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Effect of Foliar Application of Diammomium Phosphate on Morphological Characteristics and Constituents of Essential Oil of Mexican Marigold (*Tagetes minuta* L.)

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ABSTRACT

Pot trials were carried out in a greenhouse in Shiraz, Iran to determine the effect of foliar application of diammonium phosphate (DAP) on morphological characteristics and quality of essential oil (EO) of Mexican marigold (*Tagetes minuta* L.). DAP was applied at 0, 2.4, 4.8, 7.2, 9.6 and 12% (w/v). Growth parameters increased as DAP levels increased. The dry weight of shoots increased at 2.4% DAP. At 7.2% DAP, the height, leaf area, length of axillary shoots and fresh weight of aerial parts peaked. Moreover, the number of axillary shoots, number of flowers per plant and the EO yield reached a maximum at 9.6% DAP. Regarding the EO constituents, the content of dihydro tagetone and *Z*-tagetone increased as DAP level increased while *Z*-β-ocimene and *Z*-ocimenone content decreased as the DAP level increased. The role of phosphorus as a central and pivotal metabolic and regulatory nutrient element is discussed.

Keywords: Asteraceae, essential oil composition, growth parameters
Abbreviations: DAP, diammomium phosphate; **EO**, essential oil

INTRODUCTION

Asteraceae is the largest family of vascular plants with more than 23,000 species (Jeffrey 2007), rich in secondary metabolites and essential oils (EOs) (Teixeira da Silva 2003, 2004; Teixeira da Silva *et al*. 2005). The genus *Tagetes*, with the common name of marigold, consists of 30-40 species that are endemic from Arizona to Argentina (Sefidkon *et al*. 2004). Mexican marigold (*Tagetes minuta* L.) is an annual plant belongs to the Asteraceae family. It is native of grasslands and mountainous regions of South America (Dole 1999), including Argentina, Chile, Bolivia, Peru, and in the Chaco region of Paraguay (Reiche 1903; Perkins 1912; McVaugh 1943; Soule 1993). *T. minuta* grows wild from spring to early winter when it completes its life cycle (Babu and Kaul 2007). The plants reach 1 to 2 m in height. Leaves are slightly glossy green, and are pinnately dissected into 4 to 6 pairs of pinnae. The undersurface of leaves bear a number of small, punctuate, multicellular glands, orangish in color, which exude a licorice-like aroma when ruptured. Glands may also be found on the stems and involucre bracts (Dole 1999). Fractions of *T. minuta* EO have the potential for aphid control and the EO also has the potential as a natural herbicide for managing rice weeds (Singh *et al*. 2003; Tomova *et al*. 2005; Batten *et al*. 2006; Pritekel *et al*. 2006; Batish *et al*. 2007). Due to its competitive nature, *T. minuta* is resistant to natural drought and survives easily on poor soils as a weed, although it is now also cultivated as a crop for agrochemical and pharmaceutical purposes (Hulina 2008). *T. minuta* plant organs contain allelochemicals that inhibit the germination and root growth of *Acacia asak* seeds (Arif and Alhammadi 2008). "New World" people were said to use *T. minuta* as a flavorful beverage, medicinal tea, and a condiment since pre-contact times (Rees 1817). *T. minuta* is commercially grown and harvested for its EOs which are used in the flavor and perfume industry as "*Tagetes* oil". The EO is used in perfumes, and as a flavor component in most major food products, including cola beverages, alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments, and relishes (Craveiro *et al*. 1988). *Tagetes* EO is used to treat chest infections, coughs and catarrh, dilating bronchi, facilitating the flow of mucus and dislodging congestion and can be used in cases of skin infections (Chamorro *et al*. 2008; Meshkatalsadat *et al*. 2010). It has a healing effect on wounds, cuts, calluses and bunions (Singh *et al*. 2002). *T. minuta* is rich in many secondary metabolites, including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes, and aromatics (Rodriguez and Mabry 1977). Various studies on *T. minuta* reported that there are variations in the EO composition according to the harvesting location, the growth stage and the different parts of the plant (Ester *et al*. 2008). EO compositions of *T. minuta* L. have biological activities against certain pathogens (Kéita *et al*. 2000) and are well known for their biocidal properties (Gillij *et al*. 2008). Also, *T. minuta* has been reported to have antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Katerere *et al*. 2012). It is used in the treatment of colds, diarrhoea and suspected liver ailments (Rios and Recio 2005). *A. spinosus* was used in the treatment of menorrhagia, gonorrhoea, eczema and colic (Azhar *et al*. 2004) and is also cited in the treatment of diabetes (Katerere and Eloff 2005). Previous studies showed that the secondary compounds in *Tagetes* are effective deterrents of numerous organisms, including fungi, fungi pathenogenic on humans, bacteria, round worms in general, trematodes (Wang *et al*. 2003), nematodes (Hamawi *et al*. 2004), and numerous insect pests through several different mechanisms (Scrivanti *et al*. 2006; Krueger *et al*. 2010; Meshkatalsadat *et al*. 2010). The anti-

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microbial activity of five secondary compounds in *T. minuta* (β-ocimene, dihydrotagetone, tagetone, *Z*-ocimenone, and *E*-ocimenone), when tested on 40 strains of bacteria and fungi, showed that the EO of *T. minuta* had a 100% inhibitory effect on Gram-positive bacteria, a 95% inhibitory effect on Gram-negative bacteria, and a 100% inhibitory effect on fungi (Hethelyi *et al*. 1986; Cespedes *et al*. 2006). Hudson (1990) tested many different secondary compounds for antiviral activity, and determined that thiophenes demonstrated the greatest antiviral action at the lowest doses, and with the least toxicity overall. Of the thiophenes, molecules with two or more thiophene units showed the highest activity. Hudson (1990) tested 32 thiophenes, evaluated their efficacy and determined the 10 most effective ones. Atkinson *et al*. (1964) were the first authors to report thiophenes in *T. minuta*. A comparison of Atkinson's results to those of Hudson shows that 7 of 10 most effective antiviral thiophenes are found in *T. minuta*. Fractions of *T. minuta* EO have the potential for aphid control and also the potential as a natural herbicide for managing rice weeds (Shahzadi *et al*. 2010). Despite variations in their relative concentrations, the main constituents of *T. minuta* EOs are limonene and *cis*-ocimene (monoterpenes), dihydrotagetone, *trans*- and *cis*-tagetone, *trans*- and *cis*-tagetenone (oxygenated monoterpenoids). Other oxygenated monoterpenoids (terpinolene, carvacrol and carvone) and sesquiterpenes (β-caryophyllen, germacrene-D, γ-elemene have also been reported at lower concentrations (Vázquez *et al*. 2011).

Dihydrotagetone was the main compound of *T. minuta* oil extracted from leaves (Chalchat *et al*. 1995). Analysis of *T. minuta* EO by GC/MS showed that the main compounds were β-ocimene, *Z*-tagetenone, *E*-tagetenone, *E*-tagetone and *Z*-tagetone (Lawrence 1996).

Various isolation and instrumental techniques have been used to study the oil components of *Tagetes*, but steam distillation and chromatography have been shown to be the most adequate (Vázquez *et al*. 2011). However, hydrodistillation is still the most common extraction technique employed to obtain EOs from aromatic plants (Kákoniová *et al.* 2006; Magwa *et al*. 2006; Saroglou *et al*. 2006; Becerra *et al*. 2010; Moreno *et al*. 2010; Urzua *et al*. 2010; Buitrago *et al*. 2011). It is a laborious and time-consuming process that requires large amounts of sample. Moreover, when investigators extract EOs from a plant matrix for analysis, little attention is paid to the possibility that the extraction methods may yield different EO profiles, or even worse, sample degradation, despite it being well known that chemical reactions can occur during the distillation process (Babu and Kaul 2007). For this reason, the final composition of the product may not be representative of the original material, and the observed variations in oil composition may strongly depend on the type of distillation method used (Babu and Kaul 2005). Thus, it is important that researchers explore the various advantages and disadvantages of a given extraction or instrumental technique before carrying out an analysis.

Since Mexican marigold is an important medicinal plant, any study as related to the cultivation aspects of this plant is of prime interest. In commercial medicinal plant production, the main objective is to produce high biomass yields per hectare with high levels of secondary metabolites. Nutritional requirements have a major effect on the yield and growth of all horticultural and agronomic crops (Default *et al*. 2003). On the other hand, the level of secondary metabolites in medicinal plants may be positively or negatively affected by the kind and amount of nutrient elements. Phosphate plays a central, pivotal metabolic and regulatory role on the nexus of several physiological and biochemical processes in plants, including photosynthesis, energy conservation, inter and intracellular coordination of carbohydrate metabolism (Abel 2002) and in energy transfer (Harley 1971). These effects are exerted by phosphate both as an essential nutrient and as a signal for metabolic modulation. Phosphorus from the rhizosphere is the least accessible nutrient required by plants and its availability is rarely adequate for optimal growth because of poor content and its immobile form in the soil (Abel *et al*. 2002). Accordingly, it is speculated that foliarly applied inorganic phosphate may help plants to have a near normal mode of metabolism. This may be one of the working models for future experimentation. Of the various growth and yield characteristics of the crop, the importance in biomass and biogenesis of EO in the leaves was the most important for oil production (Sangwan *et al*. 2001).

In plants, phosphorus is required in relatively large amounts for the biosynthesis of primary and secondary metabolites, since phosphorus has essential functions as a constituent of nucleic acids and phospholipids (biomembranes) and plays a key role in the energy metabolism of cells (Marschner 2000). Moreover, phosphorus is known to have multifarious cellular functions in plants, including signalling and transmembrane metabolite flux. Secondary metabolism is highly modulated by these mechanisms (Nell *et al*. 2009).

Of the various growth and yield characteristics of the crop, the improvement in biomass biogenesis of essential oil in the leaves is the most important for oil production (Sangwan *et al*. 2001). Some chemicals when applied through foliage, are known to accelerate the absorption and assimilation mechanism of nutrients and improve the yields in several crops. It has been shown that soil application of diammonium phosphate (DAP) increases the grain yield in wheat, and also, it has enhanced the production of essential oil in rose, sage and lavender plants (Ram *et al*. 2003).

The present study was undertaken to investigate the effects of foliar application of diammonium phosphate on morphological characteristics and quality of EO of Mexican marigold.

MATERIALS AND METHODS

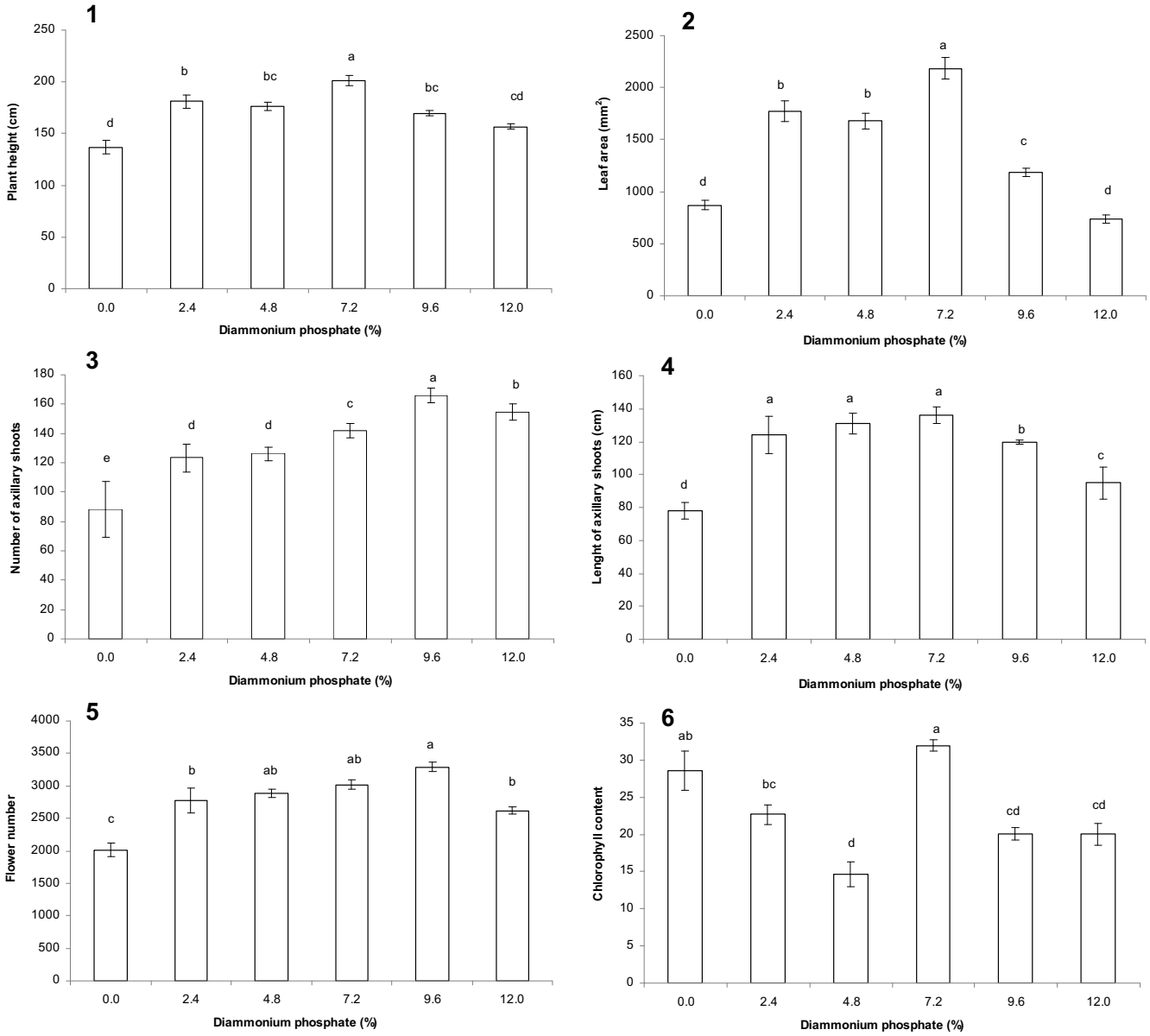
Plant material

The seeds of Mexican marigold were cultivated in a Sadra greenhouse (Shiraz, Iran) in February 2007. Plants were grown in sandy loam soil. Some of the physiological characteristics of the soil are shown in **Table 1**. The experiment was arranged as a completely randomized design (CRD) with four replications. The treatment consisted of 6 DAP levels (0, 2.4, 4.8, 7.2, 9.6 and 12%). The seedlings were hand transplanted from the nursery bed to main pots when they had 7-8 leaves on April 14th. All plants were irrigated immediately after transferring the seedlings equally among pots. Irrigation was carried out every day for one week to establish the seedlings in pots. There were 120 pots in total. DAP was sprayed at 3 stages based on Ram *et al*. (2003). First, the soil was covered by plastic in order to prevent DAP from being absorbed into the soil. Then, plants were sprayed at the stage of stem formation at 5 pm in the afternoon. The next day, the plastic was removed. The second foliar spraying was done 2 weeks later, and the third foliar spraying was done when flower-buds formed, 35 days later the second foliar spraying, and plants were at this stage for 2 weeks. Plant height, the amount of chlorophyll, the number and the length of axillary shoots and leaf area were measured at the full flowering stage. Large foliar stalks were harvested with pruning-shears, leaving about 5 cm above the ground surface. The shoots and roots fresh weight and flower number were measured. Also, chlorophyll content was measured by chlorophyll meter SPAD-502 (Konica-Minolta, Osaka, Japan). All samples were shade-dried (during 15 days). EO was extracted by subjecting flowers and leaves together (50 g) to hydrodistillation for 2 h using an all glass Clevenger-type apparatus (Goldis, Tehran, Iran), according to the method outlined by the European pharmacopoeia (Anonymous 1996). EO yield was expressed as percentage w/w on dry matter basis. The oils were dried over anhydrous Na_2SO_4 and stored in sealed vials at low temperature (4°C) before analysis. Then the oils were analyzed by GC and GC-MS. Data were subjected to variance analysis and means were compared by using Duncan's New Multiple Range Test (DNMRT).

Table 1 Some physical and chemical characteristics of the experimental soil.

EC ds m^{-1}	pH	OC[a] (%)	TN[b] (%)	P (mg kg^{-1})	K(mg kg^{-1})	Silt (%)	Sand (%)	Clay (%)
1.8	7.8	1.775	0.06	14	275	12	78	10

[a] Organic matter (OC), [b]Total Nitrogen (TN)



Effect of foliar application of DAP on plant height (**Fig. 1**), leaf area (**Fig. 2**), number of axillary shoots (**Fig. 3**), length of axillary shoots (**Fig. 4**), flower number (**Fig. 5**) and chlorophyll content (mg/g fresh weight of leaf sample) (**Fig. 6**) of *Tagetes minuta* L. Means followed by different letters are significantly different ($P \leq 0.05$), as indicated by DNMRT.

Chemicals

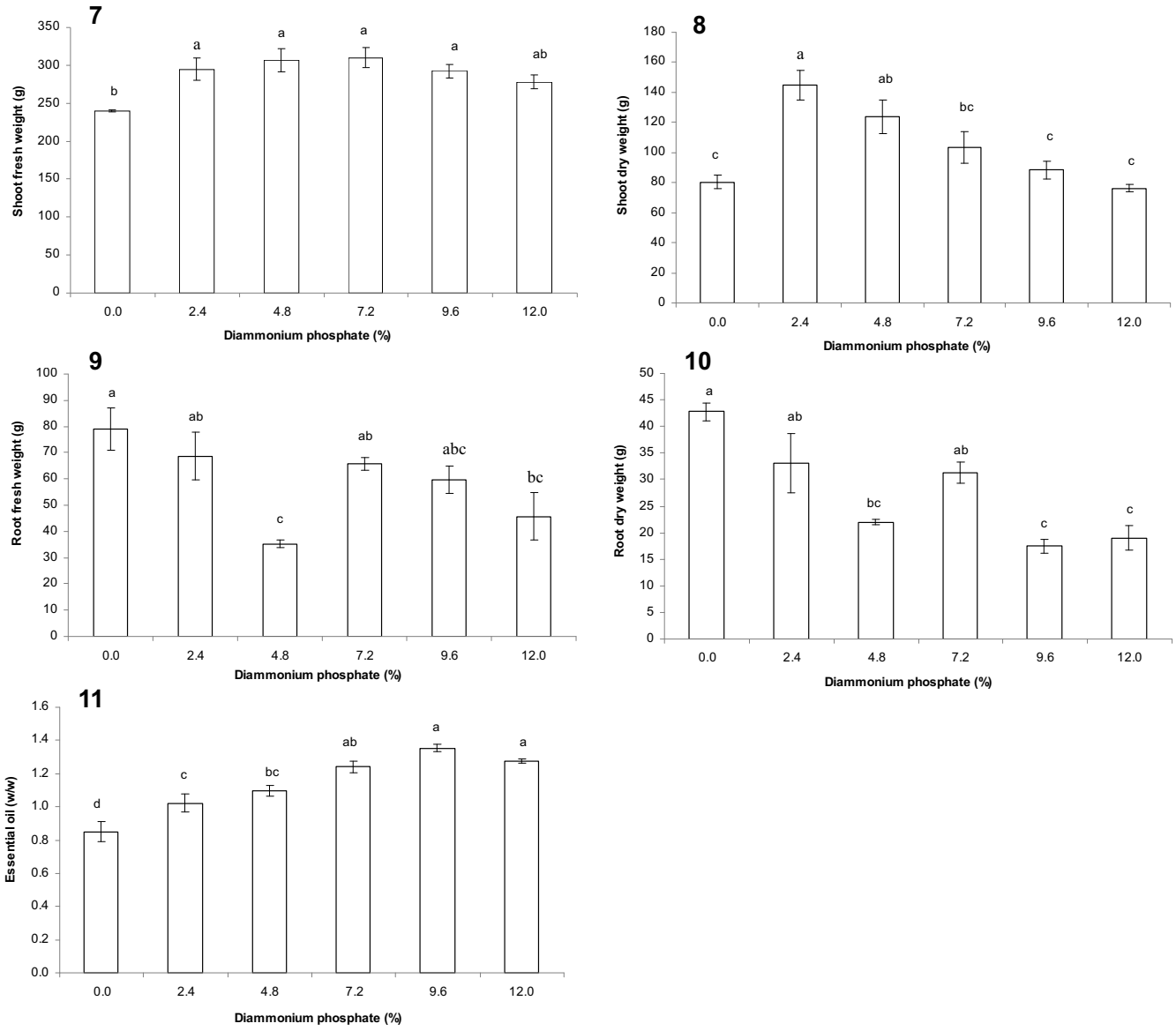
DAP was purchased from LabScan (Dubline, Ireland), and Anhydrous Na_2SO_4 was purchased from Fluka (Buchs, Switzerland). The 1-hexanol used as internal standard was purchased from Sigma-Aldrich (Buchs, Switzerland).

Hydrodistillation

After recording their fresh biomass, 50 g of each *T. minuta* L. organ (leaves, stems, flowers and seeds) were air dried during 15 days. In order to extract their EO, they were subjected to conventional hydrodistillation for 2 h. Then, extractions of all replications belonging to each treatment were mixed, and final extractions were analyzed. The hydrodistillation was performed by a simple laboratory Quikfit apparatus which consisted of a 2000 mL distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted twice with *n*-pentane and dried over anhydrous Na_2SO_4 and stored in tightly closed dark vials at 4°C until analysis. Choice of the solvent was based on its ability to extract the major constituents of the EO without loss of the high volatile components during the concentration step (Hosni *et al*. 2011). For the determination of the yield, the EO was weighed on an analytical scale. After weighing, the whole sample was re-diluted in 1 mL of the extraction solvent and 1 µL was subsequently analyzed.

Gas chromatography

Analytical gas chromatography was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-1 fused silica column (60 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was held at 40°C for 5 min and then programmed to 280°C at a rate of 4°C /min; injector and detector Flame Ionization Detector (FID) temperature was 290°C; carrier gas, helium with a linear velocity of 32 cm/s. Percentages of components were calculated by electronic integration of FID peak areas without the use of response factor correction.



Effect of foliar application of DAP on shoot fresh weight (**Fig. 7**), shoot dry weight (**Fig. 8**), root fresh weight (**Fig. 9**), root dry weight (**Fig. 10**) and essential oil content (**Fig. 11**) of *Tagetes minuta* L. Means followed by different letters are significantly different ($P \leq 0.05$), as indicated by DNMRT.

Gas chromatography-mass spectrometry analyses

The Gas chromatography-mass spectrometry analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d.). Oven temperature was 40-250°C at a rate of 4°C; transfer line temperature was 260°C; carrier gas was helium with a linear velocity of 31.5 cm/s; split ratio was 1/60; ionization energy was 70 eV; scan time was 1s; mass range was 40-300 amu.

Identification of components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds available in our laboratory and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Shibamoto 1987; Davies 1990; Adams 2001; Sefidkon *et al*. 2004; Msaada *et al*. 2007). Quantitive data (%) were obtained from the electronic integration of the FID peak areas without the use of the correction factors. Data (EO yields and other parameters) were analyzed by MSTATC using ANOVA with the least significant difference (LSD) at the 0.05 probability level.

RESULTS

The data of this investigation showed that all growth parameters were positively affected by foliar application of DAP. The plant height, the number and length of axillary shoots, the number of flowers, the fresh and dry weight of shoots, leaf area and the EO yield increased significantly by foliar application of DAP as compared to control ($P \leq 0.01$). In addition, spraying DAP affected the EO composition (**Figs. 1-11**).

The total dry matter is an important criterion for crop production. At 7.2% DAP, the height noticeably increased, and the length of plants reached 200.9 cm, but as DAP levels increased, the height of plants decreased to around 156.4 cm (at 12% DAP) (**Fig. 1**). Surprisingly, foliar spraying DAP increased leaf area of *T. minuta* when compared to control plants (869.8 mm²). However, it increased dramatically to 2184 mm² (at 7.2% DAP) before sinking to a low of 738.5 mm² (at 12% DAP) (**Fig. 2**). Although the number of axillary shoots increased from 88.25 in control plants to 166 at 9.6% DAP, it showed a dip at 12% DAP to 154 (**Fig. 3**). From the results of this research, it appeared that DAP could have the positive influence on the increasing of *T. minuta* shoot number. It can be clearly seen that the length of axillary shoots significantly increased to 136.1 cm at 7.2% DAP in comparison to control plants (78 cm), but at 2.4% (124.2 cm), 4.8% (131 cm), and 9.6% DAP (119.5

Table 2 Effect of foliar application of DAP on essential oil composition of *Tagetes minuta* L.

No.	Constituent	RI[a]	DAP (%)					
			0	**2.4**	**4.8**	**7.2**	**9.6**	**12**
1	Sabinene	979	3.5	2.7	2.7	2.84	2.4	1.84
2	*Z*-β-Ocimene	1037	12.9	11.74	9.96	10.12	9.7	7.04
3	*E*-β-Ocimene	1047	0.08		-	-	-	-
4	dihydro tagetone	1056	62.5	63.72	71.11	68.97	71.48	74.6
5	*α*-pinene oxide	1076	0.1	0.08	-	0.06	-	-
6	*cis*-thujone	1095	0.03	-	-	-	-	-
7	allo ocimene	1130	0.02	-	-	-	-	-
8	*β*-pinene oxide	1127	0.53	0.71	0.57	0.58	0.54	0.4
9	*E*-tagetone	1145	2.3	1.6	1.2	2	1.8	0.6
10	*Z*-tagetone	1155	9.02	9.9	9.1	10.36	9.95	11.2
11	methyl salicylate	1201	0.03	-	0.05	-	0.04	-
12	Verbenone	1210	0.07	-	-	-	-	-
13	*Z*-ocimenone	1233	4.58	4.07	2.43	2.05	1.47	1.8
14	*E*-ocimenone	1243	3.34	5.08	1.91	2.4	2.3	1.9
15	isoeugenyl phenylacetate	1404	0.2	-	-	-	-	0.11
16	*trans*-caryophyllene	1442	0.19	0.42	0.12	0.23	0.18	0.18
17	α-humulene	1476	0.21	-	-	0.1	-	0.15
18	Bicyclogermacrene	1518	0.18	-	-	0.68	0.09	0.11
19	Caryophyllene oxide	1609	0.07	-	-	0.03	-	-
20	neophytadiene	1842	0.21	-	-	0.03	-	-
21	Limonene	1879	0.03	0.01	0.03	0.02	0.03	0.05
22	*α*-Terpinolene	1884	0.01	0.04	0.01	-	0.02	0.04
23	*cis*-Epoxy-ocimene	1897	-	-	0.02	-	-	-
24	Linalyl propionate	1903	-	-	-	0.05	-	-
25	Piperitone oxide	1911	-	0.01	-	0.03	-	0.01
26	Heptadecane	1956	-	-	-	0.01	-	-
27	Octadecane	1984	0.02	-	0.02	-	-	0.06
28	Nonadecane	1993	-	-	-	-	0.04	0.06
29	Heneicosane	2095	-	-	-	-	0.02	0.04
30	Docosane	2286	-	-	-	-	0.01	0.01
31	Tricosane	2390	-	-	0.01	0.04	-	0.07
	Grouped compounds							
	Monoterpene hydrocarbons		16.54	14.49	12.7	12.98	12.15	8.97
	Oxygenated monoterpenes		82.87	85.17	86.34	86.5	87.54	90.51
	Sesquiterpene hydrocarbons		0.6	0.42	0.14	1.02	0.31	0.56
	Oxygenated sesquiterpenes		0.27	-	-	0.03	-	0.11
	Others		0.24	-	0.06	0.07	0.07	0.12

a Retention Index (RI), -: not detected

cm) length of axillary shoots did not show significant differences (**Fig. 4**). As regards to the number of flowers, it showed an upward trend. Control plants had the least number of flowers (2012). However, it peaked at 2771 at 2.4% DAP. The number of flowers reached a maximum at 9.6% DAP (3287), but there were not significant difference between the number of flowers at 9.6% and other levels of DAP (**Fig. 5**). On the other hand, chlorophyll content fluctuated significantly with increasing levels of DAP. The amount of chlorophyll in control plants was 28.55 though it decreased to 14.63 at 4.8% DAP and reached 32 at 7.2% DAP (**Fig. 6**). According to the fresh weight of shoots, control plants had the least amount of fresh weight of shoots (239.5 g), and spraying 2.4% DAP increased the fresh weight to 294.8 g. Though at 4.8 and 7.2% DAP, the fresh weight of shoots increased to about 310 g (**Fig. 7**). Regarding the dry weight of shoots, it was low-nearly 80.5 g in control plants, while it increased at higher concentrations and reached 144.8 g at 2.4% DAP, which can be beneficial for the production of EO. Afterwards, 12% DAP decreased the dry weight of shoots to 76.25 g, but there were no significant difference between dry weight of shoots in control plants with those of at 7.2, 9.6 and 12% DAP (**Fig. 8**). In terms of the fresh weight of root, it showed some fluctuations. The control plants had the maximum fresh weight of root (79 g). However, it decreased at 2.4 and 4.8% DAP to 68.75 and 35.25 g, respectively and then increased to 45.75 g at 12% DAP (**Fig. 9**).

According to the dry weight of root, **Fig. 10** shows a drop in the dry weight of root which decreased from 42.75 to 22 g in control plants and 4.8% DAP, respectively, but it reached 33 g at 2.4% DAP and decreased to 17.5 g at 9.6% DAP. On the other hand, the most important characteristic of medicinal plants is the EO yield. Fortunately, EO of *T. minuta* in this experiment increased significantly. As a matter of fact, there was a gradual increase in the EO yield with the increase of DAP level (**Fig. 11**). The EO yield was 0.85% in control plants. Afterwards, it peaked 1.35% at 12% DAP. As a result, there was a significant difference between the EO yield in different DAP levels, and the highest amount was obtained at 9.6% DAP. Generally, foliar spraying of 9.6% DAP increased the biosynthesis of the EO in the leaves to the extent of 0.50% over the control which was of significant importance.

EO composition

The list of detected compounds with their retention indices and relative percentages are given in **Table 2**. The results of this study in situation of constituents of the EO showed that there were 20 compounds in the EO of *T. minuta* (**Table 2**). Main compounds of EO of Mexican marigold (compounds >4%) were dihydro tagetone, *Z*-tagetone, *Z*-β-ocimene and *Z*-ocimenone. DAP application increased the amount of dihydrotagetone and *Z*-tagetone andat 12% of DAP, the amount of these compounds reached 76.4% and 11.19%, respectively. On the other hand, DAP decreased *Z*-β-ocimene and *Z*-ocimenone content, although control plants had the maximum content of these compounds roughly 13.23% and 5.53% respectively. Other compounds were in tagetes oil including sabinene, β-pinene oxide, *E*- tagetone, *E*-ocimenone, *trans*-caryophyllene, α-humulene, bicyclogermacrene, caryophyllene oxide, neophytadiene, isoeugenyl phenylacetate, verbenone, methyl salicylate, *cis*-thujone,

allo ocimene and *E*-β-ocimene. The most effective DAP levels were 7.2% and 9.6% for the production of these important compounds. Dihydro tagetone is the main compound of the EO of Mexican marigold and reached its highest amount at 12% of DAP. Previous report showed that *T. minuta* EO analyzed by GC-MS was composed mainly by *Z*-β-ocimene (40.42%), dihydro tagetone (17.64%), *Z*-tagetone (9.96%), *E*-tagetone (1.57%), *E*-tagetenone (5.3%) (Lawrence *et al.* 1985). It is well documented that phosphorus is an essential element in reproductive growth of plants (Marschner 1986) and thus, the vegetative growth and the number of flowers increased with foliar spraying of DAP which was expected in our study. Phosphorus is also known to have multifarious cellular functions in plants, including: signalling and transmembrane metabolic flux and therefore, the secondary metabolism is modulated by these mechanisms (Ram *et al*. 2003). In conclusion, it appears that phosphorus is a crucial nutrient element for Mexican marigold cultivation.

DISCUSSION

Effect of DAP on plant height

Results showed that plant height was significantly ($P \leq 0.05$) affected by foliar spraying of DAP. Plant height is one of the most important factors, and the tallest height was achieved at 7.2% DAP (200.9 cm). It showed that DAP could have positive effect on length of plants. According to research carried out by Nassar *et al*. (2004). The plant height and the number of branches of chamomile were significantly increased with increasing application of phosphorus. Singh *et al*. (2008) revealed the positive effect of phosphorous on the plant height of *Calendula officinalis* L. The effect of DAP phosphate on growth may be due to the activity of phosphate solubilization caused by the strain and increased further mineral availability uptake.

Effect of DAP on leaf area

Results showed that among the DAP treatments, the largest leaf area was recorded at 7.2% DAP, rising to 869.8 mm² at 7.2% DAP. The spray of diammonium phosphate increased leaf area and the rate of photosynthesis in the leaves of menthol mint (*Mentha arvensis*), which ultimately favoured the biosynthesis of monoterpenes in secondary metabolite plants (Ram *et al*. 2003).

Effect of DAP on the number of axillary shoots

DAP increased the number of axillary shoots, and at 9.6% DAP, the number of shoots peaked 166. Studies of El-Ghandour *et al*. (2009) demonstrated that growth parameters of *Majorana hortensis* L. such as the number of branches were positively affected by application of phosphorous. Some researchers showed that the increase in growth characters might be due to the fact that phosphate solubilizing bacteria inoculated plants were able to absorb nutrients from solution at faster rates than un-inoculated plants (Hashemabadi *et al*. 2012)

Effect of DAP on the length of axillary shoots

The length of axillary shoots at 2.4% (124.2 cm), 4.8% (131 cm), 7.2% (136.1 cm) and 9.6% (119.5 cm) DAP significantly increased in comparison to control plants (78 cm).

Effect of DAP on the number of flowers

The effect of foliar spraying of DAP on the number of was remarkable. The least number of flowers went to control plants (2012) though at 9.6% DAP, the number of flowers leaped a new peak (3287). It is well documented that phosphorus is an essential element in reproductive and vegetative growth of plants (Marschner 1986) and thus, the vegetative growth and flower numbers stimulation increased by applied DAP in was expected in our study.

Effect of DAP on chlorophyll content

As regards chlorophyll content, it varied widely. It was about 28.55 mg/g fresh weight leaf, but at 4.8% DAP it decreased significantly to 14.63 mg/g fresh weight leaf. However, the amount of chlorophyll peaked at 32 mg/g fresh weight leaf at 7.2% DAP.

Effect of DAP on fresh weight of shoots

The DAP application increased significant ($P \leq 0.05$) shoot fresh weight, and at 4.8 and 7.2% DAP shoot fresh weight increased to around 310 g. However, the lowest shoot fresh weight was obtained in control plants (239.5 g).

Effect of DAP on fresh weight of root

Regarding root fresh weight, control plants had the highest amount of fresh weight (79 g), although it decreased with foliar spraying of DAP, and dry weight hit the lowest point at 4.8% (35.25 g) DAP.

Effect of DAP on dry weight of root

According to the results, the same as root fresh weight, control plants had the highest root dry weight (42.75 g), and application of DAP decreased root dry weight. In fact, root dry weight sank to about 17.5g at 9.6 and 12% DAP.

Effect of DAP on EO

The oil of *T. minuta* has been investigated by Meshkatalsadat *et al*. (2010), who identified *Z*-β-ocimene, dihydrotagetone, *Z*- and *E*-tagetone, and *Z*- and *E*-ocimenone as the major components. The results of our analysis show that oil of *T. minuta* was particularly rich in dihydro tagetone, *Z*-tagetone, *Z*-β-ocimene and *Z*-ocimenone. However, there are reports showing that percentage of minor constituents varies. For example α-phellandrene and *o*-cymene were the major oil components from Argentina (Gill *et al*. 2000). The variations in chemical composition could be due nature of the soil, the amount of sunlight and temperature variations and the occurrence of chemotypes (Meshkatalsadat *et al*. 2010). Thus, we believe that intrinsic and external factors could have affected the content and composition of the oil of *T. minuta*.

These results are similar to those of Salardini *et al*. (1994) with pyrethrum (*Tanacetum cinerariifolium*) who reported that application of DAP increased significantly achenes and oil yield for this crop (Salardini *et al*. 1994). They are also in agreement with the data of Nikolova *et al*. (1999) and with Nible *et al*. (2005) who observed increasing biomass of chamomile. Phosphorus is also known to have multifarious cellular functions in plants, including: signaling and transmembrane metabolic flux and therefore, the secondary metabolism was modulated by these mechanisms (Ram *et al*. 2003). Saharkhiz and Omidbaigi (2008) reported that application of phosphorus in feverfew (*Tanacetum parthenium* L.) increased significantly the growth such as height, number of flowers, shoot fresh weight and EO. Trivino and Johnson (2000) reported that total yield of volatile oil of *Origanum majorana* L. was increased by application of phosphorus. Moreover, shoot fresh weight was increased two-fold by phosphor treatment as compared to the control.

Ichimura (1995) observed that DAP increased significantly the fresh weight and essential oil concentration in Sweet basil. Similar results have been noted with black cumin (*Nigella sativa*) and coriander (*Coriandrum sativum*) (Das *et al*. 1991, Ughreja and Chundawat 1992).

Foliar application of DAP may facilitate alleviation of intracellular inorganic phosphate deficiency favoring faster

flux through the normal glycolytic step, which is energy conservation and phosphoenolpyruvate availability are highly conductive to biosynthetic processes including secondary metabolite anabolism. In fact, phosphoenolpyruvate occupies a key position in serving as an isoprenogenic substrate for volatile oils in plastids (Mahmoud and Croteau 2002). Accordingly, it is speculated that foliar spraying DAP may help plants to have a near normal mode of metabolism. This may be one of the working models for future experimentation.

In conclusion, it appears that P is a crucial nutrient element for Mexican marigold cultivation. Therefore, it is strongly recommended that on sites low in available P, the crop could be supplied with adequate P. Furthermore, we suggest that the influence of DAP on the growth, chemical composition and biochemical indices of *Tagetes minuta* L. be thoroughly studied on locations with wide range of climatology, physical and chemical properties and mineralogical characteristics.

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