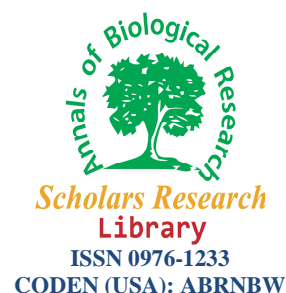




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## Role of Bio-stimulators on Seed Germination and seedling nutrient content of Pot Marigold (*Calendula officinalis* L.)

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### ABSTRACT

*Pot marigold (Calendula officinalis L.) as an important medicinal plant is used to treat skin illnesses. As bio-stimulators containing amino acids can directly or indirectly influence the physiological activities of the plants, this study was done to effects evaluation of bio-stimulators on seed germination and early growth stages of pot marigold. A factorial experiment was conducted on the basis of randomized complete design with three replicates at Institute of Medicinal Plants, ACECR. Bio-stimulators in four commercial formulations of Aminolforte, Kadostim, Fosnutren and Humiforte, and also their concentrations in six levels (0, 0.2, 0.4, 0.6, 0.8, and 1%) were the two studied factors. The studied parameters were: germination percent, germination rate, length of radical and hypocotyl, hypocotyl/radicle length ratio, fresh and dry weight of radicle, hypocotyl and seedling and also content of nutrient elements in seedlings including N, P and K. The results showed that bio-stimulators had significant effect ( $P < 0.01$ ) on all parameters. Of course, the concentration of bio-stimulators hadn't significant effect on hypocotyl length, fresh weight of radical, hypocotyls and seedling, radical dry weight and germination percent. The interaction effect had significant effect ( $P < 0.01$ ) on the studied parameters except the germination percent, and fresh and dry weight of radical. Also, content of seedlings nutrient elements including N, P and K increased with application of bio-stimulators. In conclusion, application of bio-stimulators has promoted seedling growth of pot marigold and so it is suggested that these compounds have positive effects on seedling establishment in farms.*

**Key words:** *Calendula officinalis* L., Bio-stimulators, Germination, Seedling.

### INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is a member of Asteraceae family. British names of this plant are Pot Marigold, Marygold, Garden Marigold, Marigold florets [1]. In landscape design, we can cultivate pot marigold with short plants in front of the tall plants and near lawn. Some of these plants that have special beauty near pot marigold are as follows: Titonia, Cosmos, Lupin. Complete capitula or separated florets from floral receptacle compose the medicinal part of the plant. Florets are fragrant and yellow to orange and there is not any scale among them. Width of the capitula is 4-7 cm. Lateral florets are linguiform and pistillate. Length of them is about 2 cm.

Central florets are tubular and staminate and their length is limited in 5 mm. This plant has medicinal properties especially it is used for treatment of skin illnesses [2].

The amino acids are essential constituents in all cells. In addition to their role in protein synthesis, they participate in both primary and secondary metabolic processes associated with plant development and in responses to stress. For example, glutamine, glutamate, aspartate, and asparagine serve as pools and transport forms of nitrogen, as well as in balancing the carbon/nitrogen ratio. Other amino acids such as tryptophan, methionine, proline and arginine contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones. Apart from their biological roles in plant growth, some amino acids, termed “essential amino acids,” are also important for the nutritional quality of plants as foods and feeds. This is because humans, as well as most livestock, cannot synthesize all amino acids and therefore depend on their diets for obtaining them. Among the essential amino acids, lysine, methionine, threonine, and tryptophan are considered especially important because they are generally present in low or extremely low amounts in the major plant foods. Studies on amino acid metabolism in plants have always benefited from the more advanced understanding of amino acid metabolism in microorganisms. Combined genetic, biochemical, molecular, and more recently genomics approaches coupled with administration and metabolism of various precursors as major donors of carbon, nitrogen, and sulfur, have provided detailed identification of flux controls of amino acid metabolism in microorganisms [3]. So far, bio-stimulators can be a subgroup of bio-regulators, that is, preparations with a much wider action, which regulate life processes within plants in various ways, not only through stimulation [4]. Bio-stimulators made of plants natural extracts are mainly composed of amino acids and poly peptides with low molecular weight, vitamins, enzymes and hormones (auxin, cytokinin and gibberlins), carbohydrates, betains, antioxidants and other substances and also animal extracts like mainly amino acids and peptides, and stimulator compounds of enzyme activity in plant tissues [5]. The positive effect of bio-stimulators on production, quality and growth of vegetables, *Camellia* species and forage crops is reported [18, 29]. However, some main constituents of bio-stimulators are amino acids and organic components, which can play main role in plant growth and dry mater accumulation. Because plant biomass is composed of proteins, 20-40%, and free amino acids, 0.1-1% [30, 31]. The aim of this study is to investigate the effects of bio-stimulators on seed germination of pot marigold.

## MATERIALS AND METHODS

To investigate the effect of bio-stimulators on germination seed and seedling growth of *calendula officinalis* L., a factorial experiment was conducted on the basis of completely randomized blocks design with 3 replicates in 2011 year. First factor, solution types (four commercial formulations of Aminolforte, Kadostim, Fosnutren and Humiforte) and second factor, concentrations in six levels (0, 0.2, 0.4, 0.6, 0.8 and 1%) were the arrangement of treatments. The investigated parameters were germination percent, germination rate, radicle length, hypocotyl length and hypocotyl/radicle length ratio, and fresh and dry weight of radicle, hypocotyl and seedling.

Quantity and kind of free amino acids applied in the formulation of bio-stimulators in this experiment based on the percent of total amino acids are as follows: Glysin 11.2% , Valine 5.1%, Proline 8.3%, Alanin 13.2%, Aspartic acid 4.4%, Arginine 8.3%, Glutamic acid 0.9%, Lysine 5.1%, Lucine 16.4%, Isolucine 4.4%, Phenylalanin 5.1%, Methionine 4.2%, Serin 3.9%, Treonine 0.3%, Histidine 0.3%, Tyrosin 1.5%, Glutamine 0.9%, Systeine 0.3%, Asparagine 0.4%, Tryptophan 0.4%.

### Formulation of the bio-stimulators used in experimental treatments.

Bio-stimulators*	Formulation of compounds
Aminolforte	Free amino acids 3750 mg/L, organic components 2% and total N 1.1% (Urea 0.8%, and organic N 0.3%).
Kadostim	Free amino acids 3750 mg/L, organic components 2% and total N 5% (Ammonia 0.8%, Nitric 3.1% and organic N 0.3%) and potassium (K <sub>2</sub> O) 6%.
Humiforte	Free amino acids 3750 mg/L, organic components 2% and total N 6% (Ammonia 1.4%, Urea 3.7%, Nitric 0.5%, and organic N 0.3%), potassium (K <sub>2</sub> O) 5% and phosphorous (P <sub>2</sub> O <sub>5</sub> ) 3%.
Fosnutren	Free amino acids 3750 mg/L, organic components 2% and total N 3.8% (Ammonia 2.1%, Nitric 1.4%, and organic N 0.3%) and phosphorous (P <sub>2</sub> O <sub>5</sub> ) 6%.

\*Bio-stimulators supplied by Inagrosa Industries Agro Biological are compatible to the climate of Iran.

Pot marigold seeds were obtained from Seed Technology Laboratory of Medicinal Plants Institute, ACECR and this study was conducted here. The seeds were surface-sterilized by 5 min exposure to 3% calcium hypochlorite, thoroughly rinsed with sterile distilled water and transferred to plates [6]. Twenty seeds were placed on filter paper

(Whatman #. 2) in every disinfected 9 cm diameter Petri dish. Filter papers were moistened with 9 ml of distilled water or test solution. After moistening, the Petri dishes were sealed to prevent desiccation with a plastic film (Para film, American National Can, 101 Merritt, Greenwich, CT 06836). Each treatment was applied to three Petri dishes. Then Petri dishes were kept in controlled environment chambers (plant growth chamber, Model GC-300 LT/H, JEIO Tech Co., Ltd., Seoul, South Korea) for 10 days in 24°C and photoperiod of 16 hours light and 8 hours darkness. The regularly counting of germinated seeds was conducted every 2 days and the seeds were considered germinated when the radicle was 1 mm or longer [7]. At the end of 10<sup>th</sup> day, radicle and hypocotyl length and total fresh weight of seedlings were measured and then they were taken to oven with 72°C for 48 hours to measure dry weight of seedlings. For the measurement of germination percent (GP), formula (1) and for germination rate (Rs), formula (2) was used:

(1)  $GP = 100(NG/NT)$ , where NT is total number of seeds and NG is number of germinated seeds.

(2)  $Rs = \sum_{i=1}^n (S_i / D_i)$ , where  $S_i$  is number of germinated seeds in every day,  $D_i$  is number of days to n measuring day and n determines the number of days for measuring [8].

Nitrogen, phosphorus and potassium were determined in dried leaves according to Wahing *et al.*, 1989 and Chapman and Pratt, 1961. Statistical analysis was conducted by SAS software. Also comparison of means was conducted by LSD ( $p < 0.01$ ).

## RESULTS AND DISCUSSION

The results showed that the effect of bio-stimulators on germination rate was significant ( $P < 0.01$ ) in a way that maximum germination rate with Humiforte and the least germination rate with Kadostim was obtained (Tables 1 & 2). Effect of the solution concentration on germination rate was significant ( $P < 0.01$ ) and the highest rate occurred in control (9.98) and 0.2% (9.84) and the lowest rate in 0.8% (8.94) (Tables 1 & 3). Germination rate decreased with increase in solution concentration. The interaction effect on germination rate was significant ( $P < 0.01$ ). Maximum rate of germination with 0.2% Fosnutren and minimum rate with 0.6% Kadostim was obtained (Tables 1 & 4). Effect of solution kind on germination percent was significant ( $P < 0.01$ ) in a way that maximum percent of germination with Aminolforte and minimum percent with Fosnutren occurred (Tables 1 & 2). Effect of solution concentration on germination percent was insignificant (Table 1). The interaction effect on germination percent was statistically insignificant (Table 1).

The arginine amino acid was the main compound in Aminolforte and Humiforte (11.7%). Increasing germination percent in response to arginine could be attributed to the enhancement of polyamines synthesis which may stimulate activity of hydrolytic enzymes [9]. Also application of fertilizers including ammonium nitrate, triple super phosphate, and potassium chloride increased seed germination rate and percent [10]. Increase in germination rate and percent in Humiforte and Aminolforte can be due to existence of arginine in both compounds and macro-elements of NPK in Humiforte and N in Aminolforte.

Effect of bio-stimulator on radicle length was statistically significant ( $P < 0.01$ ) in a way that highest radicle length in treatment of Aminolforte and the least length in Fosnutren was obtained (Tables 1 & 2). The effect of concentration was significant ( $P < 0.01$ ) on radicle length in a way that the highest radicle length with concentration of 0 and the least length with concentration of 1% was observed. Therefore, a decreasing trend in radicle length was observed with increasing concentration (Tables 1 & 3). The interaction effect on radicle length was significant ( $P < 0.01$ ) and the maximum radicle length with the treatment of 0.6% Aminolforte and the minimum length with 0.6% Humiforte was obtained (Tables 1 & 4). Bio-stimulators such as Aminolforte had positive effect on root growth by increasing the absorption rate of nutrients and existence of N macro-element in this compound [11]. Existence of glutamic acid (especially in Aminolforte) had positive effect on root length of *Codiaeum variegatum* L. [12]. Exogenous application of polyamine (end product of arginine) to several plant species have been shown to promote cell division, cell differentiation and general growth promotion [13, 14].

Kind of bio-stimulators had significant effect ( $P < 0.01$ ) on hypocotyl length and also, maximum and minimum hypocotyl length was observed in Kadostim and Fosnutren, respectively (Tables 1 & 2). The hypocotyl length wasn't significantly affected by the bio-stimulators concentration (Table 1). The interaction effect of solution kind and its concentration on hypocotyl length was statistically significant ( $P < 0.01$ ) and maximum hypocotyl length with treatment of 0.6% Kadostim and minimum length with 0.4% Humiforte occurred (Tables 1 & 4). Tyrosine (the most

amount of this amino acid in Kadostim) is hydroxy phenyl amino acid that is used to build neurotransmitters and hormones. Biosyntheses of cinamic acids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine. The positive effect of amino acids on yield may be due to the vital effect of these amino acids stimulation on the growth of plant cells [15]. The positive effect of amino acids on growth showed that amino acids can serve as a source of carbon and energy. When carbohydrates become deficient in the plants, amino acids are determinate, releasing the ammonia and organic acid from which the amino acid was originally formed. The organic acids then enter the Krebs cycle, to be broken down to release energy through respiration [16]. According to conducted experiments positive effects of bio-stimulators on stem growth of perennial plants is due to existence of nitrogen and potassium in Kadostim [17].

Solution kind had significant ( $P < 0.01$ ) effect on hypocotyl/radicle length ratio and maximum ratio with Fosnutren solution and minimum ratio with Aminolforte was obtained (Tables 1 & 2). Solution concentration had statistically significant ( $P < 0.01$ ) effect on hypocotyl/radicle length ratio in a way that maximum ratio with concentration of 0.6% and minimum ratio with control was obtained (Tables 1 & 3). The interaction effect was significant ( $p < 0.01$ ) on this ratio and maximum effect with 0.6% Humiforte and minimum ratio with 0.8% Fosnutren was obtained (Tables 1 & 4). The positive effect of bio-stimulators application on growth parameters of canola and sunflower seedlings was proved [18].

Effect of solution kind on fresh and dry weight of radicle was significant in a way that the most radicle fresh weight with Kadostim and the most radicle dry weight with Aminolforte, Kadostim and Fosnutren were obtained. Also, the least fresh and dry weight occurred with Fosnutren and Humiforte, respectively (Tables 1 & 2). Effect of solution concentration on fresh and dry weight of radicle was statistically insignificant. Also, the interaction effect of solution kind and concentration on fresh and dry weight of radicle was insignificant (Table 1). The same results were obtained by application of bio-stimulators on tea plant [19]. Kadostim is supposed to enhance the uptake of other applied nutrients, as well as to help the crop overcome periods of stress. This is because Bio-Synthesized Free Amino Acids (SFAAs) in this product is readily recognized by the plant cell messenger RNA, leading to an overall increase in plant metabolic activities, including enhanced photosynthesis [20].

The solution kind had significant ( $P < 0.01$ ) effect on fresh and dry weight of hypocotyls. The maximum fresh weight was observed in Humiforte and the maximum dry weight was obtained in Aminolforte, Kadostim and Humiforte. Also, the minimum fresh and dry weight was related to the treatment of Fosnutren (Tables 1&2). Effect of solution concentration on dry weight of hypocotyl was statistically significant ( $P < 0.01$ ) and maximum dry weight was related to concentration of 0, 0.2, and 0.8%. The minimum dry weight was occurred in concentration of 1% (Tables 1 & 3). The interaction effect of solution and concentration on fresh and dry weight of hypocotyl was significant ( $P < 0.01$ ) in a way that the most fresh and dry weight of hypocotyl was obtained with 0.8% and 0.6% Fosnutren, respectively (Tables 1 & 4). Also the least both parameters were observed in Fosnutren 1%. Humiforte contains coenzymes which can join macro- and micro-nutrients with the amino acid chains and transform polysaccharides into stable humic and folic acid molecules. Such humic substances have been reported to have direct and indirect effects on plant growth and productivity: direct effects are on plant metabolism through root uptake, and indirect effects through alterations to the chemical and physical properties of the soil [21]. Kadostim contains potassium which incorporated into the amino acid chains. In this bioactivity form, potassium is rapidly absorbed through the leaves. Positive effects from using this bio-fertilizer have also been observed in watermelon (yield increase by 14.5%), in 1-year-old seedlings of *Hopea odorata* of chengal hardwood group (82% more new shoot production) and in starfruit (97% fruits falling into Grade C or higher, as compared to the control with 29% of fruits lower than Grade E) [22]. Bio-stimulators had a significant effect ( $P < 0.01$ ) on fresh and dry weight of seedling in a way that the most fresh weight in Humiforte and on dry weight in Aminolforte and Kadostim and also, the minimum fresh and dry weight in Fosnutren was obtained (Tables 1 & 2). The solution concentration had significant effect ( $P < 0.01$ ) on dry weight. The highest dry weight was observed in concentrations of 0 and 0.2% and the minimum in concentration of 1% (Tables 1 & 3). The interaction effect of solution and concentration on fresh and dry weight of the seedling was significant ( $P < 0.01$ ) that the most fresh weight was related to Fosnutren with concentration of 0.8% and the highest dry weight was observed in 0.2% Aminolforte. The minimum fresh and dry weight was obtained in 0.6% Humiforte and 1% Fosnutren, respectively (Tables 1 & 4). Application of Humiforte stimulated the growth of Norway spruce [11]. Humiforte is a high-tech soluble liquid nutrient, with rapidly absorption via leaves or roots, and a high concentration of free amino acids and biologically active oligopeptides especially recommended for shock treatment [23]. The assimilation of ammonium usually occurs in the roots, whereas nitrate assimilation, depending on the species and environmental conditions, occurs either in the roots or in the leaves, after transport via the xylem [25].

Bio-stimulators had significant effect ( $P < 0.01$ ) on seedling nitrogen content in a way that the highest content was obtained in Kadostim and the lowest in Fosnutren (Tables 1 & 2). Effect of concentration on seedling nitrogen content was significant ( $P < 0.01$ ) in a way that the most percent of nitrogen was occurred in concentration of 1% (Tables 1 & 3). Interaction effect of solution kind and concentration was significant ( $P < 0.01$ ) on N content in a way that the most nitrogen percent was observed in 1% Kadostim and the least in 1% Fosnutren (Tables 1 & 4).

Effect of bio-stimulators on content of phosphorous in pot marigold seedling was significant ( $P < 0.01$ ) in a way that the most P content was Fosnutren (Tables 1 & 2). Effect of concentration was significant ( $P < 0.01$ ) on seedling P content in a way that the most content of P was obtained in concentration of 1% and the lowest content in control (Tables 1 & 3). Interaction effect of solution kind and concentration was significant ( $P < 0.01$ ) in a way that the most effective treatment on seedling P content was 1% Fosnutren (Tables 1 & 4). With consideration to formulation of compounds, it can be mentioned that the most content of P in treatment of Fosnutren is due to existence of higher concentration of this nutrient in this compound (Table 3).

Bio-stimulators had significant effect ( $P < 0.01$ ) on potassium content of seedling in a way that the most content was obtained by Kadostim and the least with Fosnutren (Tables 1 & 2). Effect of the concentration was significant ( $P < 0.01$ ) in a way that the most content of this nutrient was observed in concentration of 1% (Tables 1 & 3). The interaction effect was significant in a way that the most content of potassium was obtained by 1% Kadostim (tables 1 & 4). The most content of potassium in treatment of Kadostim can be due K concentration of formulation of compounds (Table 3).

## CONCLUSION

In this study, commercial formulations of bio-stimulators (biologically amino acid compounds) had significantly positive effect on seed germination and seedling growth of pot marigold. The effectiveness of bio-stimulators can be due to existence of amino acid compounds and nutrients such as N, P and K which can promote metabolic activities like photosynthesis and absorption and transfer of nutrients from root and leaves.

**Table 1. Analysis of variance for effects of bio-stimulators on germination parameters of *Calendula officinalis* L.**

S.O.V	df	Mean squares				
		Germination percent	Germination rate	Radicle length	Hypocotyl length	Hypocotyl/ radicle ratio
Bio-stimulators(a)	3	1176.273**	15.608**	8493.710**	220.610**	7.444**
Concentration(b)	5	142.847ns	9.561**	1720.815**	19.133 <sup>ns</sup>	2.177**
a×b	15	74.884ns	3.566**	521.858**	52.730**	0.781**
error	48	64.583	1.245	156.167	12.526	0.157
cv		9.02	12.42	24.81	11.24	29.5

\*, \*\*, ns shows significant in 5%, 1% and insignificant, respectively.

**Table 1- Continued.**

S.O.V	df	Mean squares								
		Radicle fresh weight	Hypocotyl fresh weight	seedling fresh weight	Radicle dry weight	Hypocotyl dry weight	seedling dry weight	N%	P(mg.g <sup>-1</sup> DW)	K(mg.g <sup>-1</sup> DW)
Bio-stimulators(a)	3	0.026**	0.428**	0.603**	0.0010*	0.001**	0.001**	0.050**	0.001**	1.128**
Concentration (b)	5	0.006 <sup>ns</sup>	0.055 <sup>ns</sup>	0.077 <sup>ns</sup>	0.0002 <sup>ns</sup>	0.0005*	0.0005**	0.029**	0.001**	0.050**
a×b	15	0.005 <sup>ns</sup>	0.118**	0.127**	0.0005 <sup>ns</sup>	0.001**	0.001**	0.034**	0.001**	0.131**
error	48	0.003	0.027	0.040	0.0001	0.0001	0.00008	0.007	0.000042	0.009
cv		23.53	20.25	18.1	35.28	18.75	12.37	4.49	1.85	4.89

\*, \*\*, ns shows significant in 5%, 1% and insignificant, respectively.

**Table 2-Mean comparisons of bio-stimulators effects on measured parameters\***

treatment	Germination percent	Germination rate	Radicle length (mm)	Hypocotyl length (mm)	Hypocotyl/radicle length ratio
Aminolforte	96.39 <sup>a</sup>	9.11 <sup>b</sup>	72.21 <sup>a</sup>	32.34 <sup>b</sup>	0.46 <sup>c</sup>
Kadostim	88.33 <sup>b</sup>	7.99 <sup>c</sup>	62.02 <sup>b</sup>	35.74 <sup>a</sup>	0.59 <sup>c</sup>
Humiforte	93.61 <sup>b</sup>	10.10 <sup>a</sup>	44.63 <sup>c</sup>	30.45 <sup>b</sup>	1.082 <sup>b</sup>
Fosnutren	78.05 <sup>c</sup>	8.61 <sup>b</sup>	22.62 <sup>d</sup>	27.37 <sup>c</sup>	1.87 <sup>a</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

**Table 2- Continued.**

Bio-stimulatos	Radicle fresh weight (g)	Hypocotyl fresh weight (g)	seedling fresh weight (g)	Radicle dry weight (g)	Hypocotyl dry weight (g)	Total dry weight (g)	N%	P(mg.g <sup>-1</sup> DW)	K(mg.g <sup>-1</sup> DW)
Aminolforte	0.27 <sup>a</sup>	0.75 <sup>bc</sup>	1.13 <sup>a</sup>	0.02 <sup>ab</sup>	0.06 <sup>a</sup>	0.08 <sup>a</sup>	1.87 <sup>b</sup>	0.354 <sup>b</sup>	2.07 <sup>b</sup>
Kadostim	0.28 <sup>a</sup>	0.81 <sup>b</sup>	1.21 <sup>a</sup>	0.02 <sup>a</sup>	0.06 <sup>a</sup>	0.08 <sup>a</sup>	1.93 <sup>a</sup>	0.357 <sup>b</sup>	2.15 <sup>a</sup>
Humiforte	0.23 <sup>b</sup>	1.02 <sup>a</sup>	1.23 <sup>a</sup>	0.01 <sup>b</sup>	0.06 <sup>a</sup>	0.07 <sup>b</sup>	1.85 <sup>bc</sup>	0.359 <sup>b</sup>	1.96 <sup>c</sup>
Fosnutrem	0.20 <sup>b</sup>	0.65 <sup>c</sup>	0.84 <sup>b</sup>	0.02 <sup>a</sup>	0.04 <sup>b</sup>	0.06 <sup>b</sup>	1.81 <sup>c</sup>	0.372 <sup>a</sup>	1.58 <sup>d</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

Table 3- Mean comparisons for effects of different concentrations of bio-stimulators on measured parameters\*

Concentration (%)	Germination percent	Germination rate	Radicle length (mm)	Hypocotyl length (mm)	Hypocotyl/radicle length ratio
0	93.75 <sup>a</sup>	9.98 <sup>a</sup>	70.72 <sup>a</sup>	29.85 <sup>a</sup>	0.42 <sup>d</sup>
0.2	92.50 <sup>ab</sup>	9.84 <sup>a</sup>	57.94 <sup>b</sup>	32.22 <sup>a</sup>	0.64 <sup>cd</sup>
0.4	87.92 <sup>abc</sup>	9.06 <sup>ab</sup>	49.11 <sup>bc</sup>	31.10 <sup>a</sup>	0.95 <sup>bc</sup>
0.6	89.16 <sup>abc</sup>	9.05 <sup>ab</sup>	42.57 <sup>c</sup>	32.52 <sup>a</sup>	1.50 <sup>a</sup>
0.8	86.25 <sup>bc</sup>	7.64 <sup>c</sup>	42.63 <sup>c</sup>	32.90 <sup>a</sup>	1.06 <sup>b</sup>
1	85.00 <sup>c</sup>	8.30 <sup>bc</sup>	39.28 <sup>c</sup>	30.26 <sup>a</sup>	1.43 <sup>a</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

Table 3- Continued.

Concentration (%)	Radicle fresh weight (g)	Hypocotyl fresh weight (g)	Total fresh weight (g)	Radicle dry weight (g)	Hypocotyl dry weight (g)	Total dry weight (g)	N%	P (mg.g <sup>-1</sup> DW)	K (mg.g <sup>-1</sup> DW)
0	0.27 <sup>a</sup>	0.73 <sup>b</sup>	1.00 <sup>a</sup>	0.02 <sup>a</sup>	0.06 <sup>a</sup>	0.08 <sup>a</sup>	1.81 <sup>b</sup>	0.354 <sup>c</sup>	1.89 <sup>b</sup>
0.2	0.27 <sup>a</sup>	0.84 <sup>ab</sup>	1.17 <sup>a</sup>	0.02 <sup>a</sup>	0.06 <sup>a</sup>	0.08 <sup>a</sup>	1.84 <sup>b</sup>	0.358 <sup>bc</sup>	1.91 <sup>b</sup>
0.4	0.25 <sup>ab</sup>	0.84 <sup>ab</sup>	1.15 <sup>a</sup>	0.02 <sup>a</sup>	0.05 <sup>a</sup>	0.07 <sup>ab</sup>	1.85 <sup>b</sup>	0.358 <sup>bc</sup>	1.92 <sup>b</sup>
0.6	0.24 <sup>ab</sup>	0.80 <sup>ab</sup>	1.10 <sup>a</sup>	0.02 <sup>a</sup>	0.05 <sup>a</sup>	0.07 <sup>ab</sup>	1.86 <sup>b</sup>	0.359 <sup>bc</sup>	1.92 <sup>b</sup>
0.8	0.23 <sup>ab</sup>	0.90 <sup>a</sup>	1.19 <sup>a</sup>	0.02 <sup>a</sup>	0.06 <sup>a</sup>	0.07 <sup>a</sup>	1.86 <sup>b</sup>	0.360 <sup>b</sup>	1.95 <sup>b</sup>
1	0.21 <sup>b</sup>	0.73 <sup>b</sup>	1.01 <sup>a</sup>	0.02 <sup>a</sup>	0.04 <sup>b</sup>	0.06 <sup>b</sup>	1.97 <sup>a</sup>	0.375 <sup>a</sup>	2.07 <sup>a</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

Table 4- Mean comparisons for interaction effects of different bio-stimulators and their concentrations on measured parameters.\*

Bio-stimulators	concentration	Germination percent	Germination rate	Radicle length (mm)	Hypocotyl length (mm)	Hypocotyl/radicle length ratio
Aminol forte	0	100 <sup>a</sup>	10 <sup>abcd</sup>	70.23 <sup>abc</sup>	33.407 <sup>abcdef</sup>	0.4733 <sup>g</sup>
	0.2	100 <sup>a</sup>	8.99 <sup>cdefg</sup>	73.50 <sup>ab</sup>	31.340 <sup>cdefg</sup>	0.4267 <sup>g</sup>
	0.4	96.667 <sup>ab</sup>	9.333 <sup>cde</sup>	70.31 <sup>abc</sup>	32.840 <sup>abcdef</sup>	0.4667 <sup>g</sup>
	0.6	93.333 <sup>abcd</sup>	9.220 <sup>cdef</sup>	75.32 <sup>a</sup>	34.510 <sup>abcdef</sup>	0.4533 <sup>g</sup>
	0.8	96.667 <sup>ab</sup>	8.773 <sup>cdefg</sup>	72.68 <sup>ab</sup>	32/223 <sup>bcdefg</sup>	0.4833 <sup>g</sup>
Kadostim	1	91.667 <sup>abcde</sup>	8.386 <sup>defg</sup>	71.25 <sup>abc</sup>	29.727 <sup>efghi</sup>	0.4433 <sup>g</sup>
	0	95.000 <sup>abc</sup>	9.276 <sup>cde</sup>	75.90 <sup>a</sup>	33.500 <sup>abcdef</sup>	0.4500 <sup>g</sup>
	0.2	93.333 <sup>abcd</sup>	9.000 <sup>cdefg</sup>	62.12 <sup>abcd</sup>	31.843 <sup>cdefg</sup>	0.5133 <sup>g</sup>
	0.4	80.000 <sup>ef</sup>	7.440 <sup>fg</sup>	67.11 <sup>abcd</sup>	37.803 <sup>ab</sup>	0.5567 <sup>g</sup>
	0.6	90 <sup>abcde</sup>	7.220 <sup>g</sup>	53.74 <sup>bcde</sup>	38.423 <sup>a</sup>	0.7167 <sup>efg</sup>
Humiforte	0.8	83.333 <sup>cde</sup>	7.440 <sup>fg</sup>	51.50 <sup>de</sup>	37.050 <sup>abc</sup>	0.7313 <sup>efg</sup>
	1	88.333 <sup>abcde</sup>	7.610 <sup>efg</sup>	61.76 <sup>abcd</sup>	35.827 <sup>abcd</sup>	0.5800 <sup>g</sup>
	0	85.000 <sup>bcde</sup>	9.090 <sup>cdef</sup>	58.99 <sup>abcd</sup>	26.997 <sup>shij</sup>	0.4567 <sup>g</sup>
	0.2	81.667 <sup>de</sup>	9.660 <sup>cd</sup>	28.02 <sup>fg</sup>	30.903 <sup>defgh</sup>	1.1333 <sup>def</sup>
	0.4	81.667 <sup>de</sup>	10.330 <sup>abc</sup>	9.55 <sup>g</sup>	19.533 <sup>k</sup>	2.0780 <sup>bc</sup>
Fosnutren	0.6	85.000 <sup>bcde</sup>	10.253 <sup>abc</sup>	7.78 <sup>g</sup>	24.873 <sup>ijk</sup>	3.2533 <sup>a</sup>
	0.8	68.333 <sup>fg</sup>	4.946 <sup>h</sup>	18.29 <sup>fg</sup>	28.957 <sup>fg</sup>	1.7467 <sup>cd</sup>
	1	66.667 <sup>g</sup>	7.400 <sup>fg</sup>	13.12 <sup>fg</sup>	33.013 <sup>abcdef</sup>	2.6067 <sup>ab</sup>
	0	95.000 <sup>abc</sup>	11.543 <sup>ab</sup>	77.76 <sup>a</sup>	25.520 <sup>hij</sup>	0.3233 <sup>g</sup>
	0.2	95.000 <sup>abc</sup>	11.710 <sup>a</sup>	68.14 <sup>abcd</sup>	34.807 <sup>abcde</sup>	0.5100 <sup>g</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

Table 4 continued.

treatment	concentration	Radicle fresh weight (g)	Hypocotyl fresh weight (g)	Total fresh weight (g)	Radicle dry weight (g)	Hypocotyl dry weight (g)	Total dry weight (g)	N%	P (mg.g <sup>-1</sup> DW)	K (mg.g <sup>-1</sup> DW)
Aminolforte	0	0.2396 <sup>abcdef</sup>	0.7903 <sup>cde</sup>	1.0347 <sup>cde</sup>	0.0133 <sup>def</sup>	0.0656 <sup>abcd</sup>	0.0820 <sup>abc</sup>	1.81 <sup>bcd</sup>	0.352 <sup>cd</sup>	2.08 <sup>cde</sup>
	0.2	0.2693 <sup>abcde</sup>	0.8083 <sup>cde</sup>	1.1920 <sup>bcd</sup>	0.0160 <sup>bcd</sup>	0.0663 <sup>abc</sup>	0.0860 <sup>a</sup>	1.88 <sup>bc</sup>	0.356 <sup>bcd</sup>	1.86 <sup>f</sup>
	0.4	0.2656 <sup>abcde</sup>	0.7853 <sup>cdef</sup>	1.2223 <sup>abcd</sup>	0.0143 <sup>cdef</sup>	0.0670 <sup>abc</sup>	0.0833 <sup>ab</sup>	1.90 <sup>bc</sup>	0.352 <sup>cd</sup>	1.98 <sup>cdef</sup>
	0.6	0.3146 <sup>ab</sup>	0.7867 <sup>cdef</sup>	1.1880 <sup>bcd</sup>	0.0173 <sup>bcd</sup>	0.0603 <sup>abcd</sup>	0.0786 <sup>abc</sup>	1.85 <sup>bcd</sup>	0.354 <sup>cd</sup>	2.11 <sup>bcd</sup>
	0.8	0.2920 <sup>abc</sup>	0.6350 <sup>defg</sup>	1.0583 <sup>cde</sup>	0.0163 <sup>bcd</sup>	0.0490 <sup>cd</sup>	0.0676 <sup>c</sup>	1.85 <sup>bcd</sup>	0.364 <sup>bc</sup>	2.13 <sup>bc</sup>
	1	0.2683 <sup>abcde</sup>	0.7047 <sup>cdef</sup>	1.1087 <sup>cde</sup>	0.0270 <sup>abc</sup>	0.0510 <sup>cde</sup>	0.0716 <sup>abc</sup>	1.93 <sup>b</sup>	0.349 <sup>d</sup>	2.26 <sup>b</sup>
Kadostim	0	0.2853 <sup>abc</sup>	0.6860 <sup>cdefg</sup>	1.0540 <sup>cde</sup>	0.0386 <sup>a</sup>	0.0586 <sup>abcde</sup>	0.0833 <sup>ab</sup>	1.76 <sup>cd</sup>	0.354 <sup>cd</sup>	2.13 <sup>bc</sup>
	0.2	0.3070 <sup>abc</sup>	0.7573 <sup>cdef</sup>	1.1800 <sup>bcd</sup>	0.0166 <sup>bcd</sup>	0.0583 <sup>abcde</sup>	0.0733 <sup>abc</sup>	1.83 <sup>bcd</sup>	0.354 <sup>cd</sup>	1.87 <sup>f</sup>
	0.4	0.3343 <sup>a</sup>	0.8200 <sup>cde</sup>	1.2503 <sup>abcd</sup>	0.0220 <sup>bcd</sup>	0.0566 <sup>abcde</sup>	0.0796 <sup>abc</sup>	1.92 <sup>bc</sup>	0.364 <sup>bc</sup>	1.90 <sup>f</sup>
	0.6	0.2733 <sup>abcd</sup>	0.8200 <sup>cde</sup>	1.2380 <sup>abcd</sup>	0.0153 <sup>bcd</sup>	0.0586 <sup>abcde</sup>	0.0770 <sup>abc</sup>	1.89 <sup>bc</sup>	0.360 <sup>bcd</sup>	2.13 <sup>bc</sup>
	0.8	0.2440 <sup>abcdef</sup>	0.8263 <sup>cd</sup>	1.2157 <sup>abcd</sup>	0.0190 <sup>bcd</sup>	0.0540 <sup>bcd</sup>	0.0740 <sup>abc</sup>	1.89 <sup>bc</sup>	0.355 <sup>bcd</sup>	2.27 <sup>b</sup>
	1	0.24733 <sup>abcdef</sup>	0.9307 <sup>bc</sup>	1.3563 <sup>abc</sup>	0.0123 <sup>def</sup>	0.0606 <sup>abcd</sup>	0.0743 <sup>abc</sup>	2.32 <sup>a</sup>	0.354 <sup>cd</sup>	2.60 <sup>a</sup>
Humiforte	0	0.2640 <sup>abcde</sup>	0.7113 <sup>cdef</sup>	0.9557 <sup>defg</sup>	0.0226 <sup>bcd</sup>	0.0536 <sup>cde</sup>	0.0756 <sup>abc</sup>	1.85 <sup>bcd</sup>	0.356 <sup>bcd</sup>	1.86 <sup>f</sup>
	0.2	0.2243 <sup>bcd</sup>	0.6727 <sup>cdefg</sup>	0.8840 <sup>efg</sup>	0.0240 <sup>bcd</sup>	0.0420 <sup>ef</sup>	0.0690 <sup>bc</sup>	1.81 <sup>bcd</sup>	0.357 <sup>bcd</sup>	1.91 <sup>ef</sup>
	0.4	0.1520 <sup>f</sup>	0.5513 <sup>efg</sup>	0.6850 <sup>g</sup>	0.0116 <sup>def</sup>	0.0306 <sup>fg</sup>	0.0430 <sup>de</sup>	1.80 <sup>bcd</sup>	0.356 <sup>bcd</sup>	1.93 <sup>ef</sup>
	0.6	0.2193 <sup>bcd</sup>	0.4247 <sup>g</sup>	0.6320 <sup>g</sup>	0.0193 <sup>bcd</sup>	0.0280 <sup>fg</sup>	0.0480 <sup>d</sup>	1.89 <sup>bc</sup>	0.364 <sup>bc</sup>	1.96 <sup>def</sup>
	0.8	0.1833 <sup>def</sup>	0.7970 <sup>cde</sup>	0.9600 <sup>defg</sup>	0.0233 <sup>bcd</sup>	0.0540 <sup>bcd</sup>	0.0783 <sup>abc</sup>	1.83 <sup>bcd</sup>	0.353 <sup>cd</sup>	1.98 <sup>cdef</sup>
	1	0.1603 <sup>f</sup>	0.7847 <sup>cdef</sup>	0.9297 <sup>defg</sup>	0.0283 <sup>ab</sup>	0.0510 <sup>cde</sup>	0.0816 <sup>abc</sup>	1.89 <sup>bc</sup>	0.368 <sup>b</sup>	2.10 <sup>bcd</sup>
Fosnutren	0	0.2890 <sup>abc</sup>	0.7170 <sup>cdef</sup>	0.9920 <sup>def</sup>	0.0163 <sup>bcd</sup>	0.050 <sup>cde</sup>	0.0676 <sup>c</sup>	1.83 <sup>bcd</sup>	0.353 <sup>cd</sup>	1.48 <sup>g</sup>
	0.2	0.2896 <sup>abc</sup>	1.1600 <sup>ab</sup>	1.4433 <sup>ab</sup>	0.0163 <sup>bcd</sup>	0.0646 <sup>abcd</sup>	0.0813 <sup>abc</sup>	1.82 <sup>bcd</sup>	0.364 <sup>bc</sup>	1.91 <sup>ef</sup>
	0.4	0.2620 <sup>abcde</sup>	1.2137 <sup>a</sup>	1.4600 <sup>ab</sup>	0.0146 <sup>cdef</sup>	0.0643 <sup>abcd</sup>	0.0796 <sup>abc</sup>	1.78 <sup>bcd</sup>	0.360 <sup>bcd</sup>	1.85 <sup>f</sup>
	0.6	0.1746 <sup>ef</sup>	1.1820 <sup>ab</sup>	1.3427 <sup>abc</sup>	0.0103 <sup>ef</sup>	0.0710 <sup>a</sup>	0.0836 <sup>ab</sup>	1.82 <sup>bcd</sup>	0.360 <sup>bcd</sup>	1.48 <sup>g</sup>
	0.8	0.2120 <sup>cdef</sup>	1.3323 <sup>a</sup>	1.5300 <sup>a</sup>	0.0090 <sup>f</sup>	0.0706 <sup>ab</sup>	0.0826 <sup>ab</sup>	1.88 <sup>bc</sup>	0.368 <sup>b</sup>	1.42 <sup>g</sup>
	1	0.1773 <sup>ef</sup>	0.5207 <sup>g</sup>	0.6633 <sup>g</sup>	0.0110 <sup>def</sup>	0.0190 <sup>g</sup>	0.030 <sup>e</sup>	1.71 <sup>d</sup>	0.428 <sup>a</sup>	1.36 <sup>g</sup>

\*Values in a column bearing different superscript are significantly different at 0.01 levels.

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