# The effect of compost on the growth and development of *Calendula officinalis* L. seedlings

R. Erhatić<sup>1</sup>, T. Peremin-Volf<sup>1</sup>, S. Janček<sup>1</sup> <sup>1</sup>Križevci College of Agriculture, Križevci, 48260, Croatia Presenting author email: <u>rerhatic@vguk.hr</u>

## Abstract

Marigold (*Calendula officinalis* L.) is a herbaceous plant from *Asteraceae* family. Marigold production is not very demanding, and shows results even on poor soil in comparison with other plant cultures. Substrates have an important role in flower production because they effect and maintain the whole plant system. The substrate used for marigold is usually black or white peat, coconut fiber, composted biodegradeable materials or other composts that contain necessary micro- and macroelements. The research was conducted in the spring of 2013. in the protected area of Daruvarske Toplice, Croatia. The research was aimed at determining the effect of various substrates (Leaf litter K1, composted substrate for growing champignon K2, Klasmann Potgrond (H) commercial sowing substrate S1) on the morphological properties of the growth of marigold seedlings. The observed properties are: seedling height, seedling diameter, width and length of the root, root weight and seedling weight. From the research we can conclude that the most effecient, based on all of the parameters, was the K1 substrate. Because of its favourable chemical and physical properties, easy accessability and inconsumability, as well as the low price, the chaff can be successfully used in growth of marigold seedlings.

Keywords: Marigold (Calendula officinalis L.), Substrates, Seedlings, Morphological properties

## Introduction

Marigold (Calendula officinalis) L. is a fast-growing herb that originated in southern Europe [5]. It is an annual or seldom biennial plant that blossom from May until the first autumn frosts [26]. It is grown for various purposes: as ornamental plant in gardens or garden beds or to be sold at markets as a cut flower [24]. Marigold is globally known for its medicinal importance containing various phyto-chemicals including carbohydrates, amino acids, lipids, fatty acids, carotenoids, terpenoids, flavonoids, quinones, coumarins and other constituents, showing some important biological activities like wound healing, immuno-stimulant, spasmogenic and spasmolytic, hepatoprotective, genotoxic and antigenotoxic, anti-amylase, anti-inflammatory, anti-oedematous, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-HIV and anti-cancerous, nephron-protective, prevention of oropharyngeal mucositis, hypoglycemic and gastroprotective activities with no toxic effect [28, 7, 15]. It is harvested throughout the world, and its pharmaceutical uses are described in many Pharmacopeias. The phytochemistry of *Calendula officinalis* has been extensively studied, and it mainly consists of triterpenes, flavonoids and phenolic acids [20]. Even though many reports are stressing the bioactivity of triterpenoid alcohols isolated from apolar extracts of Marigold [29], hydroalcoholic extracts look alike to folk medicine used for healing wounds. Triterpenic saponins represented in Marigold by oleanolic acid (OA) glycosides are pentacyclic triterpene compounds with a wide range of biological and medicinal properties [3]. The chemical profile of plants frequently varies in response to genetic factors and changes in the environment, such as seasonality, soil nutrients, harvest conditions, temperature, UV radiation and herbivores [8, 16]. Marigod posesses a well-developed spindle root which allows it to grow on dry soil [30]. Special feature of the marigold are yellowish or orange flowers that grow from 5 to 10 cm in diameter. In the centre of each flower head, sorted through two to three rows, fertile tubular flowers are found. The marigold fruit is a thorny curved achene, which is dark brown in color and pointed toward the edges. The flowers contain flavanoids, carotenoids, volatile oils, resins and saponins, Dried flowers contain around 0.02% of volatile oil, 5% of oils, resin, sugars etc. [26]. It is known in folk medicine for its benefitial properties. When it comes to growing, the marigold does not seek much attention, but it will yield better results when grown on humus-rich soil equipped with a drainage system. It yields very similar results both in humid and dry areas. It is considered a photophilic plant and it shows best results in areas with moderate and humid climate [25]. Marigold can be cultivated from seedlings, which is economically benefitial if it is being cultivated as an ornamental plant [12]. The technology used in production is also what affects the quality of the seedlings [4]. Substrate as a medium is very important for plant growth. Its complexity is noticeable from various types of interaction with the plant, as well as the number of components which make it up. The substrate used for marigold is usually black or white peat, coconut fiber, composted biodegradeable materials or other composts that contain necessary micro- and macroelements [21,11,14]. Modern agricultural industry endaevours to preserve the environment against the excessive nitrogen based composting, and in such endaevour it is important to stimulate microbiological activity in the soil [17]. On a global scale, in the industry of production of seedlings of therapeutic, aromatic and seasonal herbs the already existing technologies have been improved, while at the same time new, peat and compost based technologies, as well as those based on other materials of improved organic and mireal composition have been introduced [10]. It is well known that peat, a wide used substrate, is a nonrenewable resource, and diminishing availability is prompting price increases. The extensive use of peat as a substrate has led growers to consider its replacement in the medium to long term with alternative candidates achieving attention [27]. Compost is used as an organic feed or is added as a conditioner in some types of commercial substrates. Compared with substrates, the compost is richer in organic matter created by the humification. In last 10 years there has been a noticeable increase in interest for green composted materials, especially in Western Europe [2]. However, in the usage of compost there are certain limitations as, for instance, excessive salt concentration, toxicity, low porosity and unfavorable chemical and physical properties [27]. Numerous studies have shown that organic residues such as urban solid wastes, sewage sludge, pruning waste, and even green wastes following composting process can be used with very good results as growth media instead of peat [23, 1]. Geranium and marigold seedlings growth and flowering were superior in all vermicompost-based media. The higher percentage of green waste compost and green waste vermicompost in the growing medium could increase bulk density and air space; decrease total pore space and water-filled porosity; and increase pH, electrical conductivity, and macroand microelement contents [9, 18]. Leaf litter is an organic material composed of rotten leaves and other plant parts piling on forest soil. Because of its favorable physical and chemical properties it can be used as a substrate in the seedling growing [19]. With the growth of plant life cycle, leaves undergo significant structural, chemical and functional changes at different ages, altering nutrient distribution and uptake processes [22]. These changes will determine the survival strategies of the species. In this process, the use of nutrients in the environment changes with age, and the nutrient absorption capacity of plant leaves changes with time. Studies have shown that specific leaf weight, leaf nitrogen content and water use efficiency increase with the growth, but photosynthetic nitrogen use efficiency and stomatal conductance decrease [6].

## Materials and methods

The research was conducted in the spring of 2013. in the protected area of Daruvarske Toplice, Croatia. Daruvarske Toplice is a two-thousand-years-old health resort and more-than-fifty-years-old urban and architectural complex with parks. The process of leaf composting in Daruvarske Toplice is done on a special composting place. If there is a lack of compost used for horticulture, a leftover substrate for champignon production is used as a means to enrich the soil. During the preparation of the soil for seedlings the above mentioned leftover substrate is mixed toghether with the surface layer of the soil. The research was conducted in a protected area. The 4x30 m greenhouse was divided into two parts. Ventilators were set on the side walls and the top. They were set so that cold air do not blow directly onto the seedlings. The greenhouse was heated by natural gas-based floor heating system. The research was aimed at determining the effect of various substrates (Leaf litter K1, composted substrate for growing champignon K2, Klasmann Potgrond (H) commercial sowing substrate S1) on the morphological properties of the growth of marigold seedlings. The observed properties are: seedling height, seedling diameter, width and length of the root, root weight and seedling weight. The marigold were sown on March 18 th 2013. and were put into polystyrene containers with the outside temperature of 20°C. During the process of sowing a natural plant strenghtener composed of Trichoderma was added to the each substrate. The Trichoderma inhibits the growth of pathogen mycelium and stimulates the growth and the development of the root system of a plant. The experiment was conducted on randomly chosen block-settings in three repetitions. During the experiment, the day temperatures were varying from 18 °C to 25 °C, while the night temperatures were between 14 °C and 18 °C. The relative humidity was between 25 and 60%. The substrate temperature was kept between 16 °C and 23 °C. The plants sprouted four days after the sowing (22.03.2013). The substrates were maintained moderately humid during the process of cultivation. The watering was done in the morning, and during the day the plants were kept humid by sprinkling. During the growing process a systemic fungicide carbendazim (Zino, 0.05%) was used in order to prevent withering. Fungal withering of the crops appeared in the cotyledon stage, firstly in the commercial substrate Klasmann Potgrond (H) substrate S1, and after that in the composted substrate used for champignon growing compost K2. The pricking-out was done into pots for growing that were 8 cm in diameter, and that was when first real leaves appeared (11.04.2013.). The seedlings were watered with Merpan 80 WDG (20g/10 l). Merpan 80 is a fungicide based on surface action and as such blocks the negative effects of various diseases on both underground and surface parts of seedlings. The seedlings were additionally nourished with a water-soluable compost which contains 10% of nitrogen, 52% of phosphorus and 10% of potassium, and which is used to stimulate root- and flower growth.

The measuring of the seedlings was conducted 70 day after the sowing and the results are shown in Tables 3 and 4.

Chemical analysis of the composted material was conducted in Agrochemical Laboratory of College of Agriculture in Križevci.

#### **Results and discussion**

The basic chemical properties of the substrate are shown in Table 1, while those of the examined compost materials are in Table 2. Commercial substrate S1 contains medium nutritional value with all necessary microelements. The substrate was sterilized with water steam. It is a mixture of poorly degraded white peat and frostbitten black peat.

It was enriched with a polymer-based humidification agent, which works on the principle of faster, more durable and stronger water bounding. Thereby watering is reduced and so is nutrient flushing and fungal infections.

Table 1. Chemical properties of the substrate										
Substrate	pH <sub>H2O</sub> (1:10)	EC dSm <sup>-1</sup> (1:5)	Organic matter (%)	N (mg l <sup>-1</sup> )	$P_2O_5 (mg l^{-1})$	K <sub>2</sub> O (mg l <sup>-1</sup> )				
S1	5.6-6.5	0.40	85	100	130	180				

From the data collected from the composts included in research it is visible that the amount of dry matter and ash in the compost K1 is slightly higher, while the amount of water and organic matter is lower. Slightly higher amount of ash in compost K1 in respect to compost K2 is due to higher amount of dry matter and low amount of organic component, even though the humidity of both composts, and thereby the amount of dry matter as well, depends primarily on the conditions in which the composted materials were kept. In compost K1 carbon supply is slightly lower than in compost K2. That can be traced back to lower total nitrogen concentration, as well as concentration of organic matter. Also, we can notice that both composts were high in phosphorus and low in potassium, which resulted in weaker root system in compost K2, which then led to slower development of the seedlings. Compost K1 carbo out lower in nitrogen, which may be due to significantly lower organic matter content and lower water content. Compost K1 is slightly lower in carbon. We can suppose that the majority of the seedlings will develop well as long as the amount of nitrogen and potassium in the compost is around equal.

Table	e 2. Chemical	properties of t	ne compost	

**T** 1 1 2 C1

Kompost	Suha tvar (%)	рН	E.C. (µS/cm)	N (%)	K2O (%)	P (%)	H2O (%)	Ash (%)	Organic carbon (%)	Organic matter (%)
K1	59.48	7.56	139.4	1.09	1.00	2.72	40.52	70.27	17.05	29.73
K2	37.56	7.12	129.6	1.98	0.58	3.46	62.44	58.94	23.80	41.06

From the morphological features of the surface parts of the marigold seedlings shown in Table 3. we can conclude that the features taken into account (height of the plant, diameter of the plant, weight of the overhead part of the plant) gave higher results in compost K1. The highest marigold seedling was found in compost K1 (9.67 cm), while compost K2 and substrate S1 yielded lower seedlings (8.36 cm, 8.73 cm). Regarding the diameter there were no major differences. Seedlings largest in diameter were found in K1 (6.26 cm), while the seedlings smallest in diameter were found in substrate S1 (4.88 cm). The seedlings with the highest mass value were found in compost K1 (6.14 g), while the mass value was lowest in substrate S1 (4.33 g).

Table 3. Morphological	measurements of th	e above-ground	part of marigold	seedling
ruele et merphoregieu		e acore pround	part of mangola	Seeanna

Morphological characteristic	Hight of plants (cm)			Di	Diameter of plants (cm)			The weight of the overhead part of the plant (g)		
Substrate	<b>S</b> 1	K1	K2	<b>S</b> 1	K1	K2	<b>S</b> 1	K1	K2	
Mean	8.73b	9.67a	8.36b	4.88	6.26	5.75	4.33c	6.14a	4.69b	
Var	0.98	1.01	0.52	0.29	1.01	0.63	0.09	0.28	0.07	
St. Dev.	0.99	1.07	0.72	0.54	1.01	0.80	0.30	0.53	0.27	
Coef. Var. (%)	11.34	11.07	12.26	11.07	16.05	13.91	6.93	8.63	5.75	
St.Error	0.23	0.25	0.17	0.13	0.24	0.19	0.07	0.12	0.06	
Min.	7.10	8.00	6.80	3.80	5.00	4.50	4.00	5.50	4.20	
Max.	10.20	11.50	9.30	5.60	8.00	7.20	5.10	7.20	4.20	

Morphologiral measurements of the underground parts of the marigold seedlings are shown in Table 4. The seedlings with the highest mass value were found in compost K1 (0.85 g), while the mass value was lowest in compost K2 (0.38 g). There were no significant differences in the diameter and the length of the root. The plants with the highest root diameter were found in compost K1 (1.52 cm), while compost K2 gave the lowest results (0.88 cm). Seedlings with the longest root were found in substrate S1 (3.33 cm), while the root was shortest in compost K2 (2.67 cm).

Table 4. Morphological measurements of the underground part of marigold seedling										
Morphological	The diameter of the root	The rooth length	The mass of the root							
characteristic	(cm)	(cm)	(g)							

Substrate	<b>S</b> 1	K1	K2	<b>S</b> 1	K1	K2	<b>S</b> 1	K1	K2
Mean	1.32	1.52	0.88	3.33	3.30	2.67	0.63ab	0.85a	0.38b
Var	0.45	0.37	0.02	0.39	0.47	0.02	0.03	0.20	0.003
St. Dev.	0.67	0.61	0.44	0.62	0.69	0.44	0.16	0.44	0.05
Coef. Var. (%)	50.76	40.13	16.48	18.62	20.91	16.48	25.40	51.76	13.16
St.Error	0.16	0.14	0.10	0.15	0.16	0.10	0.04	0.10	0.01
Min.	0.50	0.50	0.60	2.30	2.00	0.60	0.40	0.20	0.27
Max.	0.25	2.50	1.10	4.30	4.50	1.10	1.00	1.70	0.45

# Conclusion

From the research we can conclude that the most effecient, based on all of the parameters, was the K1 substrate. Because of its favourable chemical and physical properties, easy accessability and inconsumability, as well as the low price, the chaff can be successfully used in growth of marigold seedlings.

#### References

[1] Benito, M., Masaguer, A., De Antonio and Moliner, A.: Use of pruning waste compost as a component in soi lless growing media. Bioresource Technology. Vol. 96, No5, pp. 597-603 (2005). doi: 10.1016/j.biortech. 2004.0 6.006

[2] Carlile, W.R.: The use of composted materials in growing media. Acta Horticulturae. Vol 779, pp. 321-328 (2008). doi: 10.17660/ActaHortic.2008.779.39

[3] Dlugosz, M., Markowski, M., Paczkowski, C.: Source of nitrogen as a factor limiting saponin production by hairy root and suspension cultures of *Calendula officinalis* L. Acta Physilogiae Plantarium. Vol.40, Issue: 2. No. 35 (2018)

[4] Dudaš, S., Šestan, I.: Utjecaj tehnologije uzgoja presadnica i primjene bio-algeena S-90 na kvalitetu kadifice *Tagetes patula* L. "Orange boy". Zbornik Veleučilišta u Rijeci. Vol 2, No. 1, pp. 333-342 (2014).

[5] Fernandes, E.F.A., Meloni, F., Borella, J.C., Lopes, N., P.: Effect of fertilization and harvest period on poloar metabolites of *Calendula officinalis*. Brazilian Journal of Pharmacognosy. 23. pp. 731-735 (2013)

[6] Field, C.B. and Mooney, H.A.: Leaf Age and seasonal effects on light, water, and nitrogen use efficiency in a California Shrub. Oecologia. 56 (2): 348-355 (1983). doi: 10.1007/BF00379711.

[7] Fonseca, Y.M., Vicentini, F.T.M.C., Catini, C.D., Fonseca, M.J.V.: Determination of rutin and narcissin in marigold extract and topical formulations by liquid chromatography: applicability in skin penetration studies. Quim. Nova. 33, 1320-1324 (2010)

[8] GobboNeto, L., Lopes, N.P.: Online identification of chlorogenic acids, sesquiterpene lactones, and flavonoids in the brazilian arnica *Lychnophora ericoides* Mart. (*Asteraceae*) leaves by HPLC-DAD method for their simultaneous analysis. J. Agric. Food Chem. 56, 1193-1204 (2008). doi: 10.1021/jf0728121. Epub 2008 Feb 1.

[9] Gong, X., Li, S., Sun, X., Wang, L., Cai, L., Zhang, J., Wei, L.: Green waste compost and vermicompost as peat substitutes in growing media for geranium (*Pelargonium zonale* L.) and calendula (*Calendula officinalis* L.). Scientia Horticulturae. Vol 236, pp. 186-191 (2018). doi: 10.1016/j.scienta.2018.03.051

[10] Jelačić, S., Lakić N., Beatović D., Vujošević A. (2005). Effects of different substrates on basil seedlings qual ity (*Ocimum basilicum* L.). Journal of Agricultural Sciences. 50 (2): 107-115 (2005)

[11] Lanzi, A., Incroccia, L., Pulizzi, R., Pardossi, A., Marzialetti, P.: Evaluation of some peat-alternative substrates in horticultural crops. Acta Horticulturae. Vol 807, pp. 553-558 (2009)

[12] Mihovilović, I.: Proizvodnja i prerada ljekovitog i aromatičnog bilja. Ustanova za cjeloživotno učenje Magistra. Pula (2011)

[13] Muley, B.P., Khadabadi, S., Banarase, N.B.: Phytochemical Constituents and Pharmacological Activites of *Calendula officinalis* Linn (*Asteraceae*). Tropical Journal of PharmaceuticalResearch (5): 455-465 (2009). http://dx.doi.org/10.4314/tjpr.v8i5.48090

[14] Mupambwa, H.A., Lukashe, N.S., Mnkeni, P.N.S.: Suitability of Fly Ash Vermicompost as a Component of Pine Bark Growing Media: Effects on Media Physicochemical Properties and Ornamental Marigold (*Tagetes* spp.) Growth and Flowering. Compost science & utilization. Vol. 25, pp. 48-61 (2017). https://doi.org/10.1080/1065657X.2016.1180270

[15] Nelofer, J., Khurshid Iqbal, A., Riffat, J.: *Calendula officinalis* - An Important Medicinal Plant with Potential Biological Properties. Proceedings of the Indian National Science Academy. Vol.83, Issue (4), pp. 769-787 (2017) doi: 10.16943/ptinsa/2017/49126

[16] Pavarini, D.P, Pavarini, S.P., Niehues, M., Lopes, N.P.: Exogenous influences on plant secondary metabolite levels. Animal Feed Science Techn. 176, 5-16 (2012). https://doi.org/10.1016/j.anifeedsci.2012.07.002

[17] Pohajda, I., Vaci, D.: Contemporary production of annual flowers in continental and Mediterranean Croatia. 2nd Scientific Conference VIVUS - Environmentalism, Agriculture, Horticulture, Food Production and Processing / - Naklo, Sovenija, pp. 337-345 (2013)

[18] Ponchia, G., Passoni, M., Bonato, S., Nicoletto, C., Sambo, P., Zanin, G.: Evaluation of compost and anaerobic digestion residues as a component of growing media for ornamental shrub production. Acta Horticulturae Vol. 1168, pp. 71-78 (2017). DOI: 10.17660/ActaHortic.2017.1168.10

[19] Rahimić, A., Komlen, V., Vukotić, D., Tanović, N., Aliman, J.: Utjecaj listinca na morfološka svojstva pres a dnica bosiljka (*Ocimum basilicum*). 48th Croatian & 8th International Symposium on Agriculture Dub rovnik, Croatia: 415-418 (2013)

[20] Re, T.A., Mooney, D., Antignac, E., Dufour, E., Bark, I., Srinivasan, V., Nohynek, G.: Application of the threshold of toxicological concern approach for the safety evaluation of calendula flower (*Calendula officinalis*) petals and extracts used in cosmetic and personal care products. Food Chem. Toxicol. 47, 1246–1254. (2009)

[21] Rivière, L.-M., Morel, P., Michel, J.-C., Charpentier, S.: Growing media in French horticulture. Acta Horticulturae Vol. 779, pp. 33-38 (2008)

[22] Rout, G., R., Sahoo, S.: Role of iron in planth growth and metabolisam. Reviews in Agricultural Science. 3: pp. 1-24 (2015) doi: 10.7831/ras.3.1.

[23] Simins, H.I. and Manios, V.I.: Mixing peat with MSW compost. BioCycle. Vol.31, No. 11, pp. 60-61 (1990) http://dx.doi.org/10.1100/2012/285874

[24] Simões Neri, F.C., Usberti, J.A., Usberti, R. De Oliveira Paiva, P.: Morpho-agronomic trait comparisons among Tagetes patula L. cultivars. Revista Brasileira de Horticultura Ornamental V. 18, No.1 (2012)

[25] Stepanović, B., Radanović, D., Turšić, I., Nemčević, N., Ivanec, J.: Uzgoj ljekovitog i aromatičnog bilja. Jan-Spider d.o.o. Pitomača (2009)

[26] Šilješ, I., Grozdanić, Đ., Grgesina, I. (1992). Poznavanje uzgoja i prerada ljekovitog bilja. Školska knjiga, Zagreb (1992)

[27] Tzortzakis, N., Gouma, S., Paterakis, C., Manios, T. (2012). Deployment of Municipal Solid Wastes as a Substitute Growing Medium Component in Marigold and Basil Seedlings Production. The Scientific World Journal. Vol.2012, pp. 1-6 (2012) doi: 10.1100/2012/285874

[28] Ukiya, M., Akihisa, T., Yasukawa, K., Tokuda, H., Suzuki, T., Kimura, Y.: Anti-inflammatory, anti-tumorpromoting, and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. J. Nat. Prod. 69, 1692-1696 (2006) doi: 10.1021/np068016b

[29] Wilkomisrsky, B.: Pentacyclictriterpenetriols from *Calendula officinalis* flowers. Phytochemistry. 24, 3066-3067 (1985) https://doi.org/10.1016/0031-9422 (85)80062-5

[30] Willfort, R.: LJekovito bilje i njegova upotreba. Erdut. Zagreb (2002)