Influences of Some Essential Oils on Vase-Life of Gladiolus hybrida, l. Spikes
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Abstract
Essential oils of clove, cinnamon, ginger, marjoram and fennel were evaluated against microorganisms grown in preservative solution of Gladiolus hybrida cv. Peter pears spikes compared with AgNO₃ at 10 ppm and distilled water. The essential oils were used as additives to preservative solution at 500 ppm. Data showed that, using essential oils in preservative solution significantly decreased the microbial density, spike base rot and deteriorated florets and increased spikes vase-life. The highest number of open florets and spikes water uptake was recorded when AgNO₃ and fennel oil were added to preservative solution as compared with control. Although some essential oils i.e. clove and fennel surpassed AgNO₃ in some characters, AgNO₃ prolonged the flower longevity in vase more than all essential oils.

Keywords: Gladiolus hybrida, essential oils, Biocontrol, vase-life.

Introduction
Gladioli (Gladiolus hybrida cv. Peter pears) is a genus of flowering bulbs in the iris Family (Iridaceae) which sometimes called the sword lily and native to South Africa. The species vary from very small to the spectacular giant flower spikes in commerce. The flowers are variously colored, pink to reddish or light purple with white, contrasting markings, or white to cream or orange to red. Among the most common reasons for early senescence of fresh cut spikes are both the inability of spikes to absorb water due to their rots, blockage and the short supply of carbohydrates to support respiration. Microorganisms specially bacteria which grown in preservative solution and play a great role in vase-life of flowers. The high bacterial counts in water reduced the longevity of the cut flowers [25, 11, 22, 17]. 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 and finally decreased longevity of flowers [13, 12]. 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 holding the flowers [10].

Essential oils have been investigated for their antimicrobial properties against some bacteria and fungi. The main constituents of the used essential are phenolic compounds, the antifungal mechanisms of some essential oils is due to the synthetic inhibition of DNA, RNA, Protein and polysaccarides [24, 15]. Oil constituents such as carvacrol and thymol are strong inhibitors of most pathogenic fungi [7]. Similarly, the volatile terpenes of some essential oils such as carvacrol, ρ-cymene and thymol are considered antifungal activity. Essential oils could be used in preservative solution as well as on fruits for controlling fungal and bacterial pathogens [9, 3, 4].

This research aims to study the effect of some essential oils as additives to preservative solution on the microbial density in preservative solution in order to prolong the vase-life of Gladioli spikes.

Materials and Methods
This trial was carried out to study the effect of some essential oils i.e., clove, cinnamon, ginger, marjoram and fennel as additive to preservative solution on microbial density, vase-life and quality parameters of gladioli spikes using two batches, the first on January 2nd and the second on Jul 15th during 2008.

I. Preparation of the used essential oils:
Essential oils were hydro distilled in a Clevenger's type apparatus for 6 hrs at the laboratory of Ornamental and Medicinal branch, Horticulture Department, Faculty of Agriculture, Kafir El-Sheikh University. The extracted oils were dried over anhydrous sodium sulphate to remove traces of moisture then stored in a refrigerator in the dark at 4 °C until use (European Pharmacopoeia procedure, 1983). Tween-20 "0.1% v/v" was used to dissolve the oils before adding to the preservative solution at 500 ppm [4].
II. 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. Gladioli spikes were held in glass bottles (3 flowers/bottle) containing 250 ml preservative solution including a constant glucose percentage (4%) with 10 cm depth. Then the following treatments were conducted:

1. Silver nitrate (AgNO₃) as a chemical preservative agent was used at 10 ppm of holding solution.
2. Each of essential oils of clove, cinnamon, ginger, marjoram and fennel was used alone at 500 ppm of preservative solution.
3. Untreated flowers held in preservative solution including a constant glucose percentage (4%) were employed as control treatment. Treatment was presented with three replicates (i.e., 9 cut flowers) spikes were held at room conditions (17±3°C) on January and 28±2°C on July with 60-70% and 70-80% relative humidity respectively. The following parameters were recorded:

1. Survey of microorganisms:

   Microbial survey of the flower stem surface was made just period to vase holding. Samples of lower stem parts (10 cm) were rinsed in 50 ml sterilized distilled water with shaking for 15 min. Screening of microorganisms in holding 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 [30] and malt agar medium for counts of fungi and yeasts [1]. 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, Kafr El-Sheikh University according to [2] for fungi, [5] for yeasts and [6].

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

3. Spikes vase life:

   The data of spikes were recorded four times with 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 of 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 beginning of the experiment. Also the loss in spike fresh weight with the time was determined.

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

   At the end of the experiment, spike total carbohydrates were determined according to [16]. Means between treatments were compared with Duncan’s Multiple Range Test [21].

Results and Discussion

1. Microbial survey and spike base rot:

   Using essential oils of clove, cinnamon, ginger, marjoram and fennel as preservative agents in holding solution have various effects on microbial counts (Table, 1). Generally, the used essential oils significantly reduced microbial density in holding solution compared with distilled water. The high inhibitor of microbial counts appeared when AgNO₃ at 10 ppm was added to holding solution followed by clove oil at 500 ppm in this respect when compared with the other used essential oils and distilled water. Data presented in Table (1) showed that numerous microorganisms could be isolated from spike surface and preservative solution. In respect of dominance, on the spike surface yeasts came first followed by bacteria and then fungi. The most frequent genera of fungi which isolated from spike surface and preservative solution belonged to *Botrytis*, *Fusarium*, *Mucor*, *Rhizopus*, *Aspergillus* and *Cladosporium*. While, the most isolated genera of the yeasts were *Rhodotorula* and *Torulopsis*. The used essential oils were significantly minimizing microbial counts in preservative solution specially when essential oil of clove was added to preservative solution at 500 ppm. This finding may be due to that essential oils contained specific components or antifungal compounds as phenol agents that can
inhibit the growth of certain microorganisms \cite{14}. Generally, the bacterial percentages exceed those of fungi in the preservative solution of the control treatment.

Table (1): Effect of essential oils of clove, cinnamon, ginger, marjoram and fennel on microbial counts (CFU/ml) in preservative solution of Gladiolus hybrida spikes.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Microbial counts</th>
<th>Inhibition (%)</th>
<th>Spike base rot (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fungi</td>
<td>yeasts</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Clove oil</td>
<td>43</td>
<td>267</td>
<td>178</td>
</tr>
<tr>
<td>Cinnamon oil</td>
<td>56</td>
<td>306</td>
<td>204</td>
</tr>
<tr>
<td>Ginger oil</td>
<td>62</td>
<td>359</td>
<td>212</td>
</tr>
<tr>
<td>Marjoram oil</td>
<td>49</td>
<td>289</td>
<td>176</td>
</tr>
<tr>
<td>Fennel oil</td>
<td>76</td>
<td>414</td>
<td>232</td>
</tr>
<tr>
<td>AgNO3</td>
<td>2</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Distilled water</td>
<td>166</td>
<td>1271</td>
<td>342</td>
</tr>
</tbody>
</table>

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

This may suggests that sap exudates of plant materials in preservative solution and/or the vase environment may enhance the bacterial growth rather than the fungal growth \cite{23}. Reviewing the isolated microorganisms, it was observed a qualitative but not quantitative resemble between those isolated from 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 \cite{18}. The obtained results confirmed that the preservative agents extended the vase-life of gladiolus spikes by acting as a microbial inhibitor in the preservative solution \cite{25}.

2. Vase life and water uptake of gladiolus spikes:

Data in Table (2) show that all used treatments have prolonged gladiolus spikes vase life in both batches compared with distilled water. The longest vase life is obtained from holding spikes in preservative solution containing AgNO3, clove oil, marjoram and cinnamon oils, since they recorded 15.67, 13.67, 12.67 and 12.00 days, respectively in the first batch. Whereas, the best treatments in the second batches were AgNO3, clove and cinnamon oil.

In respect of water uptake, data in Table (2) revealed that, the cumulative water absorption with the time was highest with holding spikes in preservative solution containing AgNO3, clove oil, fennel oil, marjoram oil and cinnamon oil which gave 220.33, 220.00, 215.33, 210.33 and 200.67 ml, respectively in the first batch however, the treatment of AgNO3 followed by marjoram oil then clove oil in the second batch gave 231.00, 215.33 and 207.00 ml, respectively. This ability to inhibition of microorganisms resulted in prolong vase-life of gladiolus spikes. AgNO3 prolonged the flower longevity more than used essential oils. This may be due to an alternative role of AgNO3 as inhibitor to ethylene production and action \cite{19}.

Table (2): The effect of clove, cinnamon, ginger, marjoram and fennel essential oils as additive to preservative solution on vase life and water uptake of Gladiolus hybrida spikes.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Vase life (days)</th>
<th>Water uptake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st batch</td>
<td>2nd batch</td>
</tr>
<tr>
<td>Clove oil</td>
<td>14.33 f</td>
<td>11.00 ef</td>
</tr>
<tr>
<td>Cinnamon oil</td>
<td>12.00 e</td>
<td>10.33 de</td>
</tr>
<tr>
<td>Ginger oil</td>
<td>9.33 c</td>
<td>10.00 de</td>
</tr>
<tr>
<td>Marjoram oil</td>
<td>12.67 e</td>
<td>9.67 d</td>
</tr>
<tr>
<td>Fennel oil</td>
<td>8.67 be</td>
<td>8.00 c</td>
</tr>
<tr>
<td>AgNO3</td>
<td>15.67 g</td>
<td>14.67 h</td>
</tr>
<tr>
<td>Distilled water</td>
<td>7.00 a</td>
<td>6.33 a</td>
</tr>
</tbody>
</table>

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 of essential oils to the vase preservative solution significantly increased the number of opened florets over distilled water at the four record times in both batches. Also, some treatments were similar to or less than AgNO3 in both batches. The highest number
of opened florets noticed on treated spikes with marjoram oil after 3, 6 or 9 days either in the first batch or second batch.

4. Number of deteriorated florets:

Data presented in Fig. (2) showed that the highest number of deteriorated florets was recorded in control treatment (distilled water). In contrast, clove and cinnamon oils significantly reduced the number of deteriorated florets in both batches.

Fig. (1): The effect of clove (H), cinnamon (I), ginger (J), marjoram (K) and fennel (L) essential oils as additive to preservative solution as compared with AgNO₃ (B) and distilled water (A) on opened florets of gladiolus spikes during two batches I and II.

Fig. (2): The effect of clove (H), cinnamon (I), ginger (J), marjoram (K) and fennel (L) essential oils as additive to preservative solution as compared with AgNO₃ (B) and distilled water (A) on deteriorated florets of gladiolus spikes during two batches I and II.

Fig (3): The effect of clove (H), cinnamon (I), ginger (J), marjoram (K) and fennel (L) essential oils as additive to preservative solution compared with AgNO₃ (B) and distilled water (A) on deteriorated/ opened flowers percentage during two batches I and II.

5. Deteriorated/ opened florets percentage:
A significant reduction occurred in deteriorated/ opened florets percentage when either used essential oils treatments or AgNO₃ were added to the vase solution of gladiolus spikes in both batches (Fig. 3). The highest deteriorated/ opened florets percentage resulted from treated spikes with distilled water at the four records. Whereas, the lowest percentage were obtained from spikes treated with clove oil and cinnamon oil at 6 days.

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

Data in Table (3) clear that the highest fresh weight losses were recorded when distilled water was used as a preservative solution for gladiolus spikes in both batches. There were significant differences among all used essential oils and AgNO₃ in most cases. The fresh weight of spikes holed in preservative solution including essential oils 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, and it leads to vascular occlusion [25]. The vascular occlusion causes water stress and spike wilting which results in shorter vase life [17].

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spike F.W. losses (%)</th>
<th>Total carbohydrates (Mg/g D.W.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st batch</td>
<td>2nd batch</td>
</tr>
<tr>
<td>Clove oil</td>
<td>33.11 d</td>
<td>34.52 a</td>
</tr>
<tr>
<td>Cinnamon oil</td>
<td>31.08 c</td>
<td>42.75 f</td>
</tr>
<tr>
<td>Ginger oil</td>
<td>37.10 f</td>
<td>41.92 e</td>
</tr>
<tr>
<td>Marjoram oil</td>
<td>40.55 g</td>
<td>36.87 h</td>
</tr>
<tr>
<td>Fennel oil</td>
<td>35.40 e</td>
<td>47.61 g</td>
</tr>
<tr>
<td>AgNO3</td>
<td>56.00 j</td>
<td>50.77 h</td>
</tr>
<tr>
<td>Distilled water</td>
<td>72.64 l</td>
<td>78.80 k</td>
</tr>
</tbody>
</table>

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

It is evident from data in Table (3) that total carbohydrates contents in gladiolus spikes treated with distilled water as preservative solution were reduced compared with the other treatments in both batches. The highest carbohydrates contents in the first batch noticed with spikes treated with clove oil followed by ginger oil and AgNO₃ as recorded 4.03, 3.90 and 3.87 mg/g D.W., respectively. Whereas clove oil and AgNO₃ were the best treatments at the second one. They recorded 3.44 and 3.30 mg/g D.W., respectively.

References