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Alleviation of Water Stress effects on Corn by Polyamine compounds under Newly Cultivated Sandy Soil conditions

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Abstract: Two Field experiments were carried out at private farm in newly cultivated sandy land at new Salheyia Region, El – Sharkeiya Governorate, Egypt in the summer seasons of 2014 and 2015 years, to study alleviation of water stress effects on corn by polyamine compounds. Water stress was imposed by skipping an irrigation at vegetative growth , i.e. at 35 days age (I_2) and / or at 49 days age (I_3) , tasseling and silking stage, i.e at 63 days age (I_4) and at grain filling stage, i.e at 77 days after planting date (I_5). Putrescine and specmidine was foliar applied at 0, 50 and 100 mg/ l for each compound. Water stress decreased the yield and its components. The most sensitive growth stage of corn to water stress was the vegetative growth stage at 35 days after planting, followed by the growth stage at 45 days after planting. Foliar application of polyamine compounds, i.e. putrescine and specmidine alleviated the perious water stress adverse effects on corn; increased yield and its components. The most favorable treatments to alleviate the water stress effect on yield and its components of corn plants were 100 mg/l from putrescine and/or specmidine. The data were disused in terms of interaction of water stress and polyamine compounds on corn plants.
Keywords: water stress, corn, puterscine, spermidine.

Introduction

Water used by crops varies greatly due to the variation of seasoned and locations depending mainly on the evaporative conditions on the atmosphere and the crop characteristics[1]. Thus, the knowledge of the optimum amount of water required for obtaining maximum yield and high quality is essential. Moreover, soil moisture limits the process of plant before it reaches the permanent wilting point. Drought stress affects nearly every process in the plant, where it reduces cell turgor, the size of assimilation and number of potential storage sites for produced[2]. Yield and its component of maize plant were also affected by limited water supply. Plants exposed to water stress or skipping during pre or post silking reduced the grain yield by 9 and 10% compared to conventional irrigation[1,3,4,5].

Also, most of the countries of the world are facing the problem of drought. The insufficiency of water is the principle environmental stress and to enter heavy damage in many part of the world for agriculture products [6,7, 8, 9, 10, 11,12, 5, 1,13] reported that water stress is one of the most adverse factors for plant growth and productivity. Response of plants to water stress depends on several factors, such as development stage, intensity and duration of stress and cultivar genetics [11]. The plant response is very complex because it reflects over space and time the integration of stress effects and response at all underlying levels of organization[14].

Water stress inhibits the photosynthesis of plants, causes changes in chlorophyll content and components and damage to the photosynthetic apparatus[15].

Moreover, it inhibits the photochemical activities and decrease the activities of enzyme in the calvin cycle in photosynthesis[16]. In addition, tolerance to abiotic stress is very complex due to the interact of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [17, 18, 14, 19,11].

For saving irrigation, corn cultivars that produced high yield under suitable water regime should be developed. A possible approach to minimize drought induced crop losses is the foliar spraying with polyamine compounds. The diamine putrescine and the polyamine spermidine are small aliphatic amines found in all plant cells, induced. These basic molecules which are positively charged at physiological PH, are ubiquitous in nature[20]. Because of the positive charge polyamines (i.e, petrescine and spermidine) are known to bind to negatively charged molecules e.g. nuclic acids, acidic phospholipids and various types of proteins[20]. Polyamins occur in plants in free form, bound electrostatically to negatively charged molecules and conjugated to small molecules and proteins[21].

In recent years, considerable attention has been paid to the involvement of polyamines especially putrescine (Put.) and spermidine (Spm.) in different plant development processes[21,22]. They modulate several growth and development provesses, viz-cell decision, differentiation, flowering, fruit ripening, senescence, embryogenesis and rhizogeneis[23] and are also involved in stress response[24]. In all these, previous polyamines have been ascribed various roles such as that of a new class of plant growth regulators, second hormonal messengers and as one of the reserves of carbon and nitrogen at least in cultured tisses[25]. At cellular PH values, these compounds behave as action and can interact with anionic macromolecules such as DNA, RNA, phospholipids and certain proteins[26].

The present study investigated alleviation of water stress effects on corn by foliar application with putrescine and spermidine compounds under newly cultivated sandy soil conditions.

Materials and Methods

The present investigation was carried out during the two summer seasons of 2014 and 2015 at private farm in newly cultivated sandy land at New Salheyia Region, El-Sharkeiya Governorate, Egypt to study effect of putrescine and spermidine on yield and yield components of the white maize hybrid S.C.10 under drought stress. Each experiment was laid out in split-plot design with three replications, where the main plots included the water stress treatment; meanwhile, polyamines were allocated in sub-plot. The experimental unit consisted of seven ridges, five meter long and 60 cm apart. Planting was done at Mide May in the two seasons in hills spaced 25 cm along, three Kernels per hill. Thinning to plant per hill was done at 21 days after planting. Nitrogen fertilizer was applied at rate of 120 kg N/ fed in three equal doses at 21, 28 and 35 days after planting before irrigation. Irrigation, pest control and other cultural practices were carried out as recommended by Maize Research Dept., Field Crops Research Institute, Agriculture Research center, Giza, Egypt.

Each experiment included 35 treatments which were the following treatments:

A: Irrigation treatments:

1. Irrigation every seven days as normal irrigation (I_1).
2. Skipping one irrigation at 35 days from planting (I_2) i.e vegetative growth stage.
3. Skipping one irrigation at 49 days after planting (I_3) i.e at vegetative growth stage.
4. Skipping one irrigation at 63 days after planting (I_4), i.e. at tassling and silking stage.
5. Skipping one irrigation at 77 days after planting (I_5), i.e. at grain filling stage.

B: Polyamine treatments:

1. Putrescine at two concentrations, i.e. 50 and 100 mg/l.
2. Spermidine at two concentrations, i.e. 50 and 100 mg/l.
3. 50 mg/l put. + 50 mg/l spd.
4. 50 mg/ put + 100 mg/l spd.
5. 100 mg/put + 50 mg/l spd.

6. 100 mg/l put + 100 mg/spd.
7. Control treatments (Foliar spraying with tap water).

Putrescine [$NH_2(CH_2)_3$ NH2] and spermidine [NH_2 $(CH_2)_3$ NH $(CH_2)_3$ NH_2] were sprayed twice at 50 and 60 days after planting, respectively.

At harvest date, ten graded plants where taken at random from ridges of each plot to determine yield component, i.e. plant height (cm), number of ears /plant, ears dry weight g/plant, ear diameter(cm), ear length (cm), grain index "100 grain /g" and grain and straw yields "g/plant". In addition, kernels, straw and biological yield "ton/fed" were determined from the other three ridges for each plot and then converted to yield per feddan ; where; crop index, harvest index and migration coefficient were estimated according to[27].

The data obtained were subjected to the proper statistical analysis according to[28]. For comparison between means L.S.D according to [29] at 0.05 levels was used. Combined analysis was made from the two growing seasons hence the results of the season followed similar trend were practiced according to[28].

Results and Discussion

Effect of water stress on yield and its components:

Data in table (1) show that yield and its components of corn plants were significantly affected by skipping one irrigation during the different growth stages (except crop index the effect failed to reach the significant level at 5%).

The plants received normal irrigation (I_1) showed the highest significant values of plant height, number of ears /plant, ears dry weight/plant, ear diameter, straw yield /plant, and grain; straw and biological yield /plant, whereas, the plants exposed to water stress at grain filling stage (77 days after planting) harvest the heights significant value from harvest index. On the other hand, the plants exposed to water deficit at early growth (35 and 49 days from planting) caused the greatest migration coefficient compared with normal irrigation (I_1), skipping one irrigation at tasseling and silking stage (I_4) and skipping one irrigation at 77 days from planting (grain filling stage).

Table (1): Effect of water stress on yield and components of corn plants c.v S.C.10.(Average of 2014 and 2015 seasons)

Yield and its Components / Irrigation Treatments	Plant height cm	No. of ears/ plant	Ears dry weight g/plant	Ear diameter cm	Ear length cm	Grain index 100 grains/g	Grain yield g/plant	Straw yield g/plant	Grain yield ton/fed	Straw yield Ton/fed	Biological yield ton/fed	Crop index	Harvest index	Migration coefficient
Normal irrigation (No. skipping one irrigation)	308.68	1.59	320.38	3.27	32.98	30.92	256.89	349.89	3.62	3.97	7.59	0.48	0.91	0.53
Skipping one irrigation at 35 days from planting (vegetative growth stage)	271.31	1.29	266.36	2.94	23.47	25.37	208.21	281.87	3.04	3.62	6.66	0.46	0.84	0.54
Skipping one irrigation at 49 days from planting (vegetative growth stage)	279.01	1.37	273.55	3.07	28.03	26.52	210.76	297.37	3.28	3.68	6.96	0.47	0.89	0.55
Skipping one irrigation at 63 days from planting (tasseling and silking stage)	283.81	1.59	383.81	3.12	28.98	28.43	222.23	308.7	3.41	3.76	7.17	0.48	0.91	0.53
Skipping one irrigation at 77 days from planting (grain filling stage)	305.19	1.48	304.85	3.18	30.92	29.56	240.40	320.75	3.52	3.84	7.36	0.48	0.92	0.51
L.S.D. at 5% level	2.21	0.05	6.20	0.01	1.48	0.52	10.01	9.45	0.1	0.63	0.12	n.s	0.01	0.01

These results in the soil approaches the wilting point, turgor pressure. in the plant approaches zero, cell enlargement virtually ceases and rate of cell division is markedly reduced. Skipping one irrigation at 77 days after planting (i.e. grain filling stage) was more tolerant to drought conditions than the plants exposed to drought stress at early negatitive growth stage (i.e. 35 days after planting. The decrement in plant height may be due to the direct effect of limited water supply, which reflect decreasing in metabolic products [1]. Generally the height of maize plants was decreased when plants were exposed to drought stress with decreasing number of irrigation or prolonging the irrigation intervals and these true were confirmed with the results respond by [30, 31, 32, 5,1]. With respect of dry matter accumulation in the different plant organ, it is significantly affected by irrigation treatments. It is worthy that the plants received to normal irrigation had more dry matter accumulation in corn plant parts. On the other hand, the plants exposed to missing irrigation mid or late stage of growth stages (I_4 and I_5) were less affected compared with the plants exposed to missing irrigation at early growth stage, i.e I_2 and I_3 (Table 1). Thus the plants exposed to water deficit during the critical stages of growth were negatively affected, hence, in turn, decreased the translocation of assimilating compounds from source (leaves) to other parts, especially when water deficit occurred at vegetative growth stage (I_2 and I_3) than at tasseling and silking stage, as well as, grain filling stages (I_4 and I_5). It is worthy that, the plants with irrigated normally gave the highest values of grain yield and yield attributes. Missing irrigation at 35 and 49 days after planting caused highly significant decrement in grains yield compared with missing one irrigation at 63 and 77 days after planting.

It is worthy to mention that our results are in harmony with those reported by[33,34,5,1]. Productivity production caused by drought stress may be attributed to effects of water stress on chlorophyll synthesis, normal decreases and turgor loss[35]. Regular irrigation after water stress did not recover the water stress harmful effects on productivity reduction. Moreover, the retardation of photosynthetic enzymes under water stress might cause such effects, since negative growth stage was affected by water deficit, also, water deficit induced perturbation of physiological processes at late stage critical to yield production and therefore, water deficit will be avoided at growth stage[1]. Also, water stress inhibit the photochemical activities and decrease the activities of enzymes in the Calven Cycle in photosynthesis[16]. Moreover, tolerance to abiotic stresses is very complex due to the interact of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development[14, 19,11]. Such response to drought stress might be attributed to lack of water absorbed, inadequate uptake of essential element, inhibition of meristimatic activity and/or reduction in photosynthetic capacity under such unfavorable conditions [36]. Thus, assimilates translocated to new developing far primodical were reduced and which were not enough to mention or develop this organ.

B- Effect of polyamines concentractions on yield and its components:

Date illustrated in Table (2) observed that corn plants sprayed with 50 mg/l put significantly increased plant height, number of ears/plant, ears dry weight/plant, ear diameter, ear length, grain index, grain yield/plant, straw yield/plant, grain yield/fed, straw yield/fed and biological yield/fed compared with control treatment, meanwhile caused significant decrement in migration coefficient. On the other hand, an insignificant reduction in crop index and harvest index were found when corn plants treated with 50 mg/l put compared with control treatment also. Increasing concentration of put up to 100 mg/l caused significant positive effect on plant height, number of ears/plant, ears dry weight/plant, ear diameter, grain index, grain yield/plant, straw yield/plant, grain yield/fed and biological yield/fed. On the other hand, an insignificant increase were found on ear length, straw yield/fed, and harvest index compared with 50 mg/l put.

With respect to foliar application with spermidine, data reported in table (2) indicate clearly that there are significant marked stimulatory effect on plant height, number of ears/plant, ears dry weight/plant, ear diameter, ear length, grain index, grain yield/plant, straw yield/plant, grain yield/fed, straw yield/fed and biological yield/fed compared with control treatment, however, significant marked depressive effect are shown in migration coefficient. Furthermore, increasing concentration of spermidine up to 100 mg/l caused a significant positive effect on number of ears/plant, ears dry weight/plant, ear diameter, grain yield/plant, straw yield/plant and biological yield/fed and insignificant increment in plant height, ear length, grain index, as well as grain and straw yield/fed compared with 50 mg/l spermidine treatment.

Table (2): Effect of polyamine compounds concentrations on yield and its compounds of corn plant S.C.10.(Average of 2014 and 2015 seasons)

Yield and its Components / Polyamine Concentration	Plant height cm	No. of ears/ plant	Ears dry weight g/plant	Ear diameter cm	Ear length cm	Grain index 100 grains/g	Grain yield g/plant	Straw yield g/plant	Grain yield ton/fed	Straw yield Ton/fed	Biological yield ton/fed	Crop index	Harvest index	Migration coefficient
Tap water (0.0 mg/l)	276.74	1.24	276.58	2.99	25.74	25.78	215.52	232.68	3.24	3.57	6.81	0.48	0.91	0.62
50 mg/l Put.	279.84	1.31	281.4	3.01	26.93	26.99	218.8	297.43	3.28	3.67	6.95	0.47	0.89	0.55
100 mg/l Put.	282.89	1.37	283.23	3.08	27.36	27.4	220.99	302.57	3.33	3.70	7.03	0.47	0.90	0.54
50 mg/l Spd.	287.18	1.43	281.39	3.11	27.56	27.61	221.82	307.21	3.34	3.73	7.07	0.48	0.90	0.53
50 mg/l Spd.	291.39	1.49	285.25	3.17	28.34	27.64	224.49	310.39	3.36	3.75	7.11	0.47	0.90	0.53
50 mg/l Put + 50 mg/l Spd.	285.12	1.39	286.57	3.18	28.39	27.97	224.51	310.49	3.39	3.88	7.27	0.47	0.87	0.54
50 mg/l Put + 100 mg/l Spd.	291.72	1.48	291.85	3.21	3.01	28.02	227.3	311.31	3.43	3.90	7.33	0.47	0.88	0.54
100 mg/l Put + 50 mg/l Spd.	298.40	1.53	295.92	3.23	29.70	28.13	232.25	315.9	3.49	3.91	7.40	0.47	0.89	0.54
100 mg/l Put + 100 mg/l Spd.	301.38	1.61	302.17	3.26	31.28	28.19	236.64	320.14	3.56	4.01	7.57	0.47	0.89	0.54
L.S.D. at 5% level	5.28	0.03	2.36	0.02	1.07	0.06	2.25	2.71	0.04	0.07	0.035	n.s	n.s	0.04

It is worthy that foliar application with 100 mg/l Put. + 100 mg/l Spd. on corn plants gave the height significant from yield and its components (except crop index, harvest index and migration coefficient).

It is worthy that positive effect of polyamines on yield and its components may be due to that polyamines are now considered as a new class of growth substances and also well known for their anti-senescence and anti-stress effects where their acid neutralizing and antioxidant properties, as well as to their membrane and cell wall stabilizing abilities [14]. Again, polyamines have been implicated in a large range of growth and developmental processes such as cell division, simulation, support and development of flower buds, embryogenests, fruit set and growth, fruit ripening, plant morphogenesis and response to environmental stress [37]. In addition, 38 reported that peroxidase and cellulose activities were retarted by polyamise treatments and accelerated by polyamise biosynthetic inhibitors. Also polyamines inhibits senescence in plant [38]. Thus, the filling period duration, effective filling period and filling rate increased and these processes caused on increment in yield and its components [39]. Generally, our results are in good harmony with those obtained by [39] and [40].

C: Effect of interaction between drought stress and polyamine concentrations:

The interaction between water stress treatments and foliar application with different concentrations of putrescine and spermidine caused significant effects on plant height, number of ears/plant, ears dry weight/plant, ear diameter, ear length, grain index, grain yield/plant, straw yield/plant, grain yield/fed, straw yield/fed, and biological yield/fed. On the other hand, the effect of this interaction on crop index, harvest index and migration coefficient failed to reach the significant level at 5%.

It is worthy that corn plants exposed to normal irrigation (without missing on irrigation during plant age) under foliar spraying with 100 mg/l putrescine, 100 mg/l spermidine gave the greatest values from yield and its components.

Table (3): Effect of the interaction between skipping one irrigation at different stages of growth and polyamine compounds on yield and its components of corn plant c.v S.C.10 hybrid (Average of 2014 and 2015 seasons)

Irrigation Treatment × polyamines	Yield and its Components	Plant height cm	No. of ears/ plant	Ears dry weight g/plant	Ear diameter cm	Ear length cm	Grain index 100 grains/g	Grain yield g/plant	Straw yield g/plant	Grain yield ton/fed	Strain yield Ton/fed	Biological yield ton/fed	Crop index	Harvest index	Migration coefficient
Normal irrigation (No skipping one irrigation)	Tap water (0.0 mg/l)	293	1.50	300.8	3.14	31.00	30.07	240.00	326.3	3.43	3.75	7.18	0.48	0.91	0.53
	50 mg/l Put.	297	1.50	300.43	3.17	32.48	30.15	247.00	239.3	3.50	3.80	7.30	0.48	0.92	0.52
	100 mg/l Put.	298.5	1.50	307.00	3.22	32.55	30.37	248.00	345	3.54	3.85	7.39	0.48	0.92	0.52
	50 mg/l Spd.	300.2	1.50	309.36	3.21	32.8	30.46	250.00	347	3.58	3.90	7.84	0.48	0.92	0.52
	100 mg/l Spd.	309	1.60	314.5	3.25	33.19	30.54	254.00	356	3.67	3.91	7.58	0.48	0.94	0.52
	50 mg/l Put + 50 mg/l Spd.	312	1.60	328.2	3.29	33.35	31.00	257.00	350	3.62	4.05	7.67	0.47	0.89	0.54
	50 mg/l Put + 100 mg/l Spd.	317	1.67	329.75	3.36	33.78	31.53	261.00	361	3.67	4.10	7.77	0.47	0.90	0.53
	100 mg/l Put + 50 mg/l Spd.	325	1.65	342.35	3.32	33.60	31.97	275.00	360	3.75	4.15	7.90	0.47	0.90	0.54
	100 mg/l Put + 100 mg/l Spd.	328	1.75	347.17	3.45	34.08	32.15	280.00	364.5	3.84	4.20	8.04	0.48	0.91	0.54
Skipping one irrigation at 35 days from planting (vegetative growth stage).	Tap water (0.0 mg/l)	260.7	1.00	252.33	2.77	20.3	25.11	201.6	267.10	2.92	3.43	6.35	0.46	0.85	0.54

	50 mg/l Put.	264.5	1.17	258.9	2.82	21.4	25.18	205.31	273	2.97	3.55	6.52	0.46	0.84	0.54
	100 mg/l Put.	266.6	1.25	261.70	2.94	22.59	25.29	206	278	3.01	3.62	6.63	0.45	0.83	0.54
	50 mg/l Spd.	270.9	1.33	268.70	2.94	22.82	25.35	205.9	280	3.-06	3.59	6.65	0.46	0.84	0.55
	100 mg/l Spd.	276.8	1.40	270.40	2.95	24.70	25.42	206.64	284.33	3.05	3.61	6.66	0.46	0.84	0.56
	50 mg/l Put + 50 mg/l Spd.	271.6	1.25	268	2.97	23.1	25.36	207.81	288	3.06	3.64	6.70	0.46	0.84	0.54
	50 mg/l Put + 100 mg/l Spd.	275	1.33	272	3.00	25.9	25.47	209.4	288.5	3.07	3.66	6.73	0.46	0.84	0.55
	100 mg/l Put + 50 mg/l Spd.	274.2	1.40	274	3.02	24.96	25.54	213.55	289	3.11	3.71	6.82	0.46	0.84	0.55
	100 mg/l Put + 100 mg/l Spd.	281.6	1.50	275.5	3.05	25.47	25.58	217.65	288.9	3.13	3.74	6.87	0.46	0.84	0.56
Skipping one irrigation at 49 days from planting (vegetative growth stage	Tap water (0.0 mg/l)	267.4	1.17	257.9	2.90	26.11	26.24	208.64	290.4	3.17	3.51	6.68	0.47	0.90	0.52
	50 mg/l Put.	272.5	1.25	265.1	2.94	27.63	26.32	208.8	292.5	3.20	3.58	6.78	0.47	0.89	0.53
	100 mg/l Put.	274.8	1.30	268.3	3.02	27.94	26.35	208	293.8	3.19	3.66	6.85	0.47	0.87	0.53
	100 mg/l Spd.	276.5	1.40	272.33	3.01	27.70	26.40	211.4	295.2	3.23	3.61	6.84	0.47	0.89	0.54
	50 mg/l Spd.	280.4	1.45	274.5	3.01	28	26.46	214.67	296.6	3.25	3.67	6.98	0.47	0.86	0.54
	50 mg/l Put + 50 mg/l Spd.	278.11	1.33	276.6	3.12	28.11	26.66	210.5	297.4	3.29	3.66	6.95	0.47	0.88	0.54
	50 mg/l Put + 100 mg/l Spd.	282.9	1.40	280.8	3.15	28.25	26.71	213.2	299	3.34	3.75	7.09	0.47	0.89	0.55
	100 mg/l Put + 50 mg/l Spd.	286.5	1.45	282.4	3.17	28.74	26,75	215.7	303.5	3.42	3.89	7.31	0.47	0.88	0.54
	100 mg/l Put + 100 mg/l Spd.	292	1.55	286	3.18	30.05	26.82	217.9	308	3.47	3.92	7.39	0.47	0.89	0.54

Skipping one irrigation at 63 days from planting (tasseling and silking)	Tap water (0.0 mg/l)	274.1	1.25	252.8	295	26.47	28.13	211.44	294.4	3.29	3.53	6.82	0.48	0.93	0.50
	50 mg/l Put.	278.6	1.30	260.4	298	27.81	28.24	213.70	296.3	3.30	3.60	6.90	0.48	0.93	0.51
	100 mg/l Put.	280.9	1.33	264.5	3.-06	28	28.30	219.33	301.67	3.37	3.67	7.04	0.48	0.94	0.51
	50 mg/l Spd.	282.7	1.45	265.8	3.08	28.10	28.41	218	312.45	3.30	3.64	6.97	0.48	0.91	0.50
	100 mg/l Spd.	285.2	1.50	267	3.14	28.64	28.50	212.35	318.21	3.39	3.70	7.09	0.48	0.92	0.49
	50 mg/l Put + 50 mg/l Spd.	284	1.40	274.9	3.17	29.33	28.39	223.5	304	3.40	3.89	7.29	0.47	0.87	0.52
	50 mg/l Put + 100 mg/l Spd.	286.8	1.45	281.5	3.20	30.50	28.45	228	306.75	3.46	3.95	7.41	0.47	0.88	0.53
	100 mg/l Put + 50 mg/l Spd.	288	1.55	283.6	3.22	30.19	28.69	231.60	318.64	3.57	3.98	7.55	0.47	0.90	0.52
	100 mg/l Put + 100 mg/l Spd.	294	1.60	290.8	3.25	32	28.72	233.11	325.90	3.61	4.01	7.62	0.47	0.90	0.52
Skipping one irrigation at 77 days from planting (grain filling stage)	Tap water (0.0 mg/l)	288.05	1.30	290.48	3.00	28.25	29.42	229.14	302.85	3.38	3.62	7	0.48	0.93	0.55
	50 mg/l Put.	290.60	1.33	294.9	3.05	28.95	29.47	231.9	306.90	3.42	3.67	7.09	0.48	0.93	0.55
	100 mg/l Put.	295.20	1.47	303.6	3.17	29	29.49	235	31540	3.49	3.70	7.19	9.40	0.94	0.55
	50 mg/l Spd.	309.9	1.40	298.5	3.14	29.67	29.54	236.30	319.51	3.46	369	7.15	0.48	0.94	0.54
	100 mg/l Spd.	311.6	1.50	307.5	3.25	30	29.58	239.40	320.60	3.47	3.75	7.22	0.48	0.93	0.55
	50 mg/l Put + 50 mg/l Spd.	295.8	1.45	298.8	3.20	31	29.53	240.30	327.40	3.51	4.01	7.52	0.47	0.88	0.53
	50 mg/l Put + 100 mg/l Spd.	310.6	1.60	304.6	3.25	33.25	29.59	242.50	329	3.51	4.62	7.59	0.47	0.89	0.53
	100 mg/l Put + 50 mg/l Spd.	318.7	1.60	314.8	3.27	32.80	29.67	251.70	330	3.60	4.03	7.63	0.47	0.89	0.54
	100 mg/l Put + 100 mg/l Spd.	326	1.67	326.9	3.30	35.40	2.75	257.40	335	3.75	4.05	7.80	0.48	0.93	0.55
L.S.D at 5% level		7.76	0.04	3.47	0.03	1.57	0.09	3.31	3.98	0.66	0.1	0.05	n.s	n.s	n.s.

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