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Araştırma Makalesi
(Research Article)

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Effects of Nitrogen Fixing and Phosphate Solubilizing Bacteria on Growth and Bulbs Production of Tulip Cultivars

Azot Fikseri ve Fosfat Çözücü Bakterilerin Lale Çeşitlerinin Gelişimi ve Soğan Üretimine Etkisi

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ABSTRACT

Objective: This study was under taken to determine the effects of nitrogen fixing and phosphate solubilizing bacteria on plant development, number of bulb and quality of bulb of some tulip cultivars.

Materials and Methods: The research was conducted in open field conditions, in 2013. Pink Impression, Blue Aimable and Golden Parade varieties of *Tulipa gesneriana* L. were used as plant materials. Tulip bulbs were inoculated by four different bacterial formulations comprising *Pantoea agglomerans* RK-79, *Pantoea agglomerans* RK-92, *Bacillus megaterium* TV-91C, *Bacillus subtilis* TV-17C, *Bacillus megaterium* TV-3D, *Paenibacillus polymyxa* TV-12E, *Bacillus megaterium* TV-6D, *Pseudomonas putida* TV-42A bacteria strains.

Results: According to research results, significant results were determined based on among the applications and varieties. According to applications, average maximum number mother bulbs was obtained from cultivar of Golden Parade while average maximum number bulblet was observed in Blue Aimable cultivar. The highest average maximum bulbet was obtained from formulation C application in Blue Aimable cultivar.

Conclusion: As a result of the study, it has been concluded that the bacterial formulation applications especially formulation C (RK-79 + RK-92 + TV-3D + TV-12E) application increased in the number of bulbs and quality of the cultivars.

ÖZ

Amaç: Bu çalışma azot fikseri ve fosfat çözücü bakteri formülasyonlarının bazı lale çeşitlerinde bitkisel özellikler, soğan sayısı ve soğan kalitesi üzerine etkilerini araştırmak amacıyla yapılmıştır.

Materyal ve Yöntem: Çalışma, 2013 yılında tarla koşullarında yürütülmüştür. Araştırmada bitkisel materyal olarak, *Tulipa gesneriana* L. türüne ait Pink Impression, Blue Aimable ve Golden Parade *üç farklı lale* çeşidi kullanılmıştır. Lale soğanları, *Pantoea agglomerans* RK-79, *Pantoea agglomerans* RK-92, *Bacillus megaterium* TV-91C, *Bacillus subtilis* TV-17C, *Bacillus megaterium* TV-3D, *Paenibacillus polymyxa* TV-12E, *Bacillus megaterium* TV-6D, *Pseudomonas putida* TV-42A bakteri ırklarını içeren dört farklı bakteriyel formülasyon ile aşılanmıştır.

Bulgular: Araştırma bulgularına göre, uygulamalar ve çeşitler arasında önemli sonuçlar elde edilmiştir. Belirlenen ortalama en fazla ana soğan sayısı Golden Parade çeşidinde elde edilirken ortalama en fazla yavru soğan sayısı ise Blue Aimable çeşidinde elde edilmiştir. Ortalama en fazla yavru soğan sayısı Blue Aimable çeşidinde ve formülasyon C uygulamasında elde edilmiştir.

Sonuç: Çalışma sonucunda bakteri formülasyon uygulamalarıyla, özellikle formülasyon C (RK-79 + RK-92 + TV-3D + TV-12E) uygulamasının soğan sayısı ve kalitesinin arttırılabileceği sonucuna varılmıştır.

INTRODUCTION

The tulip (*Tulipa gesneriana* L.) is a bulbous plant with flashy flowers and perennial plant in the genus *Tulipa* belonging to the family *Liliaceae*. Tulip has become one of the most important ornamental plants in the world (Kumar et al., 2013). The major goals of tulip cultivation are quality bulb production. Plant nutrition play major role in growth, flowering and bulb of tulip. The application of chemical fertilizers negatively effect on production cost and environment. Hence, there is a growing interest in looking for options that reduce the use of chemicals to maintain plant health and reduce production costs. One of among the options is the use of rhizobacteria.

The bacteria, appropriately called rhizobacteria, is located in a zone surrounding the roots of the plants or rhizosphere are known as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1981). Scientific researches involve interdisciplinary approaches to understand the adaptation of PGPR to the rhizosphere, effects on plant physiology and growth, mechanisms of root colonization, (Jeon et al., 2003) biofertilization (Minorsky, 2008), induced systemic resistance, biocontrol of plant pathogens (Van Loon, 2007; Chandler et al., 2008; Karagöz, K., ve Kotan, R. 2010), production of determinants, etc. in plant growth. Therefore, PGPR application is assigned to increase in the plant growth, yield and also soil quality (García-Fraile et al., 2012; Flores-Félix et al., 2013). There are a few studies using PGPR as plant growth promoting agent in the cultivation of ornamental plants in the world (Srivastava and Govil, 2007; Eid et al., 2009; Sharma and Kaur, 2010; Zulueta-Rodriguez et al., 2014; Parlakova-Karagoz et al., 2016).

In the bulb production, the main objective is to produce quality bulbs and the bulbs have optimal size needing the maximum number because of having the power to make better quality flowers. This increases in the commercial value of such bulbs. At the same time, these bulbs are the properties have more bulblet. Consequently, aim of this study was determined the effects of nitrogen fixing and phosphate solubilizing bacteria on plant development, number of bulb and quality of bulb of some tulip cultivars in the black inorganic mulch conditions.

MATERIALS and METHODS

The experiment was conducted in black inorganic mulch in field conditions at department of Horticulture of Agriculture Faculty, Atatürk University, Erzurum, Turkey in 2013. The soil texture was sandy-loamy and the general properties of the field soil are given in the Table 1. The region altitude is 1853 m and its climate is cold. According to the climatic values measured at the 12th Regional Directorate of Meteorology (Erzurum) between the months of January-August in 2013, the mean temperature is 6.88 °C. Annual rainfall is 28.24 kg/m^2 and average relative humidity is 66.20% (Table 2).

Table 1 The general properties of the field soil used in the experiment

Çizelge 1 *Uygulamada kullanılan tarla toprağın genel özellikleri*

Soil properties	Values
pH (1:2,5 water)	6,9
Organic matter (%)	2,48
$CaCO_3$ (%)	1,04
Texture	Sandy -Loamy
N (%)	0,002
P (ppm)	23,62
K (ppm)	996,45
Ca (ppm)	2794
Mg (ppm)	518,3

Table 2 The climatic values measured between the months of January-August in 2013 of Erzurum province (12th Regional Directorate of Meteorology (Erzurum))

Çizelge 2 *Erzurum ili 2013 yılı ocak- ağustos aylarıarasında ölçülen iklim verileri (12. Meteoroloji Bölge Müdürlüğü)*

Meteorological Elements	Months												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual average
Mean temperature (°C)	-9,5	-7,4	-0,8	7,2	11,6	15,0	19,4	19,5					6,88
Mean relative humidity (%)	83,0	89,5	75,9	64,4	63,5	57,2	50,4	45,7					66,20
Total rainfall (kg/m^2)	28,7	28,5	30,9	36,3	36,3	32,3	25,1	7,8					28,24

A total of 360 bulbs of the Pink Impression, Blue Aimable and Golden Parade cultivars belonging species *Tulipa gesneriana* L. were used in the experiments. The bulbs were selected free of wounds and rots and as homogeneous as possible in size (10 to 12 cm perimeter).

Research was established in a completely randomized design with 3 replications and there were 8 plants in each replication. All of the bacterial strains (*Pantoea agglomerans* RK-79, *Pantoea agglomerans* RK-92, *Bacillus megaterium* TV-91C, *Bacillus subtilis* TV-17C, *Bacillus megaterium* TV-3D, *Paenibacillus polymyxa* TV-12E, *Bacillus megaterium* TV-6D, *Pseudomonas putida* TV-42A) were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University (Table 3). In study, there were 5 applications: (1) Formulation A (RK-79 +RK-92), (2) Formulation B (RK-79 + RK-92 + TV-91C +TV- 17C), (3) Formulation C (RK-79 + RK-92 + TV-3D + TV-12E), (4) Formulation D (RK-79 + RK-92 + TV-6D + TV-42A) and (5) Control (untreated bacteria) (Table 4). The bulbs were planted in black inorganic mulch conditions in field; after then 5 ml of prepared bacterial formulation was injected in the planting zone onto each of the bulbs on April 17 in 2013 and the harvest of bulbs was made on July 05 in 2013. In the experiments, the grown processes of all bacterial isolates were as defined by Gunes et al. (2015). There was no nutrition application during the experiments. In addition, the flower buds formed in all applications were plucked before flower opening (Başkent, 2008) during the study and stalk height and diameter of flower were simultaneously measured. Vegetative growth of tulip plant (plant height, number of stem, area of leaf) yield and quality parameters (number of main bulbs, number of bulblets, diameter and weight of bulblets) of harvested bulbs at the end of the experiment were determined by the method described by Malta (2016). The leaf area (widest leaf) was measured using CI 202 Portable digital brand leaf area meter. Color properties of leaves (L, a* and b*) (Minolta CR-400 Colorimeter (Minolta Camera Co., Ltd., Ramsey, NJ)), chlorophyll content in green leaf (chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan)) were determined.

All data have been treated by analysis of variance, which was performed using the SPSS version 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). The means were separated by Duncan's multiple range tests. It has been set 5% to be the maximum acceptable limit to be considered a significant result.

Table 3. Bacterial strains, their host, nitrogen fixation (N) and phosphate-solubilising activity (P) properties (Kotan et al. 2009; Kotan et al. 2010; Erman et al. 2010; Karakurt et al. 2011)
Çizelge 3. *Bakteriyel ırkları, konukçuları, azot (N) fiksasyonu ve fosfat (P) çözme özellikleri (Kotan et al. 2009; Kotan et al. 2010; Erman et al. 2010; Karakurt et al. 2011)*

İsolate No	Bacterial strains (Diagnosed MIS results)	SIM	Isolated from	N	P	Siderophore
RK-79	*Pantoea agglomerans*	0.762	Rosaceae sp. (Malus L.)	+	+	-
RK-92	*Pantoea agglomerans*	0.889	Rosaceae sp. (*Pyrus* L.)	+	S+	-
TV-17C	*Bacillus subtilis*	0.677	Rosaceae *sp. (Rubus L.)*	S+	W+	-
TV-12E	*Paenibacillus polymyxa*	0.551	Poaceae sp. (*Triticum* L.)	S+	+	-
TV-42A	*Pseudomonas putida*	0.113	Poaceae sp. (*Triticum* L.)	W+	W+	+
TV-91C	*Bacillus megaterium*	0.474	Poaceae sp. (*Triticum* L.)	+	W+	-
TV-3D	*Bacillus megaterium*	0.563	Poaceae sp. (Secale L.)	S+	+	-
TV-6D	*Bacillus megaterium*	0.750	Poaceae sp. (*Triticum* L.)	+	+	-

(SIM: Similarity index; +: Positive; S+: Strong positive; W+: Weak positive; -: Negative)

Table 4 Applications created in the study and their codes
Çizelge 4 *Çalışmada oluşturulan uygulamalar ve uygulama kodları*

Code of Application	Applications
Formulation A	RK-79 +RK-92
Formulation B	RK-79 + RK-92 + TV-91C +TV- 17C
Formulation C	RK-79 + RK-92 + TV-3D + TV-12E
Formulation D	RK-79 + RK-92 + TV-6D + TV-42A
Control	Control (Uninoculated)

RESULTS and DISCUSSION

The nitrogen fixing and phosphate solubilizing bacteria were basically evaluated to determine their characteristics and suitability in terms of plant development, yield of bulb and quality of bulb of tulip cultivars in the black inorganic mulch conditions.

Plant Height (cm)

Different bacteria combinations have generally been important on the plant height in the cultivars. Most of the PGPR isolates increased in plant height (Minorsky, 2008; Bhattacharyya and Jha, 2012). The plant height was maximum (19.62 cm) in Pink Impression and minimum (15.71 cm) in Golden Parade. While an average the longest plant height (26.38 cm) in the Pink Impression cultivar was obtained in Formulation D application, an average the shortest plant height (12.50 cm) in the same cultivar was determined with Formulation A application (Table 5). Van Der Meulen-Muisers et al. (1996) has reported that height of stem is one of the most important quality criteria in tulips. Pink Impression cultivar and formulation D applications have given important results in terms of plant height. Gezgin (2007), reported that plant height ranged from 15 cm to 60 cm in tulip. Results of our study appear to be consistent with the findings of the researcher.

Number of Stem (number/plant)

'Cultivar' and 'application' factors were found significant ($p<0.001$) on the number of stem parameter in bacteria applications when compared to the control. Dole and Wilkins (1999) have determined that tulips grown for commercial bulb production are vegetative propagated. Le Nard and De Hertogh (1993) and Dole and Wilkins (1999) have determined that the average propagation rate of most tulip cultivars is between two and three bulbs per year. In our study, number of stem representing the amount of bulblet was the greatest in Blue Aimable among all cultivars in formulation B application. The finding of formulation B is the height when comparing to the previous studies in terms of amount of bulblet (Table 5).

Flower Stalk Height (cm)

It was observed that 'applications', 'cultivar' factors and interaction of the 'application and cultivar' had significant effects (at $p< 0.01$) on flower stalk height in tulip. Maximum flower stalk height (9.68 cm) was recorded in the Pink Impression cultivar. However, flower stalk height values were decreased in Pink Impression when compared to the control. It was determined that the longest flower stalk height was reported in Blue Aimable with the formulation C application when compared to the control (Table 5). It was indicated by Gezgin (2007) that stalk height varies generally between 5-50 cm in tulips. Results of our study are good fit to the researcher result. Nevertheless, it can be concluded that the early varieties have short stalk height when examined the average flower stalk height of all cultivars in the research. And, it can be expressed that this case may emerge from using different cultivars.

Flower Stalk Diameter (mm)

There were no statistically ($p>0.05$) differences in terms of flower stalk diameter at bacteria formulation applications in all cultivars (Table 5).

Leaf Surface Area (cm^2)

'Applications', 'cultivar' factors and interaction of the 'application and cultivar' had significant effects (at $p< 0.01$) on area of leaf. The average maximum area of leaf (44.18 cm^2) was determined in formilation C application. Similar results in relationship with increasing leaf surface area have been reported in Pelargonium plants (Göre and Altın, 2006). According to the control application, the maximum leaf surface area (46.32 cm^2) was obtained from Golden Parade tulip cultivar (Table 5). Ürgenç (1998) stated that bulb does not grow well due to lack of photosynthesis, if the leaves of plant are cut off. Golden Parade is tulip cultivar that have a maximum leaf surface area as well as the maximum number of main bulb. It is stated that there is a direct relationship between the two parameters. Rees (1971), stated that they result in lower growth rate and less amount of bulblet, reduction of the main bulb scales and leaf surface area. The results of our study were in accordance with this information. By means of these results, it can be concluded that different bacteria formulations have been different influences and depending on tulip cultivars (Table 5).

Color/ Greenness of the Leaf

The average maximum lightness (positive (+) L* value), greenness (negative (-) a* value) and yellowness (positive (+) b* value) of tulip leaves were 46.64, 12.95, and 19.38, respectively. In terms of the average maximum leaf color, Golden Parade cultivar was in the same group with Pink Impression cultivar. For Pink Impression cultivar, the highest L*, a* and b* values (greenness) were observed in formulation C (Table 5).

Leaf Chlorophyll Reading Value (LCRV)

The average maximum LCRV (44.30) was determined in Blue Aimable cultivar and in control application. However, control application was in the same group with formulation D application. The average minimum LCRV (38.37) was observed in formulation A application if LCRV is analyzed on the basis of applications (Table 2). Increasing in nitrogen rate obtained by bacteria applications has positively effect on the growth of plant (Table 5). It can be interpreted that this effect has caused the decline of leaf chlorophyll reading value.

Number of Main Bulbs (number/plant)

'Cultivar' factor had also significant effects (at $p< 0.01$) on number of main bulbs in the study. The average maximum

number of main bulbs (6.20 number/plant) determined for tulips cultivars were obtained from control application in Golden Parade (Table 6). Atay (1996), emphasized that production with bulbs are the most common propagation method for flower bulbs to retain the horticultural characteristics of the cultivars and to be greater ease and speed of propagation. Başkent (2008), reported that main bulb disintegrates; bulb and bulblet a bunch of formed the previous season remain in bulbous irises and tulips. He stated that the largest of these has reached a size that will create blooming. Consequently, it can be concluded that number and quality of bulb can be improved with bacteria formulation applications in depending on the cultivar factor.

Number of Bulblets (number/plant)

Number of bulblets on the effect of 'applications', 'cultivar' factors and interaction of the 'application and cultivar' were significant at p = 0.01. The average maximum number of bulblet (31.20 number/plant) was determined in Blue Aimable cultivar. The average maximum number of bulblet (26.89 number/plant) was observed in formulation A application if number of bulblet is analyzed on the basis of applications (Table 6). Le Nard and De Hertogh (1993), stated that the main objective of bulb production is cultivation salable size of bulb with low cost, good quality and large number of bulbs. Depending on the cultivar, it can be achieved the conclusion that bulb production can be increased in using different bacteria formulation applications. Thus, cost of production of bulb which is the most important input for the production of tulips can be reduced.

Diameter Bulblets (mm)

It was determined that 'applications' factor and interaction of the 'application and cultivar' were found insignificant (at p>0.05) while the 'cultivar' factor had significant effects (at p< 0.01) on diameter bulblets (Table 6). Le Nard and De Hertogh (1993), reported that temperatures in the time range from in flower initiation and in harvest have critical importance for tulip cultivation. They stated that if the weather is unusually hot and there is not enough moisture in the soil in this period, the plant is to keep it short this time and commercially valuable bulbs remain small, not overgrowth. During the period in which the experiment was conducted, irrigation needs of plants has been corrected by rigorously following. As a reason for 'applications' factor and interaction of the 'application and cultivar' were found insignificant (at p>0.05) on diameter bulblets, and it can be shown as hot weather is likely in April-July.

Weight of Bulblets (g/number)

There were no statistically (p>0.05) differences in terms of weight of bulblet at bacteria formulation applications in all cultivars. The average maximum weight of bulblet (8.41 g/number) was recorded in Golden Parade cultivar (Table 6).

CONCLUSIONS

Using PGPR can be provided reduction of application of industrial fertilizers which are potential pollutants on environment. Considering all of which exert a positive influence on vegetative development of tulip and increasing in the production bulblet; it was concluded that the applications could be reduced the cost of production material. Thereby, these results will contribute to the continuation of production and market developments. Effect on the increase in the number of tulip bulblets of our study, it can be get reference by growers in production and this will result in more bulbs production. And so, demand for tulip bulbs of our country will be able to meet and imports of tulip bulbs would be reduced. Further, national income will be greatly improved by increasing of the amount of exports.

Table 5. The effect of applications on parameters of vegetative growth of tulip (*Tulipa gesneriana* L.) plants
Çizelge 5. *Lale (Tulipa gesneriana L.) bitkilerinin vejetatif büyüme parametreleri üzerine uygulamalarının etkisi*

Applications	Plant height (cm)				Number of stem (number/plant)				Leaf surface area (cm²)			
	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean
Formulation A	12.50 c*	17.20 ^{ns}	14.10 ^{ns}	14.60 ^{NS}	4.32 ^{ns}	4.73 b*	4.20 ^{ns}	4.42 B***	32.23 b***	14.50 c***	43.26 b*	30.00 C***
Formulation B	17.19 bc	17.5	14.62	16.44	4.63	6.40 a	4.70	5.24 A	34.31 b	32.23 a	50.54 ab	39.03 B
Formulation C	20.00 ab	19.52	14.74	18.08	3.17	4.11 b	4.28	3.86 B	63.67 a	27.88 ab	40.98 b	44.18 A
Formulation D	26.38 a	18.32	16.99	20.56	3.89	4.38 b	3.32	3.86 B	39.56 b	25.71 b	54.46 a	39.91 B
Control	22.00 ab	13.75	18.11	17.95	3.28	4.94 b	3.76	3.99 B	36.84 b	18.13 c	42.36 b	32.44 C
Mean	19.62 A*	17.26 AB	15.71 B	17.53	3.86 B***	4.91 A	4.05 B	4.27	41.32 B***	23.69 C	46.32 A	37.11

Applications	Color/ greenness of the leaf											
	L*				a*				b*			
	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean
Formulation A	36.34 c***	42.84 ^{ns}	46.45 ^{ns}	41.87 B*	-9.23 c***	-11.00 ^{ns}	-12.15 ^{ns}	-10.79 ^{NS}	14.26 c***	13.04 ^{ns}	15.27 ^{ns}	14.19 AB*
Formulation B	39.00 bc	34.67	42.29	38.65 B	-9.91 c	-8.59	-11.77	-10.09	12.71c	9.44	13.91	12.02 B
Formulation C	56.26 a	34.39	49.26	46.64 A	-12.95 a	-8.86	-13.04	-11.62	19.38 a	10.21	18.05	15.88 A
Formulation D	44.29 b	35.41	43.98	41.23 B	-11.67 ab	-7.34	-12.24	-10.42	16.32 b	10.17	14.73	13.74 AB
Control	42.03 bc	44.62	40.65	42.43 AB	-10.45 bc	-10.78	-10.73	-10.65	12.83 c	15.23	12.94	13.67 AB
Mean	43.58 A***	38.38 B	44.53 A	42.16	-10.84 B***	-9.31 C	-11.99 A	-10.71	15.10 A***	11.62 B	14.98 A	13.90

Applications	Flower stalk height (cm)				Flower stalk diameter (mm)				Leaf Chlorophyll Reading Value (LCRV)			
	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean
Formulation A	7.16 c***	7.33 a*	6.18 ^{ns}	6.89 C***	7.68 ^{ns}	6.97 ^{ns}	8.07 ^{ns}	7.57 A**	35.05 b**	41.20 ^{ns}	38.86 ^{ns}	38.37 BC*
Formulation B	7.53 c	8.38 a	7.69	7.87 BC	7.01	7.32	5.38	6.57 B	36.38 b	44.63	34.55	38.52 BC
Formulation C	4.00 d	8.75 a	7.20	6.65 C	7.82	7.32	6.39	7.17 AB	33.20 b	46.52	29.11	36.28 C
Formulation D	10.38 b	7.84 a	7.11	8.44 B	6.42	6.16	6.78	6.45 B	34.88 b	47.89	39.69	40.82 AB
Control	19.33 a	4.25 b	9.10	10.89 A	8.89	7.57	7.46	7.97 A	45.70 a	41.25	42.25	43.07 A
Mean	9.68 A***	7.31 B	7.46 B	8.15	7.57 ^{NS}	7.07	6.81	7.15	37.04 B***	44.30 A	36.89 B	39.41

ns: non-significant at p>0.05, * Significant at P<0.05, ** Significant at p< 0.01, *** Significant at p< 0.001; difference between the means shown with the same letter in a column is not significant.

Table 6. The effect of applications on parameters of harvested tulip (*Tulipa gesneriana* L.) bulbs
Çizelge 6. *Hasat edilmiş lale (Tulipa gesneriana L.) soğan parametreleri üzerine uygulamaların etkisi*

Applications	Number of main bulbs (number/plant)				Number of bulblets (number/plant)				Diameter bulblets (mm)				Weight of bulblets (g/number))			
	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean	Pink Impression	Blue Aimable	Golden Parade	Mean
Formulation A	2.00 ^{ns}	1.67 ^{ns}	6.33 ^{ns}	3.33 ^{NS}	18.00 b**	33.00 ^{ns}	19.67 b*	23.56 AB*	14.72 ^{ns}	16.67 ^{ns}	22.13 ^{ns}	17.84 ^{NS}	1.94 ^{ns}	2.67 ^{ns}	8.05 ^{ns}	4.22 ^{NS}
Formulation B	2.33	2.00	6.00	3.44	27.67 a	30.33	19.33 b	25.78 A	13.15	17.79	22.28	17.74	1.65	3.02	8.11	4.26
Formulation C	1.67	2.33	6.33	3.44	22.33 ab	30.67	27.67 a	26.89 A	12.97	17.71	24.81	18.5	1.43	3.17	7.65	4.08
Formulation D	2.33	3.00	5.00	3.44	21.67 ab	33.00	21.00 ab	25.22 A	14.29	18.31	23.14	18.58	1.20	3.17	8.11	4.43
Control	2.00	1.33	7.33	3.56	15.67 b	29.00	17.00 b	20.56 B	14.78	18.34	22.84	18.65	1.78	3.10	10.1	4.99
Mean	2.07 B***	2.07 B	6.20 A	3.44	21.07 B***	31.20 A	20.93 B	24.4	13.98 C***	17.76 B	23.04 A	18.26	1.76 C***	3.03 B	8.41 A	4.40

ns: non-significant at p>0.05, * Significant at P<0.05, ** Significant at p< 0.01, *** Significant at p< 0.001; difference between the means shown with the same letter in a column is not significant.

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