Influence of Foliar Spray with Paclobutrazol or Glutathione on growth, Flowering and Chemical Composition of *Calendula officinalis* L. Plant.

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**Abstract:** The objective of this study was to evaluate the effect of using paclobutrazol or glutathione with levels of 0, 50, 100 and 150 mg/l on plant growth, flowering and some chemical composition of *Calendula officinalis* L. plant grown at the greenhouse of National Research Centre, Giza, during two successive seasons of 2003-2004 and 2004-2005. The obtained data indicated that foliar application of most paclobutrazol treatments significantly decreased plant height compared with control treatment while number of branches, fresh weight and dry weight of leaves per plant were increased. All growth parameters were significantly promoted by foliar application of glutathione. In most collections foliar application of glutathione at 150 mg/l gave the highest significant increase in number of flowers/plant, fresh weight of flowers (g/plant) and dry weight of flowers (g/plant) followed by paclobutrazol 100 mg/l compared with untreated plants. Foliar application of glutathione at 150 mg/l recorded the highest values of chlorophyll a and chlorophyll b. However, paclobutrazol and glutathione showed no significant increase in carotenoids contents. The maximal value of total carbohydrates (37.64%) was obtained when 150 mg/l glutathione was applied followed by paclobutrazol (31.42%) at level of 100 mg/l and total nitrogen percentage had the same trend.

**Key words:** *Calendula officinalis* L., paclobutrazol (PCB), glutathione (GSH), Growth, Yield, Chemical composition.

**INTRODUCTION**

Genus *Calendula* includes 15 species, native to Central Europe and the Mediterranean region, is commonly cultivated in India[7]. *Calendula officinalis* L family Asteraceae, commonly known as marigold is one of the most well-known and versatile herbs in western medicine[8]. It is grown widely across Europe and North America as an ornamental and medicinal plant. This species is known to have anti-septic and anti-inflammatory activities. It is an aromatic, erect, annual herb up to 1.5 m in height[9]. In the traditional system of medicine, the bright orange petals are an excellent remedy for inflammation and skin diseases[10] and antiseptic[11]. Marigold is an important winter flowering annual plant that is mainly used in planting flowerbeds in different types of gardens. In addition to its landscape use and as a source of colour, it is also one of the main sources of natural yellow pigments used in several industries and in the bird-feed for poultry production[12]. Plant growth regulators are widely used for modifying plant growth and development of many agricultural crops. Paclobutrazol has been found to inhibit, specifically, the three oxidative steps of the gibberellin precursor ent-kaurene to ent-kaurenoic acid[13]. Thus, paclobutrazol blocks the biosynthesis of the active gibberellin GA$_1$[14] and therefore, decreases plant growth and development. The morphological response to paclobutrazol is the reduction in internode length and this effect has been observed in herbaceous[22]. Paclobutrazol has proven its efficacy in the reduction of height and promotion of flowering and fruiting in pear by increasing number of spurs/branch. A part from reduction in plant height, paclobutrazol increased leaf N, P, Ca and Mg[4]. Paclobutrazol increases number of flowers and their longevity in chrysanthemum[28]. Glutathione is the most important non-protein thiol present in cell animal as well as in plants and bacteria. It was discovered by Hopkins in 1921 and identified as tripeptide γ-L-glutamyl-L-cysteinylglycine. In plants, the physiological significance of glutathione may be divided into two categories: sulfur metabolism and defense. Glutathione is the predominant non-protein thiol and it is an important pool of reduced sulfur[23] and it regulates sulfur uptake at root level[19]. Reduced glutathione and reduced ascorbate, the two major water-soluble antioxidants in photosynthetic and non-photosynthetic tissues, reacting directly or indirectly with reactive oxygen species[6,9]. Contribute to maintain the integrity of cell structures and the proper functions of various metabolic pathways[31]. In addition to its effects on
expression of defense genes[11,32], glutathione may also be involved in redox control of cell division[24]. Glutathione plays a crucial role in controlling and maintaining the intracellular redox state[21].

The aim of the present work is to evaluate the influence of foliar application with different concentrations of paclobutrazol or glutathione individually on vegetative growth parameters and the main constituents of Calendula officinalis L. plant were taken into consideration.

MATERIALS AND METHODS

Pot experiment were carried out at the green house of the National Research Centre, Cairo, Egypt during two successive seasons (2003-2004, 2004-2005).

Calendula officinalis L. seeds were secured from Medicinal and Aromatic Plants Research Section, Ministry of Agriculture. Seeds were planted in pots (30 cm in diameter) filled with 12 kg of loamy clay soil (mechanical analysis: sand 24%, silt 47 % and clay 29 %) at 15th and 10th October 2003 and 2004, respectively in complete randomized design with three replicates. Each replicate consisted of three plants. Water requirements were regularly fulfilled according to weather conditions. Each pot was fertilized twice with 1.5 g nitrogen as ammonium nitrate (33.5% N) and 1 g potassium sulfate (48% K2O). These fertilizers were applied at 30 and 60 days from sowing. Phosphorous as calcium superphosphate (15.5% P2O5) was mixed with soil before sowing and control sprayed with distilled water.

Foliar application of paclobutrazol and glutathione was carried out two times (45 and 55 days from sowing) as foliar spray to cover completely the plant foliage. The flowers were collected weekly starting 85 days after sowing. Meanwhile, two plant samples were taken from each treatment (at 75 and 150 days from sowing).

Plant heights (cm), number of branches, fresh and dry weights (g/ plant) of leaves were recorded.

The leaves were dried in an electrical oven at 50 °C till constant dry weight before chemical analysis, while the photosynthetic pigments were also determined in fresh green leaves (on dry matter basis) at vegetative growth. Chlorophyll a, b and total carotenoids were determined according to the methods described by v.Wettstein[31]. Total carbohydrate in the dried material was determined by using phenol sulphuric acid method[12]. Total nitrogen was determined (on a dry matter basis) using the modified Micro Kjeldahl method according to A.O.A.C.[1].

Data of the two successive seasons were subjected to statistical analysis of variance and the combined analysis for the two seasons were calculated according to Snedecor and Cochran[29], since the results showed the same leaning.

RESULTS AND DISCUSSIONS

Effect on vegetative growth: Data presented in Tables 1 and 2 indicate that foliar application of all paclobutrazol treatments on Calendula officinalis L. plants significantly decreased plant height compared with control treatment except plants received 50 mg/l at first sample that did not show significant effect on plant height. On the other hand, number of branches, fresh weight and dry weight of leaves per plant were increased by paclobutrazol application in the first and second samples compared with control treatments. The highest values were obtained in plants treated with 100 mg/l. The decrease in plant height may be explained as paclobutrazol blocks the biosynthesis of the active gibberellin GA1[33] so the morphological response to paclobutrazol is the reduction in internode length and this effect has been observed in herbaceous[22]. Our results are in agreement with Singh[26] who reported that the shortest plants on marigold and the highest number of secondary branches per plant, fresh leaf biomass and dry leaf biomass were obtained with 10 and 15 mg paclobutrazol per plant. Also he added that paclobutrazol could be used at 10 and 15 mg per plant to reduce the height of pot plants and to increase leaf biomass for pharmaceutical preparation.

Table 1: Effect of paclobutrazol and glutathione on the vegetative growth of Calendula officinalis L. plant at the first sample. (average of two seasons).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height/ plant</th>
<th>No. of branches</th>
<th>F. W. of leaves/plant</th>
<th>D.W. of leaves/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>28.4</td>
<td>3.67</td>
<td>33.1</td>
<td>3.22</td>
</tr>
<tr>
<td>P 50</td>
<td>27.2</td>
<td>5.33</td>
<td>45.1</td>
<td>3.53</td>
</tr>
<tr>
<td>P100</td>
<td>25.5</td>
<td>8.33</td>
<td>62.8</td>
<td>5.77</td>
</tr>
<tr>
<td>P150</td>
<td>24.4</td>
<td>6</td>
<td>44.1</td>
<td>3.53</td>
</tr>
<tr>
<td>G50</td>
<td>34.5</td>
<td>4.67</td>
<td>69.43</td>
<td>7.15</td>
</tr>
<tr>
<td>G100</td>
<td>37.6</td>
<td>5</td>
<td>76.5</td>
<td>7.22</td>
</tr>
<tr>
<td>G150</td>
<td>39.6</td>
<td>7.33</td>
<td>94</td>
<td>8.6</td>
</tr>
<tr>
<td>L.S.D</td>
<td>1.71</td>
<td>1.54</td>
<td>4.69</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 2: Effect of paclobutrazol and glutathione on the vegetative growth of Calendula officinalis L. plant at the second sample (average of the two seasons).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height/plant</th>
<th>No. of branches</th>
<th>F. W. of leaves/ plant</th>
<th>D. W. of leaves/ plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>39.23</td>
<td>6.33</td>
<td>171.67</td>
<td>35.56</td>
</tr>
<tr>
<td>P 50</td>
<td>36.4</td>
<td>8</td>
<td>179.35</td>
<td>38.93</td>
</tr>
<tr>
<td>P100</td>
<td>32.27</td>
<td>11.67</td>
<td>192.51</td>
<td>42.09</td>
</tr>
<tr>
<td>P150</td>
<td>29.1</td>
<td>9.33</td>
<td>158.54</td>
<td>33.66</td>
</tr>
<tr>
<td>G50</td>
<td>42.57</td>
<td>7.67</td>
<td>182.47</td>
<td>39.54</td>
</tr>
<tr>
<td>G100</td>
<td>44.63</td>
<td>8.33</td>
<td>208.3</td>
<td>43.4</td>
</tr>
<tr>
<td>G150</td>
<td>48.57</td>
<td>9.67</td>
<td>234.03</td>
<td>47.29</td>
</tr>
<tr>
<td>L.S.D</td>
<td>1.66</td>
<td>1.1</td>
<td>10.29</td>
<td>2.82</td>
</tr>
</tbody>
</table>
Regarding to the effect of glutathione, data in Tables (1and 2) emphasized that all growth parameters (plant height, number of branches and consequently leaves fresh and dry weight) of *Calendula officinalis* were significantly promoted by foliar application of glutathione especially at 150 mg/l. The same trend was observed in the second sample (Table 2). Foliar application of glutathione (150 mg/l) surpassed pacloputrazol (100 mg/l) treatments for all growth parameters at first and second sample. In this concern, Noctor *et al.*[20] reported that the amino acid cysteine is a component of the antioxidant glutathione. The influence of cysteine availability on glutathione levels reflects the importance of glutathione as a reservoir of reduced sulfur[4]. Also Häussl[3] suggested that glutathione functions as a storage pool for excess cysteine and principal form in which organic sulfur is transported in many plants[5]. These results are in agreement with those reported by Talaat and Aziz[20] who found that the application of glutathione at 50, 100 and 200 mg/l significantly increased plant height, number of branches / plant, fresh and dry weights of *Matricaria chamomilla*.

**Effects on flowering growth:** Data in Table (3) reveal that foliar application of paclobutrazol and glutathione treatments on *Calendula officinalis* L. plants significantly increased number of flowers/plant, fresh weight of flowers (g/plant) and dry weight of flowers g/plant compared with control treatments through cuts of Januarys, Februarys and Marches. Foliar application of glutathione at 150 mg / l gave the highest significant increase in flowering growth followed by paclobutrazol 100 mg/l compared with untreated plants. The comparison between paclobutrazol and glutathione reveal the superiority of glutathione at all levels of application. On the other hand, at April cuts, all treatments showed opposite trends except glutathione at 150 mg/l that recorded 111.9%, 108.8% and 112.1% as the number of flowers/plant, fresh weight of flowers (g/plant) and dry weight of flowers (g/plant), respectively relative to control treatment.

In support of these results, significant influence of paclobutrazol in increasing number of flowers has been experimentally substantiated by de Baerdemaeker *et al.*[10] and Singh *et al.*[28]. Paclobutrazol at 20 mg/plant significantly increased number of flowers in the first and
second flush and also total number of flowers/plant, however, fresh and dry weight of flowers/plant were found maximum with 40 mg paclobutrazol/plant drench followed by 20 mg/plant[27]. Also, Singh[28] reported that Paclobutrazol at 10 mg/plant could be applied to enhance flowering and increase the yield of large seeds.

Regarding to glutathione, our results could be explained by the findings obtained by Häusler[14] who suggested that glutathione functions as a storage pool for excess the amino acid cysteine. Buwalda et al.[3] indicated that the promotion effect of glutathione might be due to its effect as a reservoir of reduced sulfur as the amino acid cysteine which is a component of the antioxidant glutathione and is the principal form in which organic sulfur is transported in many plants[23].

Effects on chemical composition: Data presented in Table (4) show that foliar application of paclobutrazol and glutathione significantly affected the photosynthetic pigments content of the leaves of Calendula officinalis plants. Foliar application of paclobutrazol significantly increased chlorophyll a and chlorophyll b at 100 mg/l and glutathione at 150 mg/l, which recorded the highest values of chlorophyll a and chlorophyll b. On the other hand, no significant increase was found in carotenoids contents.

In this concern, Noctor and Foyer[21] found that glutathione plays a crucial role in controlling and maintaining the intracellular redox system. Hell and Bergmann[17] reported that efficient regulation of glutathione pool is thought to be particularly important in chloroplast metabolism, in which it provides the redox buffering capacity vital for efficient photosynthesis and is involved in processing the oxidizing that are inevitably formed as a result of light capture and subsequent electron transport.

With respect to the response of total carbohydrates to paclobutrazol and glutathione, data in table (4) reveal such stimulatory effect on the accumulation of total carbohydrates in the plants of calendula, e.g. the percentage was 26.30% in case of untreated samples and increased sharply to 33.51% in case of 50 mg/l glutathione treatment and reached maximum 37.64% by increasing the concentration to 150 mg/l. However in case of paclobutrazol treatments the maximal value of total carbohydrates (31.42%) was obtained when 100 mg/l was applied. Total nitrogen percentage had the same trend by application of paclobutrazol and glutathione. Enhancement effects of paclobutrazol and glutathione on growth parameters are in line to the results obtained by Mehouachi et al.[19] who reported that foliar spray with paclobutrazol increased total carbohydrate concentration of shoots and roots of citrange rootstock seedlings in comparison with the control. Talaat and Aziz[30] reported that foliar application of glutathione to chamomile plants significantly increased total sugars as well as total nitrogen percentages in the herb.

Thus, it could be recommend that foliar application with glutathione (150 mg/l) and also paclobutrazol (100 mg/l) individually to improve the growth, flowering and in turn the value of Calendula officinalis chemical composition.

Histological Abbreviations:

- c.c. : Chromatin Clumps.
- f.e. : Follicular Epithelium.
- f.s. : Free Sperms.
- p.n. : Pycnotic nuclei.
- s.b. : sperm bundles.
- sc. : spermatocytes.
- sg. : spermatagonia.
- st. : spermatids.
- s.v. : seminal vesicle

REFERENCES