



Biochemical and Physiological Response of Marigold (*Tagetes Erecta* L.) to Foliar Application of Salicylic Acid and Potassium Humate in Different Soil Growth Media

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Abstract

Marigold flowers have great importance in the horticultural sector as an ornamental plant that is used not only in flavoring of foods, but is also important in the pharmaceutical and medicinal industries. The current study was carried out in pots under greenhouse conditions to evaluate the response of marigold plants to foliar application of salicylic acid (SA) and potassium humate (KH) using two growth media. Physiological and biochemical parameters were extensively evaluated along with the oil and beta carotene production potential of marigold plants. Different concentrations of SA (50, 100, 150, and 200 mg L⁻¹) and KH (500, 1000, 1500, and 2000 mg L⁻¹) were applied as foliar application in different soil growth media. Applied SA and KH treatments significantly increased all studied vegetative growth parameters except plant height, which remained nonsignificant. Enzymatic antioxidant activities were also enhanced under applied treatments of SA and KH. These activities were increased in the case of KH up to 1500 mg L⁻¹, whereas this trend was recorded up to 200 mg L⁻¹ in the case of SA with the exception of catalase. Photosynthetic parameters and chlorophyll fluorescence were decreased with increased doses of SA up to 200 mg L⁻¹, but this trend was typically the opposite for KH in both growth media. The oil productivity of marigold flowers increased up to 100 and 1000 mg L⁻¹ of SA and KH, respectively, whereas the highest oil yield was recorded after application 1000 mg L⁻¹ of KH. Therefore, production of marigold flowers could be achieved using poor growth media like sand, and further studies concerning different alternative growth media, particularly ecofriendly and cheap materials, are needed.

Keywords Antioxidants · Beta-carotene · Catalase · Peroxidase · Electrolyte leakage

Data Availability Statement

Not applicable

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Biochemische und physiologische Reaktion der Ringelblume (*Tagetes erecta* L.) auf die Blattapplikation von Salicylsäure und Kaliumhumat bei unterschiedlichen Bodensubstraten

Zusammenfassung

Ringelblumen haben im Gartenbau eine große Bedeutung als Zierpflanze, die nicht nur zum Aromatisieren von Lebensmitteln, sondern auch in der pharmazeutischen und medizinischen Industrie verwendet wird. Die vorliegende Studie wurde in Töpfen unter Gewächshausbedingungen durchgeführt, um die Reaktion von Ringelblumenpflanzen auf die Blattapplikation von Salicylsäure (SA) und Kaliumhumat (KH) unter Verwendung zweier Kultursubstrate zu bewerten. Physiologische und biochemische Parameter sowie die potenzielle Öl- und β -Carotin-Produktion der Ringelblumenpflanzen wurden eingehend untersucht. Verschiedene Konzentrationen von SA (50, 100, 150 und 200 mg l⁻¹) und KH (500, 1000, 1500 und 2000 mg l⁻¹) wurden als Blattapplikation bei verschiedenen Bodensubstraten ausgebracht. Die Behandlungen mit SA und KH führten zu einem signifikanten Anstieg aller untersuchten vegetativen Wachstumsparameter mit Ausnahme der Pflanzhöhe, die nicht signifikant blieb. Auch die enzymatischen antioxidativen Aktivitäten wurden durch die Anwendung von SA und KH erhöht. Diese Aktivitäten waren im Falle von KH bis zu 1500 mg l⁻¹ erhöht, während dieser Trend im Falle von SA bis zu 200 mg l⁻¹ verzeichnet wurde, mit Ausnahme der Katalase. Die photosynthetischen Parameter und die Chlorophyll-Fluoreszenz nahmen mit steigender SA-Dosis bis zu 200 mg l⁻¹ ab, während dieser Trend bei KH bei beiden Nährböden typischerweise umgekehrt war. Die Ölproduktivität der Ringelblumenblüten nahm bis zu 100 bzw. 1000 mg l⁻¹ SA und KH zu, während der höchste Ölertrag nach Anwendung von 1000 mg l⁻¹ KH verzeichnet wurde. Daher könnte die Produktion von Ringelblumenblüten auch mit schlechten Kultursubstraten wie Sand erreicht werden, und es sind weitere Studien zu verschiedenen alternativen Kultursubstraten, insbesondere zu umweltfreundlichen und kostengünstigen Materialien, erforderlich.

Schlüsselwörter Antioxidantien · β -Carotin · Katalase · Peroxidase · Elektrolytleckage

The marigold or African marigold plant (*Tagetes erecta* L.) belongs to the Asteraceae family, a widespread and very large family of flowering plants, and this species of the genus *Tagetes* is native to Mexico (Yasheshwar et al. 2017). The marigold plant has a high propagation rate, rapid growth, and short lifecycle, and this ornamental plant is usually used in the cut-flower trade (Chitraprabha and Sathyavathi 2018). Due to its high beta carotene content, marigold can be added to poultry feed to improve egg yolk pigmentation, fat, and skin (Laosinwattana et al. 2018; Madanan et al. 2021). The bloom concentrate of marigold can also be used for cotton fabrics (Harlapur et al. 2020) or floral wastes for compost and biofuel production (Dutta and Kumar 2022). Extracted nano-based lutein from the petals of marigold is used as surfactant (Jivan and Abbasi 2019). The flowers of marigold are used in folk medicine as a therapeutic agent (Barhoi et al. 2022) or as health promoters for gastrointestinal diseases such as stomatitis, diarrhea, and dyspepsia, and for skin protection, (Meurer et al. 2019; Rodrigues et al. 2019; Kumar et al. 2020a; Gul et al. 2022).

There are several species of marigold, including the common marigold (*Calendula officinalis*), French marigold (*Tagetes patula*), marigold (*Tagetes erecta*), lemon marigold (*Tagetes tenuifolia*), and Mexican mint marigold (*Tagetes lucida*; Chitrakar et al. 2019). These marigold plants are considered one of the most popular edible flowers from ancient times, which are added to the world cuisine for

desirable flavor and fragrance in foods and beverages as well as for making visually plain dishes more appealing (Chitrakar et al. 2019). These edible flowers are considered to be a source of natural antioxidants (Mikołajczak et al. 2020; Gul et al. 2022), valuable phytonutrients (Skrajda-Brdak et al. 2020), bioactive compounds (Takahashi et al. 2020) like carotenoids (Rodrigues et al. 2019), and functional raw materials (Chen et al. 2020). Therefore, applied growth promoters like salicylic acid (SA) and potassium humate (KH) can enhance the nutritional value of marigold flowers.

Several amendments, such as SA and KH, have been used to promote horticultural production and increase growth of crops like marigold. The main effects of applied SA and KH in agricultural production may include their impacts as stimulators. Several studies on exogenous application of SA have confirmed that SA is an important regulator of various biochemical and physiological processes related to plant growth and development (El-Beltagi et al. 2017; Ali 2021; Ding and Ding 2020; Gorni et al. 2020; El-Hady et al. 2021). These included studies on the effect of SA on accumulation of primary metabolites in many plants such as yarrow (*Achillea millefolium* L.; Gorni and Pacheco 2016; Gorni et al. 2020), black mustard (*Brassica nigra*; Ghassemi-Golezani et al. 2020), and *Ajuga integrifolia* (Abbasi et al. 2020); and studies on the mitigating role of SA under drought stress (de Andrade et al. 2020; Sedaghat et al. 2020), nutrient stress (Deus et al.

2020; Es-sbihi et al. 2020), salinity stress (Abdoli et al. 2020), and pathogen stress (Sofy et al. 2021). Concerning the impact of applied SA on marigold plants, a few published studies have investigated the SA-induced changes to growth, flowering, and flavonoid production (Pacheco et al. 2013) and shown it to promote hormone content, growth, and flowering in marigold (Basit et al. 2018), and to reduce the effects of salinity (Abou El-Ftouh et al. 2018; Abd El Gayed 2020) and drought (Gholinezhad 2020; Mohamed et al. 2021).

Humic substances are considered the main pool of soil organic carbon (more than 60%), which results from microbial transformations of plant and animal matter (Stevenson 1994). These humic substances can regulate plant growth and development under stressful and non-stressful conditions (Bulgari et al. 2019). Treating plants with humate is considered a promising approach, leading to improvements in seed germination, root growth, adventitious rooting, shoots and leaves, uptake of nutrients and water, and activity of enzymatic antioxidants (Aalipour et al. 2020; Torun and Toprak 2020). Potassium humate (KH) is a commercial product resulting from the reaction of potassium salt with humic acid to produce complex humic substances (Howladar 2018; Torun and Toprak 2020). Exogenous seed dressing (Ullah et al. 2020) or soil (Abdo et al. 2020) or foliar application of KH improved plant productivity (Shyala et al. 2019). Previous studies have investigated exogenous application of potassium humate on marigold, e.g., Shyala et al. (2019), and there is an urgent need for more investigations concerning this topic.

Production of seedlings in horticultural nurseries is a very important process, particularly for production of expensive seedlings and difficult-to-germinate seeds (Chrysargyris et al. 2020). The components of the growth medium are very important for this production, and a special growth medium is needed particularly when the available soils are contaminated or suffer from problems like salinity, alkalinity, heavy clay, or other conditions. In addition, foliar application of applied stimulants or nutrients is, in this case, better than soil application for overcoming these obstacles. Therefore, good growth media should provide seedlings with adequate support, nutrients, and water, allowing exchange of oxygen between the roots and the outside atmosphere as well as high cation-exchange and water-holding capacity (Mahmoud et al. 2019; Roehrdanz et al. 2019; El-Beltagi et al. 2022; Shalaby et al. 2022).

Several materials have been applied as candidate culture substrates for growth media and were incorporated into the media in various ratios, such as peat with different forms, e.g., cocopeat (Ameri et al. 2020) and peat moss (Roehrdanz et al. 2019), as well as perlite (Rady and Rehman 2016; Feng et al. 2020), sycamore pruning waste (Ameri et al. 2020), vermiculite (Sato et al. 2020), and sand (Mahmoud et al. 2019), beside solid organic waste including compost from waste (Roehrdanz et al. 2019), biochar (Prasad et al. 2018), biochar–compost mixture (Radin et al. 2018), and spent coffee grounds (Chrysargyris et al. 2020). Therefore, a suitable planting medium is considered a basic requirement to achieve a proper yield and high profits as well as having a vital impact on the quality of seedlings and their productivity (Mahmoud et al. 2019).

Therefore, the aim of this study was to evaluate the role of foliar application of SA and KH on marigold under conditions of different soil growth media. The efficacy of SA and KH in promoting physiological and biochemical parameters of marigold plants also was investigated.

Materials and Methods

Plant Materials and Preparation of Growth Media

Seeds of the local marigold variety (Mandarin) were obtained from the Research Centre of Medicinal and Aromatic Plants, Dokki, Giza, Egypt. Seedlings of marigold (35 days old) 15 cm in length were produced from pure seeds in the nursery of the Faculty of Agriculture at Kafrelsheikh University (latitude 31°15'47 N and longitude 30°57'14 E). Seeds were cultured in a foam tray (209 cells) in the presence of cocopeat and vermiculite (1:1; v/v) as a popular growth medium (M2), which is expensive compared to cocopeat and sand (1:1; v/v) as an alternative and cheap medium (M1). Seedlings were cultured in plastic pots 25 cm in diameter filled with cocopeat+sand or cocopeat+vermiculite as two different treatments according to germination or growth medium. Pots were cultured on 14 March 2019 and kept under black net (50% shading). Seedlings were watered using fresh water manually every 5 days using 10L watering cans, and the same water volume was applied to each pot. The plants were harvested by 1 June 2019.

Table 1 Main chemical analysis of growth media in the experiment

Growth medium	pH	EC (dS m ⁻¹)	Organic matter (g kg ⁻¹)	Available nutrients (mg kg ⁻¹)					
				N	Fe	K	Zn	Cu	P
M1	7.58	0.30	15.6	252	10.5	546	46.5	0.77	13.59
M2	6.44	0.41	18.0	308	43.45	1452	60.5	1.49	19.64

M1 cocopeat and sand, M2 cocopeat and vermiculite, EC electrical conductivity

Analysis of Soil Growth Media

The analyses of growth media, including chemical analyses, were performed before the experiment (Table 1). Soil electrical conductivity (EC) or salinity was measured in a 1:5 ratio using an EC meter (MI 170, Milano, Italy), whereas pH was measured in a 1:1 ratio using a pH meter (Jenway 3510, Staffordshire, UK). Organic matter content was determined according to Nelson and Sommers (1996) using the dichromate oxidation method. According to Bremner and Mulvaney (1982), the available N was determined using the micro-Kjeldahl method, whereas available phosphorus was measured according to Olsen and Sommers (1982). Available potassium was determined by flame photometer (Jenway PFP7, Staffordshire, UK), whereas available copper, iron, and zinc were quantified using an atomic absorption spectrophotometer (Avanta E, GBC, Victoria, Australia) according to Page et al. (1982).

Treatments of Growth Promoters

After 10 days from transplanting, seedlings were treated separately with two growth promoters at different concentrations: for SA 0, 50, 100, 150, and 200 mg L⁻¹; for KH 0, 500, 1000, 1500, and 2000 mg L⁻¹. KH was purchased from FMC, China (water solubility >98%, humic acid 80%, potassium 8–12% [K₂O], zinc 100 ppm), whereas SA purchased from Loba Chemie pvt. Ltd., India (purity 99.5%). Seedlings were foliar sprayed with growth promoters three times during the growth season at an interval of 15 days. Growth parameters including plant height, number of branches, root length (the longest root), and root fresh and dry weight were recorded at the end of the experiment on 1 June. Six samples or plants from each treatment were chosen randomly to determine the previous parameters for three replicates.

Analysis of Photosynthetic Parameters

Chlorophyll *a* (Chl *a*) and *b* (Chl *b*) and carotenoids were determined in the fully expanded young leaves. Chlorophyll content was extracted from leaf tissue by grinding to a fine powder in a mortar with liquid nitrogen and adding 100 mg to a 2 mL Eppendorf tube. Thereafter, 1 mL of 80% acetone was added and the powder was homogenized by inverting for 10 min in ice using a shaker. The absorbance was measured at 470, 649, and 665 nm using a spectrophotometer (double-beam UV/visible spectrophotometer Libra S80PC, Cambridge, UK). Chlorophyll and carotenoid concentrations were calculated from the spectrophotometric data using the formulae of Lichtenthaler and Welburn (1983). There were three replicates within each treatment. Chlorophyll fluorescence parameters were

measured using a portable chlorophyll fluorometer (OS30P, Labo Amirica, USA). The minimal and maximal fluorescence (F_0 and F_m) were measured for 30 min in dark-adapted leaves using light of <0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and after a 1 s saturating pulse (>3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the same leaves, respectively. According to Dewir et al. (2005), maximal variable fluorescence ($F_v = F_m - F_0$) and the photochemical efficiency of photosystem II (F_v/F_m) were calculated for dark-adapted leaves. Each treatment had four single-leaf replications.

Biochemical Assessment of Antioxidant Enzymatic Activities

To determine antioxidant enzyme activities, 0.5 g of fully expanded young leaves was homogenized in liquid nitrogen with 3 mL of extraction buffer (50 mM TRIS buffer pH 7.8 containing 1 mM EDTA-Na₂ and 7.5% polyvinylpyrrolidone) using a prechilled mortar and pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant, which was re-centrifuged at 12,000 rpm for 20 min at 4°C, was used for the total soluble enzyme activity assay using an ultraviolet-160A spectrophotometer (160 A, Shimadzu, Kyoto, Japan). These enzymes included catalase (CAT; EC 1.11.1.6), polyphenol oxidase (PPO; EC 1.10.3.1), and peroxidase (POX; EC 1.11.1.7) activities. These enzymes were measured at 240, 495, and 470 nm, respectively, according to Aebi (1984), Malik and Singh (1980), and Hammer-schmidt et al. (1982).

Electrolyte Leakage

Measurement of electrolyte leakage (EL) was performed according to Whitlow et al. (1992) and Szalai et al. (1996). The initial values of EC were recorded using an Acromet AR20 EC meter (Fisher Scientific, Chicago, IL, USA), whereas the final values were measured for each flask to calculate the EL for each bud as the initial conductivity/final conductivity $\times 100$.

Oil Content and Yield in the Plant

The oil content in marigold plants was measured by hydrodistillation in the Clevenger apparatus using dried plant (flower + leaves + shoots). After 3 h of extraction, the oil yield of each plant was detected by the oil volume collected in the burette. Oil percentage was calculated according to following formula:

$$x = \frac{\text{Oil weight (g)}}{\text{Plant weight (g)}} \times 100$$

Table 2 Effect of different growth media, salicylic acid, and potassium humate at different concentrations on some plant growth parameters

Treatment	Plant height (cm)		Number of flowers per plant		Number of branches per plant	
	M1	M2	M1	M2	M1	M2
<i>Salicylic acid dose (mg L⁻¹)</i>						
0	81.6 def	82.8 cdef	49.50 bc	49.00 bc	7.7 e	13.0 ab
50	79.5 fg	68.5h	57.50 bc	80.50 ab	11.3 bcd	13.0 ab
100	77.3 fg	78.8fg	56.90 bc	30.40 c	7.3 e	12.3 abc
150	83.0 cdef	75.5 fgh	55.20 bc	41.00 c	9.0 de	12.0 abc
200	73.5 gh	89.5 bc	32.30 c	33.20 c	9.0 de	12.0 abc
<i>Potassium humate (mg L⁻¹)</i>						
0	81.6 def	82.8 cdef	49.50 bc	49.00 bc	7.7 e	13.0 ab
500	92.6 b	82.6 cdef	100.20 a	23.50 c	14.7 a	9.0 de
1000	104.3 a	88.3 bcde	93.50 a	27.90 c	14.3 a	11.0 bcd
1500	81.5 ef	89.1 bcd	63.10 bc	25.00 c	7.7 e	8.7 de
2000	81.8 def	80.3 fg	80.90 ab	30.30 c	10.0 cde	12.0 abc
<i>Significance</i>	**	**	**	**	**	**

Means within a column for one medium followed by the same letter are not significantly different according to Duncan’s multiple range test at $P \leq 0.05$

SA salicylic acid, KH potassium humate, M1 cocopeat + sand, M2 cocopeat + vermiculite

Biochemical Analysis of Flowers

According to the method of Nagata and Yamashita (1992), β -carotene was determined in flowers by separately homogenizing 1.0g fresh weight of the sample with 10ml of an acetone–hexane mixture (2:3) for 2 min to uniform mass. Samples were maintained in an ice–water bath to prevent overheating. The homogenates were centrifuged at 5000rpm for 10min at 20°C. Using a spectrophotometer (Varian Cary 50 Scan, UV/VIS spectrophotometer, USA), the absorbance spectrum of each supernatant was measured and the absorption maxima were read at 453, 505, 645, and 663 nm. β -carotene content was calculated from the following equation:

$$\beta - \text{carotene}(\text{mg } 100 \text{ ml}^{-1}) = 0.216 A_{663} - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453}$$

Statistical Analyses and Experimental Design

The experiment was set up in a completely randomized factorial design with two factors (two types of media and nine levels of SA and KH) and two-way analysis of variance (ANOVA). The means and ANOVA were calculated using CoStat (version 6.311) statistical CoHort software (Berkeley, CA, USA). The mean separations were carried out using Duncan’s multiple-range tests. Significance was determined at $p \leq 0.05$.

Results

Plant Growth Parameters

Marigold growth parameters investigated in the current study included plant height and the total number of branches and flowers per plant in two different growth media. The growth media also clearly impacted on the growth parameters of marigold based on the concentration of SA or KH, as presented in Table 2. The highest significant values in number of flowers and branches per plant (100.2 and 14.7) were recorded for the growth medium M1 at 500mg L⁻¹ KH, whereas this trend was obtained at 50–100mg L⁻¹ in the case of SA application (Table 2). A different trend was observed for the growth medium M2: applied SA recorded the highest values (80.5 and 13.0) of flowers and branches per plant at a dose of 50 mg L⁻¹ SA.

The best results of marigold growth parameters were associated with lower concentrations of SA and KH for both growing media (M1 and M2), i.e., 50mg L⁻¹ for SA and 1000mg L⁻¹ for KH. In general, the values of growth parameters were higher for M2, which contains vermiculite, compared to M1. The M2 growth medium (cocopeat + vermiculite) recorded better results under foliar application of SA and KH compared to M1 (cocopeat + sand). The highest number of flowers per plant (102.2 and 80.5) was recorded for M1 and M2 at applied concentrations of 500 and 50mg L⁻¹ of KH and SA, respectively.

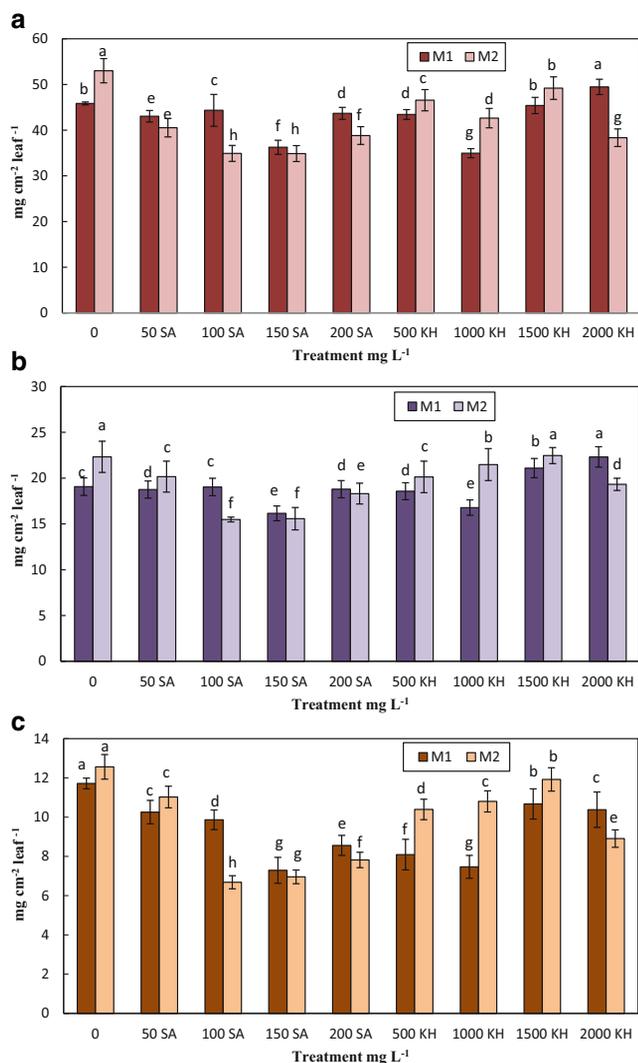


Fig. 1 Effect of different growth media (*M1* and *M2*) and salicylic acid (*SA*) and potassium humate (*KH*) at different concentrations on chlorophyll and carotenoid content of marigold plants: **a** chlorophyll *a*, **b** chlorophyll *b*, **c** carotenoids (*M1* = cocopeat + sand; *M2* = cocopeat + vermiculite); least significant difference (medium \times treatment) for Chl *a*, *b*, and carotenoids were 3.90, 3.20, and 1.90, respectively, with high significance for each

Photosynthetic Parameters and Chlorophyll Fluorescence

In this study, quantitative measures of photosynthetic parameters (i.e., Chl *a*, *b*, and carotenoids) were considered to be useful in investigating the response of marigold plants to different doses of SA and KH under two growth media conditions (Fig. 1). Concerning SA, the leaf content of Chl *a*, *b*, and carotenoids was decreased by increasing the applied dose up to 200 mg L⁻¹, whereas these parameters were increased by increasing the applied concentration of KH up to 1500 mg L⁻¹. It is also worth mentioning that the alternative growth medium (*M1*) shows a continuous increase in these

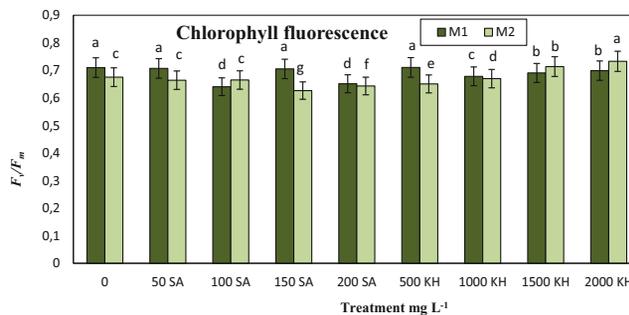


Fig. 2 Effect of different growing media (*M1* and *M2*) and salicylic acid (*SA*) and potassium humate (*KH*) at different concentrations on photochemical efficiency of photosystem II (F_v/F_m) of marigold plants (*M1* = cocopeat + sand; *M2* = cocopeat + vermiculite); least significant difference (medium \times treatment) for chlorophyll fluorescence was 0.10 with no significance

parameters upon increasing KH up to 2000 mg L⁻¹, whereas in the ideal growth medium (*M2*), this increase was only up to 1500 mg L⁻¹. This difference may be due to the impact of vermiculite on the plants and their nutritional value. In general, the same trend could be observed for carotenoids, where there was a continuous decrease in carotenoid content with increasing applied doses of SA in both of the two different growth media, whereas the opposite was recorded for the applied KH in both media. It could be noticed that the values of Chl *a*, *b*, and carotenoids were the same in different growth media (*M1* and *M2*) with an increasing applied dose of SA, but at the highest applied concentration of KH (2000 mg L⁻¹), the curve leveled off, with a higher value for *M1* compared to *M2*. The same trend was also observed for chlorophyll fluorescence, as presented in Fig. 2 at different concentrations, on F_v/F_m of marigold plants for each growth medium.

Enzymatic Antioxidant Activities and Electrolyte Leakage

The general observation from Fig. 3 is that growing medium *M2*, which contains vermiculite, has higher values of antioxidants compared to *M1* (sand growth medium). These results may confirm that these foliar promoters (SA and KH) do not stress candidate plants. In general, for each growth medium, the enzymatic antioxidants were decreased with increasing applied SA concentration (like carotenoids), whereas they were increased for KH application. From the data in Fig. 3, it is apparent that more studies under different stressful conditions are required. The studied enzymatic antioxidant activities are known antioxidants that plants generate under non-desirable or stress conditions and the plants generate high contents of these enzymes, which represents a result of stress from the treatments. The enzymatic antioxidant activities, including CAT, POX, and PPO activities,

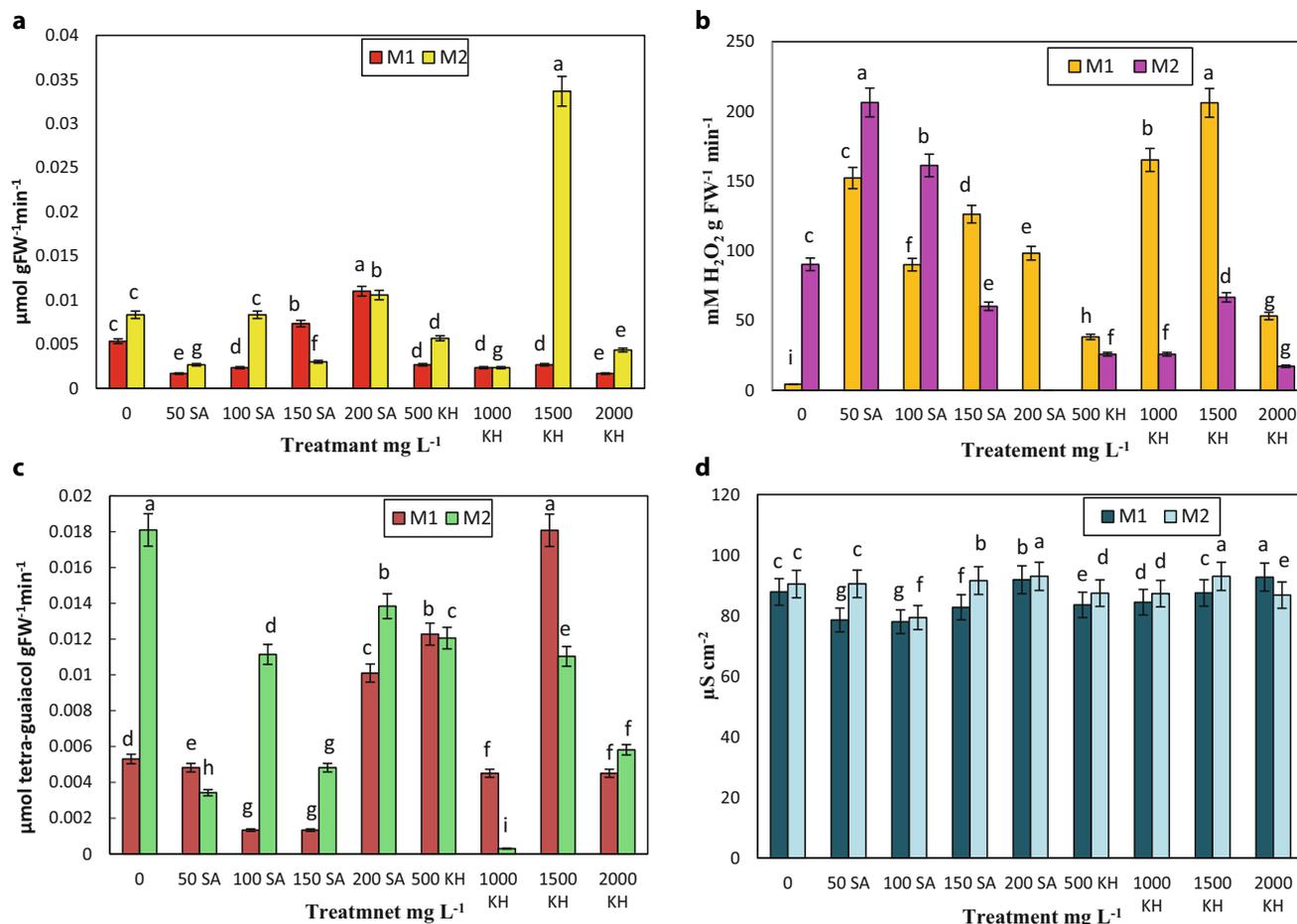


Fig. 3 Effect of different growing media (*M1* and *M2*) and salicylic acid (*SA*) and potassium humate (*KH*) at different concentrations on level of antioxidant enzyme activities and electrolyte leakage of marigold plants, **a** polyphenol oxidase (*PPO*), **b** catalase (*CAT*), **c** peroxidase (*POX*), **d** electrolyte leakage (*M1* = cocopeat + sand; *M2* = cocopeat + vermiculite); least significant difference (medium \times treatment) for electrolyte leakage was 8.90 with no significance, whereas for *PPO*, *CAT*, and *POX* is was 0.003, 10.86, and 0.002, respectively, with high significance for each

in addition to EL, were measured in this study as presented in Fig. 3. To distinguish among treatments, the enzymatic antioxidant activities and EL were evaluated. In general, the results of the enzymatic activities and EL show that different treatments have not stressed plants in the growing media. In Fig. 3d, there is a clear trend of decreasing EL values for SA and KH treatments and the same trend, in general, for other enzymatic antioxidant activities (i.e., *PPO*, *CAT*, and *POX*), as presented in Fig. 3a–c, respectively.

Oil Production and Beta Carotene

It could be noticed that growth medium *M1* allows the oil yield to increase at low applied doses of SA and KH, whereas at higher doses, *M2* was the best at allowing this increase in productivity. Based on the fact that flowers marigold are edible, the economic value of this ornamental plant is represented in the beta carotene and oil content

of the flowers. As shown in Fig. 4, the results indicate that there is a significant difference between the two growth media and among different SA levels. A significant difference among KH doses could also be noticed (Fig. 4) for both oil content and yield. Oil content was increased by SA and KH application up to 150 and 1000 mg L⁻¹, respectively, and the same trend was recorded for oil yield at the same levels. Comparing the results of different levels of SA and KH in Fig. 5, it can be seen that both SA and KH have the same trend. This response included an increase in the beta carotene content in flowers by increasing levels of SA and KH up to 200 and 2000 mg L⁻¹, respectively. The beta carotene content of marigold for both growth media was similar, indicating that growth medium *M1*, which contains sand, could be successfully used as alternative to *M2* or vermiculite.

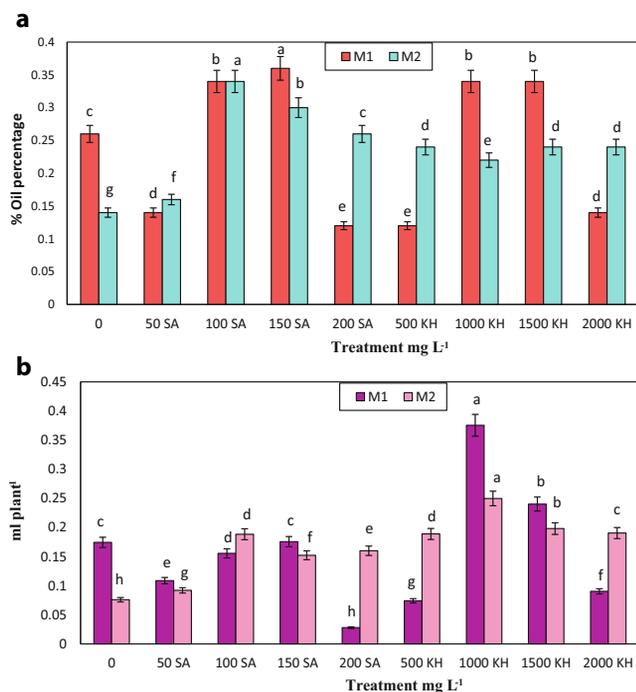


Fig. 4 Effect of different growing media (*M1* and *M2*) and salicylic acid (*SA*) and potassium humate (*KH*) at different concentrations on oil production of marigold flowers: **a** oil percentage, **b** oil yield (*M1* = cocopeat + sand; *M2* = cocopeat + vermiculite); least significant difference (medium \times treatment) for oil percentage and oil yield was 0.07 and 0.016, respectively, with high significance for each

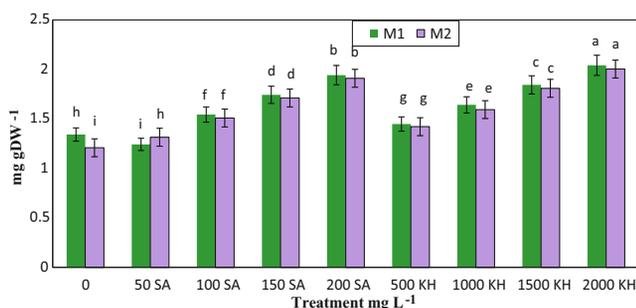


Fig. 5 Effect of different growing media (*M1* and *M2*) and salicylic acid (*SA*) and potassium humate (*KH*) at different concentrations on beta carotene of marigold plants (*M1* = cocopeat + sand; *M2* = cocopeat + vermiculite); least significant difference (medium \times treatment) for beta carotene was 0.011, with no significance

Discussion

Effect of SA and KH on Growth Parameters

This study assessed two factors, including two applied growth promoters (SA and KH) and two growth media (*M1* as alternative medium and *M2* as common medium). This study includes the impact of applied treatments on growth parameters, photosynthetic parameters, the antioxidant system, and oil productivity. This study handled

the production of marigold plants because these plants represent an important ornamental, medicinal, and pharmaceutical crop. Marigold crop is considered an economic ornamental crop that produces brownish yellow, bright yellow, orange to brown flowers which containing beta carotene. A lot of benefits have been reported regarding the importance of marigold, including using their edible flowers and coloring agent, as well as in the medicinal and pharmaceutical industries (Shafique et al. 2021). These flowers are used as garlands on many social and religious occasions in several countries (Chitrakar et al. 2019). They can also be used as an effective repellent in organic farming due to their herbicidal, fungicidal, bactericidal, and insecticidal properties (Laosinwattana et al. 2018). Therefore, this study focused on marigold as a promising ornamental crop and the production of marigold plants in the horticultural sector.

For production of healthy and proper marigold plants, the current study compared a popular growth medium (*M2*; cocopeat + vermiculite) to an alternative growth medium (*M1*; cocopeat + sand). These studied growth media represent one which is very common in Egypt (*M2*) but expensive compared to the second and alternative growth medium (*M1*), which has sand as the main component and is available in Egypt. There are several kinds of growth media, which contain, in general, an organic source or peatmoss beside another component like perlite, vermiculite, sand, or rice husk. Based on the chemical composition of different growth media, it could be noticed that the medium with vermiculite (*M2*) has some benefits and problems, which was reflected in the obtained results. The benefits of vermiculite may include its high nutrient content, but the main problem is its high moisture retention and content, which causes a lot of pathogens. In the current study, all growth parameters of marigold have high significance upon comparison between the two growth media (*M1* and *M2*), as presented in Table 2. Based on the chemical composition of each medium (Table 1), the main difference between the growth media include the organic matter, nitrogen, and potassium contents, but this difference may not be significant. Although growth medium *M1* (as an alternative growth media) has a lower content of organic matter, N, and K, these contents maybe enough for growing plants, whereas the main problem of growth medium *M2* is the high soil moisture content, which impedes growth and uptake of other nutrients in cultivated plants.

Growth media have been become a vital component in the production of plants in the horticultural sector under the greenhouse system worldwide, as an alternative to soil or soilless farming. There is an urgent need for research into new components of the current growing media beside peat, which is already used as a substrate for cultivation in the professional greenhouse growing system. Peat is the main

component in the soilless system and has many problems, so the use rate of peat in these growing media should be reduced (Feng et al. 2020). The main factors controlling the growth media components in soilless farming include the irrigation and fertilization system and their efficiency, rooting medium, and the source of nutrients in media (Vandecasteele et al. 2018; Aly et al. 2019).

In the current study, foliar application of KH at low doses (from 500 to 1000 mg L⁻¹) achieves, in general, the highest values for vegetative growth of marigold plants (Table 2). A strong relationship between foliar applied levels of KH and marigold plant growth is reported in the literature (e.g., Shyala et al. 2019), as also for other crops like eggplant (Howladar 2018), *Brassica oleracea* var. *acephala* (Abbey et al. 2018), cotton (Ullah et al. 2020), and *Elaeagnus angustifolia* (Torun and Toprak 2020). This result may be explained by the fact that the KH promoter has dual benefits due to the nutritional role of humic substances and K nutrient in cultivated plants (e.g., Pal and Ghosh 2010; Mohammadipour et al. 2012). These benefits include enhancing enzyme activities, membrane permeability, and hormonal activity in cultivated plants, as well as increased water-holding capacity, nutrient uptake, and yield. Foliar application of potassium humate increases plant height, number of branches, dry weight, and yield of cultivated plants, as reported by Mohamed et al. (2018).

In this study, the role of applied SA in enhancing growth parameters could be seen, with increasing doses up to 50–150 mg L⁻¹; this result was distinguished from that for the growth medium of cocopeat + vermiculite. This result may reflect the high nutritional value of vermiculite compared to sand, which is considered an inert growing substance. For both growth media and in general, the inhibitory effect of SA at higher concentrations up to 200 mg L⁻¹ could be shown in Table 2. The role of SA in promoting the growth parameters is shown in Table 2, which mainly depends on plant species, different stages of plant development, and on the concentration of SA used (Arif et al. 2020). The low exogenous dose of SA (up to 100 mg L⁻¹) used in this study may interact with nutrients and other phytohormones and significantly contribute to metabolism as well as plant growth (Rasheed et al. 2020). In other words, SA may improve gas exchange, compatible solutes and secondary metabolites, and photosynthetic pigments, thereby increasing plant growth and biomass production (Saheri et al. 2020). A few studies have been published concerning the promotive role of SA in producing marigold plants, as reported by Pacheco et al. (2013), Ibrahim (2017), and Basit et al. (2018) using doses up to 276, 200, and 120 mg L⁻¹, respectively. Pacheco et al. (2013) reported that SA has stimulator effects on various physiological processes related to plant growth and development of marigold. It is well known that KH is

a natural material resulting from the interaction between potassium and humic acid. This material has the ability to improve different soil properties (biological, physical, and chemical) beside the soil dynamic of nutrients. KH has a significant role in plant nutrition through its vital role in promoting plant growth. Exogenous application of SA encourages germination of seeds, plant flowering, upregulates the photosynthetic rate, and increases the activity of nonenzymatic, enzymatic, and antioxidants processes under normal and changing environments (Tucuch-Haas et al. 2017; Arif et al. 2020; El-Hady et al. 2021).

Effect of SA and KH on Antioxidants and Photosynthetic Pigments

It is well documented that K⁺ acts as a key regulator for more than 60 enzymes within the plant system by controlling the activation of these enzymes as well as controlling the opening and closing of leaf stomata (Kumar et al. 2020b). So, the important role of KH in supporting photosynthetic activity (Chl *a*, Chl *b*, and carotenoids) of marigold plants in different growth media is clear (Figs. 1 and 2). It seems possible that these results are due to the dual direct and indirect impact of both humate and potassium ions. Whereas the highest applied dose (2000 mg L⁻¹ KH) may cause an increase in these photosynthetic parameters particularly in the case of M1 (sand and cocopeat), this increase was limited to 1500 mg L⁻¹ KH in the case of M2 (vermiculite and cocopeat). There was a highly significant interaction between all previous photosynthetic parameters and KH treatments. The observed increase in chlorophyll fluorescence in both growth media was recorded at the highest KH dose. It is difficult to explain this result, but it might be related to the nonsignificant interaction between growth media and KH treatments. The antagonistic interactions between magnesium (Mg) and potassium when K⁺ is applied at high doses may cause the decrease of studied photosynthetic activities, because Mg is an essential component of chlorophyll (Xie et al. 2020).

Concerning the impact of SA application on the content of photosynthetic pigments (i.e., chlorophyll *a* and *b*), carotenoids, and chlorophyll fluorescence, a decrease in Chl *a*, Chl *b*, carotenoid content, and chlorophyll fluorescence of marigold plants with increasing levels of SA was observed (Figs. 1 and 2). This result is somewhat counterintuitive compared to the results of Basit et al. (2018), who reported that these parameters were increased by increasing the applied doses of SA up to 120 mg L⁻¹. A possible explanation is that the current work applied a dose up to 200 mg L⁻¹, which may inhibit these parameters. Bayat et al. (2012) also reported that exogenous SA applied to marigold decreased EL but nonsignificantly increased chlorophyll content as well as the number of flowers per plant and plant

height. On the other hand, at low doses of applied SA, this acid may promote formation of photosynthetic pigments and their derivatives, increase the production of energy and the respiration rate for more pigment synthesis, and regulate the production of green color in foliage and flowering (Basit et al. 2018).

As biochemical parameters, enzymatic antioxidant activities (CAT, PPO, and POX) and EL were measured to evaluate the impact of SA on growing marigold plants. The CAT activity was significantly increased by application of SA up to 50 mg L⁻¹, recording the highest level of CAT (150 and 200 mM H₂O₂ g⁻¹ FW min⁻¹ for the two growth media, respectively; Fig. 3). Concerning PPO and POX activities, the values of both enzymes were increased by increasing the applied SA up to 200 mg L⁻¹ and the same trend was observed for EL for both growth media. It has been confirmed under salinity and oxidative stress that exogenous application of SA enhances the activity of some enzymatic antioxidants such as CAT, POX, and superoxide dismutase (SOD), by scavenging reactive oxygen species (ROS) and free radicals (Arif et al. 2020).

Potassium is the main regulator of overall plant growth, although this nutrient is not found in any chemical structure of plant proteins (Guo et al. 2017), and also controls several plant reactions by activation of their enzymes (Kumar et al. 2020b). Hence, it could conceivably be hypothesized in the current study that these are remarkable findings about the impact of foliar application of KH at different concentrations on the antioxidant capacity of marigold leaves (Fig. 3). This finding has important implications for developing growth media, which show the highest values of antioxidants enzymes (CAT, PPO, and POX) up to 1500 mg L⁻¹ under sand media (M1). The important role of KH has been confirmed under different stresses in many cultivated plants like kale (Abbey et al. 2018) and eggplant (Howladar 2018).

Effect of SA and KH on Oil Productivity and Beta Carotene

The oil produced from marigold flowers is considered an important product of this ornamental plant. In a recent study, foliar application of 150 mg L⁻¹ SA resulted in highly significant increases in the essential oil content (60%) and yield (58.8%) of marigold flowers in both growth media as compared to the control (Fig. 4). This result may be due to the action of SA, which may act as a chemical elicitor by enhancing the production of many secondary metabolites including phenolic compounds, alkaloids, flavonoids, and phytoalexins (Gorni and Pacheco 2016). It is somewhat surprising that no significance among the interactions between growth media and treatments was noted in this condition. Foliar application of SA up to 200 mg L⁻¹ signifi-

cantly increased the beta carotene content of marigold flowers (Fig. 5). These results confirm the association between exogenous foliar application of SA and beta carotene content in marigold flowers. These findings also match those observed in earlier studies (Abou El-Ftough et al. 2018; Abd El Gayed 2020), which reported that foliar application of SA up to 300 mg L⁻¹ increased the marigold flower beta carotene content. Therefore, exogenous foliar application of SA at low doses might be recommended for some medicinal crops under field conditions due to the improvement of phytochemical and agronomic traits, particularly for ecofriendly farming practices (Gorni et al. 2020).

The oil of marigold flowers is one of the main target products beside beta carotene. Therefore, a stimulator or promoter that can enhance this productivity is needed. The results of this study are in keeping with previous observational studies which confirmed that foliar application of KH increased the essential oil content of many crops such as basil (El-Sayed et al. 2015) and *Nepeta* species (Mohamed et al. 2018). These results agree with the findings of previous studies and the current study, which confirmed that KH doses increased both oil content and yield (Fig. 4). The oil content was increased by applied KH up 1000 mg L⁻¹, whereas the increase in the beta carotene content in flowers extended up to 2000 mg L⁻¹. This increase in oil content may be linked to the ability of KH to solubilize and transport nutrients for cultivated plants, which promotes uptake of water and nutrients by roots (Mohamed et al. 2018; Maraai et al. 2019).

In this work, SA was selected to evaluate different foliar concentrations (from 50–200 mg L⁻¹) in marigold plants and their content of oil. The role of SA in enhancing growth and oil production has been confirmed for some medicinal plants in the Asteraceae family, including safflower (Chavoushi et al. 2020), feverfew (*Tanacetum parthenium* L. Bip.; Ahmadi et al. 2020), sunflower (Shatoori et al. 2020), and marigold (Pacheco et al. 2013; Basit et al. 2018; Gholinezhad 2020), as well as under different stresses (Wani et al. 2017) like soil salinity (Rasheed et al. 2020), drought (Sedaghat et al. 2020), and nutrient stress (Es-sbihi et al. 2020; Madanan et al. 2021).

Conclusion

The best growth parameters could be achieved for marigold plants in general using doses of 100 and 1500 mg L⁻¹ of SA and KH, respectively. This trend may differ based on which parameter we require from nursery production of this important ornamental plant. The obtained values of all photosynthetic parameters and chlorophyll fluorescence decreased with increasing doses of SA up to 200 mg L⁻¹, but this trend was the opposite for KH in both growth media.

This may be due to the role of K in regulating several physiological processes, particularly photosynthesis, working of stomata, and activating more than 60 enzymes. The antioxidant enzymes activities were enhanced by increased foliar application of SA up to 200 mg L⁻¹ (except CAT), but up to 1500 mg L⁻¹ for KH. Furthermore, the beta carotene content of marigold plants was increased by increasing both SA and KH up to 200 and 2000 mg L⁻¹ in different growth media. On the other hand, best oil productivity (percent and yield) from marigold plants was obtained using doses of 100 and 1000 mg L⁻¹ of SA and KH, respectively. Based on the findings of this study, the common growth medium (cocopeat and vermiculite), which is expensive, could be replaced with the alternative growth medium (cocopeat and sand), which showed a nonsignificant difference between growing media (M) and treatments (T), particularly for beta carotene production. Further studies are needed to evaluate other cheap growing media and higher concentrations of foliar application of SA and KH.

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Conflict of interest T.A. Shalaby, N.A. El-Newiry, M. El-Tarawy, M.E. El-Mahrouk, A.Y. Shala, H.S. El-Beltagi, A.A. Rezk, K.M.A. Ramadan, W.F. Shehata, and H. El-Ramady declare that they have no competing interests.

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