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Effect of Simulated Acid Rain and Auxin on chlorophyll 'a' and chlorophyll 'b' content in the leaves of *Capsicum frutescens* var. sweet magic

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Abstract

Chlorophyll is a pigment which is present in almost entire plant kingdom. It is green in color. Photosynthesis, the food formation process of the plant takes place in presence of chlorophyll. This food is utilized in various physiological activities taking place inside the plant body. During this process, synthesis of organic compounds takes place and light energy is converted into chemical energy. It is said to help in detoxifying the blood, odor control, healing of wounds, keeping gut healthy, providing energy, improving immune system and cancer prevention. Chlorophyll 'a' and chlorophyll 'b' absorbs light of different wavelengths. Chlorophyll 'a' absorbs violet-blue and red light in the visible part of the spectrum. Chlorophyll 'b' absorbs light in blue part of the spectrum. During electron transport chain chlorophyll 'a' donates electrons and act as primary electron donor. It transfers energy to the reaction centre P_{700} and p_{680}. Chlorophyll 'b' expands absorption spectrum of plants and thus helps in the process of photosynthesis. However, Acid rain leaches out the chlorophyll content of the plant thus affecting the growth of the plant.

In order to study the cumulative impact of Acid rain, Auxin and combinations of Acid rain and Auxin concentrations on the chlorophyll in 'Sweet Magic' variety of Capsicum frutescens. experiments were carried out. Accordingly plants were treated with normal water (control set), lab prepared acid rain of pH 5, pH 4 and pH 3 concentration; Auxin solution of $1x10^{-7}$, $1x10^{-6}$ and $1x10^{-5}$ M concentration and combination of acid rain and auxin of pH $5.0+1x10^{-5}$, pH $5.0+1x10^{-6}$, pH $5.0+1x10^{-7}$, pH $4.0+1x10^{-5}$, pH $4.0+1x10^{-6}$, pH $4.0+1x10^{-7}$ and pH $3.0+1x10^{-5}$, pH $3.0+1x10^{-6}$, pH $3.0+1x10^{-7}$ M concentrations. Tests were done after plant age of 45 days every fortnight i.e. after 45 days, 60 days, 75days, 90 days and 105 days. Maximum Chlorophyll 'a' content was observed at the plant age of 105 days treated with pH $4.0+1x10^{-7}$ M (390.21% of control) and maximum Chlorophyll 'b' content was observed at the plant age of 105 days treated with pH 5.0+1x10-5M concentration (609.82 of control).

Keywords: Acid rain, auxin, chlorophyll 'a', chlorophyll 'b' Interactive, *Capsicum frutescens*, Sweet Magic.

Introduction

Chlorophyll is a green pigment involved in photosynthesis. It is sensitive to light and absorbs energy from light and use it to convert light energy in to chemical energy and carbohydrates are formed. Chlorophyll is found in almost all photosynthetic organisms. Cyanobacteria, algae and green plants have chlorophyll. Chlorophylls are magnesium porphyrin compounds. The porphyrin ring consist of four pyrrole rings joined together by bridges. A long chain of carbon atom called as phytol tail is attached to porphyrin ring at fourth pyrrole ring. Molecular formula of chlorophyll a is $C_{55}H_{72}$ O_5 N_4 Mg and of chlorophyll b is $C_{55}H_{70}$ O_6N_4Mg. It consist of magnesium porphyrin 'head', which is hydrophilic and a phytol tail which is lipophilic in nature. In chlorophyll b there is a -CHO group instead of a $-CH_3$ group at the third C-atom in second pyrrole ring. Both chlorophyll 'a' and 'b' are essential for the process of photosynthesis. After receiving a quantum of light chlorophyll 'a' molecule gets excited . It expels an electron carrying extra energy, this electron takes part in further chemical reactions.

Chlorophyll 'b' also absorbs light, though in different part of the spectrum and thus both help in the process of photosynthesis. Enrichment of vegetables, fruits and cereals with chlorophyll is important to improve the quality of food and therefore, experiments were conducted to increase chlorophyll content in Capsicum frutescens. var. sweet magic.

Effect of acid rain on physiology of plants has been studied by different workers. Simona Ioana et. al. (2009)[1] noticed that the germination and the growth decreases when the pH of acid rain decreases under stress of simulated acid rain.

Zabawi, et.al., (2008) [2] studied the effect of simulated acid rain on germination and growth of rice plants. Wood and Bormann (1975)[3] showed a significant increase in leaching of potassium, magnesium and calcium from leaves of plant bean and Sugar maple after exposure to the rain of pH 2.3. A significant increase in foliar leaching of calcium in leaves of Micofiana topaccun was noticed by Fairflax and Lepp (1975)[4] after exposure to pH 3.8. Hindawi et al (1980)[5] demonstrated lower contents of nitrogen, calcium, magnesium and phosphorus in foliage of Phaseolus vulgaris when exposed to simulated acid rain. The inhibitory effects of simulated acid rain of pH 2.6 was reported through a reduction in leaf area ratio (LAR)(Norbey and Luxmoore (1983)[6].

Chappelka et. al. (1985)[7] showed significant gaseous pollutant and rain pH interaction occurred for leaf area increase, mean unit leaf rate(ULR)and chlorophyll content in yellow poplar (Liriodendron tulipifera L.) seedlings. Ferrenbaugh (1976)[8] noted that average chlorophyll content of leaves was lower after events of rain of pH 2.0 compared with higher pH levels when seedlings of Phaseolus vulgaris were exposed to sulphuric acid solutions of various pH levels.

Hindawi et. al. (1980)[5] reported that the microscopic observations of the injured trifoliates of Phaseolus vulgaris, which resulted due to the exposure to the acid mist , revealed that the palisade cells were plasmolysed and the chloroplast lost structural integrity. Kumaravelu and Ramanujam (1998)[9] showed the impact of simulated acid rain on growth, photosynthetic pigments, cell metabolites and leaf characteristics of green gram. Zhou,et.al (2002)[10] worked on the stress effects of simulated acid rain on three woody plants- Osmanthus fragrana, Chimonanthus praecox and Prunus persica. The results showed that maximum change in chlorophyll content was noticed in Osmanthus fragrana and less change was noticed in Prunus persica. Cao, et.al. (2010)[11] found that when treatment of rape plant was done with weak acidic water (acid rain of pH 4.0 to 5.0), the growth was stimulated up to some extent in comparison to control. But it had no effect on leaf chlorophyll content, photosynthetic characters, plant biomass and yield. But when acid rain concentration was increased, leaf chlorophyll content, photosynthetic rate decreased gradually. Shaukat and Khan (2008)[12] reported that simulated acid rain treatment of pH 3.0 and 4.0 reduced the chlorophyll 'a' and 'b' content significantly in comparison to control in tomatoes (Lycopersicon esculentum mill.).

Chongling et.al. (1995)[13] noticed the effect of simulated acid rain on ecophysiological characterstics of mung bean and maize and found that the chlorophyll content, chlorophyll 'a' and 'b' values, leaf viability of mung bean and maize and the pH leaf sap and soil are positively correlated with the pH simulated acid rain. Shan et. al.(1996)[14] found a reduction in net photosynthetic rate per unit chlorophyll a+b content in Pinus armandi exposed to acidic rain. Kumaravelu and Ramanujam (1998)[9] showed the impact of simulated acid rain on growth, photosynthetic pigments and leaf characteristics of green gram (Vigna radiata cv. ADT-1 and CO^{-5}). In cultivar ADT-1 after 5 showers at pH 2.5 chlorophyll contents decreased whereas in cultivar CO^{-5} decrease was noticed after 10 showers of acid rain. Raj et. al. (2003)[15] reported significant decrease in chlorophyll content due to lowering in pH level of acidic rain in Triticum aestivum cv. raj.3077. Devpura and Khan (2003)[15] also found decreased chlorophyll content with decreasing pH levels of acidic rain in Phaseolus aureus. Kumari and Tomar (2009) [16]worked on the effect of simulated acid rain on chlorophyll and ascorbic acid contents of Mentha piperita L. (peppermint). The chlorophyll content was 18.35 mg/gm of leaves compared to control sample where it was 23.076mg/gm of leaves at pH 3 simulated acid rain (Simona Ioanna et.al. ,2009)[1].

The effect of acid rain has been studied by some other workers also on different plants. [27-32] However the effect of acid rain and auxin has not been studied on chlorophyll content in the leaves of *Capsicum frutescens* var. sweet magic.

Objective of the Study

The present work is done to study the effect of acid rain on chlorophyll 'a' and chlorophyll 'b' content of *Capsicum frutescens* .Chlorophyll is very much important for plant for the process of photosynthesis, and is responsible for food formation in plants. It was also studied, if auxin can counter the damage done by the acid rain on the chlorophyll content if any.

Material and methods

Electronic pH meter was used to prepare the acidic water (acid rain) of desired concentrations i.e. pH 5.0, pH 4.0 and pH 3.0 for treatment of plants. Sulphuric acid (H2SO4) and nitric acid (HNO3) is mixed in proportion of 7:3 volume by volume in distilled water.(Lee J.J.,1981)[17]. For preparing molar solution of Auxin (IAA) (10^{-4} Molar), 17.5 mg of Auxin is dissolved in one liter (1000 ml) of distilled water. For preparing Auxin solution of different concentrations ($1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ M) dilution is done accordingly. To study the amount of chlorophyll in the plant leaves of *Capsicum frutescens* of 'Sweet magic' variety, a set of quality seeds were sown in the prepared soil. Healthy seedlings of 15 days were planted in the soil for experiment. In addition of regular watering, the sets of plants were treated with spray of respective Auxin solutions after attaining age of thirty days. Chlorophyll content of the leaves was measured on attaining plant age of 45 days.. These 45 days old plants were given four spray of 10 ml. each of identified treatment (twice a week). This

process was repeated at the plant age of 60, 75, 90 and105 days after every fortnight.

Estimation of Chlorophyll Content

Quantification of chlorophyll content is done as per protocol developed by Holm (1965)[18]. 100 mg of fresh leaves of *Capsicum frutescens* var. Sweet magic are homogenised with 1ml of 80 % chilled acetone in mortar pestle over the ice pack. Homogenised solution is taken in an eppendorf tube and centrifuged at 2000 rpm for 10 minutes. Pellet are extracted twice with 500 µl of 80% acetone and final volume is made up to 3 ml. Absorbance is taken at 644 and 662 nm in PC based UV. VIS spectrophotometer (Perkin Elmer Lemda 2S, german made) against 80% acetone solution as blank (80% acetone).

The chlorophyll content is quantified through following formulas-

Chlorophyll (a) mg/gm = ((9.78 x A662) – (0.99 x A644)) x V/1000 x fresh weight

Chlorophyll (b) mg/gm = ((21.4 x A644) – (4.64 X A662)) x V/1000 x fresh weight

Ratio of chlorophyll a / chlorophyll b was observed in mg /gm.

Results and discussion

For determining the effects of pre decided values of acidic water(Acid Rain), Auxin and combination of Acid waters and Auxin on the amount of chlorophyll 'a' and 'b' of sample plants leaves, the tests were conducted at the sample plant age of 45,60,75,90 and 105 days in all cases as shown in the tables.

Table-1 reflects how the plants responded when sprinkled with water of different pre determined value of acidity (pH 3.0, 4.0 and 5.0) in terms of amount of chlorophyll 'a' and chlorophyll 'b' (mg/gm) present in the leaves of Sweet magic variety of *Capsicum frutescens*.

Chlorophyll content

The chlorophyll 'a' , when sprinkled with water of acidity (pH 3.0) is 77.58 %, 96.16 %, 72.27 %, 104.14 % and 111.44 % of control values, with water of acidity (pH 4.0), the content is 97.88 %, 120.87%, 51.78 %, 78.40 % and 98.88 % and is 148.48 %, 98.76 %, 103.13 %, 107.84 % and 153.24 % of control values when exposed to acidic water of pH5.0 respectively.

Chlorophyll 'b' Content

Content of chlorophyll 'b' at the plant age of 45, 60, 75,90 and 105 days is 69.35 %, 84.95 %, 106.22 %, 84.40% and 85.44 % of controls when water of pH 3.0 was used for treatment, with acid rain (pH 4.0), it is 84.64%, 50.66%, 86.12%, 55.97% and 79.64 %. With the spraying of water of acidity (pH 5.0) is done, it comes to 93.22%, 80.88%, 121.05%, 95.97 % and 127.93% of the control values.

Table-2 shows the results obtained at the application of $1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ M Auxin on chlorophyll 'a' and chlorophyll 'b' in the leaves of 'Sweet Magic' variety of *Capsicum frutescens*.

Chlorophyll 'a'

When sample plants were exposed to the sprinkling of $1X10^{-5}$ M Auxin at the predetermined plant age, the chlorophyll 'a' amounts to 119.24 %, 76.66 %, 85.71 %, 84.97 % and 88.14 % of control. At the application of $1x10^{-6}$ M, it is 380.30 %, 130.17 %, 93.61 %, 113.68 % and 248.78 % of control and at $1x10^{-7}$ M, it is 153.03 %, 160.40 %, 81.01 %, 114.7657 % and 269.09 % of control.

Chlorophyll 'b'

The Chlorophyll 'b' amounts to 121.83 %, 116.43 %, 99.62 %, 263.90 % and 116.80% of control when sample plants were treated with Auxin $1X10^{-5}$ M. The chlorophyll 'b' of sample plants treated with $1x10^{-6}$ M auxin is 161.46 %, 78.59 %, 111.77 %, 205.91 % and 346.75 % of control. When sample plants were exposed to $1x10^{-7}$ M auxin, it is 173.93 %, 77.79 %, 86.41 %, 254.09 % and 332.66 % of the control.

Table-3 shows chlorophyll 'a' and chlorophyll 'b' content after the sample plants were subjected to pre determined combined treatments of acidic waters and auxin solutions on the leaves of 'Sweet magic' variety of *Capsicum frutescens*.

Chlorophyll' 'a'

Table shows value of chlorophyll 'a' when sample plants are subjected to acidic water and Auxin solution of (pH 3.0+1x 10^{-5} M) to be the 186.97 %, 200.65%, 128.21%, 94.33% and 82.07%, at the treatment with water and Auxin (pH 3.0+1x 10^{-6} M), the chlorophyll 'a' pigment is 235.3%, 172.56%, 101.33%, 95.32% and 158.13% with acidic water and auxin (pH 3.0+1x10^{-7}), the chlorophyll 'a' is 211.67%, 90.18%, 106.39%, 98.47%, 238.66 % of the control.

Treating with acidic water and auxin solution (pH 4.0+1x10^{-5} M), value of chlorophyll 'a' amounts to 288.64%, 209.17%, 84.99%, 108.01%, 267.41% of contol values. Treatment with acidic water and auxin solution (pH 4.0+1x10^{-6}), chlorophyll 'a' value stands at 47.27%, 159.30%, 62.87%, 131.77%, 272.92% of control values. Treatment with acidic water and auxin (pH 4.0+1x 10^{-7}M) , the chlorophyll 'a' content are 68.03%, 143.50%, 148.82%, 140.68% and 390.21% of the control values.

When the sample plants are treated with acidic water and auxin (pH 5.0+1x 10^{-5}M), value of chlorophyll 'a' comes 133.03%,168.73%, 119.53%, 103.78%, 251.92 % of control value. Treatment of acid rain and auxin (pH 5.0+1x10^{-6} M), the chlorophyll 'a' content of sample plant leaves comes to 113.48%, 158.52%, 95.36%, 130.87%, 382.55%.Treatment of acidic water and auxin (pH 5.0+1x10^{-7}M) value of chlorophyll 'a' measures 89.55%, 63.91%, 81.31%, 129.34%, 302.51% of the control values.

Chlorophyll 'b'

Table 3 shows that chlorophyll 'b' is 150.98%, 121.12%, 114.93%, 230.33% and 338.82% of control values on subjecting the sample plants to treatment with acidic water and Auxin solution of (pH 3.0+1x10^{-5} M).The values are 122.10%, 129.02%, 104.02%, 361.51%, 441.30% on treatment of plants with acidic water and Auxin solution of (pH 3.0+1x10^{-6} M).Subjecting the treatment of acid rain and auxin (pH 3.0+1x10^{-7} M), the chlorophyll 'b' comes to 114.82%, 59.36%, 90.43%, 309.81%, 432.19% of the control values.

Combination of acidic water and Auxin solution (pH 4.0+1x10^{-5}M) treatment, gives the values of chlorophyll 'b' as 113.28%, 112.42%, 107.08%, 326.42% and 249.94% of control values. With acid rain and auxin (pH 4.0+1x10^{-6} M), chlorophyll 'b' is 125.80%, 55.92%, 112.82%, 236.23%and 576.92%of controls and 115.21%, 69.26% , 153.40%, 284.28 % and 178.34% of the control at treatment with acid rain and auxin (pH 4.0+1x10^{-7}M).

Sample plants with spray of combination of acidic water (simulated acid rain) and auxin (pH 5.0 1x10^{-5}M) gives value of chlorophyll 'b' as 166.65%, 98.00%, 145.74%, 365.16% and 609.82 % of control value. Treatment of acidic water and Auxin (pH 5.0+1x10^{-6} M), shows the chlorophyll 'b' as 177.55%, 156.04%, 95.60%, 268.18% and 223.08% of control values. Treating the sample plants lot with acid rain and auxin (pH 5.0+1x10^{-7}M) the chlorophyll b content comes to 138.12%, 122.61%, 134.26%, 357.48%and 394.67% of the control.

A variable trend is observed at all the pH values (pH 3.0, 4.0 as well as at pH 5.0). A steep hike in content is observed from 90-105 days. Decrease in chlorophyll content at higher levels of acidity may be attributed to the fact that in presence of mineral acid, chlorophyll 'a' gives magnesium free compound-phytyl phaeophorbide-a (phenophytin-a) [Agarwal, O.P. (2000)][19].

As per Morrison (1984)[20] leaching of magnesium causes decrease in chlorophyll content. Kumari and Tomar, 2009,[16] also observed reduction in chlorophyll-a and chlorophyll-b contents is dependent on pH of simulated acid rain and treatment duration. Toxic ions and chemicals produced by acid rain may be the reason for reduction in chlorophyll content. As per Kukarskikh et. al (1995)[21] reduction in chlorophyll content can be due to the reduction in the number of PS-I and PS-II reaction centers in the plants under treatment.

There are non linear trends for chlorophyll-'a' at all concentrations of auxin. Chlorophyll-'a' is maximum at 105th day of treatments with pH 4.0+1x10^{-7} M. The values increase in zig-zag pattern with other maxima at the 60th day of all the combinations except pH 3.0+1x10^{-7} M and pH5.0 +1x10^{-7} M combinations. Increase may be due to the increased effectiveness of the auxin component. For chlorophyll-'b', maximum content is seen at the 45th day at all the concentrations and then there is variable pattern.

IAA acts is a coenzyme and hence plays an important role in the formation of chlorophyll [Jacobs (1979)][22] . Abdul-El-Wahab (1997) [23] found that at 500 ppm treatment IAA increased chlorophyll-'b' content in apple nursery plant. According to Zhong et. al., 2009,[24] greening and seedlings exposure to light was promoted by the transcription factor EIN and basic helix-loop-helix (bHLH). Both these factors were found to regulate chlorophyll biosynthesis through several promoters. Some of these promoters include transcription factor EIN3G of PORA and PORB. It is also found that PIFI targeted the promoter of PORC. Together these three POR genes encoded different information for a rate limiting enzyme in chlorophyll biosynthesis (Schallar, 2012)[25]. When 100 ppm IAA treatment given to periwinkle (Catharanthus roseus G. Don) prominent increase in chlorophyll-'a' and 'b' content was observed by Nagiub et. al. (2003)[26].

Conclusion

Chlorophyll-'a' is maximum at 105th day of treatments with pH 4.0+1x10^{-7} M. Change in chlorophyll-'b' content is through V- turn at prominent combination of pH 5.0+1x10^{-5} M; the maximum value is at the two ends (45th and 105th day). The increase in chlorophyll content may be attributed to higher potential of auxin. Higher concentration of auxin enhances chlorophyll content, through ethylene hormone synthesis. The intermediacy of transcription factors like EIN-3 and promoters PORA and PORB, promotes chlorophyll biosynthesis (Schaller, 2012) [25] leading to increase in chlorophyll content.

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Table.1: Effect of Simulated Acid Rain (pH 3.0,4.0,5.0) on chlorophyll 'a' and 'b'content (mg/gm f.wt. ± SD) in the leaves of *Capsicum frutescens* var. sweet magic

Treatment	PLANT AGE (In days)									
	45		60		75		90		105	
	a	B	a	b	a	b	a	B	a	B
Control	0.220 ±0.00	0.870 ±0.05	0.510 ±0.12	0.580 ±0.15	0.550 ±0.08	0.350 ±0.08	0.370 ±0.03	0.270 ±0.06	0.480 ±0.04	0.280 ±0.03
3.0	0.170 ±0.08	0.600 ±0.21	0.490 ±0.07	0.490 ±0.06	0.440* ±0.02	0.370 ±0.05	0.390 ±0.03	0.220 ±0.03	0.530 ±0.05	0.240 ±0.03
4.0	0.220 ±0.01	0.730** ±0.01	0.620 ±0.04	0.300* ±0.04	0.290* ±0.10	0.300 ±0.04	0.290 ±0.05	0.150 ±0.05	0.470 ±0.12	0.220 ±0.05
5.0	0.330* *±0.04	0.810 ±0.04	0.550 ±0.09	0.470 ±0.14	0.570 ±0.07	0.420 ±0.05	0.440 ±0.05	0.250 ±0.05	0.730* *±0.04	0.360* *±0.03

N.B. **=.01 Level of significance *= .05 Level of significance

Table.2: Effect of Auxin ($1x10^{-5}$,$1x10^{-6}$,$1x10^{-7}$M) on chlorophyll 'a' and 'b' content (mg/gm f. wt. ± SD) in the leaves of *Capsicum frutescens* var. sweet magic

Treatment	PLANT AGE (In days)									
	45		60		75		90		105	
	a	B	a	b	A	B	a	B	a	B
Control	0.220 ±0.00	0.870 ±0.05	0.510 ±0.12	0.580 ±0.15	0.550 ±0.08	0.350 ±0.08	0.370 ±0.03	0.270 ±0.06	0.480 ±0.04	0.280 ±0.03
$1x10^{-5}$M	0.260 ±0.07	1.050 ±0.52	0.390 ±0.11	0.680 ±0.12	0.470 ±0.10	0.350 ±0.04	0.310 ±0.04	0.700** ±0.14	0.420 ±0.12	0.330 ±0.16
$1x10^{-6}$M	0.840** ±0.11	1.400** ±0.08	0.670 ±0.10	0.460 ±0.07	0.520 ±0.05	0.390 ±0.09	0.420 ±0.05	0.550** ±0.02	1.190** ±0.2	0.980** ±0.21
$1x10^{-7}$M	0.340** ±0.07	1.510* ±0.20	0.820** ±0.09	0.450 ±0.03	0.450 ±0.03	0.300 ±0.01	0.430 ±0.07	0.670** ±0.13	1.290* ±0.47	0.940** ±0.28

N.B. **=.01 Level of significance *= .05 Level of significance

Table.3: Interactive Effect of Simulated Acid rain (pH 3.0,4.0,5.0) and Auxin ($1x10^{-5}$,$1x10^{-6}$,$1x10^{-7}$M) on chlorophyll 'a' and 'b' content (mg/gm f. wt. ± SD) in the leaves of *Capsicum frutescens* var. sweet magic

Treatment	PLANT AGE (In days)									
	45		60		75		90		105	
	A	B	A	b	a	B	A	B	A	B
Control	0.220 ±0.00	0.870 ±0.05	0.510 ±0.12	0.580 ±0.15	0.550 ±0.08	0.350 ±0.08	0.370 ±0.03	0.270 ±0.06	0.480 ±0.04	0.280 ±0.03
3.0+1x1 0^{-5}	0.410 ±0.17	1.310** ±0.12	1.030** ±0.19	0.710 ±0.05	0.710 ±0.07	0.400 ±0.04	0.350 ±0.06	0.620** ±0.08	0.390 ±0.13	0.950** ±0.09
3.0+1x1 0^{-6}	0.520 ±0.27	1.060 ±0.36	0.880** ±0.06	0.750 ±0.38	0.560 ±0.01	0.360 ±0.05	0.350 ±0.04	0.960** ±0.10	0.760 ±0.35	1.240** ±0.19
3.0+1x1 0^{-7}M	0.470** ±0.12	0.990 ±0.68	0.460 ±0.08	0.350 ±0.10	0.590 ±0.03	0.320 ±0.11	0.360 ±0.05	0.820** ±0.11	1.140 ±0.50	1.220* ±0.47
4.0+1x1 0^{-5}	0.640** ±0.24	0.980 ±0.68	1.070** ±013	0.650 ±0.06	0.470 ±0.13	0.370 ±0.07	0.400 ±0.08	0.870** ±0.12	1.280 ±0.59	0.700** ±0.21
4.0+1x1 0^{-6}	0.100 ±0.08	1.090 ±0.80	0.820** ±0.13	0.330 *±0.01	0.350 * ±0.05	0.390 ±0.07	0.490** ±0.01	0.630** ±0.11	1.300* ±0.46	1.630** ±0.22
4.0+1x1 0^{-7}M	0.150** ±0.04	1.000 ±0.38	0.740** ±0.04	0.400 ±0.08	0.820 ±0.18	0.530 ±0.11	0.520** ±0.04	0.750** ±0.07	1.870** ±0.10	0.500 ±0.38
5.0+1x1 0^{-5}	0.290** ±0.03	1.440** ±0.09	0.870 ±0.25	0.570 ±0.09	0.660 ±0.06	0.510 * ±0.02	0.380 ±0.07	0.970** * ±0.08	1.200* ±0.28	1.720* ±0.53
5.0+1x1 0^{-6}	0.250 ±0.07	1.540 ±0.47	0.810** ±0.05	0.910 ±0.14	0.530 ±0.05	0.330 ±0.02	0.480** ±0.04	0.710** ±0.08	1.830** ±0.04	0.630** ±0.19
5.0+1x1 0^{-7}M	0.200 ±0.03	1.200 ±0.27	0.330 ±0.05	0.710 ±0.08	0.450 0.08±	0.470 ±0.02	0.480** ±0.06	0.950** ±0.10	1.450** ±0.11	1.110* ±0.31

N.B. **=.01 Level of significance *= .05 Level of significance

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