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EVALUATION OF SOME FUNGAL AND BACTERIAL CULTURE FILTRATES IN CONTROLLING PRESERVATIVE SOLUTION MICROORGANISMS AND GRAY MOULD ROT OF *GLADIOLUS HYBRIDA* CV. PETER PEARS SPIKES

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ABSTRACT: ***This work was carried out at the Faculty of Agriculture, Kafr El-Sheikh University using two batches, the first on January 2nd and the second on July 15th during 2008 season. Vase-life of Gladiolus hybrida cv. Peter pears spikes is determined by many reasons. Among the most common reason for early senescence of fresh spikes is the inability of spikes to absorb water due to their rot and blockage by microorganisms. Culture filtrates (CFs) of Epicoccum nigricum, E. purpurascens, Trichoderma viride, Trichoderma harzianum and Bacillus subtilis were evaluated to abscission this reason compared to $AgNO_3$ at 10ppm. Using CFs in preservative solution significantly decreased the rots of spikes base by minimizing microbial density. Deteriorated florets also were decreased. In contrast, vase-life of gladioli spikes was significantly increased. Highest number of open florets and water uptake of gladioli spikes were recorded when silver nitrate, culture filtrates of B. subtilis were added to preservative solution comparing to the control. Although some culture filtrates i.e. Bacillus subtilis and Epicoccum nigrum surpassed AgNO3 in some characters, AgNO3 prolonged the flower longevity more than CFs.***

Gray mould rot of gladioli caused by Botrytis cinerea is considered also one of the most postharvest dangerous diseases of gladioli spikes. The aforementioned CFs were tested for their effectiveness against Botrytis cinerea in-vitro and in-vivo. The antifungal activity of CFs at the used three different concentrations (10, 20 and 30%) was compared in vitro on the basis of linear growth inhibition of Botrytis cinerea. Highly significant effect was always achieved with culture filtrates of B. subtilis, E. nigricum and E. purpurascens. In vivo control capability of culture filtrates (CFs) of E. nigricum, E. purpurascens and B. subtilis at concentrations of 10, 20 and 30% against gray mould of gladioli spikes under 20±2˚C showed that culture filtrate of B. subtilis at both concentrations 20 and 30% significantly prolong vase-life of gladioli spikes up to 15.6 and 13.3 days respectively compared with control (7.0 days). Culture filtrate of B. subtilis and E. nigricum at 20 and 30% played a role in retarding the development of the grey mould symptoms caused by B. cinerea on gladioli spikes.

Key Words: ***Gladiolus hybrida, Culture filtrates, Biocontrol, gray mould and Botrytis cinerea.***

INTRODUCTION

Gladioli spikes suffer from relatively short vase-life. Among the most common reasons for early senescence of fresh cut spikes are both the inability of spikes to absorb water due to the blockage of their vessels and the short supply of carbohydrates to support respiration. The inability of spikes to absorb water is a very common reason for premature wilting (Halevy and Mayak (1979); Zagory and Reid, 1986 and Loon *et al.*, 1998). Kofranek and Paul (1974), Mayak *et al.* (1977), Doorn *et al.* (1986), Zagory and Reid, 1986 and Doorn and Witte (1994), reviewed that microorganisms specially the bacteria which grown in preservative solution play a great role in vase-life of flowers. They also reported that a major cause of poor keeping quality of many cut flowers is attributed to rots of spikes and plugging of vascular tissues by bacteria.

Historically, there are many methods to reduce the negative effect of these microorganisms on the longevity of flowers. For example, Silver salts in preservative solutions have been shown to be an effective for extending flower longevity and quality (Reid *et al.,* 1980, Awad *et al.,* 1986 and Younis, 1991). Also, adding $AgNO_3$ in preservative solution significantly decreased stem rot and the microbial density and increased the vase-life of rose flowers (Nowak and Rudnicki, 1990) and Tuberose (Menesy and El-Gremi, 1996). Another example, adding sucrose to preservative solution either alone or with $AgNO_3$ greatly improved flower opening and consequently vase-life of Tulip and Daffodil (Doss, 1986), *Triteleia laxa* – Benth (Han *et al.,* 1990) and Carnation and Rose (Younis, 1991). In an attempt to use biological methods at this field Menesy and El-Gremi, 1996 used biologically produced antimicrobial crude namely CPs-4 which extracted from cell free culture filtrates of *Pseudomonas* sp. They reported that the addition of CPs-4 to preservative solution resulted in decreasing of microbial density and correlative extension in the Tuberose flower longevity. Likewise, Hoogerwerf *et al.* (1992), Yakinova (1998), Petridou *et al.* (1999) and Knee (2000) showed that adding antimicrobial agents to the cut flower preservative solution could inhibit the microbial growth and prevent the obstruction of the vascular system in the stem, and subsequently, prolong the vase life of the flowers.

Postharvest losses of roses may reach very high values depending on species, harvest methods, length of storage, and marketing conditions (Sommor, 1985). Gray mould rot on many commodities caused by *Botrytis cinerea* is considered one of the most determinal postharvest disease, causing losses of horticultural crops (Cline and Bardsly, 1984, McCain and Welch, 1982 and Carre, 1984). *Botrytis cinerea* can invade pre-harvest, during the flowering stage, through wounds, through contact of senescing flower parts, or through direct penetration of the skin and remain quiescent until conditions become favorable for its growth and development. Biocontrol agents such as *Trichoderma harzianum* was used to control this disease on roses (Elad *et al.,* 1993). They reported that *T. harzianum* reduced

development of gray mould on flowers and branches by 70%. Latorre *et al*.(1997) similarly reported that *Trichoderma harzianum* provided partial control of *Botrytis* bunch rot of 'Thomson Seedless' table grape . Also, El-Kazzaz *et. al.* (1999) used cell free culture filtrates of *Bacillus subtilis, Pseudomonas* sp., and *Trichoderma harzianum* as biocontrol agents for controlling gray mould rot of apple fruit disease. They reported that these cell free culture filtrates gave sufficient control of this disease either *in vitro* or *in vivo*. *Epiccocum* sp., *Trichoderma* spp., *Ulocladium* sp. and *Pseudomonas* sp. significantly controlled gray mould rot on lettuce (Card *et al.,* 2002). Similarly *Bacillus subtilis, Pseudomonas* sp., *Trichoderma asperellum* and Pharmaplant-turbo significantly controlled gray mould rot of Navel orange fruits caused by *Botrytis cinerea* (Belal *et al*., 2006).

The present study aims to study the effect of culture filtrates of certain biocontrol agents as additives to preservative solution on the microbial density in preservative solution and against gray mould rot in an attempt to prolong the vase-life of gladioli spikes.

MATERIALS AND METHODS

This work was carried out at the Faculty of Agriculture, Kafr El-Sheikh University using two batches, the first on January 2nd and the second on Jul 15th during 2008 season.

A. Evaluation of the used CFs as additives to preservative solution against microbial density which caused spike base rot in order to prolong vase life of gladioli spikes:

A.1. Preparation of culture filtrates of the used biocontrol agents:

Biocontrol agents which used at this study were *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *Trichoderma harzianum* and *Bacillus subtilis. Epicoccum nigricum, E. purpurascens* and *Trichoderma viride* were kindly obtained from Plant Pathology Dept. Agric. Research Institute, Giza, Egypt. *Trichoderma harzianum* and *Bacillus subtilis* were previously isolated and identified (El-Kot and Belal, 2006 and El-Kot and Hegazi, 2008).Culture filtrates of these biocontrol agents were prepared using methods described by El-Bghdady (1993).

A.2. Plant material and vase establishment:

Uniform gladioli (*Gladiolus hybrida* cv. Peter pears) spikes free from diseases, mechanical injury and insect injuries were obtained from local wholesale distributor. Spikes were cut under aseptic conditions to 60 cm length. Cut gladioli spikes were held in glass bottles (3 spikes/bottle) containing 250 ml preservative solution including a constant glucose percentage (4%) with 10cm depth. Then the following treatments were conducting:

a. Silver nitrates ($AgNo_3$) as a chemical preservative agent were used at 10ppm of preservative solution.

b. Each of culture filtrates (CFs) of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* were used alone at 30% of preservative solution.

c. Untreated spikes were held in preservative solution including a constant glucose percentage (4%) were employed as control treatment.

Each treatment was presented with three replicates (i.e., 9 cut flowers) each spikes were held at room conditions (17±3˚C) on January and 28±2˚C on Jul with 60-70% and 70-80% relative humidity respectively. The following parameters were recorded:

1. Survey of microflora:

Microbial survey of the flowering stem surface was made just period to vase preservative, samples of lower stem parts (10cm) were rinsed in 50 ml sterilized distilled water with shaking for 15 min. Screening of microorganisms in preservative solution was done at the beginning of wilting using serial dilutions. Portions of the obtained suspensions (0.1 ml) were plated on to standard-plate-count agar medium for bacterial count (Seeley and Vandemark,1981) and malt agar medium for counts of fungi and yeasts (Abd-ElHfez *et al.,* 1996). Counts were registered as cfu (colony forming unit) after 72 h incubation period at 27±1˚C. Samples of the arising single colonies with different phenotypic and microscopic features were picked up on slants for further possible identification studies. Identification of the isolated fungi, yeasts and bacteria were carried out at microbiological branch, Agricultural Botany Department, Faculty of Agriculture, KafrelSheikh University according to Alexopoulus, 1979 for fungi, Barnett and Pankhurst, 1974 for yeasts and Bergy´s manual for bacteriology, 1984.

2. Spike base rot caused by microorganisms grown in preservative solution were recorded as cm rot on spikes of gladioli.

3. Spikes vase life:

The data of spikes were recorded four times through three days intervals. The total numbers of both opened and deteriorated florets were recorded for times after 3, 6, 9 and 12 days from the beginning the experiment and percentage of deteriorated / opened florets were calculated. Vase life of each spike was considered terminated when the number of deteriorated florets surpassed the number of the opened ones.

4. Water uptake and spike fresh weight losses:

Water uptake was used as a criterion for spike quality by determination the relationship between the cumulative water absorption of each individual spike and the time after the experiment beginning. Also the loss in spike fresh weight with the time was determined.

5. Total carbohydrates:

At the end of the experiment, spike total carbohydrates were determined according to Herbert *et al*. (1971)

6. Mean of opened florets number.
7. Mean of deteriorated florets number.
8. Percentage of mean of deteriorated florets number/ mean of opened florets number.

B. Evaluation of the used CFs against gray mould rot caused by *Botrytis cinerea* in order to prolong vase life of gladioli spikes

B.1. Isolation, identification and pathogenicity tests of gladiolus pathogenic fungi

Samples of rotted stored gladioli spikes were collected from different flowers market at Alexandria and Kafr El-Sheikh Governorates, Egypt . Small pieces of rotted roses were surface sterilized using 0.3% sodium hypochlorite for 2min., washed several times with sterilized distilled water, air dried and then put on PDA medium. After 2-3 days of incubation at 20±2˚C, the isolates were purified by the hyphal-tip method according to Booth (1977) and the pure cultures were transferred on PDA. Cultural, morphological and microscopical properties were considered to identify the isolated pathogens according to Barnett and Barry (1972). Pathogenicty tests were performed on gladioli spikes and isolated pathogenic fungi were maintained on PDA slants at 4 ˚C for further experiments according to the method described by Moline and Locke (1993).

B.2. *In vitro* experiments

The antifungal activity of the aforementioned culture filtrates against *Botrytis cinerea* was determined *In vitro* as inhibition percentages in the growth of mycelium. The inhibition of mycelial growth was tested by agar dilution technique using three culture filtrates concentrations, i.e. 10,20 and 30%. They were metered to hand warm Botrytis Minimal Agar Medium (BMA) [glucose 10g/l,K_2Hpo_4 1.5 g/l, KH_2PO_4 2g/l,$(NH_4)_2SO_4$ 1.0g/l, $MgSO_4(7H_2O)$ 5.0 g/l, agar 20g/l]. Then small agar disc, originated from 8 days old pure culture of the pathogen, was placed into the center of agar plates. Petri dishes were closed with parafilm, and incubated at 20±2 ˚C. Three Petri-dishes were used for each treatment. Evaluation was carried out when the pathogen overgrew the control agar plates. The growth of mycelia on treated plates was compared with that of the control.

B.3. *In vivo* experiments

The three culture filtrates showed high antifungal activity (*E. nigricum, E. purpurascens* and *B. subtilis*) against *B. cinerea in vitro* were chosen to be used *in vivo* studies. They were used at three different concentrations (10, 20 and 30%). Sterile distilled water was used as control. Gladiolus spikes free from mechanical injury, scratches and wounds or bruises were selected, and surface sterilized by spraying with 0.3% sodium hypochlorite for 3 min., then washed several times in sterilized distilled water and air-dried. Then sprayed by a suspension of $5x10^5$ spores of *B. cinerea* per ml and kept at 20±2˚C for 6

hours. After those gladioli spikes were sprayed by the chosen culture filtrates (*E. nigricum, E. purpurascens* and *B. subtilis*)as mentioned above (10, 20 and 30%). Also sterile distilled water was used as check treatment. The treated gladiolus roses were kept at 20±2°C.Three flowers per treatment with a complete randomization of vase in each test were test. End of flower longevity was considered when about 75%of florets showed rot.

Means between treatments were compared with Duncan's Multiple Range Test according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

A. Evaluation of the used CFs as additives to preservative solution against microbial density which caused spike base rot in order to prolong vase life of gladioli spikes:

The effect of culture filtrates of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* as additive to preservative solution on microbial counts, spike base rot, vase life, water uptake, number of opened florets, number of deteriorated/ opened florets percentage, Spike fresh weight losses percentage and total carbohydrates contents of gladioli spikes was studied at this trial.

A.1. Microbial survey and spike base rot:

Using culture filtrates (CFs) of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* as preservative agents in preservative solution have various effects on microbial counts (Table, 1). Generally, the used culture filtrates significantly reduced microbial density in preservative solution compared with distilled water. The high inhibitor of microbial counts appeared when AgNo3 at 10 ppm was added to preservative solution followed by CF of *Bacillus subtilis* at 30% of preservative solution. This inhibition of microbial counts in preservative solution resulted in reducing spike base rot of gladioli spikes (Table, 1). In this study, numerous microorganisms could be isolated from spike surface and preservative solution. In respect of dominance, on the spike surface yeasts came first and followed by bacteria and then fungi. The most frequent genera of fungi which were isolated from spike surface and preservative solution belonged to *Mucor, Rhizopus, Aspergillus, Cladosporium* and *Fusarium.* While, the most isolated genera of the yeasts were *Rhodotorula* and *Torulopsiss*. Generally, in preservative solution of the control treatment, the bacterial percentages exceed those of fungi. This may suggests that sap exudates of plant materials in preservative solution and/or the vase environment may enhance the bacterial rather than the fungal growth (Woltering, 1987). Reviewing the isolated microorganisms, it was observed a qualitative but not quantitative resemble between those isolated spike surface and those from the preservative solution of the control treatments. This leads to presume that plant materials are the source of vase including contamination (Menesy and El-Gremi, 1996).The obtained results confirmed that preservative agents were

extend the spikes of gladiolus vase-life by its role as a microbial inhibitor in preservative solution (Zagory and Ried, 1986). It is suggested that the principal mechanism of *B. subtilis* isolates is inhibition of the fungus by production of antibiotics such as surfactin, fungycin, mycosubtilin and bacillomycin (Kowall *et al*., 1998) reported. These compounds are amphilic, membrane active surfactants and peptide antibiotics with specific antimicrobial potential. Morilkawa *et al.,* 1992, Peltola *et al.,* 2001 and McGrath, 2004 reviewed that surfactin and pumilacidin are bioactive cyclic peptides produced by some Bacillus isolates. Also, *T. harzianum* and *T. viride* works through different mechanisms, i.e. production of gliotoxin (Abd El-Moity, 1981). Protease production is common among micro organisms, including fungi, among which *Trichoderma* spp. are well-known producers. *Trichoderma* spp. has been found to manifest a direct effect on enzymes of *B. cinerea* by secretion of proteolytic enzymes; it produces protease on leaves in the presence of the pathogen, and the protease reduces *B. cinerea* germination and subsequent disease development (Elad and Kapat, 1999). The hydrolytic enzymes produced by *B. cinerea,* endo-PG and exo-PG, were partially deactivated by protease from the T39. Similarly *E. nigrum* known to be produced four carotenoids pigments (Fobben and Olga Gribanovski-Sassu, 1968). These carotenoids may be caused inhibition of microbial growth in preservative solution.

Table (1): Effect of culture filtrates of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* on microbial counts (cfu/ml) of *Gladiolus hybrida* cv. Peter pears spikes preservative solution.

Treatments	Microbial counts				% Inhibition	Spike base rot (cm)
	Fungi	Yeasts	Bacteria	Total		
Epicoccum nigrum	27	143	118	288 e	83.81 b	1.4 d
E. purpurascens	29	159	123	311 c	82.51 d	1.9 c
Trichoderma viride	33	148	119	300 d	83.13 c	1.7 c
T. harzianum	37	178	134	349 b	80.38 e	2.2 b
Bacillus subtilis	24	117	111	252 f	85.83 b	0.9 e
$AgNO_3$	2	7	13	22 g	98.76 a	0.5 f
Distilled water	166	1271	342	1779 a	00.00 f	3.1 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

2. Vase life and water uptake of gladioli spikes:

Data in Table (2) show that all used treatments have prolonged gladioli spikes vase life in both seasons compared with distilled water (control treatment). The longest vase life is obtained from spikes holded in preservative solution containing AgNO3, culture filtrate of *B. subtilis* and *E. nigrum*, since they recorded 15.67, 14.33 and 13.67days, respectively in the first season and 14.67, 12.67 and 12.00 respectively in the second season.

In respect of water uptake, data in Table (2) revealed that, the cumulative

water absorption with the time was highest with the spikes holded in preservative solution containing culture filtrate of *B. Subtilis* and $AgNO_3$ as gave 232.00 and 220.33 ml, respectively in the first season and the treatments of $AgNO_3$ and culture filtrate of *B. subtilis* in the second season as gave 231.00 and 222.00 ml, respectively.

The ability of culture filtrates to prolong vase life of gladioli spikes could be due to its fatal influence on wide range of microorganisms, since high bacterial counts in the vase water can shorten flower longevity (Knee, 2000). Many studies indicated that, high bacterial counts in water reduced the longevity of the cut flowers (Zagory and Reid, 1986; Stamps and McColley, 1997 and Loon *et al*., 1998). The water conducting tubes in the stem (xylem vessels) become plugged by bacteria, yeast, and/or fungi, which are living in the water or on the flower, and proliferate in the containers preservative the flowers (Doorn, 1997). These microorganisms and their chemical products plug the stem ends and restrict water absorption. They continue to multiply inside and eventually block the stem tubes (Doorn *et al*., 1991 and Doorn *et al*. 1995) finally decreased longevity of flowers.

Table (2): The effect of culture filtrates of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* as additive to preservative solution on vase life and water uptake of *Gladiolus hybrida* cv. Peter pears spikes

Treatments	Vase life (days)		Water uptake (ml)	
	1st batch	2nd batch	1st batch	2nd batch
Trichoderma viride	8.00 ab	7.00 bc	153.00 d	172.67 c
T. harzianum	7.80 ab	6.77 b	125.00 b	100.33 b
Epicoccum nigrum	13.67 f	12.00 fg	182.00 f	217.00 i
E. purpurascens	11.00 d	9.33 d	130.00 c	200.67 f
Bacillus subtilis	14.67 f	12.67 g	232.00 k	222.00 j
$AgNO_3$	15.67 g	14.67 h	220.33 j	231.00 k
Distilled water	7.00 a	6.33 a	100.67 a	84.00 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

3. Number of opened florets:

It is quite clear from Fig (1) that addition aforementioned treatments to the vase preservative solution significantly increased the number of opened flowers over distilled water at the four record times in both seasons. Also, some treatments were similar to or less than $AgNO_3$ in both seasons. The highest number of opened florets noticed on spikes treated with culture filtrate of *B. subtilis* after 3, 6 or 9 days in the first season, and culture filtrate of *E. nigrum* after 9 or 12 days, culture filtrate of *Bacillus subtilis* after 12 days in the second one when compared to other treatments. These results are agreement with Al-Humaid (2004) who reported that the addition of Biocide to preservative solution has improved the post-harvest quality of the spikes by stimulating the flower opening rate that would otherwise lead to

carbohydrate depletion in the spikes due to consumption of carbohydrate by newly opened flowers. Moreover, improving the flower opening rate probably requires an external supply of carbohydrates to recover the consumption of carbohydrate by newly opened flowers.

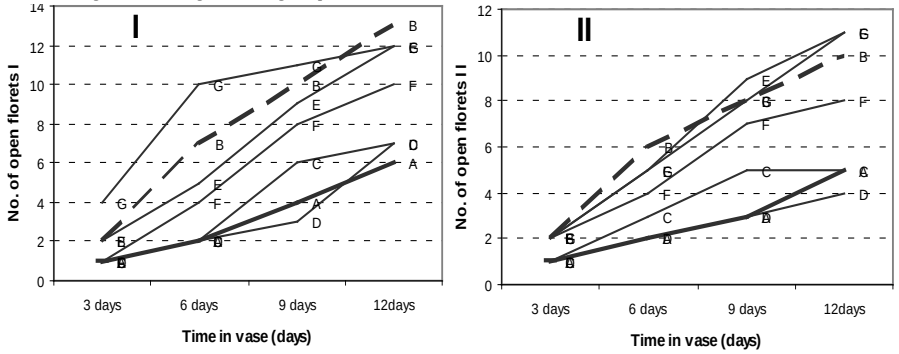

Fig. (1): The effect of culture filtrates of *Trichoderma viride* (C) , *T. harzianum* (D),*Epicoccum nigricum*(E), *E. purpurascens* (F) and *Bacillus subtilis*(G) as additive to preservative solution compared with $AgNO_3$ (B) and distilled water (A) on opened florets of *Gladiolus hybrida* cv. Peter pears spikes during two seasons I and II.

4. Number of deteriorated florets:

Generally, data presented in Fig. (2) Showed that the highest number of deteriorated florets was recorded in control treatment (distilled water). In contrast culture filtrate of *T. harzianum* significantly reduced the number of deteriorated florets in both seasons.

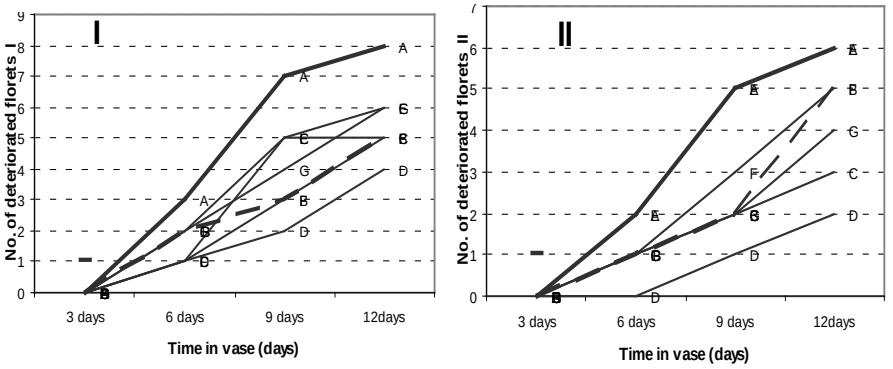

Fig. (2): The effect of culture filtrates of *Trichoderma viride* (C) , *T. harzianum* (D),*Epicoccum nigricum* (E), *E. purpurascens* (F) and *Bacillus subtilis*(G) as additive to preservative solution compared with $AgNO_3$ (B) and distilled water (A) on deteriorated florets of *Gladiolus hybrida* cv. Peter pears spikes during two seasons I and II.

5. Deteriorated/ opened flowers percentage:

A significant reduction occurred in deteriorated/ opened flowers percentage when either used treatments or $AgNO_3$ were added to the vase solution of gladioli spikes in both seasons (Fig. 3). The highest deteriorated/ opened florets percentage in the first season resulted from spikes treated with culture filtrate of *T. viride* and distilled water at the four records. Whereas, in the second one the highest deteriorated/ opened florets percentage were recorded on spikes treated with distilled water at four records and the lowest ones were recorded on spikes treated with culture filtrate of *T. harzianum*.

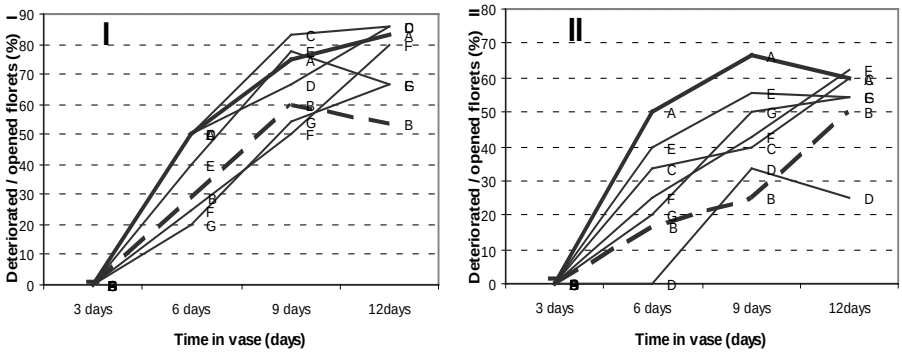

Fig. (3): The effect of culture filtrates of *Trichoderma viride* (C), *T. harzianum* (D),*Epicoccum nigricum*(E)*, E. purpurascens* (F) and *Bacillus subtilis*(G) as additive to preservative solution compared with $AgNO_3$ (B) and distilled water (A) on deteriorated/ opened florets percentage of *Gladiolus hybrida* cv. Peter pears spikes during two seasons I and II.

6. Spike fresh weight losses percentage and Total carbohydrates contents:

It is clear from data presented in table (3) that the highest fresh weight losses were observed when distilled water was used as a preservative solution for gladioli spikes in both seasons. There were significant differences among all used culture filtrates and $AgNO_3$ in most cases. The fresh weight of spikes treated with these treatments continued to increase then declined thereafter due to flower wilting. These results may be explained by the fact that these agents may be increased water absorption which allowed more increase in fresh weight on the contrast of this, distilled water increased bacterial population in the spike subsequently, it leads to vascular occlusion (Zagory and Reid, 1986). The vascular occlusion causes water stress and spike wilting which results in shorter vase life (Loon *et al.,* 1998).

It is evident from data in Table (3) that total carbohydrates contents in

gladioli spikes treated with distilled water as preservative solution were reduced compared with the treatments in both season. The highest carbohydrates contents (3.90 Mg/g D.W.) in the first season noticed with spikes treated with culture filtrate of *B. Subtilis*. Whereas culture filtrate of *Bacillus subtilis* followed by culture filtrate of *Epicoccum nigrum* and $AgNO_3$ were the best treatments at the second season. They recorded 3.72, 3.51 and 3.30 Mg/g D.W., respectively.

Table (3): The effect of culture filtrates of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* as additive to preservative solution on spike fresh losses (%) and total carbohydrates (Mg/g D.W.) of *Gladiolus hybrida* cv. Peter pears spikes.

Treatments	Spike F.W. losses (%)		Total carbohydrates (Mg/g D.W.)	
	1st season	2nd season	1st season	2nd season
Trichoderma viride	42.92 h	52.34 i	2.80 c	3.00 abc
T. harzianum	62.86 k	70.38 j	2.68 b	2.85 ab
Epicoccum nigrum	30.41 b	35.00 a	3.81 g	3.51 bc
E. purpurascens	50.02 i	40.39 d	2.91 d	3.00 abc
Bacillus subtilis	25.67 a	38.66 c	3.90 h	3.72 c
$AgNO_3$	56.00 j	50.77 h	3.87 gh	3.30 bc
Distilled water	72.64 l	78.80 k	2.57 a	2.25 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level.

B. Evaluation of the used CFs against gray mould rot caused by *Botrytis cinerea* in order to prolong vase life of gladioli spikes

At this trial the aforementioned culture filtrates were used to control of gray mould rot of gladioli spikes during two batches at 20±2˚C

B.1. Isolation, identification and pathogenicity tests of gladiolus pathogenic fungi:

Isolation trials were carried out on rotted gladioli spikes collected from different flowers market at Alexandria and Kafr El-Sheikh Governorates, Egypt. Results showed that the following fungi were isolated during this studied and identified as *Botrytis cinerea, Aspergillus* sp., *Penicilluim* sp., *Alternaria* sp., *Cladosporium* sp. and *Stemphyllium* sp. The high frequent isolated fungi was *B. cinerea* (67.4%) followed by *Penicilluim* sp. (15.3%), Aspergillus sp.(7.5%), *Alternaria* sp.(3.6%), *Cladosporium* sp.(3.2%) and *Stemphyllium* sp.(2.8%). Pathogenicity tests indicated that all isolated fungi were pathogenic to gladioli spikes causing various types of rots. This finding was in parallel with Cline and Bardsly (1984), McCain and Welch (1982) and Carre (1984). They reported that *Botrytis cinerea* is considered one of the most determinal postharvest disease, causing losses of horticultural crops.

B.2. *In vitro* experiments

The antifungal activity of culture filtrates of *Epicoccum nigricum, E. purpurascens, Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* at 10,20 and 30% on *Botrytis cinerea* was determined *In vitro* as inhibition percentages in the growth of mycelium. The inhibition of mycelial growth was tested by agar dilution technique.

It is quite clear from data presented in table (4) that the different used culture filtrates caused various degrees of inhibition to diameter growth of *B. cinerea.* The highest inhibition of diameter growth (100%) of *B. cinerea* was observed when culture filtrate of *B. subtilis* at 30% was added to media but the lowest inhibition of diameter growth (30%) was recorded when culture filtrate of *T. harzianum* at 10%. Generally, 30% culture filtrate caused highest inhibition of *B. cinerea* than 20% and 10%. Results also showed that culture filtrate of *B. subtilis*, *E. nigricum* and *E. purpurascens* were the best treatments in this respect.

Table (4): The inhibition percentage of diameter growth of *Botrytis cinerea* caused by culture filtrates of *Epicoccum nigrum*, *Epicoccum minitans*, *Trichoderma harzianum, Trichoderma viride* and *Bacillus subtilis*.

Treatments	Concentration (%)	Growth diameter (cm)	% of inhibition
Epicoccum nigrum	10	3.7	58.88 g
	20	2.1	76.66 k
	30	1.1	87.77 n
Epicoccum purpurascens	10	4.1	54.44 e
	20	2.6	71.11 j
	30	1.8	80.00 m
Trichoderma harzianum	10	6.3	30.00 b
	20	5.2	42.22 c
	30	3.8	57.77 f
Trichoderma viride	10	4.4	51.11 d
	20	2.9	67.77 i
	30	2.0	77.77 l
Bacillus subtilis	10	3.4	62.22 h
	20	1.8	80.00 m
	30	0.0	100.00 o
Control (distilled water)	-	9.0	00.00 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level

B.3. *In vivo* experiments

The three culture filtrates showed high antifungal activity (*E. nigricum, E. purpurascens* and *B. subtilis*) against *B. cinerea in vitro* were chosen to be used *in vivo* studies. They were used at three different concentrations (10, 20 and 30%). Sterile distilled water was used as control.

Data presented in table (5) indicated that vase-life of gladioli spikes was

significantly prolonged when sprayed with culture filtrates of *E. nigricum, E. purpurascens* and *B. subtilis* at 10, 20 and 30% after 6 hours from artificially inoculated with the causal agent of grey mould rot, *B. cinerea.* The longest (15.6 days) vase-life of gladioli spikes was recorded when treated with 30% culture filtrates of *B. subtilis* while the lowest vase-life (9.8 days) was observed when gladioli spikes sprayed with culture filtrates of *E. purpurascens* at 10% compared with control (7.2 days), (Table, 5).

Table (5): Effect of culture filtrate of *Epicoccum nigrum, Epicoccum purpurascens* and *Bacillus subtilis* on vase-life of *Gladiolus hybrida* cv. Peter pears spikes during storage at 20±2˚C

Treatments	Concentration (%)	Vase-life	
		1st batch	2nd batch
Epicoccum nigrum	10	10.4 c	10.9 c
	20	12.3 e	12.4 f
	30	13.8 h	14.1 h
Epicoccum purpurascens	10	10.1 b	9.8 b
	20	11.6 d	11.7 d
	30	12.9 f	13.2 g
Bacillus subtilis	10	11.6 d	12.2 e
	20	13.2 g	13.3 g
	30	15.3 i	15.6 i
Control (distilled water)	-	7.2 a	7.4 a

The number in the same column means followed by the same letter are not significantly different according to DMRT at 0.05 level

These inhibition of diameter growth of *B. cinerea* and control capability of the used culture filtrates to the causal agent grey mould rot of gladioli spikes could be explained according to; Andrews, (1985), Leifert *et al*.(1995), Kowall *et al*. (1998), Elad (1996) and Seddon and Schmitt, 1999) who reported that the principal mechanism of *B. subtilis* isolates is inhibition of the fungus by production of antibiotics such as surfactin, fengycin, mycosubtilin and bacillomycin. These compounds are amphilic, membrane active surfactants and peptide antibiotics with specific antimicrobial potential. Morilkawa *et al.,* 1992 and Peltola *et al.,* 2001 also reviewed that surfactin and pumilacidin are bioactive cyclic peptides produced by some *Bacillus* isolates. Similarly, *T. harzianum* and *T. viride* works through different mechanisms, i.e. production of gliotoxin and other metabolites (Abd El-Moity, 1981). An *Epicoccum* sp. has previously been shown to suppress *B. cinerea* on bean (Hannusch & Boland 1996). It was found that biocontrol efficacy was independent of the atmospheric environment when evaluated at temperatures ranging from 20-28°C and relative humidities ranging from 90-100%. Under these conditions, the isolate reduced grey mould by 100%. Mechanisms of action were attributed tocompetition for nutrients or antibiosis (Hannusch & Boland 1996).

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تقييم استخدام رواشح بعض المزارع الفطرية والبكتيريه كعوامل مقاومة حيوية لميكروبات محلول الحفظ والعفن الرمادى على الشماريخ الزهرية للجلاديولس صنف بيتر بيرز.

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الملخص العربى

اجرى هذا البحث بكلية الزراعة–جامعة كفر الشيخ على مجموعتين الاولى فى الثانى من يناير٢٠٠٨ والثانية فى ١٥ يوليو من نفس العام.

يتحكم فى مدة بقاء شماريخ الجلاديولس فى الفازة عدد من العوامل، اهم هذه العوامل التى تؤدى الى التدهور السريع للشماريخ بعد القطف هو عدم قدرتها على امتصاص الماء نتيجه لتعفن قاعدة الشمراخ وانسداد الاوعيه نتيجه نمو الكائنات الدقيقه بها. تم تقييم رواشح مزارع بعض الفطريات والبكتيريا مثل ايبيكوكم نيجركم *Epicoccum nigricum* ، ايبيكوكم بوربيوراسنس *E. purpurascens* ، تريكودرما فيريدى *Trichoderma viride* ، تريكودرما هارزيانم *Trichoderma harzianum*، باسيللس ستللس *Bacillus subtilis* كاضافات الى محلول الحفظ للحد من تاثير هذا المسبب مقارنة باضافة نترات الفضه بتركيز ١٠ جزء فى المليون.

أدى استخدام هذه الرواشح الفطرية والبكتيرية كإضافات إلى محلول الحفظ الى خفض معنوى فى عفن قاعدة الشمراخ عن طريق تقليل الكثافة الميكروبية في المحلول، كذلك قلل من تدهور الازهار. على العكس من ذلك، حدثت زيادة معنوية في كل من مدة بقاء الزهرة فى الفازة، تفتح الازهار، معدل امتصاص الشماريخ للماء عندما استخدمت كل من نترات الفضة، راشح مزارع بكتيريا *Bacillus subtilis* كإضافات إلى محلول الحفظ مقارنة بالكنترول. كذلك أوضحت النتائج انه بالرغم من تفوق بعض رواشح المزارع البكتيريه والفطرية مثل *Bacillus subtilis* ، *Epicoccum nigricum* على نترات الفضه فى بعض الصفات، إلا أن نترات الفضة أطالة عمر الشماريخ أكثر من الرواشح.

يعتبر العفن الرمادى المتسبب عن فطر بوتريتس سيناريا *Botrytis cinerea* من اخطر الامراض التى تصيب شماريخ الجلاديولس. تم اختبار تأثير الرواشح السابقة في مقاومة هذا المرض على شماريخ

الجلاديولس. حيث تم اختبار مقدرة هذه الرواشح على تثبيط نمو الفطر المسبب للمرض على مستوى المعمل عن طريق إضافة الرواشح المختلفة الى بيئة نمو الفطر بثلاثة تركيزات مختلفة هي ١٠، ٢٠، ٣٠%. اوضحت النتائج المعملية ان اكثر تثبيط لنمو هذا الفطر حدث عند اضافة راشح كل من *B. subtilis*، *E. nigricum*، *E. purpurascens* على التوالى الى بيئة نمو الفطر. لذلك فقد استخدمت هذه الرواشح الثلاثة بتركيزات ١٠، ٢٠، ٣٠% لمعاملة شماريخ الجلاديولس بعد العدوى الصناعية بالفطر *Botrytis cinerea* لمقاومة العفن الرمادى على الشماريخ المحفوظة على درجة حرارة ٢٠+٢ مْ . اوضحت النتائج ان رش هذه الرواشح الفطرية والبكتيرية على شماريخ الجلاديولس بتركيزات ٢٠، ٣٠ % أطال معنويا مدة بقاء الزهرة فى الفازة باكثر من ٦، ١٥ ، ٣ ،١٣يوم على التوالى مقارنة بالكنترول (٧ أيام). اتضح من النتائج ان راشح كل من البكتيريا *B. subtilis* والفطر *E. nigricum* بتركيزات ٢٠، ٣٠ % لعبا دوراً مهما فى منع تطور أعراض العفن الرمادى على شماريخ الجلاديولس.

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