EFFECT OF AUXIN AND SIMULATED ACID RAIN ON THE SULPHUR CONTENT IN THE LEAVES OF *CAPSICUM FRUTESCENS* VAR. *SWEET MAGIC*

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Abstract: Sulphur compounds of plant as well as of animal origin are of immense medicinal interest as they cure a number of ailments. For instance , thiazoles are antibiotic (e.g. Penicillin) , anti-microbial (e.g. Sulphathiazoles). They are vitality factors (for instance, Vitamin – B_1) & act on central nervous system besides other functions. Compounds of plant origin are safer in comparison to synthetic compounds. Therefore, we planned to enhance sulphur contents in the plants of Capsicum. For this purpose *Capsicum frutescens* var. *sweet magic* was treated with simulated acid rain of the pH 3.0 , 4.0 & 5.0 ; auxin (indole acetic acid) solutions of 1.0 x 10^{-5} , 1.0 x 10^{-6} & 1.0 x 10^{-7} M concentrations as well as interactive effects of pH – auxin binary solutions of different combinations (3.0 + 1.0 x 10^{-5} M , 3.0 + 1.0 x 10^{-6} M , 3.0 + 1.0 $^{-7}$ M ; 4.0 + 1.0 x 10^{-5} M , 4.0 + 1.0 x 10^{-6} M , 4.0 + 1.0 x 10^{-7} M & 5.0 + 1.0 x 10^{-5} M , 5.0 + 1.0 x 10^{-6} M , 5.0 + 1.0 x 10^{-7} M) & their effect on the sulphur contents of leaves of *Capsicum frutescens* var. sweet magic were studied . Best pH for sulphur content is 3.0 [sulphur content at 60th day = 155.86 % of control] & best auxin concentration is 1.0 x 10^{-5} M[sulphur content = 141.77 % of control at 45th day] . Best combination of pH & auxin is 3.0 + 1.0 x 10^{-6} M [sulphur content = 198.85 % of control at 60th day] . Moreover , acid rain & auxin assist each other towards enhancement of sulphur content in leaves.

Keywords: *Capsicum Frutescens* var. *sweet magic*, Simulated acid rain (SAR), Auxin (indole acetic acid)

INTRODUCTION

Capsicum frutescens L. is a small shrub of family Solanaceae. It is an annual or short lived pungent perennial plant (Chopra *et al.* 1956). *Capsicum frutescens* is native of South or Central America and is believed to have been introduced in India in the middle of seventeenth century in Shimla hills . This plant is a much branched shrub, 3-4 ft. in height. *Capsicum frutescens* is of therapeutic value also. Its fruit causes burning sensation and increase in appetite. It is useful in indigestion, diarrhoea, chronic ulcers, loss of consciousness and delirium & it's juice is antimicrobial, useful in backache, stomach ache, chest trouble and cough etc. Capsicum is of great nutritive value as it contains protein, carbohydrates and ascorbic acid. Besides vitamin-A (thiamin chloride hydrochloride) some minerals are also present . Sulphur contents in plants are due to the presence of sulphur containing compounds like vitamin-A, amino acids like cysteine , cystine , methonine , biotin etc. which may be responsible for curative effect of *Capsicum frutescens* . Acid rain has multiferous effects. It has direct as well as indirect effects on organisms as it comes in contact with. When deposited in gaseous form, it causes direct damage to plants in the form of visual injury along with yellowing and tissue depigmentation known as chlorosis (Sharma, B.K., 2001). Acid precipitation affects terrestrial vegetation due to reduced rate of photosynthesis and growth (Cohen et.al.,1981), as well as increased vunerability to drought and disease. Present work is done on the *Capsicum frutescens* L.var. *sweet magic* . Effects of simulated acid rain (pH3.0, 4.0 and 5.0) and (1x10^{-5} M, 1x10^{-6} M and 1x10^{-7} M) Auxin solution and interactive effect of acid rain and Auxin (pH3.0+1x10^{-5} M, 3.0+1x10^{-6} M, 3.0+1x10^{-7} M), (pH4.0+1x10^{-5} M, 4.0+1x10^{-6} M, 4.0+1x10^{-7} M) and (pH5.0+1x10^{-5} M, 5.0+1x10^{-6} M, 5.0+1x10^{-7} M) have been studied on sulphur contents in leaves .

MATERIAL AND METHOD

Simulated acid rain of different grades of pH (3.0, 4.0, 5.0) were prepared with the help of electronic pH meter by adding mixture of H_2SO_4 and HNO_3 in ratio of 7:3 (v/v) in distilled water (Lee, J.J.,1981).
The molar solution of Auxin (IAA) (10 $^{-4}$ Molar) were prepared by dissolving 17.5 mg Auxin in 1000 ml of distilled water. Thereafter, dilution was done as required for preparing the solutions of 1x 10^{-5}, 1x10^{-6}, 1x10^{-7} M concentration of Auxin.
For the study of sulphur content in the leaves of *Capsicum frutescens* var. *sweet magic* certified seeds were sown in the soil. Besides the regular watering to plants the setwise treatment which was given to plant is detailed below in Table-A.

Table A.

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Sr. No	Set No	Treatment	Periodicity
1	1st set	Control- Water (pH6.0)	10 ml spayed twice a week
2	2nd set	Acid rain - pH 3.0	10 ml sprayed twice a week
3	3rd set	Acid rain - pH 4.0	10 ml sprayed twice a week
4	4th set	Acid rain - pH 5.0	10 ml sprayed twice a week
5	5th set	$1x10^{-5}$ M Auxin	10 ml sprayed twice a week
6	6th set	$1x10^{-6}$ M Auxin	10 ml sprayed twice a week
7	7th set	$1x10^{-7}$ M Auxin	10 ml sprayed twice a week
8	8th set	Acid rain + Auxin (pH 3.0 + $1x10^{-5}$M)	10-10 ml each sprayed twice a week
9	9th set	Acid rain + Auxin (pH 3.0 + $1x10^{-6}$M)	10-10 ml each sprayed twice a week
s10	10th set	Acid rain + Auxin (pH 3.0 + $1x10^{-7}$M)	10-10 ml each sprayed twice a week
11	11th set	Acid rain + Auxin (pH 4.0 + $1x10^{-5}$M)	10-10 ml each sprayed twice a week
12	12th set	Acid rain + Auxin (pH 4.0 + $1x10^{-6}$M)	10-10 ml each sprayed twice a week
13	13th set	Acid rain + Auxin (pH 4.0 + $1x10^{-7}$M)	10-10 ml each sprayed twice a week
14	14th set	Acid rain + Auxin (pH 5.0 + $1x10^{-5}$M)	10-10 ml each sprayed twice a week
15	15th set	Acid rain + Auxin (pH 5.0 + $1x10^{-6}$M)	10-10 ml each sprayed twice a week
16	16th set	Acid rain + Auxin (pH 5.0 + $1x10^{-7}$M)	10-10 ml each sprayed twice a week

Estimation of Sulphur

Quantification of sulphur was done by turbiditory method as according to C.C.Mitchell (1992). 100 mg dried and finely powdered leaves were taken in 100 ml borosil beaker. 2 ml concentrated HNO_3 was added in it and each of the beaker covered with watch glass and allowed to stand over- night or till frothing subsides. Covered beakers were placed on hot plate at 125^0c for one hour. After one hour removed the beaker from hot plate and cooled. Added 1 ml of 30% H_2SO_4 and digested at the same temperature. Heated and added H_2O_2 till digest is clear. After the digestion of sample, removed watch glass and evaporated to dryness at 80^0C (residue should be colourless or white). Now added dilute HNO_3 or HCl or a combination of two and also added deionised water to dissolve the residue and make up the volume depending upon requirement of analytical procedure .Now, kept the solution in eppendorf tubes and centrifuged at 3000 rpm for 15 minutes. 1ml of each standard and digested sample is taken in test tubes. Added 22 ml AcOH – H_3PO_4 solution and mixed on stirrer. Added 0.5ml of $BaSO_4$ seed suspension, 1ml of $BaCl_2$ solution and mixed each tube exactly for same length of time on a vertex. Added 1 ml of gum acacia solution and mixed again.

Allowed to stand for 30 minutes. Mixed each sample uniformly just prior to reading absorbance or transmittance on a spectrophotometer set at 440 nm wave length. The amount of sulphur is calculated with the help of calibration curve.

RESULT AND DISCUSSION

Effect of simulated acid rain of different concentrations (pH 3.0, 4.0 and 5.0) on sulphur content in the leaves of *Capsicum frutescens* var. '*Sweet magic* is shown in Table-1. When the treatment of pH 3.0 is given, sulphur content is 117.80%, 155.86%, 132.18%, 121.43% and 97.48% of control at the age of 45, 60, 75, 90 and 105 days respectively. At pH 4.0, the sulphur is 76.59%, 57.47%, 73.68%, 66.58%, 57.47% of control, and sulphur content is 91.77%, 101.38%, 116.63%, 100.35% and 88.10% of control at pH 5.0 concentration at plant age of 45, 60,75,90 and 105 days respectively.

Table-2 shows the effect of treatment of $1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ M auxin on sulphur content in the leaves of *Capsicum frutescens* var. *Sweet magic*. When the treatment of $1x10^{-5}$ M auxin is given to the plants, the sulphur content is 141.77%, 74.71%, 91.40%, 89.02% and 93.33% of the control; On treatment with $1x10^{-6}$ M auxin, the sulphur content is 108.29%, 74.71%, 72.38%, 102.67% and 99.56% of control and at $1x10^{-7}$ M , the sulphur content is 110.49%, 115.79%, 92.12%, 104.02% and 94.21% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

The Interactive effect of treatment of simulated acid rain of pH 3.0 , 4.0 & 5.0 and auxin ($1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$) on sulphur content in the leaves of *Capsicum frutescens* var. *Sweet magic* is shown in table-3. On treating the plants with acid rain and auxin (pH $3.0+1x10^{-5}$ M), the sulphur is 101.40%, 110.19%, 81.71%, 83.38% and 62.10% , at the treatment of acid rain and auxin (pH $3.0+1x10^{-6}$ M), the sulphur are 146.34%, 198.85%, 98.81%, 105.51% and 100.92% and when the plants undergo a treatment of acid rain and auxin (pH $3.0+1x10^{-7}$ M) , the sulphur is 133.35%, 154.10%, 90.47%, 103.24% and 100.80% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

At the treatment of acid rain and auxin (pH $4.0+1x10^{-5}$ M), the sulphur content is 132.99%, 121.01%, 73.37%, 102.23% and 111.94% , when the treatment of acid rain and auxin (pH $4.0+1x10^{-6}$ M) is given , sulphur content is 99.94%, 139.08%, 107.98%, 105.25% and 107.27% and the sulphur content are 161.34%, 183.30%, 106.79%, 116.32% and 118.29% at the plant age of 45, 60, 75, 90 and 105 days respectively at the treatment of acid rain and auxin (pH $4.0+1x10^{-7}$ M).

When made to undergo treatment with acid rain and auxin (pH $5.0+1x10^{-5}$ M), the sulphur content in the leaf of the plant are 111.22%, 89.50%, 103.52%, 89.24% and 94.69% , at the treatment of acid rain and auxin (pH $5.0+1x10^{-6}$ M), the sulphur content are 140.49%, 136.17%, 91.76%, 93% and 75.68% and at the treatment of acid rain and auxin (pH $5.0+1x10^{-7}$ M), the sulphur content is 109.88%, 103.07%, 117.15%, 112.29% and 110.98% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

For var. *sweet magic*, maximum sulphur content is found on 45^{th} day at pH 3.0 [155.86 % of the control] . Minimum sulphur content at 45^{th} day was found at pH 4.0 [57.47% of control]. At pH 5.0 , significant increase at 45^{th} day . The reason for higher concentrations of sulphur in treated plant may be the sulphuric acid component of acid rain (Sharma, 1990) which may interact with sulphur metabolism. . Higher sulphur content at earlier age might have been due to the greater accumulation in smaller plants.

In leaves of var. *sweet magic*, maximum sulphur content is found at $1x10^{-5}$ M auxin at the 45^{th} day to significant level [141.77% of control]. At all the concentrations after that, there is an initial decline at the 75^{th} day, followed by variable increase. Variable pattern of change in sulphur content at different concentrations of auxin treatment with the number of days can be reasoned on account of interaction of auxin with plant metabolism and dependence of yield of biosynthesis on variable condition like temperature, wind, water contents sunlight and sun heat.

For var. *sweet magic*, maximum sulphur content is found on 60^{th} day at pH $3.0+1x10^{-6}$ M [198.85% of control] . Thus it is the optimum combination for sulphur content. Pattern of change is irregular. Minimum significant combination is pH $3.0+1x10^{-5}$ M at 105^{th} day. The reason for higher concentrations of sulphur in treated plant may be the sulphuric acid component of acid rain (Sharma, 1990) in combination which may interact with sulphur metabolism. Effect of auxin to increase sulphur metabolism might have assisted effect of acid rain to increase sulphur content. High sulphur content at earlier plant age is due to greater accumulation at juvenile stage.

CONCLUSION

Sulphur contents increase with increase in H^+ ion concentration in acid rain due to the presence of H_2SO_4 in acid rain. Auxin also interacts with metabolism of plants which may enhance synthesis of sulphur compounds. Combination of acid rain & auxin show increase in sulphur contents due to sulphur content in acid rain & interaction of auxin with sulphur metabolism.

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Table 1. Effect of simulated acid rain (pH 3.0, 4.0, 5.0) on sulphur content(µg/100mg dry wt ± SD .) in the leaves of *Capsicum frutescens* var. *sweet magic*

Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	5.47 ± 0.50	4.35 ± 0.32	6.43 ± 0.42	7.62 ± 0.30	8.35 ± 0.30
3.0	6.44* ± 0.20	6.78** ± 0.15	8.50** ± 0.45	9.25* ± 0.73	8.14 ± 0.39
4.0	4.19* ± 0.39	2.50** ± 0.33	4.74* ± 0.54	5.07** ± 0.44	4.80** ± 0.48
5.0	5.02 ± 0.30	4.41 ± 0.39	7.50 ± 1.33	7.65 ± 1.18	7.35 ± 1.03

NB **=.01 level of significance *=.05 level of significance

Table 2. Effect of Auxin ($1x10^{-5}$,$1x10^{-6}$,$1x10^{-7}$ M) on Sulphur content (µg/100mg dry wt., ± SD) in the leaves of *Capsicum frutescens* var. *sweet magic*

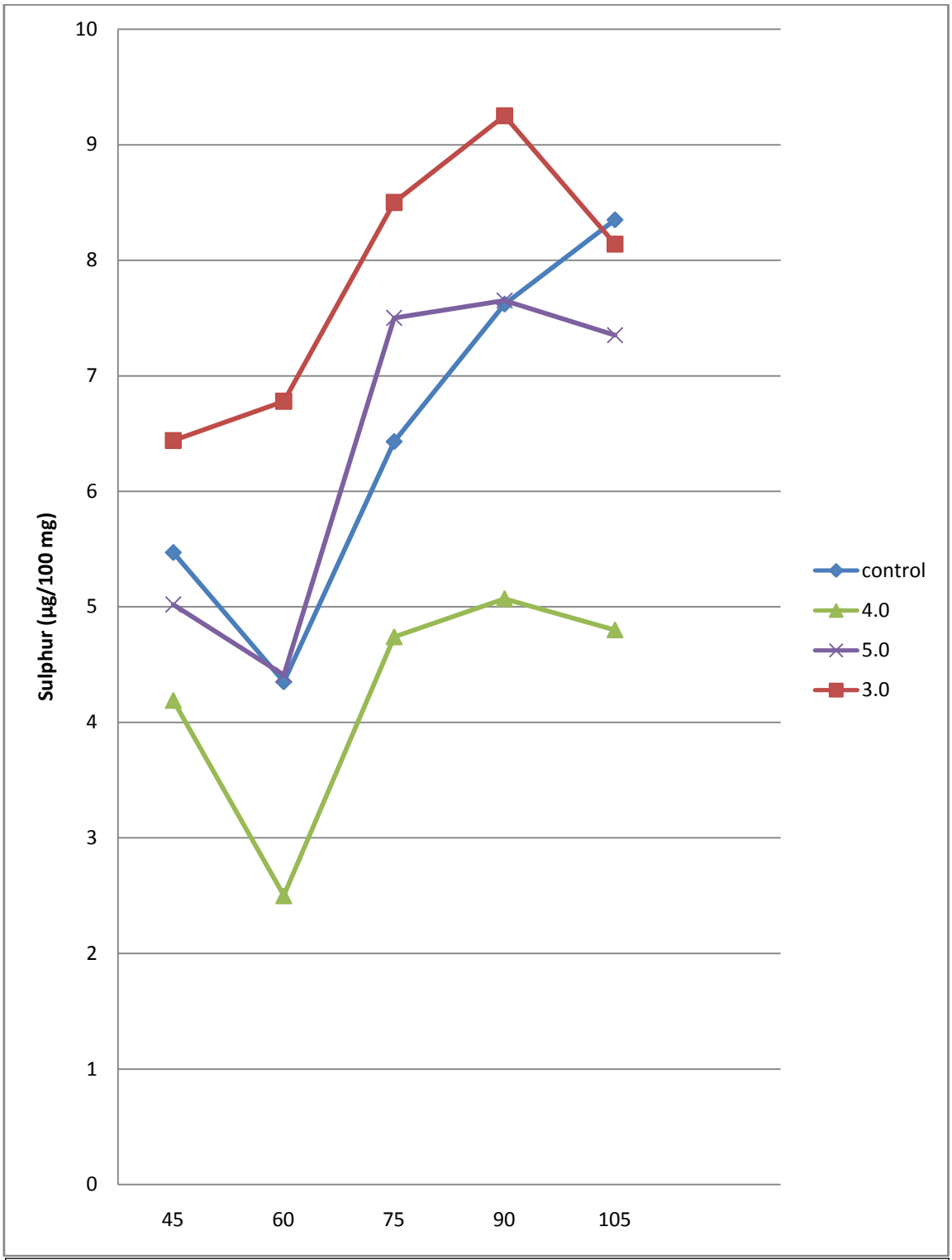
Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	5.47 ± 0.50	4.35 ± 0.32	6.43 ± 0.42	7.62 ± 0.30	8.35 ± 0.30
$1x10^{-5}$ M	7.75** ± 0.61	3.25** ± 0.25	5.88 ± 0.21	6.78 ± 0.28	7.79 ± 0.12
$1x10^{-6}$ M	5.92 ± 0.31	3.45** ± 0.25	4.66* ± 0.81	7.82 ± 0.17	8.31 ± 0.48
$1x10^{-7}$ M	6.04 ± 0.79	5.04 ± 0.29	5.93 ± 0.34	7.93 ± 0.79	7.86 ± 0.28

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

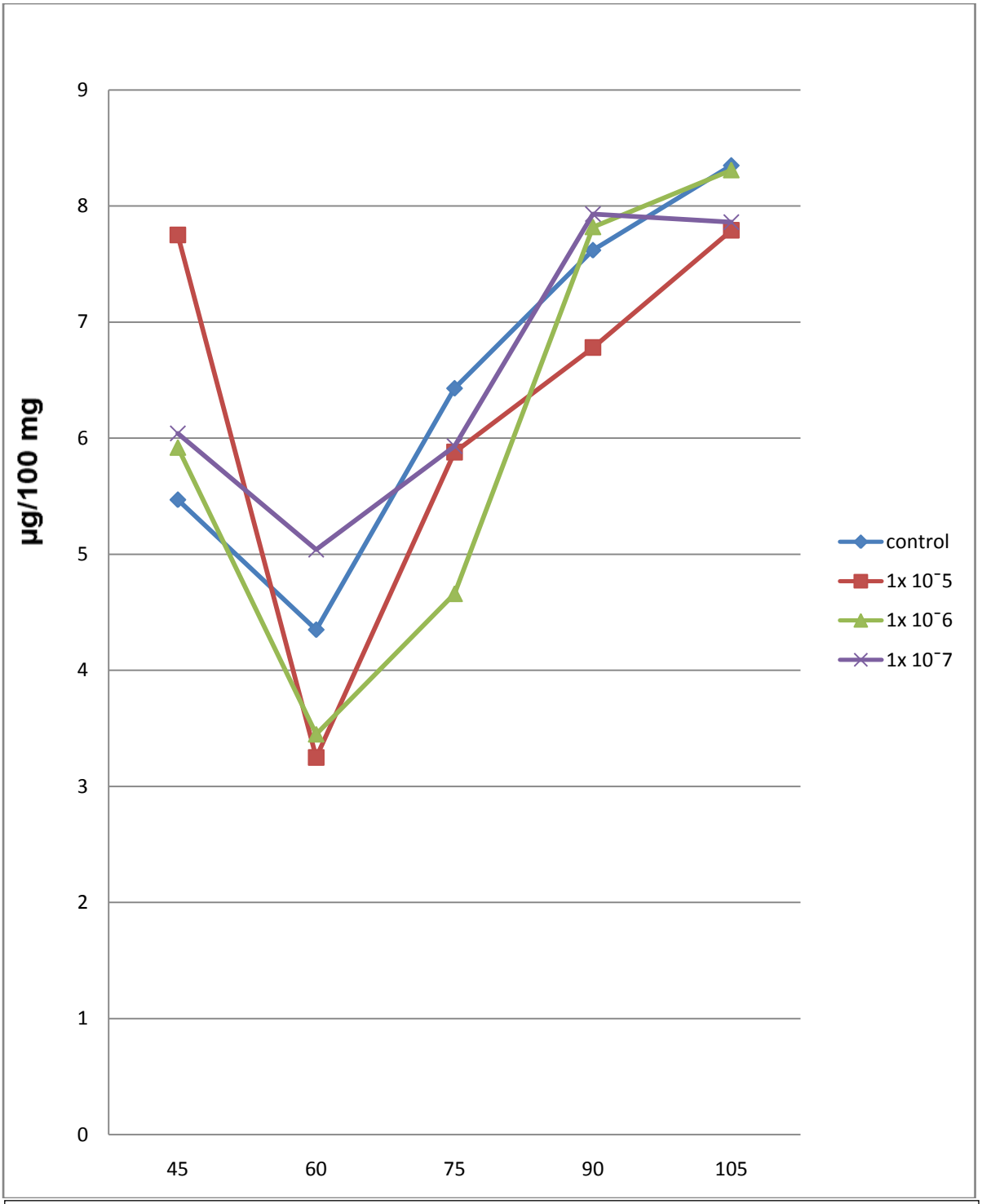
Table 3. Interactive Effect of simulated acid rain (pH3.0,4.0,5.0) and Auxin (10^{-5},10^{-6},10^{-7}M) on sulphur content(µg/100mg dry wt ± SD.) in the leaves of *Capsicum frutescens* var. *sweet magic*.

Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	5.47	4.35	6.43	7.62	8.35
	± 0.50	± 0.32	± 0.42	± 0.30	± 0.30
3.0 +1X10^{-5} M	5.54	4.79	5.26*	6.35*	5.18**
	± 0.39	± 0.38	± 0.18	± 0.47	± 0.29
3.0 + 1x10^{-6} M	8.00*	8.65**	6.36	8.04	8.42
	± 0.81	± 0.67	± 0.36	± 0.61	± 0.51
3.0 +1x10^{-7} M	7.29*	6.70*	5.82	7.87	8.41
	± 0.52	± 1.01	± 0.42	± 0.45	± 0.51
4.0 +1X10^{-5} M	7.27**	5.27	4.72**	7.79	9.34
	± 0.43	± 0.87	± 0.43	± 0.75	± 0.65
4.0 + 1x10^{-6} M	5.46	6.05**	6.95	8.02	8.95
	± 0.54	± 0.45	± 0.84	± 1.48	± 0.83
4.0 +1x10^{-7} M	8.82**	7.97**	6.87	8.86*	9.87*
	± 1.04	± 0.78	± 0.66	± 0.47	± 0.54
5.0 +1X10^{-5} M	6.08	3.89	6.66	6.80	7.90
	± 0.56	± 0.57	± 0.74	± 0.47	± 0.86
5.0 + 1x10^{-6} M	7.68**	5.92	5.90	7.09	6.32*

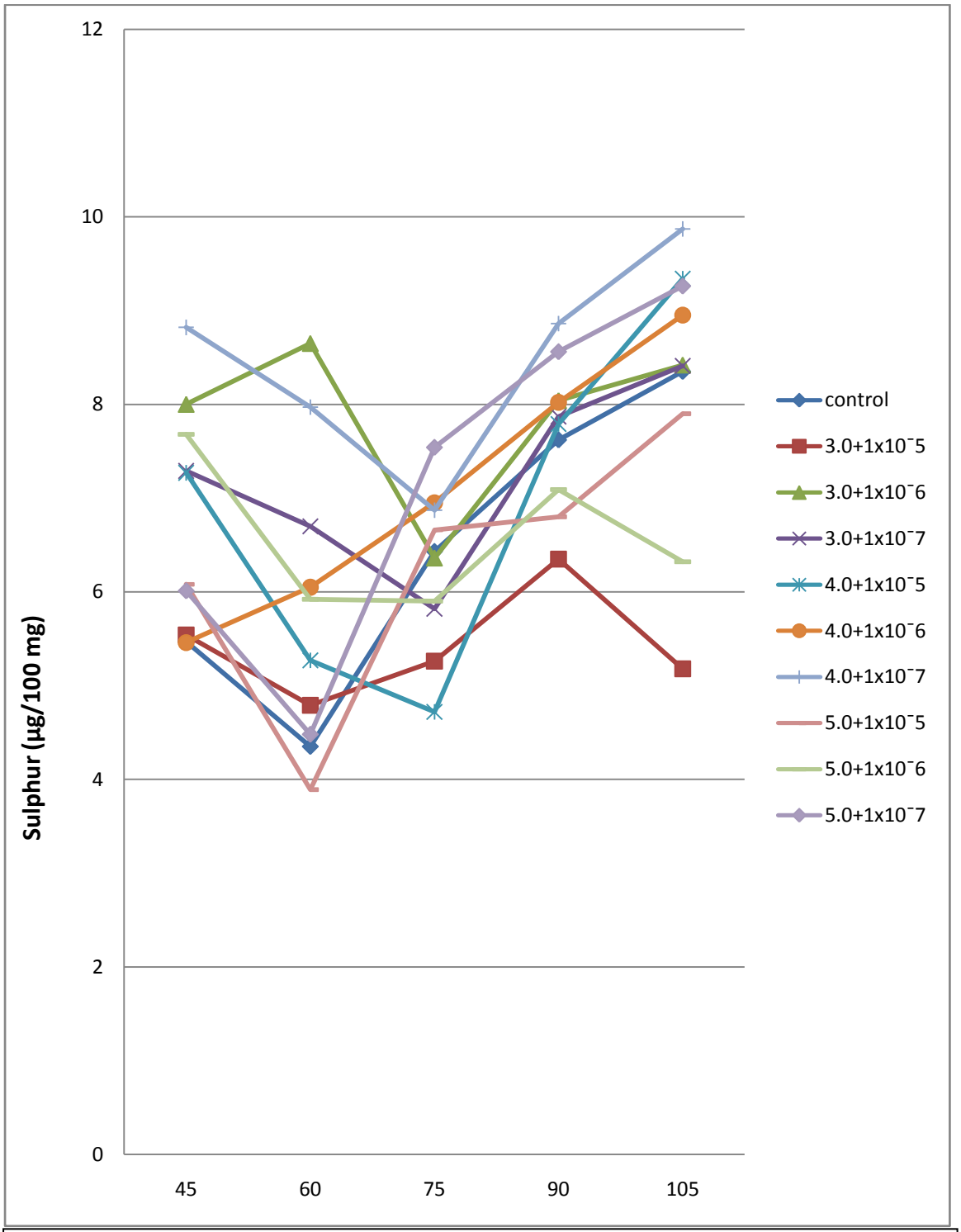
	± 0.43	± 0.98	± 0.53	± 0.36	± 0.96
5.0 +1x10^{-7} M	6.01	4.48	7.54	8.56	9.26
	± 0.85	± 0.95	± 0.64	± 1.27	± 0.62
N.B.	**=.01 level of significance *=.05 level of significance				



Graph No 1: Effect of simulated Acid rain (pH3.0, 4.0 & 5.0) on Sulphur content (µg/100mg dry wt.) in the leaves of *Capsicum frutescens* var. *Sweet magic* at different plant age



Graph No 2:Effect of Auxin (1x10 $^{-5}$, 1x 10^{-6}, 1x 10^{-7} M) on Sulphur content (µg/100 mg dry wt.) in leaf of *Capsicum frutescens* var. *Sweet Magic* at different plant age



Graph No:3: Interactive Effect of acid rain (pH 3.0, 4.0 & 5.0) and Auxin ($1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ M) on Sulphur content (µg/100mg dry wt.) in leaf of *Capsicum frutescens* var. *Sweet Magic* at different plant age

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