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Effect of Growth Hormones i.e., GA_3 and Kinetin and Heavy Metal i.e., $Pb(NO_3)_2$ on the Seedlings of *Cucumis sativus* L.

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Abstract: Effect of growth hormones i.e., kinetin and gibberellic acid as well as heavy metal i.e., $Pb(NO_3)_2$ were studied pertaining to external and internal morphology of *Cucumis sativus* L. Moreover, the histomorphology of leaf was also studied. The following concentrations of growth hormones i.e., 40 ppm kinetin, 200 ppm GA_3 and heavy metal i.e., 100 ppm $Pb(NO_3)_2$ and 200 ppm $Pb(NO_3)_2$ were applied individually. In combinations, they were 40 ppm kinetin, 100 ppm $Pb(NO_3)_2$, 40 ppm kinetin+200 ppm $Pb(NO_3)_2$, 200 ppm GA_3+100 ppm $Pb(NO_3)_2$ and 200 ppm GA_3+200 ppm $Pb(NO_3)_2$. In the external morphology, applied kinetin revealed reduction in the length of internode and petiole, which was accompanied by expansion in diameter. Furthermore, it increased the fresh and dry weight. Applied GA_3 registered increase in length and inhibition in diameter and decrease in fresh and dry weight. Application of $Pb(NO_3)_2$ treatments showed decrease in length as well as in fresh and dry weight, which was accompanied by some expansion in diameter. The results of mixed doses of $Pb(NO_3)_2$ with kinetin and GA_3 were more or less similar as observed in individual doses of kinetin and GA_3. In the leaf, fresh and dry weight, leaf area and number of leaves were increased with kinetin. Contrarily $Pb(NO_3)_2$ treatments revealed inhibition in fresh and dry weight and leaf area. Moreover, number of stomata/mm^2, stomatal index and %age of open stomata showed increase with kinetin. In the internal morphology of internode, the epidermis, cortical collenchyma and parenchyma, cambial region, xylary region and pith showed increase with kinetin and decrease with GA_3. In the petiole, the epidermis, collenchymatous hypodermis and xylary elements showed expansion with kinetin and $Pb(NO_3)_2$ treatments, while inhibition was observed with GA_3. In the case of leaf, width of epidermal cells, size and number of mesophyll cells and width of xylem elements revealed increase with kinetin, while rest of the treatments showed no significant effect.

Key words: *Cucumis sativus* L., internode, petiole, leaf, xylary region, hormones, GA_3, Kin, heavy metal, $Pb(NO_3)_2$

INTRODUCTION

Hormone mediated control of plant growth and development involves both synthesis and response[1]. Cytokinins are hormones, which are associated with plant growth and development. They take part in the control of cell division, chloroplast development, shoot inhibition, growth and leaf senescence[2]. Cytokinins stimulate growth by expansion rather than by elongation[3]. Ronzhina[4] reported that in *Cucurbita pepo* lower concentration of cytokinins enhanced cell division of mesophyll. Gibberellic acid causes stem elongation, which is accompanied by inhibition in diameter[5,6]. In *Lens culinaris* L., treatment with GA_3 increased the number of internodes, which lead to axis elongation[7]. Heavy metals are growth inhibitors and are said to be involved in blocking the essential physiological processes[8-10]. Chaudhry and Qurat-ul-Ain[11] working on *Phaseolus vulgaris* L., reported inhibition in the growth of leaves as well as in the mesophyll with the application of $Pb(NO_3)_2$. $Pb(NO_3)_2$ has inhibitory effects whereas kinetin and GA_3 are well known for enhancement of growth. The present study was undertaken to see whether the inhibitory effects of $Pb(NO_3)_2$ can be revealed by kinetin and GA_3.

MATERIALS AND METHODS

The main purpose of the present study was to observe the effect of growth hormones i.e., kinetin and GA_3 as well as heavy metal i.e., $Pb(NO_3)_2$ on the external and internal morphology of first internode as well as petiole and leaves of *Cucumis sativus* L. (Cucurbitaceae). The following concentration of growth hormones and heavy metal were used, 40 ppm kinetin, 200 ppm GA_3, 100 ppm $Pb(NO_3)_2$, 200 ppm $Pb(NO_3)_2$, 40 ppm

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kinetin+100 ppm $Pb(NO_3)_2$, 40 ppm kinetin+200 ppm $Pb(NO_3)_2$, 200 ppm GA_3+100 ppm $Pb(NO_3)_2$, 200 ppm GA_3+200 ppm $Pb(NO_3)_2$. Plants were grown in earthenware pots in the month of May and 27 μL of each hormonal treatment was given on apical meristem. $Pb(NO_3)_2$ was applied in soil along with water. Plants were removed from the pots after 15 days. In external morphology, length, diameter, fresh and dry weight of internode, petiole and leaves moreover number of leaves, leaf area and SLA was studied. In the internal morphology, 1 cm long pieces of internode, petiole and leaf were fixed in Corney's modified fluid. Material was dehydrated and cleared in tertiary butyl alcohol grades, infiltrated and embedded in paraffin wax. The embedded material was processed in transverse planes with rotary microtome (10-15 μm). The material was then passed through desending series of xylene stained with safranine and fast green and mounted in Canada balsam. The material was observed under microscope. Data obtained was compared with control as well as among themselves. All data was subjected to statistical analysis[12].

RESULTS

External morphology: The internode and petiole of *Cucumis sativus* L., registered inhibitory effect on the length with applied 40 ppm kinetin, which was followed by significant expansion in diameter and increased fresh and dry weight after 15 days when compared with control. Contrarily, applied 200 ppm GA_3 showed well marked increase in the length of internode and petiole, which being 46.15 and 30%, respectively (Table 1 and Fig. 1). However, it was followed by inhibition in diameter and decreased fresh and dry weight. Application of 100 ppm and 200 ppm $Pb(NO_3)_2$ showed significant decrease in the length i.e., 41 and 49.03% in case of internode and for petiole it was 37.4 and 42.12%, respectively, which was accompanied by expansion in diameter and decreased fresh and dry weight (Table 2).

The application of mixed doses of 40 ppm kinetin+100 ppm $Pb(NO_3)_2$ and 40 ppm kinetin+200 ppm $Pb(NO_3)_2$ showed decreased length (Fig. 2). However, it was followed by expansion in diameter and increased fresh and dry weight. Contrarily, 200 ppm GA_3+100 ppm $Pb(NO_3)_2$ and 200 ppm GA_3+200 ppm $Pb(NO_3)_2$ showed increased length, reduction in diameter and decreased fresh and dry weight in the internode as well as in the petiole. In the control only 3 leaves were observed. However, with extraneous 40 ppm kinetin and 200 ppm GA_3 the number of leaves observed were 4 (Fig. 1). In 200 ppm GA_3+100 ppm $Pb(NO_3)_2$ 5 leaves were observed. The number of leaves remained constant in the remaining treatments. Fresh weight, dry weight, leaf area and specific leaf area was increased only with the application of kinetin. All the remaining treatments showed decrease in all the above mentioned parameters (Table 3). The stomata observed were anomocytic and anisocytic, which were 90 and 10%, respectively (Fig. 3). All the treatments exhibited decrease in the number of epidermal cells/mm^2 over control. Number of stomata/mm^2 was increased only with the application of kinetin. Length of guard cells increased with the application of GA_3 and its mixed doses with $Pb(NO_3)_2$. Width of guard cells and stomatal pore was increased with kinetin, $Pb(NO_3)_2$ and its mixed doses with kinetin. Length of stomatal pore showed increase

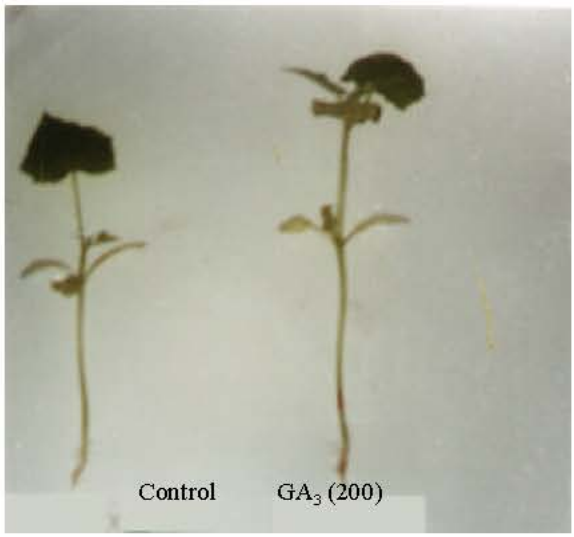

Fig. 1: Effect of 200 ppm GA_3 showing the increased length of internode

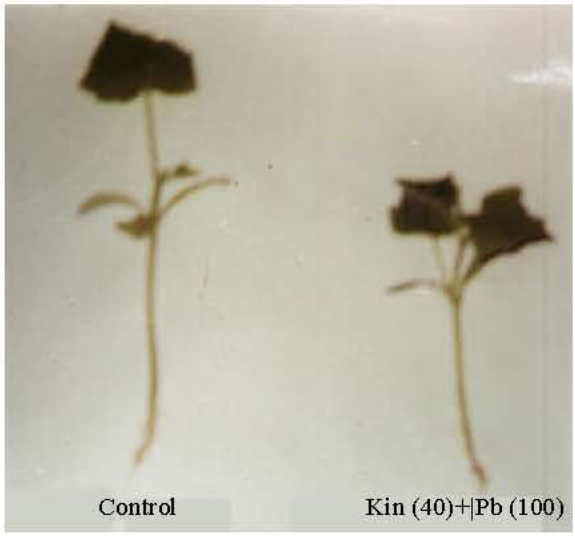

Fig. 2: Effect of 40 ppm kinetin+100 ppm $Pb(NO_3)_2$ on the length of petiole

Table 1: Effect of growth hormones and heavy metal on the external morphology of internode1

Treatments	Length of internode 1 (cm)	Diameter of internode 1 (cm)	Fresh weight of internode 1 (g)	Dry weight of internode 1 (g)
Control	2.60±1.950	0.525±0.393	0.367±0.152	0.050±0.035
40 ppm Kinetin	2.35±1.762	0.700±0.675	0.405±0.228	0.077±0.049
200 ppm GA_3	3.80±2.470	0.475±0.356	0.354±0.300	0.045±0.105
100 ppm Pb	1.51±0.731	0.568±0.543	0.275±0.045	0.035±0.006
200 ppm Pb	1.32±0.343	0.549±0.506	0.270±0.029	0.033±0.003
40 ppm Kinetin+100 ppm Pb	1.87±1.180	0.650±0.637	0.388±0.043	0.068±0.168
40 ppm Kinetin+200 ppm Pb	1.56±0.743	0.600±0.525	0.370±0.114	0.060±0.029
200 ppm GA_3+100 ppm Pb	3.55±2.137	0.460±0.412	0.339±0.191	0.040±0.075
200 ppm GA_3+200 ppm Pb	3.09±2.006	0.440±0.243	0.330±0.146	0.038±0.063

Table 2: Effect of growth hormones and heavy metal on the external morphology of petiole of leaf 1

Treatments	Length of petiole (cm)	Diameter of petiole (cm)	Fresh weight of petiole (g)	Dry weight of petiole (g)
Control	2.35±1.762	0.678±0.506	0.200±0.0307	0.024±0.0045
40 ppm Kinetin	2.30±1.632	0.800±0.600	0.269±0.1320	0.033±0.0072
200 ppm GA_3	3.05±2.287	0.580±0.504	0.189±0.0840	0.020±0.0420
100 ppm Pb	1.47±0.957	0.700±0.562	0.156±0.0220	0.012±0.0032
200 ppm Pb	1.35±0.787	0.686±0.006	0.148±0.0142	0.009±0.0020
40 ppm Kinetin+100 ppm Pb	2.20±1.732	0.755±0.543	0.218±0.1260	0.027±0.0080
40 ppm Kinetin+200 ppm Pb	1.97±1.237	0.715±0.506	0.208±0.0780	0.025±0.0067
200 ppm GA_3+100 ppm Pb	2.86±1.950	0.520±0.375	0.178±0.0645	0.018±0.0250
200 ppm GA_3+200 ppm Pb	2.60±1.650	0.485±0.262	0.164±0.0320	0.016±0.0037

Table 3: Effect of growth hormones and heavy metal on the external morphology of the leaf 1

Treatments	Fresh weight of 1st leaf (g)	Dry weight of 1st leaf (g)	Area of 1st leaf (sq. cm)	Specific leaf area of 1st leaf	No. of leaves
Control	0.475±0.1960	0.0636±0.0001	1015.90±1.12	15973.2	3
40 ppm Kinetin	0.489±0.0210	0.0652±0.0090	1049.50±1.09	16096.0	4
200 ppm GA_3	0.466±0.0004	0.0630±0.0060	1000.95±1.28	15874.6	4
100 ppm Pb	0.443±0.0050	0.0625±0.0040	965.91±1.92	15454.5	3
200 ppm Pb	0.440±0.0010	0.0620±0.0220	948.21±1.67	15293.7	3
40 ppm Kinetin+100 ppm Pb	0.470±0.0008	0.0634±0.0040	1010.91±1.82	15944.9	3
40 ppm Kinetin+200 ppm Pb	0.460±0.0005	0.0633±0.0120	1009.00±1.90	15939.9	3
200 ppm GA_3+100 ppm Pb	0.450±0.0017	0.0629±0.0010	989.70±1.44	15734.5	5
200 ppm GA_3+200 ppm Pb	0.448±0.0110	0.0626±0.0030	980.00±2.30	15654.9	3

Table 4: Effect of growth hormones and heavy metal on epidermis of leaves

Treatments	No. of epidermal cells/ mm^2	No. of stomata/ mm^2	Size of guard cells (µm) Length	Size of guard cells (µm) Width	Size of stomatal pore (µm) Length	Size of stomatal pore (µm) Width	Stomatal index	%age of open and closed stomata Open	%age of open and closed stomata Closed
Control	74.00 ±0.23	33.50 ±0.34	86.34 ±0.54	50.60 ±0.004	59.66 ±0.021	34.00 ±0.27	36.57	91.66	8.34
40 ppm Kinetin	73.80 ±0.60	35.50 ±0.006	86.09 ±0.42	52.17 ±0.17	54.93 ±0.712	34.88 ±0.02	37.90	92.57	7.43
200 ppm GA_3	73.10 ±0.42	32.00 ±0.54	87.56 ±0.27	50.52 ±0.023	60.02 ±0.014	33.73 ±0.026	35.80	92.80	7.20
100 ppm Pb	71.25 ±0.42	30.50 ±0.72	84.00 ±0.09	50.82 ±0.43	52.31 ±0.041	34.66 ±0.07	35.23	70.95	29.05
200 ppm Pb	69.20 ±0.27	28.90 ±0.90	83.95 ±0.20	50.70 ±0.39	52.00 ±0.117	34.48 ±0.05	34.73	68.24	31.76
40 ppm Kinetin+ 100 ppm Pb	73.30 ±0.021	32.79 ±0.23	85.67 ±0.28	51.62 ±0.71	53.00 ±0.129	34.79 ±0.54	36.29	89.00	11.00
40 ppm Kinetin+ 200 ppm Pb	73.19 ±0.24	32.35 ±0.41	85.00 ±0.81	51.46 ±0.32	52.98 ±0.012	34.69 ±0.60	36.06	88.74	11.26
200 ppm GA_3+ 100 ppm Pb	72.48 ±0.27	31.78 ±0.24	87.22 ±0.94	45.50 ±0.031	59.66 ±0.140	32.08 ±0.72	35.70	88.77	11.25
200 ppm GA_3+ 200 ppm Pb	71.60 ±0.24	30.99 ±0.47	87.00 ±0.54	42.26 ±0.27	59.60 ±0.402	31.91 ±0.49	35.54	86.94	13.06

only with GA_3. Applied 40 ppm kinetin showed increase in the value of stomatal index. The remaining treatments showed decrease in comparison to control. Applied 40 ppm kinetin and 200 ppm GA_3 showed more or less same percentage of open and closed stomata when compared with control, while Pb $(NO_3)_2$ treatments showed maximum number of closed stomata. In the mixed doses the %age of open and closed stomata were more or less similar (Table 4 and Fig. 3).

Internal morphology: The epidermal cells of internode, petiole as well as of leaf did not show any marked

Table 5: Effect of growth hormones and heavy metal on the internal morphology of Internode 1

Treatments	Width of epidermal cells (µm)	Width of collen-chyma cells (µm)	No. of cortical layers	Width of cortical region (µm)	Width of cortical paren-chyma cells (µm)	No. of vascular bundles	No. of layers of cambium	No. of metaxylem elements in one strand	Width of metaxylem elements (µm)	Width of cellular region of pith (µm)	Width of fistular region of pith (µm)
Control	31.40	31.60	8	270.00	78.00	7	5	3	39.30	420.15	328.00
	±0.009	±1.21		±2.19	±2.81				±2.09	±6.65	±5.50
40 ppm Kinetin	32.60	33.70	9	349.00	89.60	8	6	4	49.30	550.79	310.90
	±1.00	±2.10		±1.97	±1.73				±2.60	±5.31	±5.00
200 ppm GA_3	30.90	30.00	8	260.50	76.00	6	5	3	38.80	370.90	342.90
	±0.91	±1.07		±1.78	±2.40				±1.71	±6.35	±4.97
100 ppm Pb	31.80	32.40	8	330.20	83.00	7	5	2	43.30	501.00	324.00
	±0.51	±1.57		±1.65	±2.71				±2.80	±6.42	±4.01
200 ppm Pb	31.60	31.90	7	321.00	81.60	7	5	2	42.00	496.00	326.00
	±0.93	±2.39		±1.65	±1.97				±2.13	±6.34	±4.70
40 ppm Kinetin	32.40	33.40	8	335.20	86.80	8	6	3	46.30	539.00	316.00
+100 ppm Pb	±1.29	±1.25		±1.91	±1.74				±2.06	±5.69	±3.38
40 ppm Kinetin	32.20	32.90	7	333.29	83.50	8	6	3	43.90	526.10	320.90
+200 ppm Pb	±2.06	±2.00		±1.59	±1.62				±2.65	±5.81	±4.10
200 ppm GA_3	30.50	29.60	7	258.80	74.60	6	5	3	38.30	365.10	346.00
+100ppm Pb	±2.00	±2.00		±1.02	±1.37				±2.59	±5.01	±3.00
200 ppm GA_3	30.00	29.10	7	250.40	74.00	6	5	3	38.00	359.20	350.50
+200 ppm Pb	±1.21	±2.01		±2.00	±1.02				±2.09	±5.17	±3.70

Table 6: Effect of growth hormones and heavy metal on the internal morphology of petiole of leaf 1

Treatments	Width of epidermal cells (µm)	No. of layers of hypodermis	Width of collen-chymatous hypodermal cells (µm)	Width of petiole (µm)	No. of vascular bundles	No. of strands of xylem elements in a single bundle	No. of metaxylem elements in a single strand	Width of metaxylem elements(µm)
Control	24.30	2	24.30	1593.00	6	3	3	32.30
	±1.63		±0.29	±3.47				±3.30
40 ppm Kinetin	24.70	2	25.00	1750.00	7	3	3	36.80
	±0.39		±0.20	±3.65				±0.98
200 ppm GA_3	24.20	2	24.20	1556.90	5	3	3	31.10
	±0.30		±0.10	±2.10				±1.35
100 ppm Pb	24.30	2	24.60	1682.30	5	3	3	34.80
	±0.29		±0.31	±2.80				±1.90
200 ppm Pb	24.30	2	24.60	1670.90	5	3	3	33.60
	±0.21		±0.24	±3.59				±2.00
40 ppm Kinetin	24.60	2	24.80	1735.90	7	3	3	36.00
+100 ppm Pb	±0.15		±0.26	±0.85				±1.71
40 ppm Kinetin	24.60	2	24.70	1706.90	7	3	3	35.00
200 ppm Pb	±0.28		±0.01	±5.00				±1.62
200 ppm GA_3	24.00	2	24.00	1549.00	5	3	3	30.80
+100 ppm Pb	±0.21		±0.30	±1.98				±1.59
200 ppm GA_3	23.90	2	23.80	1537.30	5	3	3	29.80
+200 ppm Pb	±0.26		±0.31	±2.78				±2.00

Table 7: Effect of hormones and heavy metal on the internal morphology of leaf 1

Treatments	Width of adaxial epidermal cells (µm)	Width of abaxial epidermal cells (µm)	No. of palisade layer	No. of palisade cells in 100 µm		Length of palisade cells (µm)		Width of palisade cells (µm)		Width of spongy mesophyll (µm)	No. of strand of xylem elements in main vein	No. of xylem elements in main vein	Width of meta xylem elements in main vein (µm)
				Outer	Inner	Outer	Inner	Outer	Inner				
Control	30.60	25.80	2	50	47	45.00	26.90	17.80	19.90	98.20	4	11	33.60
	±0.89	±1.09				±0.24	±1.26	±0.60	±0.60	±0.16			±0.33
40 ppm Kinetin	31.00	26.00	2	58	56	46.00	27.30	19.00	21.80	101.10	4	11	36.50
	±0.67	±0.15				±0.45	±0.71	±0.07	±0.49	±0.65			±0.90
200 ppm GA_3	29.70	25.10	2	48	45	43.60	25.80	16.80	18.80	94.60	3	11	32.80
	±1.09	±0.55				±0.76	±1.00	±0.59	±0.59	±0.07			±0.19
100 ppm Pb	28.80	24.30	2	45	38	41.60	24.80	16.00	17.50	90.90	3	11	32.10
	±1.20	±0.69				±0.89	±0.30	±0.60	±0.11	±0.16			±0.24
200 ppm Pb	28.60	24.10	2	43	36	41.00	24.40	15.80	17.00	89.80	3	11	31.10
	±0.80	±0.29				±0.31	±0.26	±0.45	±0.17	±0.06			±0.96
40 ppm Kinetin	30.20	25.50	2	49	46	44.60	26.20	17.50	19.60	96.30	3	11	33.40
+100 ppm Pb	±0.67	±0.31				±0.20	±0.36	±0.50	±0.91	±0.81			±0.54
40 ppm Kinetin	29.90	25.30	2	48	44	44.00	26.00	17.20	19.00	95.50	3	11	33.00
+200 ppm Pb	±0.71	±0.52				±0.37	±0.61	±0.67	±0.17	±0.65			±0.28
200 ppm GA_3	29.60	24.80	2	47	42	43.20	25.40	16.70	18.40	92.60	3	11	32.60
+100 ppm Pb	±0.96	±0.17				±0.41	±0.001	±0.71	±0.23	±1.69			±0.45
200 ppm GA_3	29.40	24.60	2	46	39	42.80	24.90	16.50	18.00	91.80	3	11	32.30
+200 ppm Pb	±0.80	±0.37				±0.37	±0.01	±0.40	±0.45	±0.71			±0.54

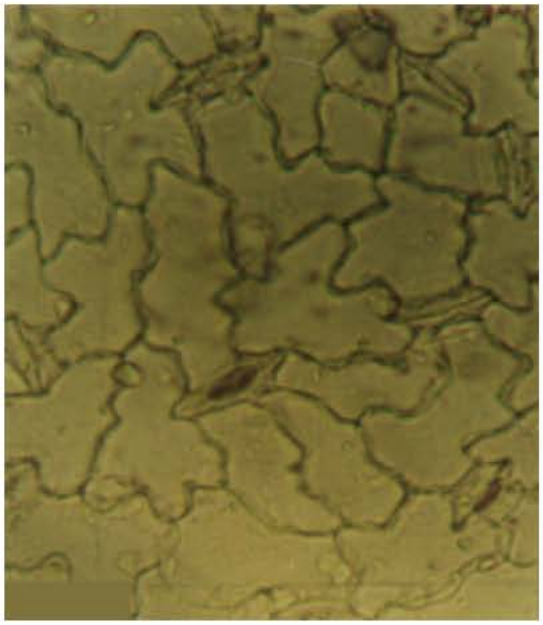

Fig. 3: 200 ppm $Pb(NO_3)_2$ treatment showing the maximum number of closed stomata

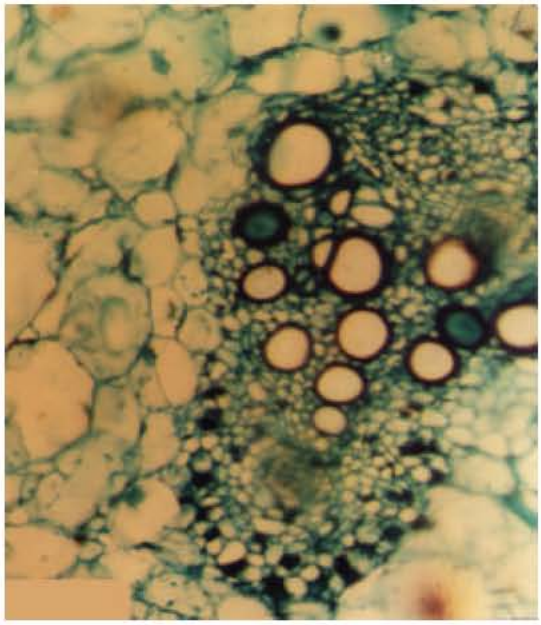

Fig. 4: Effect of 40 ppm kinetin on the cortical cells and xylem elements of internode

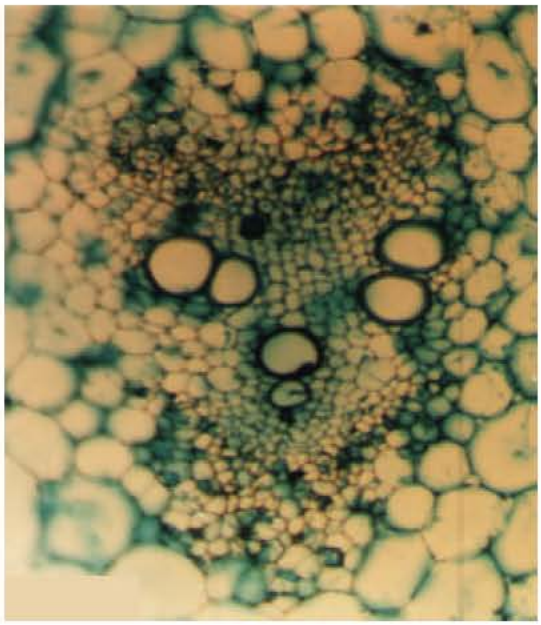

Fig. 5: T.S. of internode showing vascular cambium and xylem elements following treatments with 40 ppm kinetin

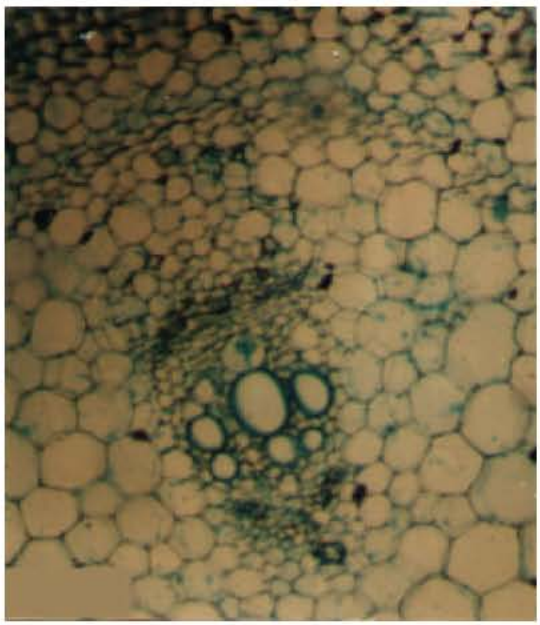

Fig. 6: Effect of 200 ppm GA_3 on cortical cells and vascular bundle of internode

response with the application of hormones and heavy metal. In the internode 1, width of collenchyma cells registered expansion with 40 ppm kinetin, 100 ppm and 200 ppm Pb $(NO_3)_2$, 40 ppm kinetin+100 ppm Pb $(NO_3)_2$ and 40 ppm kinetin+200 ppm $Pb(NO_3)_2$. Contrarily, the application of 200 ppm GA_3, 200 ppm GA_3+100 ppm Pb $(NO_3)_2$ and 200 ppm GA_3+200 ppm Pb $(NO_3)_2$ showed inhibition (Table 5). The number of cortical layers increased with the application of kinetin, whereas 200 ppm GA_3, 100 ppm Pb $(NO_3)_2$ and 40 ppm kinetin+100 ppm Pb $(NO_3)_2$ revealed no effect. On the other hand, inhibition

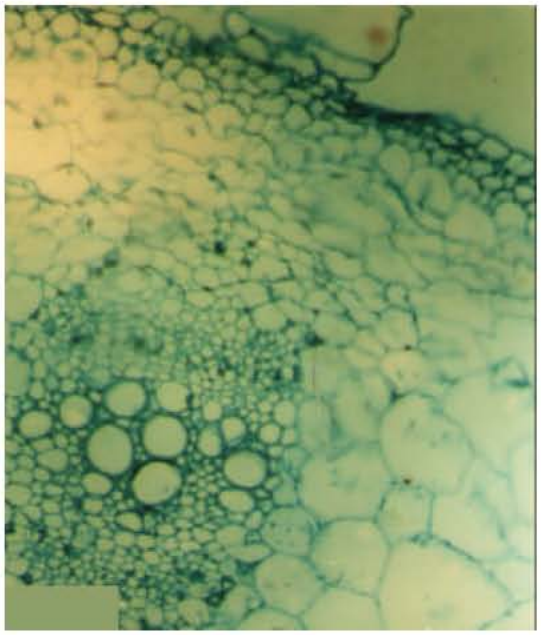

Fig. 7: Effect of 40 ppm kinetin+200 ppm Pb $(NO_3)_2$ on vascular bundle (bicollateral) of petiole.

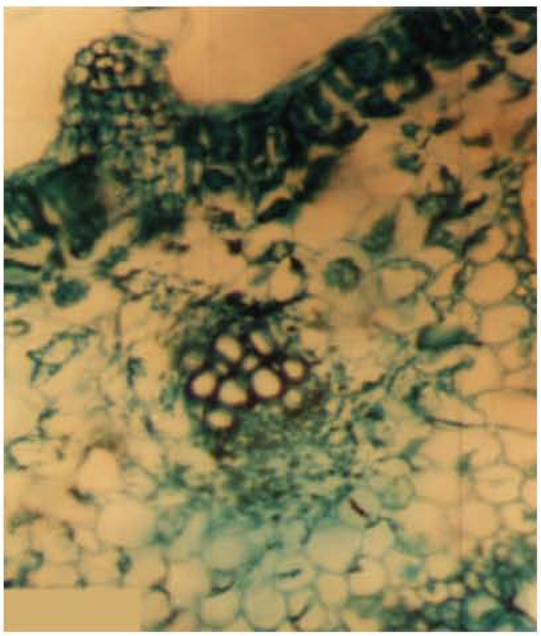

Fig. 8: Effect of 40 ppm kinetin+200 ppm Pb $(NO_3)_2$ on vascular bundle (bicollateral) of petiole

was observed with 200 ppm Pb $(NO_3)_2$, 40 ppm kinetin+200 ppm $Pb(NO_3)_2$, 200 ppm GA_3+100 ppm Pb $(NO_3)_2$ and 200 ppm GA_3+200 ppm Pb $(NO_3)_2$. Width of cortical region and consequently cortical parenchyma cells showed maximum expansion with extraneous kinetin and the mixed doses of $Pb(NO_3)_2$ with kinetin (Table 5 and Fig. 4). An insignificant expansion was recorded with Pb $(NO_3)_2$ treatments, while rest of the doses proved to be inhibitory. The vascular bundles observed in control were 7. They increased up to 8 with 40 ppm kinetin, 40 ppm kinetin+100 ppm Pb $(NO_3)_2$ and 40 ppm kinetin+200 ppm Pb $(NO_3)_2$, thus showing the enhancement of cell divisions (Table 5). The application of kinetin and its mixed doses with Pb $(NO_3)_2$ showed a positive effect on the vascular cambium thus increasing its layers (Fig. 5). The rest of the doses revealed no response. The number of metaxylem elements in one strand increased with kinetin whereas inhibition was registered with Pb $(NO_3)_2$ treatments (Table 5 and Fig. 4). In all the remaining doses the number remained constant. Even the mixed doses of kinetin proved to be ineffective. Width of metaxylem elements registered expansion with kinetin, Pb $(NO_3)_2$ treatments and the mixed doses of Pb $(NO_3)_2$ with kinetin. Contrarily, GA_3 and its mixed doses proved to be inhibitory (Table 5 and Fig. 6). The cellular region of pith was increased with kinetin, Pb $(NO_3)_2$ treatments and its mixed doses with kinetin, which was followed by corresponding decrease in fistular region (Table 5).

In the petiole, the collenchymatous hypodermal layers remained constant in all the treated plants thus showing no effect. Furthermore, the width of these cells revealed insignificant response (Table 6). The width of petiole showed inhibition with GA_3 and GA_3+Pb $(NO_3)_2$ treatments. Number of vascular bundles increased with kinetin and kinetin+Pb $(NO_3)_2$ treatments. The rest of the treatments showed reduction in number when compared with control (Table 6). Number of strands of xylem elements in a single bundle and number of metaxylem elements in single strand remained constant in all the treatments. The width of metaxylem elements showed expansion with kinetin, Pb $(NO_3)_2$ treatments and the mixed doses of kinetin with Pb $(NO_3)_2$. Contrarily, inhibition in width was observed with the application of GA_3 and its mixed doses with Pb $(NO_3)_2$ treatments (Table 6 and Fig. 7).

In the leaf, the width of adaxial and abaxial epidermal cells showed negligible increase with the application of kinetin (Table 7), whereas all the remaining treatments showed insignificant inhibition. The palisade layers remained constant in all the treatments i.e., 2 (Fig. 8). In both palisade layers, the number of palisade cells in 100 μm, length of palisade cells as well as the width showed expansion with applied kinetin. All the remaining treatments registered inhibition. The only effective dose was kinetin for instance spongy mesophyll showed increase with extraneous kinetin (Table 7). All the other treatments showed insignificant inhibition when

compared with control. The number of xylem strands showed decrease except with applied kinetin. No effect was observed in the number of xylem elements. Similarly, width of metaxylem elements responded only with kinetin, whereas the rest of the doses showed inhibition (Table 7).

DISCUSSION

External morphology: In the present study applied kinetin revealed decrease in the length of internode and petiole. However, it was accompanied by remarkable expansion in diameter. This shows that cytokinins block the increase in length and elongation zone and promote expansion[11]. Applied GA_3 showed marvelous increase in length and inhibition in diameter (Table 1 and Fig. 1). This may be due to decrease in the concentration of available sugars, which are utilized in extension growth[13]. Application of $Pb(NO_3)_2$ showed inhibition in length with some increase in diameter (Table 2). The inhibitory effects of heavy metals are reported by many workers[8,11,14,15]. The mixed doses of kinetin+$Pb(NO_3)_2$+further decreased the length and expanded the diameter (Fig. 2), both of these doses are well known for their effects on length[9]. The mixed doses of GA_3+Pb $(NO_3)_2$ showed increased length and narrow diameter in spite of the presence of Pb $(NO_3)_2$ (Table 2). This shows the dominating effect of GA_3 where length is concerned[16]. Fresh and dry weight of internode and petiole increased with kinetin and its mixed doses with Pb $(NO_3)_2$ (Table 1). This may be due to increase in the concentration of cytokinins[17]. Furthermore, in the leaf, fresh and dry weight increased only with the application of kinetin (Table 3). Weight gain increases with the application of kinetin[18]. The rest of doses showed reduction in weight. Leaf area and specific leaf area showed expansion with kinetin. This may be due to the wall loosening enzymes, which are activated by the application of hormones[19]. Applied GA_3 reduced leaf blade (Fig. 1)[6]. Application of Pb $(NO_3)_2$ and its mixed doses with kinetin and GA_3 registered inhibition in leaf area and SLA, $Pb(NO_3)_2$ causes inhibitory effects[9]. Number of leaves increased with kinetin, GA_3 and GA_3+100 ppm $Pb(NO_3)_2$ (Fig. 1). This increase may be attributed to the enhancement of apical meristematic activity and increase in the number of nodes[20]. No effect was registered with the rest of the doses (Table 3). Majority of stomata was anomocytic, however anisocytic type was also observed (Fig. 3). Plants may have more than one type of stomata but a particular type is always dominant[21]. The number of stomata/mm^2 registered increase with kinetin and decrease with GA_3 (Table 4). This may be due to increase/decrease in the leaf area. The remaining treatments showed inhibition, which may be due to inhibitory effect of Pb $(NO_3)_2$[22]. The length of guard cells increased with GA_3 and its mixed doses with Pb $(NO_3)_2$ (Table 4). This increase may be due to some enzymatic activities[23]. Width of guard cells and stomatal pore increased with kinetin, Pb $(NO_3)_2$ and its mixed doses with kinetin. This is in response to increased ambient humidity[24]. The remaining doses showed inhibition. Length of stomatal pore increased with GA_3. This may be due to the rapid increase in respiratory rate[25]. Application of kinetin and GA_3 increased the %age of open stomata. Stomatal opening is attributed to the end result of solute accumulation in guard cells[26,27]. The application of heavy metal leads to decreased number of open stomata[28], which have been registered in the present work (Table 4 and Fig. 3).

Internal morphology: The epidermal cells of both internode and petiole did not respond well with any treatment (Table 5). Wardlaw[29] has reported similar effects. Width of collenchymatous hypodermal cells showed expansion with kinetin (Table 5). Kinetin not only allows cell expansion but also enhanced cell division[30]. Likewise, $Pb(NO_3)_2$ treatments also registered expansion and the mixture of $Pb(NO_3)_2$ with kinetin showed increase in width (Table 6). Kinetin is well known to promote expansion growth. Although $Pb(NO_3)_2$ causes toxicity and inhibition[31], which was more or less not observed in the present work. Contrarily, GA_3 and its mixed doses with Pb $(NO_3)_2$ revealed inhibition. This may be due to GA_3[32] as revealed in external morphology that this hormone causes elongation, which is accompanied by inhibition in diameter (Table 1).

In the internode, number of cortical layers increased with kinetin. Kinetin stimulates cell division and development has been reported by Werner *et al.*[33]. Width of cortical region and cortical parenchyma cells registered expansion with kinetin (Fig. 5). The growth response to cytokinins is mainly due to enhanced cell division[34]. Similarly, expansion was recorded with Pb $(NO_3)_2$ treatments (Table 5). The present work is not in accordance with Halls and Williams[35] who observed some deleterious effects with extraneous Pb $(NO_3)_2$. The mixed doses of kinetin with Pb $(NO_3)_2$ also showed expansion in width. This shows the dominating effect of kinetin, which regulates cell division[36]. Applied kinetin and its mixed doses with Pb $(NO_3)_2$ registered increase in the number of vascular bundles representing promotion of cell division. Similar results were observed by Aloni[37]. GA_3 and its mixed doses revealed inhibition (Table 5 and Fig. 6). Chaudhry and Khan[16] have reported inhibition with applied GA_3. Number of cambial layers showed increase with kinetin and also with its mixed doses

(Table 5 and Fig. 5). This may be attributed to applied kinetin. Cytokinins have a crucial role in the formation and maintenance of procambial cells[38]. The remaining treatments showed no effect. Number of metaxylem elements in one strand mostly increased with kinetin thus revealing enhanced cell division[30]. The Pb $(NO_3)_2$ treatments revealed inhibition[15]. Kinetin as mentioned earlier is very effective in causing expansion growth[39] and in the present work also kinetin and its mixed doses exhibited expansion in the width of metaxylem elements (Fig. 4). Furthermore, Pb $(NO_3)_2$ treatments also showed expansion in width (Table 5). This is not in harmony with the reports given by Wu *et al.*[31] who observed inhibition with $Pb(NO_3)_2$. Contrarily, GA_3 and its mixed doses showed inhibition (Fig. 6), thus revealing dominating action of GA_3[40]. An expansion in the cellular region of pith was observed with kinetin and its mixed doses. This may be due to increase in the width of cells. Cell number and cell division activities are regulated by cytokinin levels[18]. The Pb $(NO_3)_2$ treatments also showed expansion, which is not in harmony with the reports given by Brown *et al.*[10]. According to these workers Pb $(NO_3)_2$ has inhibitory effect on the growth. GA_3 and its mixed doses revealed inhibition. Jones and Moll[41] working on lettuce hypocotyls have given similar reports. Width of fistular region of pith showed expansion with GA_3 and its mixed doses, which may be due to inhibition of cellular region (Table 5). Ozeki and Komamine[42] have reported that GA_3 has no role on transverse growth but in the present work significant inhibition has been registered.

The width of petiole showed expansion with kinetin and its mixed doses. Chaudhry and Khan[16] reported increase in the width with applied kinetin. Pb $(NO_3)_2$ causes expansion. Although Khan and Scullion[43] reported that heavy metals are growth inhibitors but in the present work no inhibition was recorded, so far as width is concerned. Contrarily, GA_3 and its mixed doses revealed inhibition, this may be due to applied GA_3, which is well known for decreasing transverse growth[44]. Number of vascular bundles increased with kinetin as well as its mixed doses. This may be due to enhanced cell division[45]. All the remaining doses showed inhibition. Number of strands of xylem elements in a single bundle and number of metaxylem elements in a single strand remained constant with the application of growth hormones and heavy metals i.e., 3 (Table 6). Width of metaxylem elements showed expansion with kinetin and its mixed doses with Pb $(NO_3)_2$ (Table 6 and Fig. 7). Similar reports have been given by Chaudhry and Rasheed[39]. Pb $(NO_3)_2$ treatments showed expansion. Hall and Williams[35] have reported that Pb $(NO_3)_2$ causes reduction in growth parameters, however in the present work no inhibition was noted.

In the leaf, width of adaxial and abaxial epidermal cells increased with kinetin, which may be attributed to the expansion of leaf area. Exogenous cytokinins stimulates cell expansion and cell division in cotyledons has been reported by Stoynova *et al.*[3]. The rest of doses showed inhibition (Table 7). Number of palisade layers remained constant in all the treated plants i.e., 2. In both palisade layers, number of palisade cells in 100 μm showed increase moreover length as well as width showed increase with kinetin. This again may be due to expanded leaf area. Leaf growth depends upon the concentration of cytokinins[46]. GA_3 revealed inhibition. This may be due to the narrowing of leaf[6]. Pb $(NO_3)_2$ registered reduction[47]. Width of spongy mesophyll registered increase with kinetin. Cytokinins enhanced cell division of mesophyll has been reported by Ronzhina[4]. GA_3 showed decrease in width, which may be due to decrease in leaf area (Fig. 8). Awan *et al.*[48] reported that GA_3 increased the width of leaf tissue, but the present work is not in harmony with the reports of the above mentioned workers. Pb $(NO_3)_2$ treatments and all the mixed doses showed inhibition. Application of Pb $(NO_3)_2$ causes inhibition in the growth of mesophyll has been reported by Chaudhry and Qurat-ul-Ain[11]. Number of strands of xylem elements in main vein remained constant with kinetin. However, inhibition was recorded with rest of doses (Table 7). Number of xylem elements in main vein remained constant in all the treated plants. Width of xylem elements showed expansion with kinetin. Exogenous cytokinins stimulate cell expansion[3]. Application of GA_3, Pb $(NO_3)_2$ and the mixed doses registered inhibition (Table 7 and Fig. 8). This shows the inhibitory effect of GA_3 and Pb $(NO_3)_2$ which has been reported by Hernandez[7], Hall and Williams[35].

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