room temperature and all vases were covered with cotton plug as previously mentioned. Cut stems in vases were monitored daily and when wilt symptoms were developed (three days after dipping basal leafy stem cuts in inoculated vase solution), disease severity for each bacterial species was calculated. To fulfill Koch's postulates, re-isolations were conducted, and identification of the recovered bacteria was performed as previously mentioned where confirmed the associated bacterial species.

Disease assessment:

According to Vakalounakis and Fragkiadakis (1999), five disease ratings were assigned as follows: 0 = No symptoms; 1 = Light yellowing of leaves, light or moderate rot on basal stem; 2 = Moderate or severe yellowing of leaves and severe rot basal stem, 3 = Moderate wilting and desiccation, curling, necrosis and rot basal stems, 4= Dead by severe wilting, yellowing, desiccation, graying, curling, and necrosis and leaves drop. Then, percentage of disease severity (DS%) was calculated according to the formula adopted by Song *et al*. (2004).

$$\text{Disease severity (DS\%)} = \frac{\sum (\text{scale} \times \text{number of plants infected})}{\text{highest scale X total number of plants}} \times 100$$

3. Effect of the tested antimicrobial agents to control wilt of A. aethiopicus cut stems under natural infection:

Three materials *i.e.,* sodium hypochlorite (SHC) 40% (NaOCl), citric acid (CA) pure ($C_6H_8O_7$) and pure 8-hydroxy quinoline citrate (8-HQC) singly and at different concentrations as well as their mixtures with the antibiotic oxytetracycline hydrochloride (Ox) were tested in treatments according to Kantharaj *et al*. (2018), as follows:

Treatments

T_1	Sodium hypochlorite (SHC) at 2, 5 and 10 ppm.
T_2	Citric acid (CA) at 200, 500 and 1000 ppm.
T_3	8-hydroxy quinoline citrate (8-HQC) at 50, 100 and 200 ppm.
T_4	SHC (at 2, 5 and 10 ppm) + oxytetracycline hydrochloride (Ox) at (1000 ppm).
T_5	CA (at 200, 500 and 1000 ppm) +Ox (at 1000 ppm).
T_6	8-HQC (at 50, 100 and 200 ppm) + Ox (at 1000 ppm).
Control	distilled water

All agents were dissolved in tap water with 10 g/l sucrose, each vase contained 500 ml of the tested antimicrobial agent solution. *A. aethiopicus* cut stems were prepared, and the experiment was conducted as previously mentioned under pathogenicity test. Then, *A. aethiopicus* cut stems in vase treatments were monitored for any wilt symptoms. All antimicrobial agents were obtained from El-Gomhouria Company, Alexandria branch.

3.1. Effect of the teste materials on bacterial and fungal proliferation in A. aethiopicus vase solution:

When wilt symptoms began to appear on the control, a one ml of vase solution from all replicates was collected, five days after dipping the basal portion of cut stems in the different antimicrobial agents treatments of solutions. Then, the serial dilution method–pour plate technique was applied to count bacteria on nutrient agar according to Fung (2009) and Reynolds (2015) and also, the detected fungal colonies were also counted according to Jowkar *et al*. (2012). However, bacterial

count at the 5th day of the treatment was below the limit to apply the counting equation (Fung, 2009), so, samples were taken again from control and all treatments on the seventh day for bacterial and fungal counting. After the serial dilutions, one ml of the fifth dilution was plated onto nutrient agar in plates. Then, inoculated plates were incubated at 30 ± 1°C for 24 h. Number of bacteria was counted through the number of colonies formed after incubation and expressed as Colony Forming Units/ml (CFU/ml). Reduction percentage (R%) in bacterial and fungal colonies was calculated according to Ferreira *et al*. (1991) as follows:

$$\text{Reduction \% (R\%)} = \frac{\text{Control- Treatment}}{\text{Control}} \times 100$$

3.2. Effect of antimicrobial agents on bacterial and fungal occurrence in A. aethiopicus cut stems:

On the seventh day of treatment, one cm long segments were cut from the leafy cut stem ends in each vase treatment for isolation of the accompanied bacterial and fungal species as previously mentioned according to Li *et al*. (2012), Rafi and Ramezanian (2013) and Jowkar *et al*. (2012). Occurrence (%) of the associated fungal species was calculated according to Braithwaite *et al.* (2006), while occurrence of the associated bacteria was assigned as + and – according to Reynolds (2015).

3.3. Effect of antimicrobial agents on physiology and vase life of A. aethiopicus cut stems:

At the end of experiment, after 14 days of dipping cut stems in antimicrobial agents treatments, the following assessments were conducted According to Safeena *et al*. (2014), Hashemabadi *et al*. (2015) and Amin (2017) as follows:

$$\text{Cut stems weight loss (g)} = \frac{\text{Initial fresh weight} - \text{Final fresh weight}}{\text{Initial fresh weight}} \times 100$$

Cut stem water uptake (g):

It is the difference between the weight of the vase solution at the end of the vase life and weight of the vase solution at the beginning of trial.

Cut stem vase life (days):

Vase treatments with leafy cut stems were monitored daily and foliage vase life was recorded as the number of days until 20% of the foliage showed symptoms of wilting, yellowing, desiccation, graying, curling, and necrosis and leaves drop.

Statistical analysis:

The obtained data were statistically analyzed using Statistix program, and means comparisons were conducted using least significance difference (LSD) at 5% level of significance according to Snedecor and Cochran (1989). Pearson's correlation coefficient was conducted using Excel, version, 2016.

Results

1. Fungal and bacterial species associated with A. aethiopicus vase wilt.

Data presented in Table (1) show that five fungal species i.e., *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and *F. solani*, were found to be associated with *A. aethiopicus* leafy cut stem wilt in the surveyed vases. Also, six bacterial species, *i.e.*, *Bacillus subtilis*, *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Streptomyces* sp., and *Streptobacillus* sp. were also associated with *A. aethiopicus* leafy cut stem vase wilt.

However, *Fusarium oxysporum* was the most frequent and constituted 32.30% of the surveyed plants. Also, all bacteria spp., were the most frequent colonies and recovered with mean CFU number of $105.33x10^5$ bacterial species, respectively. (Table 1).

Table 1. Frequency of fungi and bacterial species associated with *A. aethiopicus* vase wilt.

Fungal species	Frequency (%)	Bacterial species
Alternaria alternata	25.16* b	*Bacillus subtilis*
Aspergillus flavus	10.32 d	*Bacillus sp.*
A. niger	14.21 cd	*Pseudomonas sp.*
Fusarium oxysporum	32.30 a	*Streptococcus sp.*
F. solani	18.01 c	*Streptomyces sp.*
-	-	*Streptobacillus sp.*
Total	100.00	The total number of bacteria $105.33x10^5$ CFU**
LSD. at 0.05	5.61	-

*Values followed by different letter (s) are significantly different at 5% level

**CFU (Colony Forming Unit/ milliliter) = Bacteria / ml, estimated by the serial dilution method.

2- Pathogenicity of the recovered fungal and bacterial species:

Data in Table (2) show that all fungal and bacterial species investigated were able to induce infection on the inoculated *A. aethiopicus* leafy cut stems with different severity degrees. The developed vase wilt disease severity (DS) with the tested fungal species ranged between 45.00 and 90.00% while DS with the tested bacterial species ranged between 35.00 and 86.67 % for the tested bacterial species. However, *F. oxysporum* was the most virulent and showed 90.0% DS, followed by *A. alternata* which caused 87.5% DS and *F. solani* with 59.17% DS. Meanwhile, Streptomyces sp. was the most virulent tested bacterial species that showed the highest wilt DS, values being 86.67% followed by *Pseudomonas* sp. and *Streptococcus* sp. with 65.0 and 61.67% DS, respectively (Table 2).

Table (2): Mean vase wilt disease severity on *Asparagus aethiopicus* leafy cut stems during pathogenicity.

Bacterial species		Fungal species	
Bacillus subtilis	35.00* c	*Alternaria alternata*	87.50 a
Bacillus sp.	35.83 c	*Aspergillus flavus*	51.67 bc
Pseudomonas sp.	65.00 b	*A. niger*	45.00 c
Streptococcus sp.	61.67 b	*Fusarium oxysporum*	90.00 a
Streptomyces sp.	86.67 a	*F. solani*	59.17 b
Streptobacillus sp.	37.50 c	-	-
Control	0.00		0.00
Overall mean	53.61 B	Overall mean	59.72 A
LSD. at 0.05	7.28		10.16
	3.56		

** Values followed by different letter (s) are significantly different at 5% level.

3. Effect of antimicrobial agents on controlling A. aethiopicus vase wilt:

3.1. Effect of antimicrobial agents on bacterial and fungal numbers in A. aethiopicus vase solutions:

Data presented in Table (3) show that all tested antimicrobial agents caused significant reduction in the number of bacterial and fungal proliferation in vase solutions of the different treatments, however, this effect was more pronounced with mixing antimicrobial agents with oxytetracycline hydrochloride (1000 ppm). Meanwhile, 8-hydroxy quinoline citrate (8-HQC) at 200 ppm + oxytetracycline hydrochloride (1000 ppm) showed reduction percentages of 84.29 and 51.72 % in bacterial and fungal numbers, respectively. Also, sodium hypochlorite (SHC) at 10 ppm + oxytetracycline hydrochloride (1000 ppm) recorded reduction in the numbers of bacteria and fungi that reached 85.25 and 42.44%, respectively. This was followed by citric acid (CA) at (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) which showed 83.31 and 28.32 %, reduction in bacterial and fungal numbers, respectively while the remaining treatments showed lower effect. Also, it is evident from Figure (1) that all tested antimicrobial agents succeeded in decreasing the associated bacterial and fungal proliferation and the associated *A. aethiopicus* cut stem wilt.

Table (3): Effect of antimicrobial agents on bacterial and fungal numbers in vase solutions with *Asparagus aethiopicus* leafy cut stems, 7 days after dipping the basal stem in vase treatment solutions under natural infection.

Treatment		Number of bacteria			Number of detected fungal colonies		
		Number $\times 10^5$ (CFU*/ ml)	Reduction (%)	Mean of Reduction (%)	Number	Reduction (%)	Mean of Reduction (%)
SHC	2 ppm	40.87 bc**	48.01#	60.25	17.20 b	09.88	10.66
	5 ppm	33.57 de	57.29		17.11 b	10.47	
	10 ppm	19.30 gh	75.45		16.89 bc	11.63	
CA	200 ppm	36.00 cd	55.54	55.08	18.22 ab	05.75	06.32
	500 ppm	45.37 b	43.97		18.11 ab	06.32	
	1000 ppm	27.73 ef	65.75		18.00 ab	06.90	
8-HQC	50 ppm	21.67 fg	72.33	67.75	15.44 cd	19.19	20.54
	100 ppm	36.87 cd	52.92		15.11 de	20.93	
	200 ppm	17.23 ghi	77.99		15.00 de	21.51	
SHC	2 ppm + Ox	14.13 hi	82.49	83.59	11.78 f	38.37	40.12
	5 ppm + Ox	13.70 i	83.02		11.56 f	39.53	
	10 ppm + Ox	11.90 i	85.25		11.00 fg	42.44	
CA	200 ppm + Ox	33.83 de	54.83	72.11	13.89 e	27.75	27.94
	500 ppm + Ox	16.33 ghi	78.19		13.88 e	27.75	
	1000 ppm + Ox	12.50 hi	83.31		13.78 e	28.32	
8-HQC	50 ppm + Ox	14.10 hi	81.79	82.91	09.89 gh	48.85	50.38
	100 ppm + Ox	13.43hi	82.65		09.50 gh	50.57	
	200 ppm + Ox	12.17 i	84.29		09.33 h	51.72	
Overall Mean control		78.48 a			19.20 a		
LSD. at 0.05		6.88			1.45		

SHC= Sodium hypochlorite, CA= Citric acid, 8-HQC= 8-hydroxy Quinoline citrate and Ox= Oxytetracycline hydrochloride antibiotic (at 1000 ppm)

*CFU (Colony Forming Unit/milliliter) = Bacteria/ml, estimated by serial dilution method.

** Means followed by different letter (s) are significantly different at 5% level.

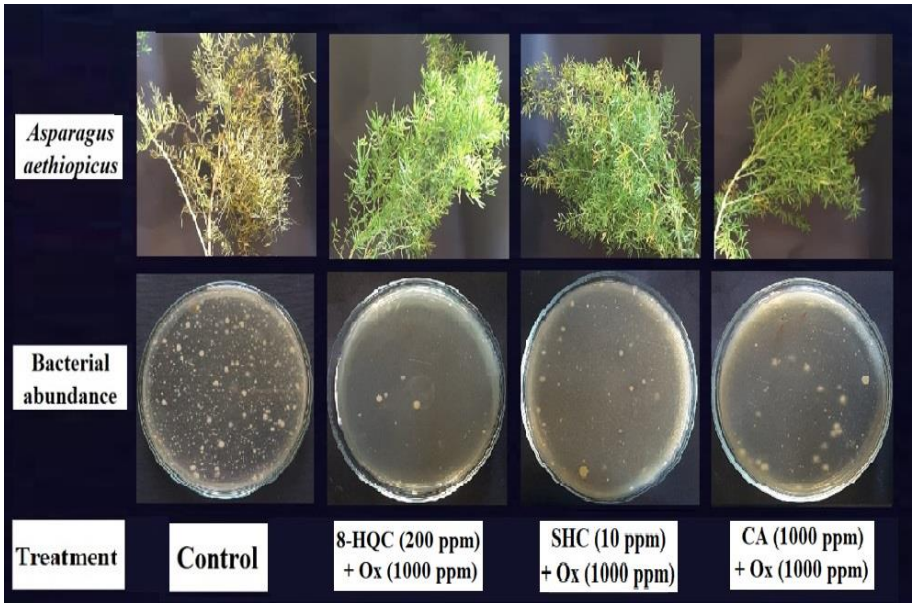

Figure (1): Effect of 8-hydroxy quinoline citrate at 200 ppm + oxytetracycline hydrochloride (1000 ppm), sodium hypochlorite at 10 ppm + Ox (at 1000 ppm) and citric acid at 1000 ppm + Ox (1000 ppm) on *Asparagus aethiopicus* vase disorder caused by bacterial and fungal, and proliferation bacterial on nutrient agar.

Concerning the associated bacterial species, data in Table (4) show that six species of bacteria were isolated and identified from the collected samples. These associated detected species were *Bacillus subtilis*, *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Streptomyces* sp. and *Streptobacillus* sp. However, occurrence of these species was differed with the different treatments' where *Bacillus subtilis*, *Pseudomonas* sp. were the most frequent bacteria over the different treatments. Meanwhile, while the six bacterial species were occurred in control and in CA treatments, only two bacterial species, *i.e., Bacillus subtilis and Pseudomonas* sp. were occurred in 8-HQC + ox treatment (Table 4).

Regarding the associated fungal species, data in Table (5) show that five fungal species were found to be associated with *A. aethiopicus* leafy cut stem wilt where *Alternaria alternata* and *F. oxysporum* were the most frequent fungi over the different treatments. Moreover, data indicated that 8-HQC + Ox treatments showed the lowest mean fungal frequencies for all detected fungal species. However, the 8-HQC 200 ppm + Ox treatment was the most effective to inhibit the associated fungi and showed the lowest fungal frequencies for all species (Table 5).

Table (4): Effect of antimicrobial agents on the occurrence of the associated bacterial species in *Asparagus aethiopicus* cut stem in treated vases under natural infection.

Treatment		*Bacillus subtilis*	*Bacillus* sp.	*Pseudomonas* sp.	*Streptococcus* sp.	*Streptomyces* sp.	*Streptobacillus* sp.
SHC	2 ppm	+	-	+	+	+	+
	5 ppm	+	-	+	+	-	-
	10 ppm	+	-	+	-	-	-
CA	200 ppm	+	+	+	+	+	+
	500 ppm	+	+	+	+	+	+
	1000 ppm	+	+	+	+	+	+
8-HQC	50 ppm	+	-	+	-	+	+
	100 ppm	+	-	+	-	+	+
	200 ppm	+	+	+	+	-	-
SHC	2 ppm + Ox	+	-	+	-	-	-
	5 ppm + Ox	+	-	+	+	-	-
	10 ppm + Ox	+	-	+	+	-	-
CA	200 ppm + Ox	+	+	+	-	-	-
	500 ppm + Ox	+	+	+	+	-	-
	1000 ppm + Ox	+	+	+	+	-	-
8-HQC	50 ppm + Ox	+	-	+	-	-	-
	100 ppm + Ox	+	-	+	-	-	-
	200 ppm + Ox	+	-	+	-	-	-
Control		+	+	+	+	+	+

SHC= Sodium hypochlorite, CA= Citric acid, 8-HQC= 8-hydroxy Quinoline citrate and Ox= Oxytetracycline hydrochloride antibiotic (at 1000 ppm), (+) Positive growth, (-) No growth (absence of bacteria)

Table (5): Effect of antimicrobial agents on occurrence of the associated fungal species in *Asparagus aethiopicus* cut stem in treated vases under natural infection.

Treatment		*Alternaria alternata*	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium oxysporum*	*Fusarium solani*
SHC	2 ppm	22.33*	15.12	15.06	25.04	19.35
	5 ppm	20.51	14.39	14.46	26.95	18.92
	10 ppm	20.31	11.04	14.74	25.87	13.09
	Mean	21.05 A*	13.51 AB	14.75 A	25.95 A	17.12 A
CA	200 ppm	21.62	18.52	16.62	24.16	19.08
	500 ppm	16.05	16.75	16.90	18.03	19.68
	1000 ppm	13.70	16.51	15.34	19.20	19.43
	Mean	17.12 ABC	17.26 A	16.29 B	20.46 ABC	19.30 AB
8-HQC	50 ppm	20.25	13.47	13.47	27.44	16.58
	100 ppm	21.21	11.95	13.80	27.27	13.80
	200 ppm	18.49	11.71	15.08	24.51	13.13
	Mean	19.98 AB	12.38 AB	14.12 A	26.41 A	14.50 BC
SHC	2 ppm + Ox	17.40	9.89	10.07	20.15	09.89
	5 ppm + Ox	14.97	9.98	11.05	19.07	09.98
	10 ppm + Ox	10.07	06.36	06.36	10.07	03.33
	Mean	14.15 BC	8.75 BC	9.16 B	16.43 BC	7.74 D
CA	200 ppm + Ox	18.86	10.47	14.93	23.62	12.97
	500 ppm + Ox	19.90	11.27	12.89	21.60	11.53
	1000 ppm + Ox	18.94	12.11	11.95	21.66	09.39
	Mean	19.23 AB	11.28 B	13.26 AB	22.29 AB	11.30 CD
8-HQC	50 ppm + Ox	13.89	8.33	11.11	16.67	11.11
	100 ppm + Ox	11.62	8.84	08.84	14.65	08.84
	200 ppm + Ox	10.53	3.03	07.50	10.53	03.03
	Mean	12.01 C	6.73 C	9.15 B	13.95 C	7.66 D
Control		22.41	19.14	16.95	27.81	19.93
LSD. at 0.05		8.75	7.04	6.75	9.59	7.05

SHC= Sodium hypochlorite, CA= Citric acid, 8-HQC= 8-hydroxy Quinoline citrate and Ox= Oxytetracycline hydrochloride antibiotic (at 1000 ppm) * Means of treatments followed by different letter (s), for each single fungus are significantly different at 5% level.

3.2. Effect of antimicrobial agents on the physiological traits and vase life of A. aethiopicus cut stems:

Foliage vase life was monitored daily until the end of the experiment, 14 days after treatment with antimicrobial agents and the numbers of days until 20% of the foliage wilting, yellowing, desiccation, graying, curling, necrosis, and leaves drop were recorded. Also, foliage weight loss and water uptake were also calculated at the end of experiment and data are presented in Table (6). It is evident from the tabulated data that all treatments succeeded to decrease weight loss and enhanced water uptake and vase life of the treated *A. aethiopicus* twigs and the effect was increased with increasing the concentration (Table 6). However, treatment of 8-hydroxy quinoline citrate (200 ppm) + oxytetracycline was the most effective and decreased twigs weight loss to be a low as 4.05 % compared to 44.37 % for the untreated control. Also, this treatment enhanced water uptake and vase life to be as high of 44.90 g. and 14.50 days compared to 16.81 g. and 5.44 days for the control of the previous parameters, respectively. This was followed by sodium hypochlorite (10 ppm) + oxytetracycline hydrochloride (1000 ppm) and citric acid at (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) which showed 9.40 %, 43.32 g and 14.0 days, and 26.79 %, 40.78 g and 9.67 days for the previous parameters, respectively. However, treatments with antimicrobial agents only without oxytetracycline hydrochloride showed lower effect for all the previous parameters (Table 6).

4. Correlation between the bacterial and fungal reductions of treatments and the physiological traits and vase life of A. aethiopicus cut stems.

It is evident from Figure (2) that strong correlations to vase life ($r = 0.85 - 0.96$) were existed between the bacterial and fungal reductions exerted by the different antimicrobial agents treatments and the associated weight loss, water uptake, and vase life of *A. aethiopicus* cut stems, basal, dipped in the treatment solutions. The correlation types were very similar for both the bacterial and fungal reduction effect, however, there were positive correlations with vase life and water uptake, but negative correlation with cut stem weight loss (Figure 2).

Table (6): Antimicrobial effect of agents at different concentrations on physiological traits and vase life of *Asparagus aethiopicus* cut stems, base dipped in treatment solutions.

Treatment		Weight loss (%)		Water uptake (g)		Vase life (days)	
SHC	2 ppm	25.36 ef	20.48* C	30.47 j	34.42* D	06.17 ij	08.10* DE
	5 ppm	24.30 efg		35.43 i		09.00 fg	
	10 ppm	11.78 k		37.36 gh		09.33 efg	
CA	200 ppm	32.57b	31.10 A	25.38 l	27.39 D	06.17 ij	07.22 D
	500 ppm	30.90 bc		27.32 k		07.17 hij	
	1000 ppm	29.82 bcd		29.48 j		08.33 gh	
8-HQC	50 ppm	27.45 cde	24.95 B	35.44 i	36.45 C	10.83 cde	10.11 C
	100 ppm	25.23 efg		36.50 hi		08.50 gh	
	200 ppm	22.18 fg		37.42 gh		11.0 cd	
SHC	2 ppm + Ox	21.54 gh	17.76 D	41.57 cd	42.44 A	09.83 defg	11.39 B
	5 ppm + Ox	22.35 fg		42.44 bc		10.33 def	
	10 ppm + Ox	9.40 k		43.32 b		14.0 a	
CA	200 ppm + Ox	22.44 fg	24.97 B	38.46 fg	39.56 B	7.33 hi	08.94 D
	500 ppm + Ox	25.69 ef		39.45 ef		9.83 defg	
	1000 ppm + Ox	26.79 de		40.78 de		9.67 defg	
8-HQC	50 ppm + Ox	18.05 hi	12.65 E	41.56 cd	43.09 E	12.0 bc	13.28 E
	100 ppm + Ox	15.85 i		42.82 bc		13.33 ab	
	200 ppm + Ox	04.05 h		44.90 a		14.50 a	
Control		44.37 a		16.81 m		05.44 k	
LSD. at 0.05		3.86	1.93	1.55	0.78	1.54	0.77

SHC= Sodium hypochlorite, CA= Citric acid, 8-HQC= 8-hydroxy Quinoline citrate and Ox= Oxytetracycline hydrochloride antibiotic (at 1000 ppm), (*) Mean of treatment

Values followed by different letter (s), for each single parameter, are significantly different at 5% level.

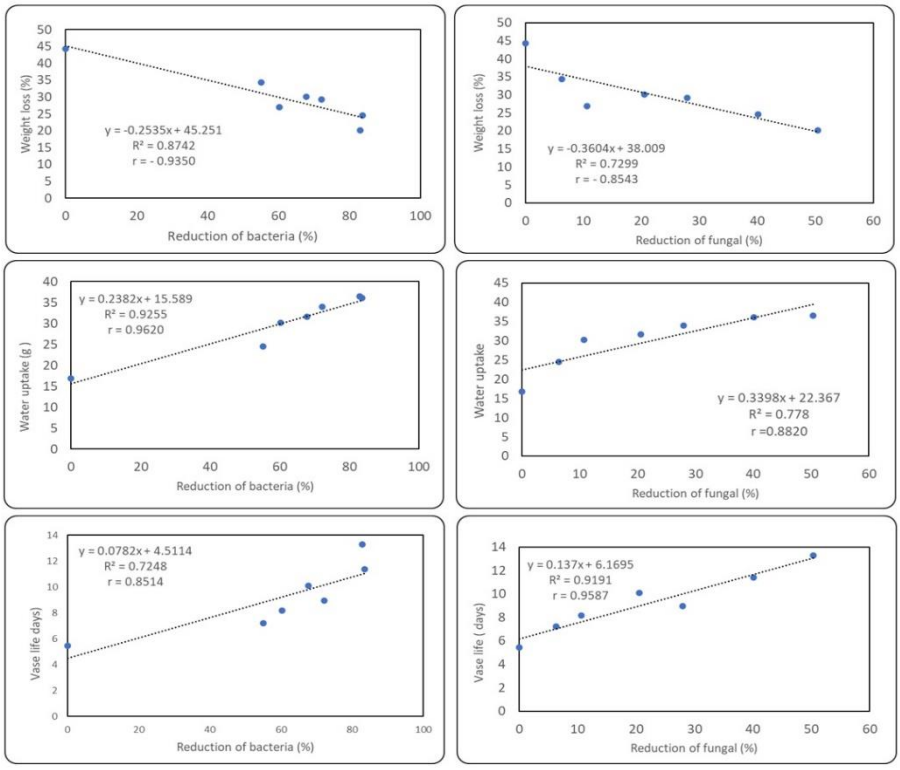

Figure (2): Correlation between the bacterial and fungal reductions of treatments and the physiological traits and vase life of *Asparagus aethiopicus* cut stems.

Discussion

Vase life was normally terminated when the foliage became wilted, and the stems collapsed. Poor water relations in leafy stems can be the result of microbial or physiological occlusions in the vascular system Byung-Chun *et al*. (2010).

In this respect, Coutinho and Wingfield (2017) and Zeiss *et al*. (2019) mentioned that the presence of pathogenic agents (bacterial and/or fungal ones) and their metabolites often lead to vascular siege in cut stems damaging the important cellular processes and accelerates the death of the infected stems.

In the present study, several bacterial and fungal species were found to be associated with *A. aethiopicus* cut stem wilt and its vase life. This included six

bacterial species, *i.e., Bacillus subtilis, Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Streptomyces* sp. *Streptobacillus* sp., and also five fungal species, *i.e., Alternaria alternata, Fusarium oxysporum, F. solani, Aspergillus flavus, and A. niger.* However, *Bacillus subtilis*, *Pseudomonas* sp., *Alternaria alternata* and *F. oxysporum* were the most frequent bacterial and fungal species. These results are in harmony with several investigators (Van-Doorn 2012; Jowkar *et al.*, 2013; Rida *et al*., 2016; Coutinho and Wingfield 2017 and Sarhan *et al*., 2018).

The usage of some antibiotics such as oxytetracycline was commonly used to control cut stem decay in vases resulted by plant pathogens, however, this problem was not fully resolved. Also, preservative solutions (antimicrobial agents) in vase were useful for reducing the risk of such pathogens in some studies (Li *et al*., 2017 and Kantharaj *et al*., 2018).

The antimicrobial agents are chemical substances used for treatment microorganisms to destroy, deter, render harmless, or exert a controlling effect on any harmful organism, or, a group of substances including preservatives, disinfectants, germicides, antibiotics and pesticides used for the control of harmful organisms. Several antimicrobial agents such as sodium hypochlorite, citric acid and 8-hydroxyquinoline citrate are used in vase solutions in order to control decay to increase the vase life of several cut flowers (Rida *et al*., 2016 and He *et al*., 2018).

In the present study the three tested antimicrobial agents, *i.e.,* 8-hydroxy quinoline citrate (8-HQC), sodium hypochlorite and citric acid significantly reduced number of bacterial and fungal numbers in the different vase treatments where *A. aethiopicus* cut stem basal ends were dipped and this effect was increased by increasing the antimicrobial agent concentration. However, 8-HQC at 200 ppm + oxytetracycline hydrochloride (1000 ppm) showed reduction percentages of 84.29 and 51.72 % in bacterial and fungal numbers, respectively. Also, sodium hypochlorite at 10 ppm + oxytetracycline hydrochloride (1000 ppm) recorded bacterial and fungal reductions that reached 85.25 and 42.44 %, respectively. This was followed by citric acid (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) which showed 83.31 and 28.32%, reductions in bacteria and fungi numbers. However, antimicrobial agent treatments without oxytetracycline hydrochloride showed lower reduction effect than antimicrobial agents amended with oxytetracycline hydrochloride. These results are in harmony with those recorded by Erin *et al*. (2014), Hema *et al*. (2015), Malakar *et al*. (2017) and Balieiro *et al*. (2018).

The effect of 8-HQC may be due to that 8-hydroxyquinoline contains a hydroxyl group which has a pH value that could affect the bacterial and fungal growth (Malakar *et al*., 2017). Also, sodium hypochlorite contains chlorine atoms that may enhance the efficacy of that compound. Also, citric acid effect probably

due to its pH effect, in addition to the presence of the effective antibiotic oxytetracycline hydrochloride (Hema *et al*., 2015).

On the other hand, all antimicrobial agents treatments succeeded to decrease weight loss and enhanced water uptake and vase life of the treated *A. aethiopicus* twigs and this effect was increased with increasing the concentrations of the antimicrobial agents. Meanwhile, treatment with 8-HQC (200 ppm) + oxytetracycline (1000 ppm) was the most effective and decreased twigs weight loss to be a low as 4.05 % compared to 44.37 % for the untreated control. Also, this treatment enhanced water uptake and vase life to be as high of 44.90 g and 14.50 days compared to 16.81 g and 5.44 days for the control of the previous parameters, respectively. This was followed by sodium hypochlorite (10 ppm) + oxytetracycline hydrochloride (1000 ppm) and citric acid at (1000 ppm) + oxytetracycline hydrochloride (1000 ppm) treatments.

Meanwhile, strong correlations to vase life (r = 0.85 - 0.96) were existed between the bacterial and fungal reductions exerted by the different antimicrobial agents treatments and the associated weight loss, water uptake, and vase life of *A. aethiopicus* cut stems basal dipped in the treatment solutions. The correlation types were very similar for both the bacterial and fungal reductions, however, there were positive correlations with vase life and water uptake, but negative correlation with cut stem weight loss.

These results are in consistence with Van-Doorn (2012) and Younis *et al*. (2018) who indicated that accompanied bacterial and fungal proliferation in vase solutions lowering their lifetime and causing insufficient water uptake due to physical blockage of xylem vessels as vessels has been infected. Consequently, the obtained enhancement for physiological aspects and vase life may be due to increasing water uptake that led to the longevity of the leafy stems. This is, also, in harmony with Elgimabi and Sliai (2013) who showed that the vase life of Taif rose cut flowers was prolonged by 8-HQS treatments. In addition, Farahat *et al.* (2014) reported that leaf water content was the highest in a solution containing 125 ppm 8-HQS.

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تقييم فعالية بعض المضادات الميكروبية فى خفض الحمل الميكروبى لنبات الأسبرجس الخشن ومردود ذلك على طول العمر فى المزهرية

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يعتبر اطالة العمر القصير للمزهريات مشكلة رئيسية في صناعة نبات الأسبراجس الخشن المقطوف. أظهرت الدراسة الحالية أن العديد من الأنواع البكتيرية والفطرية وجدت مرتبطة بذبول سيقان الأسبراجس وتقصير عمره في المزهريه "الفازه". وشمل ذلك سته أنواع من البكتيريا، وهى (*Bacillus subtilis, Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Streptomyces* sp. *Streptobacillus* sp.) ، وكذلك خمسه أنواع فطرية، وهى (*Alternaria alternata, Fusarium oxysporum, F. solani,* *Bacillus subtilis,* *Aspergillus flavus, and A. niger*) ومع ذلك، كانت الأنواع (*Pseudomonas* sp., *Alternaria alternata and F. oxysporum*) هي الأنواع البكتيرية والفطرية الأكثر تكراريه في العزل. تم أختبار ثلاث مركبات وهى 8-هيدروكسي كينولين سترات (8-HQC)، هيبوكلوريت الصوديوم ، وحمض الستريك. نتج عن إستخدام كل منهم خفض معنوى في عدد كل من المستعمرات البكتيرية وتكرار النمو الفطرى في مختلف المعاملات عند غمس سيقان الأسبراجس الخشن المقطوفة في محاليلها. وقد ثبت زيادة هذا التأثير مع زيادة تركيز المركبات المختبره سالفه الذكر. ومع ذلك، أظهر8-هيدروكسي كينولين سترات (8-HQC) عند ٢٠٠ جزء في المليون + أوكسي تترا سيكلين هيدروكلوريد (١٠٠٠ جزء في المليون) اعلى نسب انخفاضا معنوي بلغ ٨٤,٢٩ و ٥١,٧٢٪ في عدد البكتيريا والفطريات على التوالي. كما سجل هيبوكلوريت الصوديوم بتركيز ١٠ جزء في المليون + أوكسي تترا سيكلين هيدروكلوريد (١٠٠٠ جزء في المليون) انخفاضا في عدد البكتيريا والفطريات بلغ ٨٥,٢٥٪ و ٤٢,٤٤٪ على التوالي. تبع ذلك حمض الستريك (١٠٠٠ جزء في المليون) + أوكسي تترا سيكلين هيدروكلوريد (١٠٠٠ جزء في المليون) الذي أظهر ٨٣,٣١٪ و ٢٨,٣٢٪، انخفاضا في عدد البكتيريا والفطريات بينما كانت معاملات المضادت الميكروبية المختبرة بدون أوكسي تترا سيكلين هيدروكلوريد الاقل تاثيرا. أدت جميع معاملات المركبات المختبره سالفه الذكر الى تقليل فقدان الوزن وتحسين امتصاص الماء واطاله حياة المزهرية لسيقان نبات الأسبراجس الخشن المقطوفة، وزاد هذا التأثير مع زيادة التركيز. وفي الوقت نفسه، كان إستخدام المعاملة 8-HQC (٢٠٠ جزء في المليون) + أوكسي تترا سيكلين (١٠٠٠ جزء في المليون) هى الأكثر فعالية وأقل انخفاضا في وزن السيقان المقطوفة بنسبة ٤,٠٥٪ مقارنة بـ ٤٤,٣٧٪ للمجموعة غير المعالجة "الكنترول". كما عززت هذه المعاملة امتصاص الماء واطاله حياة المزهرية لتصل إلى ٤٤,٩٠ جم و ١٤,٥٠ يوم مقارنة بـ ١٦,٨١ جم و ٥,٤٤ يوم للمجموعة غير المعالجة "الكنترول" في المعاملات السابقة على التوالي. وتلي ذلك هيبوكلوريت الصوديوم (١٠ جزء في المليون) + أوكسي تترا سيكلين هيدروكلوريد (١٠٠٠ جزء في المليون) ثم حمض الستريك عند (١٠٠٠ جزء في المليون) + أوكسي تترا سيكلين هيدروكلوريد (١٠٠٠ جزء في المليون).