

Research Article

Salicylic acid an emerging growth and flower inducing hormone in marigold (*Tagetes sp. L.*)

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Abstract

Salicylic acid (SA) is an emerging plant growth regulator that acts as signaling molecule in plants under biotic and abiotic stresses. SA also exerts a stimulatory effect on different physiological processes of plant growth but its association with leaf pigments and flowering is less known. Current experiment was conducted to evaluate the effect of exogenous application of different doses of SA on marigold (*Tagetes sp.*) in greenhouse condition. Marigold (*Tagetes sp. L.*) plants were randomly divided in 4 groups and treated exogenously with four different concentrations of SA (T₀: 0 (only water), T₁: 40, T₂: 80 and T₃: 120 mg/L). The solutions were sprayed on aerial parts of plant after 60 days of sowing. Results analysis showed that T₃ (120 mg/L SA solution) showed maximum number of leaves plant⁻¹ (30.38), highest plant height (50.63 cm), more number of inflorescence, greater stem diameter (7.84 mm), maximum fresh weight of flowers (11.90 g), and maximum dry weight of flower (1.25 g). Whereas, minimum number of leaves (22.74), lowest plant height (40.8 cm), less number of inflorescence, smaller stem diameter (4.75 mm), minimum fresh flower weight (7.13 g), and minimum dry flower weight (0.7 g) were observed in T₀. Furthermore, various leaf pigments were found higher in T₃. Present study concluded that T₃ treatment of SA improved leaf pigments and morphometric parameters in Marigold. From the aforementioned results, it is suggested that 120 mg/L concentration of SA should be sprayed exogenously before flowering stage, on marigold plants for better growth and flower production.

Keywords: Flower production; Growth variables; Leaf pigments; Marigold; SA concentration; *Tagetes spp*

Introduction

Marigold is the most commonly grown ornamental plant, botanical nomenclature is *Tagetes erecta* L. and locally known as Genda, which belongs to family *Asteraceae*

or *Compositae*. It is extensively used for general purposes, religious functions and other ceremonies [1]. It is usually grown in pots or in beds for mass display as well as in mixed borders for decorative purpose

[2]. Marigold has lots of varieties, varying in plant height, flower size, yield and quality. Its flowers are usually big with globular shaped [3, 4]. Cultivation of marigold is easy due to its wide adaptability to various soils and climatic conditions. It is an annual economic plant species utilized in raw or in processed forms in modern medicinal industry, worldwide. In addition to the edible uses (i.e. coloring and flavoring agent of food), marigold also contain active ingredients and compounds that have wide applications in nylons and manufacturing dyes industries [5] and in pharmacy [6]. Active ingredients of marigold are produced and stored in its flowers, most important of which are water-soluble carotenoid, flavonoids, essential oil, and mucilaginous compounds [7]. However, flavonoids of inflorescences play a key role in the pharmacological activity and in most cases they are categorized for quercetin and rutin compounds [3, 4]. Its seeds contain 15-20 % oil, 45-60 % of which constitute calendic acid [8].

Salicylic acid (SA) is a plant hormone and act as an antioxidant, produced by root cells. It play a crucial role in regulating certain physiological processes in plants such as growth, germination, photosynthesis and ion absorption and act as an important signaling molecule to various environmental stresses [9]. SA also contributes in the regulation of biological processes in plants and is accepted as endogenous growth regulator due to its phenolic nature [10]. It play a key role in thermogenesis (heat generation in staminate region of flower up-to 14°C compared to normal) in Arum lily as natural inductor, which encourages flowering in many angiosperm's such as *Annonaceae*, *Araceae*, *Aristolochiaceae*, *Cyclanthaceae*, and *Nymphaeaceae* family plants. It controls ion uptake by roots and create fragrance in flower to attract insect for pollination [10, 11]. The experimental results of previous researchers showed influence of SA in regulation of gene expression signals in the passage of Arabidopsis leaf senescence.

Besides this, SA might function as a gravitropism inhibition regulator of fruit ripening [12]. SA that play a key role in plant growth regulation and development is actually a hormone-like substance [10, 11] which defensive effects in contrast to abiotic stress factors such as deadly metals [13], low temperature, heat stress and oxidative harm [14] has been confirmed. The role of SA in bringing salt tolerance has been studied in many plant species. It is also reported that SA bring tolerance from salinity in tomato crop [15], carrot [16] and changes its expression in plants in response to different environmental stresses [9, 12]. We predict that exogenous application of SA will improve the leaf pigments and subsequent growth parameters and hence flowering will be enhanced. The main objective of this investigation was to study the effect of new generation hormone (SA) on the vegetative growth and flowering of marigold and to know the effect of SA on leaf pigments and production of marigold flower, because the role of SA in defensive response is widely explored but its role in leaf pigments and flowering was neglected.

Materials and methods

Experimental site

A research was held under uniform condition in a greenhouse at Directorate of Floriculture, DHRD, National Agricultural Research Center, Islamabad (33.6701° N latitude, 73.1261° E longitude).

Experimental procedure

Seeds of marigold (*Tagetes sp*) were brought from Gurr Mandi Peshawar, grown in trays under green net in semi shade, without temperature control. Experiment was laid out in a completely randomized design and 48 plants were randomly divided in 4 groups (T₀, T₁, T₂ and T₃), each group contain 12 plants and 4 replicates (n=3). After 35 days, the seedlings were transplanted into 5 L pots filled with dry leaf mold, soil and sand in a ratio of 1:1:1 (v/v/v). The pots were placed in green house and irrigated daily to rescue humidity of the substrate throughout the experiment. Spraying of SA (Aldrich, St.Louis, MO,

USA) was performed on day 60 of seed sowing (before the reproductive stage) on aerial parts of marigold. The spray was repeated after 1 week (day 67). The 4 different concentrations of SA solutions in T₀, T₁, T₂ and T₃ were 0, 40, 80 and 120 mg/L, respectively [17]. A total of 100 ml solution was sprayed on each plant, each time. The inflorescences were initiated and harvested after 90 days of sowing (DAS) with the appearance of the 1st flowers held twice in a week until plant senescence (120 DAS). The data regarding various variables in each treatment was calculated and average was taken. Studied traits include, number of leaves plant⁻¹, which were calculated by counting total no of leaves per plant. Plant height (cm) was noted from the soil surface to the tip of the plant, measured by measuring tape. Number of inflorescence was observed by counting total number of flowers plant⁻¹. Stem diameter (mm) was calculated at the base of the stem with vernier caliper [7]. Fresh weight of flower (g) was noted with digital balance (Shimadzu, model AY220, Japan), while dry weight of flower (g) was determined by drying inflorescences in an oven at 40°C with air circulation until constant weight was achieved [5].

Leaf pigments

Leaf pigments were determined according to the procedure reported in [18, 19] and using the following equations.

$$\begin{aligned} \text{Chlorophyll-a } (\mu\text{g/ml}) \\ &= 12.25A_{663.6} - 2.55A_{646.6} \end{aligned}$$

$$\begin{aligned} \text{Chlorophyll-b } (\mu\text{g/ml}) \\ &= 20.31A_{646.6} - 4.91A_{663.6} \end{aligned}$$

$$\begin{aligned} \text{Total chlorophyll } (\mu\text{g/ml}) \\ &= 17.76A_{646.6} + 7.34A_{663.6} \end{aligned}$$

$$\begin{aligned} \text{Carotenoids } (\mu\text{g/ml}) \\ &= 4.69A_{440.5} - 0.267\text{Chl-a} \\ &\quad + \text{Chl-b} \end{aligned}$$

Statistical analysis

The data recorded for various variables was subjected to analysis of variance (ANOVA) suitable for completely randomized design using statistics 8.1 software package (Statistix®, Analytical Software Inc, Tallahassee FL, USA). Significant findings were tested by least significant difference

(LSD) [13]. $P < 0.05$ was considered significant [20].

Results and discussion

Number of leaves plant⁻¹

Leaves play a very important role in photosynthesis which results in an increased yield. Our findings indicate that a different concentration of SA influences the number of leaves per plant. The highest number of leaves at each harvest were recorded in treatment T₃ (30.38), followed by T₂ (26.7), which was at far with T₁ (25.33) while lowest number of leaves (22.74) was recorded in control (T₀) (Figure 1A). SA due to its defensive aspect induced a protective mechanism in plants physiology under unfavorable environment, especially in response to different pathogens and abiotic stresses. SA fixed functions of certain enzymes directly and defensive control genes also induce precise changes in chloroplast structure and leaf number which play a vital role in plant energy status. Subsequently, plant uses two photo-systems that reduce NADPH and generate ATP thus used enough energy to form organic compounds (assimilates), translocation and storage of which enable plants to increased number of leaves beneficially. SA is a phenolic nature compound and its application on zinnia produced profound increase in number of leaves. Moreover, it is involved in regulation of growth processes of plants, such as in ornamental plants, and stimulate leaves in young shoots [21]. Similarly, [5] revealed that number of leaves in African violet increases with higher concentration of SA.

Plant height (cm)

Height of marigold plant was influenced by various levels of SA foliar application. Our result showed that maximum plant height was observed in treatment T₃ (50.63 cm), followed by T₁ (47.13 cm) and T₂ (44.1 cm). While, minimum plant height (40.8 cm) was noted in plants treated with (T₀) tap water (Figure 1B). SA is a phenolic compound that enables plants to survive under challenging soil and environmental

situations. SA plays key roles in regulation of various physiological and developmental processes of plants [21]. Different concentration of SA increases most of nutritional and hormonal regulation in plants [22]. Increased plant height in marigold with the application of SA could be the increased rubisco chemical action and photosynthetic rate. According to [23],

SA cause an increase in plant growth with increasing cell division in both stem and root, hence increasing plant height (~23%) under greenhouse and field condition. Furthermore, foliar application of SA treatment on African violets increased length of petioles and improved height in onion [7].

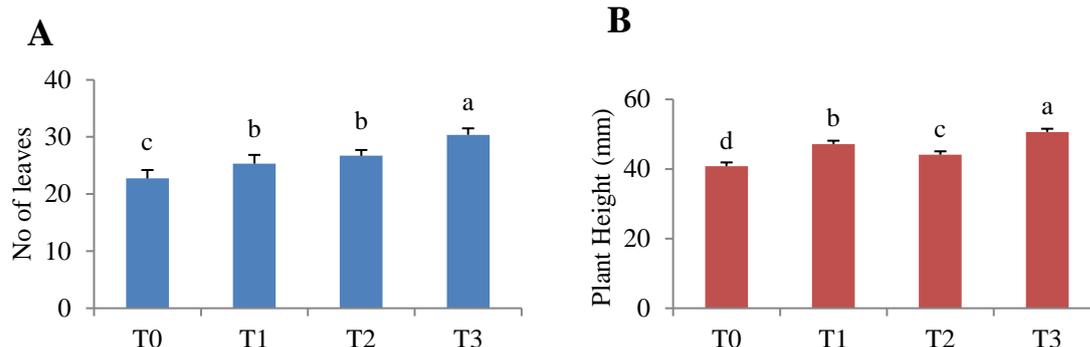


Figure 1. Effect of exogenous spray of different levels of SA on (A) Number of leaves and (B) plant height (cm). Data presented as ^{a-d} Means ± SD, bars lacking a common superscript differ significantly from one another ($P \leq 0.05$). T₁, T₂, T₃ are different treatment groups while T₀ is control

Stem diameter (mm)

The data presented in (Figure 2A) revealed highly significant result for stem diameter in marigold plant exposed to different level of SA. Highest stem diameter was recorded in T₃ (7.84 mm) followed by T₂ (6.5 mm) and T₁ (5.7 mm). While the thinnest stem diameter was recorded in treatment T₀ (4.75 mm). The increase in stem diameter by applying SA may be attributed to the enhanced absorption of ions and minerals by plant. SA also improves plant performance by formation of certain enzymes in plant, thus stimulating chlorophyll synthesis and photosynthetic activities, which progresses plant growth [9]. Hence, application of SA concentrations causes an increase in stem diameter (Figure 2A) and plant height (Figure 1B) [24]. Similarly, [25] reported that different treatments of acetyl SA on potato plants encouraged plant growth and number of leaves per plant.

Number of inflorescence plant⁻¹

SA foliar application showed significant

difference for number of inflorescence per plant (Figure 2B) in marigold (*Tagetes sp.*). Maximum number of inflorescence was recorded in treatment T₃ (9.7) followed by T₂ (7.7) and T₁ (6.33) while minimum number of inflorescence was noted in T₀ (5.00). These results are in line with [5] who reported that increased concentration of SA results in greater number of inflorescences per plant. SA is considered as new generation hormone, which induces thermogenesis in staminate region of flower up to 14°C, that induces and boost flowering in plant [10, 11]. These results are in agreement with those of [27] who have studied the effect of exogenous SA application on growth of *Calendula officinalis* under salinity stress. Similarly, [5] described that foliar application of SA in Saintpaulia, cause an increase in the number of flowers. SA enhances transcription and translation of mRNA and protein [26] that help in developing new groups of isozymes enhance the number of flower buds [27].

Fresh flower weight (g)

Fresh weight of inflorescence of marigold was influenced by spraying various concentration of SA solution on marigold (*Tagetes sp*). Maximum fresh weight was recorded in treatment T₃ (11.90 g) followed by T₂ (10.1 g) and T₁ (8.3 g), while minimum fresh flower weight (7.13 g) was recorded in T₀ (Figure 2C). According to [28], SA might have changed the bio-physical characteristics of plant cell wall. SA and auxin have a synergistic effect to promote photosynthesis and favored translocation of phot-assimilates into flowers. Present results are in agreement with the results of [5] in marigold and [29] in tuberose. Furthermore, SA enhances cell division in stem and leaves which is a leading cause of increase in number of inflorescence [7].

Dry flower weight (g)

Application of exogenous SA also influenced significant variations in dry weight of inflorescence. Maximum dry weight of marigold flower was recorded in treatment T₃ (1.25 g) followed by T₂ (1.09 g) and T₁ (1.01 g), while minimum dry weight was recorded in T₀ (0.7 g) (Figure 2D). Application of SA significantly increased the dry weight of flower by improving photosynthetic efficiency [9], stabilization of chlorophyll and assimilates translocation from source to sink [27] which ultimately enhanced dry weight of flower [28]. Furthermore, SA acts as defense hormone that could reduce the abiotic stress in leaves which ultimately leads to increase amount of dry matter contents production in marigold flowers [30]. Similar results were also reported by [31] in marigold.

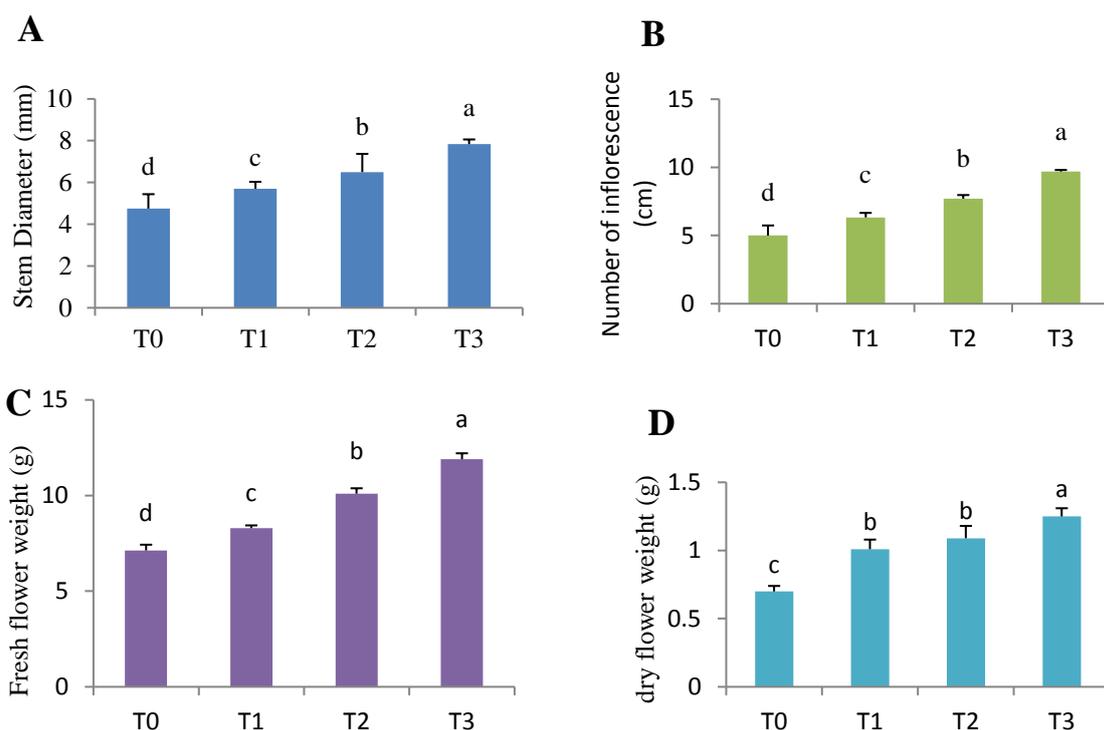


Figure 2. Effect of exogenous spray of different levels of SA on (A) stem diameter (mm) (B) number of inflorescence (cm) (C) fresh flower weight (g) and (D) dry flower weight (g). Data presented as ^{a-d} Means ± SD, bars lacking a common superscript differ significantly from one another ($P \leq 0.05$). T₁, T₂, T₃ are different treatment groups while T₀ is control

Leaf pigments

Effect of SA exogenous application on leaf pigments is shown in (Figure 3). It is evident from the (Figure 3A) that there is a positive association between SA application and chlorophyll-a content of leaf. Higher chlorophyll-a content ($20.62 \mu\text{g}\cdot\text{ml}^{-1}$) was recorded in T₃, while lower ($13.87 \mu\text{g}\cdot\text{ml}^{-1}$) was recorded in T₀. Furthermore, statistical analysis showed a significant increase with increase in level of SA application. Effect of exogenous application of SA on leaf chlorophyll-b pigment is shown in (Figure 3B). An increase in level of SA application significantly increases chlorophyll-b content. However highest chlorophyll-b content was recorded in T₃ ($16.58 \mu\text{g}\cdot\text{ml}^{-1}$) as compared to T₀ ($5.88 \mu\text{g}\cdot\text{ml}^{-1}$). Furthermore, leaf total chlorophyll pigments were also influenced by SA application. A positive association between leaf total chlorophyll content and SA were observed. Moreover, higher trend of total

chlorophyll ($37.21 \mu\text{g}\cdot\text{ml}^{-1}$) was observed in T₃, while lower in T₀ ($19.76 \mu\text{g}\cdot\text{ml}^{-1}$) (Figure 3C). The carotenoid contents were also increased by SA application on marigold plants as shown in (Figure 3D). In comparison with T₀ ($1.07 \mu\text{g}\cdot\text{ml}^{-1}$), T₃ showed higher concentration of leaf carotenoid pigments ($7.67 \mu\text{g}\cdot\text{ml}^{-1}$). SA enhances cell division in leaf surface [32] and effect photosynthetic pigments and their derivatives [33, 34] which have a direct relationship with cell division and leaf pigment contents [35]. SA molecules also increased respiration rate and production of energy for synthesis of more pigments. SA increases stomatal transpiration which is responsible for regulating growth, production, green color in foliage and flowering [11]. The pathway of biosynthesis of photosynthetic pigments and their derivatives are linked together and have direct co-relationship with hormones [36].

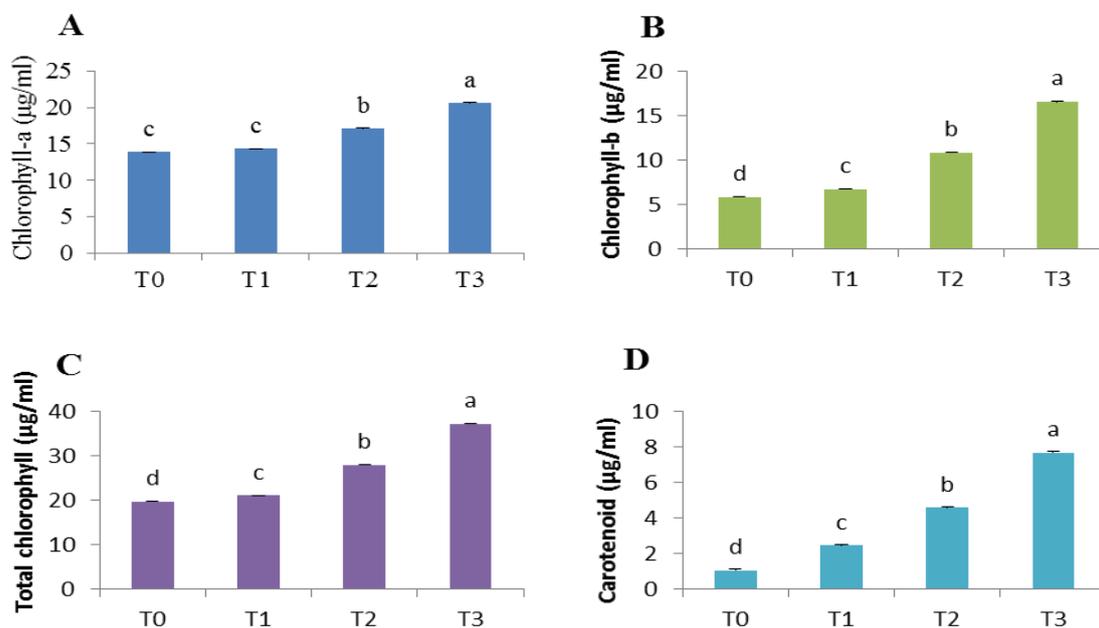


Figure 3. Effect of exogenous spray of different levels of SA on (A) Leaf chlorophyll-a ($\mu\text{g}/\text{ml}$) (B) Leaf chlorophyll-b ($\mu\text{g}/\text{ml}$) (C) Leaf total chlorophyll ($\mu\text{g}/\text{ml}$) and (D) Leaf carotenoid ($\mu\text{g}/\text{ml}$). Data presented as ^{a-d} Means \pm SEM, bars lacking a common superscript differ significantly from one another ($P \leq 0.05$). T₁, T₂, T₃ are different treatment groups while T₀ is control

Conclusions

In this study, results showed that SA plays an important role in plant growth, influencing leaf pigments and enhancing flower quality. The best result was recorded in treatment T₃ (120 mg/L) closely followed by T₂ (80 mg/L). This study provides deep understanding and new role of new generation hormone (SA) in flower induction, leaf pigments, and growth of marigold plant, thus could help the researchers to investigate the molecular approach in future research.

Authors' contributions

Conceived and designed the experiments: A Basit & K Shah, Performed the experiments: A Basit, K Shah, MA Khalid & F Khan, Analyzed the data: A Basit, K Shah, MU Rehman, L Xing & I Ahmed, Contributed materials/ analysis/ tools: A Basit, K Shah & N Alam, Wrote the paper: A Basit, K Shah, X Zuo & M Han.

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