

EFFECT OF FOLIAR APPLICATION OF HUMIC ACID AND BENZYLADENINE ON GROWTH AND FLOWERING OF POT MARIGOLD (*Calendula officinalis* L.)

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(Received: June 26, 2018; Accepted for Publication: December 4, 2018)

ABSTRACT

The experiment was conducted at the Horticultural Department, College of Agriculture, Duhok University, during growing season from October, 2017 to April, 2018. To study the effect of humic acid and benzyladenine on the growth and flowering development of pot marigold, the seedlings which growth of transplanted in to plastic bag which were spray with three concentrations of humic acid (HA) (0, 0.05, 0.1 and 0.15) g l⁻¹ and benzyladenine (BA) (0, 50, 100 and 150) mg l⁻¹. The results show that treated plants with HA at 0.15 g l⁻¹ led to positive effect in no. of leaves/ plant, fresh weight of roots/ plant, days required to inflorescence bud initiation, no. of days remain inflorescence on the plant, no. of ray flowers/ inflorescence, dry weight of inflorescence and vas life, also it was noticed that the no. of lateral branches, (E-W) plant spread, fresh weight of vegetative growth/ plant, no. of days required to convert inflorescence bud to full inflorescence and inflorescence diameter was observed at 150 mg l⁻¹ of BA.

KEYWORDS: *Calendula officinalis*, Humic Acid, Benzyladenine, Vegetative and Flowering Growth.

INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is an annual herbaceous plant and a member of Asteraceae family. It's known as pot marigold, common marigold, garden marigold, English marigold, or Scottish marigold. It is cultured as rock gardens, borders, flower beds and balcony plants (Golestani *et al.* 2013). Rigane *et al.* (2013) illustrated that the pot marigold originated from Mediterranean countries, Eastern and Southern Europe and planted usually in North America, Eastern Europe, Germany and India. Pot marigold yellow and orange flowers rich by vitamin A, flavonoids, carotenoids, essential oils and mucilage substances. As well as it grown as an ornamental plant it has many medicinal benefits blood refiner, blood sugar decreasing, anti-inflammatory, skin anti-fungal and anti-viral characters (Mohammad and Kashani, 2012).

Plant fertilization is necessary for increasing yield and quality. The mineral fertilizer increase crop yield, while excessive its uses can be have reverse environmental influences, such applications result in the increase amount of heavy metals, especially Cd, Pb, and As in the soil (Atafar *et al.*, 2010). Therefore, it is necessary to use other resources of nutrients for plants which

cause highest proportion of yields with least environmental influences. Humic acid is a complex heterogeneous blend of organic material product from the decay of plant and animal residue (MacCarthy *et al.*, 1990). Humic acid is one of organic fertilizers has been applied for research in different scope of agriculture, like soil chemistry, fertility, plant physiology in addition to environmental sciences because of the various effects on plant growth (Tan, 1998). Gadimov *et al.* (2007) have reported that the potassium humate enhance in plants growth quality and tolerance of plant to drought, saline, cold, diseases and pests infection. Arancon *et al.* (2003) who explained that HA improve growth of marigolds (*Tagetes patula* L. 'Antigua Gold'), also enhanced yield and quality of flowers of gerbera (Nikbakht *et al.*, 2015). Highest fresh weight of flowers was appeared by using of humic acid on (*Calendula officinalis* L.) flowers (Mohammadipour *et al.* 2012). Fan *et al.* (2014) reported that foliar treatment of humic acid on chrysanthemum increased the growth and development that enhance net photosynthetic rate due to the high content of chlorophyll. Humic acid at high concentrations increased flowering and yield of gladiolus (Baldotto and Baldotto, 2013).

Plant growth regulators (PGRs) are an organic complex, either natural or synthetic which regulate or controls one or more particular physiological processes in the plant. The use of plant growth regulators is one of the factors in increasing the growth, yield and flower quality of plants (Nuvale *et al.*, 2010). The most influences of cytokinins in plants are stimulation of cell division and enlargement, and the delaying of senescence (Mooney and Van Staden, 1986). Benzyladenine has recently used to improve the quality of different ornamental plants (Han, 2001). Eraki (1994) in his study on *Hibiscus sabdarifja* L. plants stated that use of BA significantly increased plant height, number of branches in additional of fresh and dry weights of leaves. Mutui *et al.* (2004) stated that the adding of BA at 25 or 50 mg l⁻¹ increased the number of days to full opening of primary florets and delayed the start of flower senescence of *Alstroemeria* cut flowers. Foliar application of BA on pot marigold increased growth of different organs, active constituent's productions and total carbohydrates content as compare to the untreated plants (Menesi *et al.* 1991). BA causes increase in general growth on croton compared with control treatment as reported by (Rawia and Bedou, 2006).

This study was designed to evaluate the effect of Humic acid and Benzyladenine on vegetative growth and flowering yield of pot marigold.

MATERIALS AND METHODS

The study was carried out in the Horticultural Department, College of Agriculture, University of Duhok, Kurdistan Region, Iraq, during growing season from October, 2017 to April, 2018. Local seeds of pot marigold were sowing in basin after four weeks of germination seedling were transplanted in to the nursery bags size (12x30) cm were filled with a mixture of media that consists of garden sand and sheep manure and petmose 1:1:1 (v/v) with 1 plant/ bag. Three concentrations of humic acid (HA) (0.05, 0.1 and 0.15) g l⁻¹ and benzyladenine (BA) (50, 100 and 150) mg l⁻¹ were used as well as to control. Plants sprayed by these materials three times within ten days intervals starting after two weeks of seedlings transplanting. All agricultural and horticultural practices were achieved for the plants

under the study. All plants were harvested in the April, vegetative and flowering characters measured, after then dried in oven at 70°C for 72 hours, then dry plant parameters measured. Randomized Complete Block Design (RCBD) was used for perform this experiment and statistical analysis by using SAS program (SAS, 2007).

The study parameters included the following:

1 –Vegetative growth properties: -

Number of leaves/ plant, no. of main branches, no. of lateral branches, (N-S) plant spread (cm), (E-W) plant spread (cm), total chlorophyll in leaves (SPAD), fresh weight of vegetative growth/ plant (g), fresh weight of roots/ plant (g), dry weight of vegetative growth/ plant (g), dry weight of roots/ plant (g) and roots length (cm).

2- Flowering growth properties:-

Days required to inflorescence bud initiation, no. of days required to convert inflorescence bud to full inflorescence (after 50% from bud initiation), no. of days remain inflorescence on the plant, no. of inflorescences/ plant, length of inflorescence stalk (cm), inflorescence diameter (cm), no. of ray flowers/ inflorescence, fresh weight of inflorescence (g), dry weight of inflorescence (g) and vas life (days).

RESULTS

1- Vegetative growth properties

The results in Table (1) Clearly showed the effect of humic acid and benzyladenine on some characters of pot marigold. It appeared that there are significant increases in number of leaves/ plant which reached to (150.55 leaves) when plants sprayed with 0.15 g l⁻¹ humic acid as compared to the other concentrations and untreated plant (control), the second concentration of humic acid (0.1 g l⁻¹) had a significant increase in the no. of main branches (9.17 branches) was noticed as compared to the other treatments. Whereas, spraying pot marigold plants with BA at 150 mg l⁻¹ gave the best no. of lateral branches (26.50 branches) as compared to the control which produce least lateral branches (19.50). The (N-S) and (E-W) plant spread wasn't reached to the significant level when plants sprayed with different concentrations of humic acid and benzyladenine but it significantly increased as compared to control treatment.

Table (1): Effect of humic acid and benzyladenine on number of leaves/ plant, no. of main branches, no. of lateral branches, (N-S) plant spread and (E-W) plant spread of pot marigold.

conc.	Number of leaves/ plant	No. of main branches	No. of lateral branches	(N-S) plant spread (cm)	(E-W) plant spread (cm)
Control	106.00 d	5.39 c	19.50 b	31.50 b	10.66 b
HA 0.05 g l ⁻¹	135.89 b	6.17 bc	25.77 a	35.62 ab	24.22 a
HA 0.1 g l ⁻¹	123.55 c	9.17 a	26.00 a	36.49 a	24.03 a
HA 0.15 g l ⁻¹	150.55 a	7.05 b	25.78 a	34.49 ab	28.58 a
BA 150 mg l ⁻¹	107.61 d	6.28 bc	21.39 b	35.38 ab	24.62 a
BA 100 mg l ⁻¹	127.17 bc	6.67 bc	25.50 a	36.69 a	27.42 a
BA 150 mg l ⁻¹	124.11 c	7.50 b	26.50 a	34.93 ab	29.33 a

* Mean with the same letter and each parameter are not significantly different at 0.05 level according to Duncan multiple range test.

Table (2) Explains that the total chlorophyll content in leaves of pot marigold was significantly influenced by various concentrations of humic acid and benzyladenine compared to control that gave lower content of it. Spraying plants with 150 mg l⁻¹ BA caused significant increase in the amount of fresh weight of vegetative growth/ plant (48.43 g) as compared to untreated plants which reached to (31.93) g, while, a significant increase in the fresh weight of roots/ plant (19.17 g) was

obtained since the plants sprayed with 0.15 g l⁻¹ humic acid compared to plants treated with 50 mg l⁻¹ BA and control. Dry weight of vegetative growth/ plant was not significantly affected by the different concentrations of humic acid and benzyladenine but it was significantly increase as compared to the control, meanwhile humic acid at 0.1 g l⁻¹ recorded best values (4.41 g) and (34.53 cm) of dry weight of roots/ plant and roots length respectively, as compared to other treatments and control.

Table (2): Effect of humic acid and benzyladenine on total chlorophyll in leaves, fresh weight of vegetative growth/ plant, fresh weight of roots/ plant, dry weight of vegetative growth/ plant, dry weight of roots/ plant and roots length of pot marigold.

conc.	Total chlorophyll in leaves (SPAD)	Fresh weight of vegetative growth/ plant (g)	Fresh weight of roots/ plant (g)	Dry weight of vegetative growth/ plant (g)	Dry weight of roots/ plant (g)	Roots length (cm)
Control	33.58 b	31.93 c	7.96 c	3.32 b	2.80 cd	22.58 d
HA 0.05 g l ⁻¹	39.68 a	38.02 bc	17.13 a	6.47 a	3.63 a-c	31.25 ab
HA 0.1 g l ⁻¹	41.08 a	40.60 b	18.55 a	6.24 a	4.41 a	34.53 a
HA 0.15 g l ⁻¹	41.87 a	42.81 ab	19.17 a	6.97 a	4.23 a	32.67 a
BA 50 mg l ⁻¹	40.50 a	42.11 ab	8.46 c	6.10 a	2.29 d	26.21 b-d
BA 100 mg l ⁻¹	43.02 a	41.55 ab	15.85 ab	7.56 a	3.71 ab	29.80 a-c
BA 150 mg l ⁻¹	39.87 a	48.43 a	11.31 bc	6.45 a	2.86 b-d	25.53 cd

* Mean with the same letter and each parameter are not significantly different at 0.05 level according to Duncan multiple range test.

2- Flowering growth properties

The data present in Table (3) Shows that the spraying pot marigold plants with different concentrations of humic acid and benzyladenine led to reduce the no. of days required to inflorescence bud initiation, the best treatment (least days required to inflorescence bud initiation) (55.88 days) was noticed when plants sprayed with 0.15 g l⁻¹ humic acid as compared with control which needed to (81.33) days. Although the first and second concentrations of humic acid and first concentrations of benzyladenine was not effect significantly in the no. of days required to convert inflorescence bud

to full inflorescence whilst the third concentration of humic acid (0.15 g l⁻¹) and (150 mg l⁻¹) of BA reduced significantly the no. of days required to convert inflorescence bud to full inflorescence as compared with control. At the same time spraying plants with humic acid at (0.15 g l⁻¹) caused significant increase in the no. of days remain inflorescence on the plant as compared with control which remained (5.50) days. Also humic acid at (0.1 g l⁻¹) caused significant increase in the no. of inflorescences/ plant and length of inflorescence stalk compared with untreated plants which recorded least value.

Table (3): Effect of humic acid and benzyladenine on days required to inflorescence bud initiation, no. of days required converting inflorescence bud to full inflorescence, no. of days remain inflorescence on the plant, no. of inflorescences/ plant and length of inflorescence stalk of pot marigold.

conc.	Days required to inflorescence bud initiation	No. of days required to convert inflorescence bud to full inflorescence	No. of days remain inflorescence on the plant	No. of inflorescences / plant	Length of inflorescence stalk (cm)
Control	81.33 a	7.89 a	5.50 c	18.55 d	17.19 b
HA 0.05 g l ⁻¹	64.28 b	7.00 ab	7.50 bc	25.67 a-c	20.36 ab
HA 0.1 g l ⁻¹	71.23 b	7.83 a	7.33 bc	29.33 a	23.60 a
HA 0.15 g l ⁻¹	55.88 c	5.50 c	10.33 a	26.17 ab	21.34 a
BA 50 mg l ⁻¹	64.50 b	7.33 ab	7.00 bc	20.28 d	19.82 ab
BA 100 mg l ⁻¹	65.78 b	6.33 bc	7.50 bc	22.00 cd	21.27 a
BA 150 mg l ⁻¹	69.83 b	5.33 c	8.00 b	24.83 bc	21.45 a

* Mean with the same letter and each parameter are not significantly different at 0.05 level according to Duncan multiple range test.

Spraying pot marigold plants with different concentrations of of humic acid and benzyladenine shows there are no significant increases in the inflorescence diameter between them, but there were significantly increased as compared to the control as displayed in Table (4). The highest no. of ray flowers/ inflorescence (56.22 flowers) was observed as a result of spraying plant with 0.15 g l⁻¹ humic acid as compared to the control which produce (32.67) flowers, while, the first concentration of humic

acid (0.05 g l⁻¹) was significantly increased in the fresh weight of inflorescence (1.69 g) as compared to untreated plants (1.04) g. Data also showed that the dry weight of inflorescence and vas life was significantly affected by the concentrations of humic acid, the highest concentration of it gave maximum values of dry weight of inflorescence and vas life (0.42 g) and (7.17 days) respectively, compared to the control treatment that gave lower rates.

Table (4): Effect of humic acid and benzyladenine on inflorescence diameter, no. of ray flowers/ inflorescence, fresh weight of inflorescence, dry weight of inflorescence and vas life of pot marigold.

conc.	Inflorescence diameter (cm)	No. of ray flowers/ inflorescence	Fresh weight of inflorescence (g)	Dry weight of inflorescence (g)	Vas life (days)
Control	4.37 b	32.67 d	1.04 b	0.21 b	4.33 c
HA 0.05 g l ⁻¹	5.20 a	39.17 cd	1.69 a	0.38 a	5.17 bc
HA 0.1 g l ⁻¹	5.03 a	42.55 bc	1.63 a	0.36 a	6.00 a-c
HA 0.15 g l ⁻¹	5.07 a	56.22 a	1.31 ab	0.42 a	7.17 a
BA 50 mg l ⁻¹	5.47 a	48.50 ab	1.47 ab	0.30 ab	5.50 a-c
BA 100 mg l ⁻¹	5.20 a	46.00 bc	1.47 ab	0.38 a	6.67 ab
BA150 mg l ⁻¹	5.57 a	50.50 ab	1.62 a	0.38 a	6.17 a-c

* Mean with the same letter and each parameter are not significantly different at 0.05 level according to Duncan multiple range test.

DISCUSSION

Foliar application of humic acid had a promoting influence on the vegetative and flowering growth characters of pot marigold, since spraying plants with humic acid led to increase in number of leaves/ plant, no. of main branches, total chlorophyll in leaves, fresh weight of roots/ plant, dry weight of roots/ plant and roots length as compared to untreated plants, this effect may be due to the effects of humic substances on the growth of plants are recognized by morphological, physiological and biochemical effects (Nardi *et al.*, 2002). Abdel-Mawgoud *et al.* (2007) have mentioned that the positive effect of humic acid's ability to nutrients uptake may be because of its effect on plant dry biomass, also (Zhang and Ervin 2004) mentioned that the presence of iron in the humic acids or their colloidal nature have a real influence on the growth of different microorganisms which may excrete a range of vitamins, growth matters and antibiotics and these may active plant growth. Application Potassium-humate to the plants caused to increasing and improving growth traits of plants (Zaky *et al.*, 2006) and (Karakurt and Padem, 2009). Abolina and Tashkhadzhaev (1968) indicated that the coal-Humic fertilizers promoted the biochemical processes in plants (respiration, photosynthesis and chlorophyll content). Similar result had been observed the influences of humic substances on plant growth, showed improved effects on growth independent of nutrition (Dursun *et al.*, 2002).

In addition to the effect of humic acid on plant growth characters also it affected on flowering growth which caused increases in the no. of days remain inflorescence on the plant, no. of inflorescences/ plant, length of inflorescence stalk, no. of ray flowers/ inflorescence, fresh weight of inflorescence, dry weight of inflorescence, vas life and reduce days required to inflorescence bud initiation as compared to control treatment, this effect may be attributed as mentioned by (Azooz, 2009) and (Abd EL-Aal *et al.*, 2005) that humic acid increase photosynthesis, chlorophyll density and plant root respiration which resulted in major plant growth also the beneficial effect of humic acid on plant growth may be because to its playing as source of plant growth hormones yield. This agreed with the results found by (Ahmad, 2013) who observed that the humic acid enhance vegetative and flowering growth. Humic acid substance has auxin-like activity, improved nutrient uptake which may be responsible for the longer vase life of cut stems (Baldotto and Baldotto, 2013). This effect is agreement with Ricardo *et al.* (1993), in his studies who stated that the application of humic acid produced earlier flowering and higher yield in marigold. Application of humic at 500 mg l⁻¹ acid produced higher and healthier plant growth and enhanced flower yield and quality of gerbera (Nikbakht *et al.*, 2015).

On the other hand, The reason of improving the growth and flowering of pot marigold, in no. of lateral branches, (N-S) plant spread, (E-W)

plant spread, fresh weight of vegetative growth/ plant, dry weight of vegetative growth/ plant, no. of days required to convert inflorescence bud to full inflorescence and inflorescence diameter when plants treated with of benzyladenine as compared to control treatment, may be due to the important role of cytokinin in the regulation of the plant cell cycle and many developmental processes, also it promote growth and development of plant organs, detention of chlorophyll, translocation of nutrient (Pandey and Sinha, 1984). Al-hasnawi (2011) was observed that the cytokinins improve the nitrogen content on leaves which is essential in the formation of protein, nucleic acid, chlorophyll, enzyme, vitamins and plant hormones. cytokinin which has been found essential for gene expression at transportation level, cellular division and growth, produce naturally within plants which led to increase in flower size, plant height, breaking of dormancy, resistance to biotic and abiotic stress and branch strength (Hossein *et al.*, 2007). These results were in accordance with many reports which have shown that the cytokinins are actively stimulated earliness and delaying senescence (Mok, 1994). BA increase dry mass production by stimulates cell division and chlorophyll accumulation that help to activate photosynthesis and accumulation of dry matter (Abou Aziz, 2011). Eraki (1994a) in his study on *Hibiscus sabdariffa* L. plants stated that use of BA significantly increased plant height, number of branches in additional of fresh and dry weights of leaves. Application of BA on *Zantedeschia aethiopia* caused to increase number of leaves (Majidian, 2012). Delay senescing effect of BA help to increase post-harvest life of ornamentals as mentioned by (Subhashini *et al.*, 2011) on *Dracaena*.

CONCLUSION]

According to the results obtained in this study that the humic acid and benzyladenine had promoting effect on the growth of pot marigold which produced highest vegetative growth also it had active role in the pot marigold flowers characters as compared with control.

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كارتيكرنا رهشاندنا بهلكى يا ترشى هيوميك و بهنزل نهدينين لسهر شينبوون *Calendula officinalis L.*) و كول دانا كول بوهارى

پوخته

نهف فهكولينه هاته كرن ژههيقا جريائكى 2017ى و تا ههيقا نيسانى 2018ى، ژبو نهزمونكرنا كارتيكرنا ترشى هيوميك و بهنزل نهدينين لسهر شينبوون و كول دانا كول بوهارى. نوفى كولا هات جاندين لناف هه و دكا دا بو جار ههفتيا بو ده مئ بوينه شتل باش فهكوهاستن بو كيسكىت نايلونى، تاقيكرنا كارتيكرنا ريژيت جورا و جور ژ ترشى هيوميك (0.05, 0.1, 0.15) غم/لتر و بهنزل نهدينين (50, 100, 150) ملغم/لتر دكل كونترولى هات كرن. نهجاميت فهكولينى ديار كرن كو رهشاندنا رووهكا ب ترشى هيوميك ريژا 0.15 غم/لتر دهر نهجام هاتن ديار كرن كو زيده بونهكا بهرچاف ل ريژا بهلكا كريبه بو ههر رووهكهكى، كيشه تهر ين رها بو ههر رووهكهكى، ژمارا روژا مانا كولا لسهر رووهكهكى، ژمارا بهلكين تيشك بو ههر كولهكى، كيشه حشك يا كولا، ژمارا روژا مانا كولا ل قافكين و روژين بو كوهورينا بشكا بو كولين فهكرى هات زيده كرن. ههر هوسا هاته ديار كرن كو بترين ريژا جهقين رها، بهلافبونا رووهكهكى، كيشه تهرين كهسكاتى بو ههر رووهكهكى، رووبهري كولا و روژين بو كوهورينا بشكا كولئ بو كولا تهمام هات ديتن دمئ بكارينانا 150 ملغم/لتر ژ بهنزل نهدينينى.

تأثير الرش الورقي لحامض الهيوميك و البنزل أدنين على النمو و التزهير نبات لأقحوان *Calendula officinalis L.*

الخلاصة:

أجريت التجربة خلال فترة شهر تشرين الاول 2017 الى شهر نيسان 2018 لدراسة تأثير الحامض الهيوميك و بنزل أدنين على النمو و التزهير نبات لأقحوان. تم زراعة البذور في أحواض و بقت لمدة أربعة أسابيع لحين أصبحت بادرات و التي نقلت وزرعت في أكياس بلاستيكية و عوملت بتركيز مختلفة من الحامض الهيوميك (0.05, 0.1, 0.15) غم/لتر و بنزل أدنين (50, 100, 150) ملغم/لتر. بينت نتائج التجربة أن النباتات التي عوملت بحامض الهيوميك عند تركيز 0.15 غم/لتر أدت إلى تأثير إيجابي في عدد الأوراق/ النبات، الوزن الطري للجدور/ النبات، عدد الأيام بقاء نورة زهرية على النبات، عدد الأزهار الشعاعية/ نورة، الوزن الجاف لنورة زهرية، ومدة بقاء نورة زهرية في المزهرة و الأيام المطلوبة لبدء تكوين أول برعم نورة زهرية. كما لوحظ أن عدد الفروع الجانبية، انتشار النبات من جهة شرق للغرب، الوزن طري الخضري/النبات، قطر نورة زهرية و عدد الأيام اللازمة لتحويل برعم نورة زهرية لنورة كاملة لوحظ عند معاملة نباتات بتركيز 150 ملغم/لتر من بنزل أدنين.