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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (12): 1643-1648, 2005
ISSN 1028-8880


# Morphogenetic Effects of IAA and $HgCl_2$ on the Seedlings of *Pisum sativum* L.

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**Abstract:** Effects of heavy metals i.e., IAA and $HgCl_2$ were studied on the external and internal morphology of root, shoot and compound leaves of *Pisum sativum* Linn. The hormone and heavy metal were used individually i.e., 150 ppm IAA, 300 ppm IAA, 100 ppm $HgCl_2$ and 200 ppm $HgCl_2$ and in combination i.e., 150 ppm IAA+ 100 ppm $HgCl_2$, 150 ppm IAA + 200 ppm $HgCl_2$, 300 ppm IAA+ 100 ppm $HgCl_2$, 300 ppm IAA+ 200 ppm $HgCl_2$. In the external morphology with IAA applications inhibition in length with a corresponding increase in diameter was observed in root and shoot. Moreover, there was enhanced increase in the number of rootlets and tendrils. $HgCl_2$ showed inhibition. In combinations of IAA+ $HgCl_2$ inhibition was studied. Furthermore, $HgCl_2$ decreased the number of leaflets. In internal morphology expansion was registered in epidermis, cortex, vascular and pith region with IAA and inhibition with $HgCl_2$.

**Key words:** *P. sativum,* root, stem, compound leaves, harmone, IAA, heavy metal, $HgCl_2$

## INTRODUCTION

Plant hormones are biostimulants which promote growth[1]. They are involved in locking and unlocking of growth processes serving as raw materials building energy or enzyme components[2]. Growth hormones can stimulate the activity of key chemical processes[3]. Auxins have an important stimulatory role and regulate development by controlling growth e.g., activating root meristem and lateral root formation[4,5]. The differentiation of xylem and phloem is enhanced by IAA[6]. Furthermore, IAA exerts influence upon plant growth i.e., cell division, cell expansion and elongation[3]. However IAA inhibition has also been reported[3]. IAA causes expansion in the protoplast of mesophyll[7] and translocation carbohydrate for a longer period of time. Radial growth was enhanced in *Pisum sativum* L. in place elongation by extraneous IAA[8].

Heavy metals have direct physiological toxic effects on the plants stored or incorporated in living tissues[9]. They cause inhibition in the growth of leaves as well as in mesophyll cells. Mercury inhibited root growth[10]. In *Pisum sativum* mercury inhibited the volume of mesophyll[7]. It further effects the rate of water diffusion e.g., in maize roots and was an inhibition of water channels in cell membrane[11]. The present study will help to elucidate the deleterious effects of mercury and the reversal of these effects by IAA.

## MATERIALS AND METHODS

The aim of the present study was to observe the effects of growth hormones i.e., indole-3-acetic acid and heavy metal i.e., mercuric chloride on the external and internal morphology of seedlings of *Pisum sativum* L. (Fabaceae). Following combinations of growth hormones and heavy metals were used 150 ppm IAA, 300 ppm IAA, 100 ppm $HgCl_2$, 200 ppm $HgCl_2$, 150 ppm IAA + 100 ppm $HgCl_2$, 150 ppm IAA + 200 ppm $HgCl_2$, 300 ppm IAA+ 100 ppm $HgCl_2$ and 300 ppm IAA + 200 ppm $HgCl_2$. Plants were growth in earthenware pots in the month of November. Twenty seven microliter of each hormonal treatment was given on apical meristem. $HgCl_2$ was applied in soil along with water. Plants were removed after 15 days. For the external morphology length and diameter of root and stem were observed, moreover number of compound leaves, length and diameter of rachis was undertaken. In the internal morphology the epidermis, cortical and vascular region was studied. In the stem the width and length of cells of the above mentioned regions were dealt with. Furthermore, the rachis and mesophyll of the leaflets were observed. For this 1 cm long pieces of root, stem and compound leaf were taken and fixed in Carney's modified fluid, dehydrated, infiltrated and embedded in paraffin wax, the material was processed in transverse and longitudinal planes with rotary microtome, stained with safranine and fast green and mounted in Canada balsam. The comparative data was obtained and subjected to statistical analysis[11].

## RESULTS

### External morphology

**Root:** In the external morphology the length of root showed inhibition with IAA treatments (Fig. 1). However $HgCl_2$ treatments also showed similar results (Fig. 2). In


Fig. 1: Inhibition with IAA treatments


Fig. 2: Effects of mixed doses

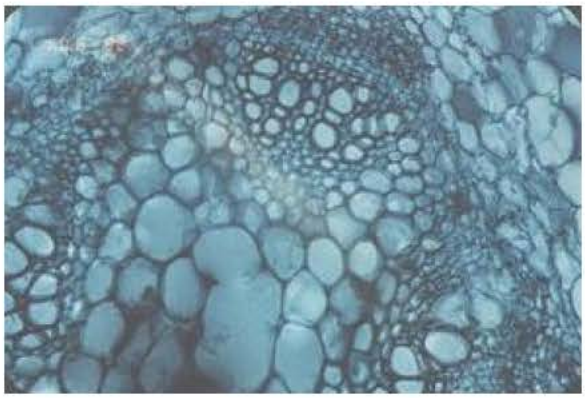

Fig. 3: TS stem showing xylem elements in 150 ppm IAA

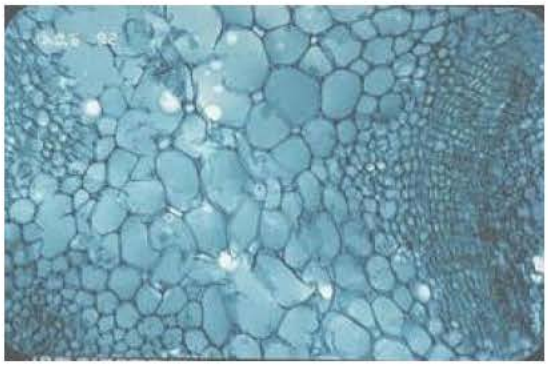

Fig. 4: TS stem showing enhancement of vascular cambium in 300 ppm IAA

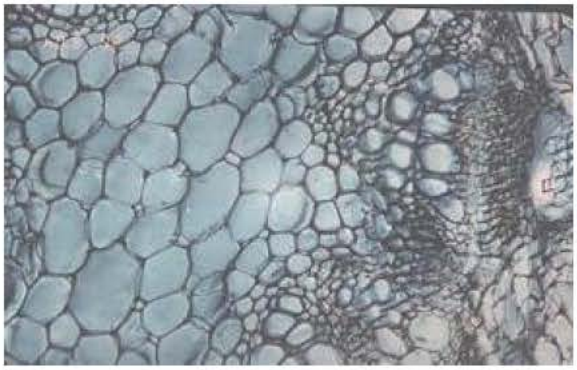

Fig. 5: TS stem showing pith region 150 ppm IAA + 100 ppm $HgCl_2$

Fig. 6: TS of leaf showing single layer of palisade mesophyll 150 ppm IAA treatments

the mixed doses also inhibition was registered. The diameter of root and showed expansion with IAA. However, a high dose of 200 ppm $HgCl_2$ showed decrease. In the combinations of mixed doses of 200 ppm $HgCl_2$ showed inhibition in diameter (Table 1). The diameter of root showed expansion with IAA, the mixed doses revealed some inhibition. Moreover, the effects of rootlets showed significant increase with 300 ppm IAA when compared with control (Table 1).

Table 1: Effects of IAA and $HgCl_2$ on the external morphology of root in *Pisum sativum* L.

| Treatments | Length of root (cm) | Diameter of root (cm) | No. of rootlets |
|---|---|---|---|
| Control | 6.50±0.081 | 0.65±0.062 | 18.0±0.909 |
| 150 ppm IAA | 5.35±0.061 | 0.75±0.170 | 20.0±0.548 |
| 300 ppm IAA | 4.56±0.084 | 0.94±0.063 | 22.0±0.953 |
| 100 ppm $HgCl_2$ | 5.00±0.650 | 0.95±0.094 | 12.0±0.860 |
| 200 ppm $HgCl_2$ | 3.25±0.060 | 0.45±0.028 | 8.0±1.149 |
| 150 ppm IAA + 100 ppm $HgCl_2$ | 5.20±0.107 | 0.65±0.107 | 10.0±1.433 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 4.89±0.855 | 0.70±0.171 | 10.0±1.286 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 4.20±0.065 | 0.85±0.076 | 16.0±1.441 |
| 300 ppm IAA+200 ppm $HgCl_2$ | 3.00±0.644 | 0.55±0.088 | 18.0±0.909 |

Table 2: Effects of IAA and $HgCl_2$ on the external morphology of stem in *Pisum sativum* L.

| Treatments | Length of stem (cm) | Diameter of stem (cm) | No. of internodes | Diameter of internodes (cm) | No. of tendrils | Length of tendril (cm) |
|---|---|---|---|---|---|---|
| Control | 6.50±0.081 | 0.75 ±0.175 | 4.00±0.642 | 0.65± 0.065 | 4.00±0.692 | 2.50±0.064 |
| 150 ppm IAA | 0.75±0.083 | 0.66 ±0.061 | 5.00±0.064 | 0.60± 0.601 | 1.00±0.212 | 2.35±0.064 |
| 300 ppm IAA | 5.00±0.645 | 0.85±0.063 | 3.00±0.574 | 0.84± 0.064 | 1.00±0.215 | 2.25±0.0405 |
| 100 ppm $HgCl_2$ | 0.30±0.060 | 0.80±0.063 | 5.00±0.061 | 0.75± 0.071 | 4.00±0.642 | 2.00±0.064 |
| 200 ppm $HgCl_2$ | 5.20±0.076 | 0.90±0.143 | 5.00±0.831 | 0.80± 0.062 | 2.00±0.461 | 1.60±0.128 |
| 150 ppm IAA + 100 ppm $HgCl_2$ | 5.25±0.509 | 0.70±0.174 | 3.00±0.575 | 0.70± 0.174 | 1.00±0.209 | 1.75±0.089 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 4.50±0.081 | 0.92±0.092 | 2.00±0.405 | 0.89± 0.095 | 1.00±0.215 | 2.00±0.410 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 6.50±0.612 | 0.99±0.099 | 3.00± 0.580 | 0.97±0.98 | 1.00±0.215 | 1.85±0.090 |
| 300 ppmIAA+ 200 ppm $HgCl_2$ | 4.50±0.133 | 0.90±0.093 | 3.00± 0.575 | 0.95±0.135 | 2.00±0.410 | 2.15±0.415 |

Table 3: Effects of IAA and $HgCl_2$ on the external morphology of leaf in *Pisum sativum* L.

| Treatments | No. of leaves | Length of rachis (cm) | Diameter of rachis (cm) |
|---|---|---|---|
| Control | 16.00±1.285 | 1.00±0.211 | 0.40±0.028 |
| 150 ppm IAA | 14.00± 1.185 | 1.00±0.212 | 0.55±0.009 |
| 300 ppm IAA | 10.00±1.430 | 1.00±0.214 | 0.53±0.081 |
| 100 ppm $HgCl_2$ | 12.00±0.861 | 0.90±0.009 | 0.50±0.080 |
| 200 ppm $HgCl_2$ | 6.00±1.430 | 1.00±0.211 | 0.45±0.006 |
| 150 ppm IAA +100 ppm Hg $Cl_2$ | 4.00±0.643 | 0.95±0.009 | 0.65±0.071 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 6.00±0.814 | 1.0± 0.211 | 1.00±0.210 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 6.00±0.0953 | 0.99±0.088 | 0.60±0.072 |
| 300 ppmIAA+ 200 ppm $HgCl_2$ | 6.00±0.285 | 1.00±0.209 | 0.30±0.580 |

Table 4: Effects of IAA and $HgCl_2$ on internal morphology of root in *Pisum sativum* L.

| Treatments | Width of epidermal cells (μm) | Width of endodermal cells (μm) | Width of cortical region (μm) | Width of metaxylem elements (μm) | Width of pith (μm) |
|---|---|---|---|---|---|
| Control | 30.00±1.001 | 10.00±0.501 | 105.00±5.020 | 51.50±1.002 | 71.5±1.861 |
| 150 ppm IAA | 31.50±1.012 | 10.50±0.250 | 115.00±0.621 | 55.60±0.620 | 75.25±5.014 |
| 300 ppm IAA | 32.00±1.021 | 10.65±0.234 | 185.00±1.707 | 65.00±0.752 | 80.00±0.496 |
| 100 ppm $HgCl_2$ | 31..25±0.354 | 10.25±0.088 | 100.00±5.02 | 51.25±0.179 | 75.00±0.439 |
| 200 ppm $HgCl_2$ | 31.20±0.379 | 10.01±0.195 | 92.50±0.438 | 51.00±0.307 | 79.45±0.589 |
| 150 ppm IAA + 100 ppm $HgCl_2$ | 30.50±0.379 | 9.99±0.297 | 155.50±0.389 | 57.50±3.450 | 70.45±0.211 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 30.25±0.322 | 10.05±0.094 | 153.45±0.144 | 50.25±0.872 | 68.55±0.042 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 29.75±0.424 | 9.95±0.004 | 153.35±0.563 | 50.35±0.178 | 70.55±0.10 |
| 300 ppmIAA+ 200 ppm $HgCl_2$ | 28.00±0.323 | 10.35±0.395 | 100.55±0.149 | 49.50±0.009 | 68.00±0.008 |

**Stem:** The length of stem showed inhibition with applied IAA. However, a corresponding increase in diameter was observed. Similarly the $HgCl_2$ treatments registered inhibition in extension growth and some increase in expansion growth (Table 2).

**Leaf:** The number of compound leaves in control was 16 after 15 days. With IAA treatments there was no effect on the number of leaves, however, $HgCl_2$ treatments showed decrease in number (Table 3). The mixed doses showed no significant effect.

## Internal morphology

**Root:** In the internal morphology of the root, the epidermal cells showed more or less no effect, epidermal cells were least effective, however, IAA treatments showed

Table 5: Effects of IAA and $HgCl_2$ on internal morphology of stem in *Pisum sativum* L.

| Treatments | Width of epidermal cells (μm) | Width of cortical region (μm) | Number of vascular bundles (μm) | Number of layers of cambium (μm) | Width of metaxylem elements (μm) | Width of pith (μm) |
|---|---|---|---|---|---|---|
| Control | 32.50 ±0.484 | 515.00±5.328 | 7 | 6 | 57.50±1.841 | 410.00±5.460 |
| 150 ppm IAA | 34.75± 0.281 | 525.00±0.420 | 8 | 6 | 60.00±2.781 | 395.00±0.241 |
| 300 ppm IAA | 170.00±0.485 | 528.00±0.434 | 8 | 6 | 61.35±2.234 | 393.00±0.241 |
| 100 ppm $HgCl_2$ | 160.00±0.349 | 520.001.297 | 6 | 6 | 58.55±2.780 | 397.00±1.364 |
| 200 ppm $HgCl_2$ | 150.00±0.486 | 518.00±0.584 | 6 | 6 | 58.50±2.303 | 398.00±1.367 |
| 150 ppm IAA + 100 ppm $HgCl_2$ | 140.00±0.677 | 522.50±0.270 | 7 | 6 | 58.85±0.962 | 400.00±0.367 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 139.00±0.302 | 521.00±0.497 | 7 | 6 | 58.75±0.394 | 405.00±0.246 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 35.00 ±1.290 | 526.75±0.487 | 7 | 6 | 60.25±0.187 | 390.00±0.348 |
| 300 ppm IAA+ 200 ppm $HgCl_2$ | 34.70 ±0.876 | 525.00±0.678 | 7 | 6 | 60.10±0.389 | 394.00±5.476 |

Table 6: Effects of IAA and $HgCl_2$ on internal morphology of Leaf in *Pisum sativum* L.

| Treatments | Width of adaxial epidermal cells (μm) | Width of abaxial epidermal cells (μm) | No. of layers of palisade cells | Width of palisade cells (μm) | No. of layers of spongy mesophyll | Width of spongy mesophyll (μm) | Width of Spongy cells (μm) | No. of strands of xylem elements in mid-vein (μm) |
|---|---|---|---|---|---|---|---|---|
| Control | 30.81±0.423 | 27.73±1.678 | 1 | 20.35±0.379 | 5 | 250.90±3.987 | 40.50±1.333 | 4 |
| 150 ppm IAA | 31.00±0.876 | 27.80±4.393 | 1 | 23.50±0.876 | 5 | 254.85±0.049 | 43.50±0.076 | 4 |
| 300 ppm IAA | 31.85±0.704 | 28.40±0.048 | 1 | 25.85±0.876 | 5 | 256.50±0.049 | 45.75±0.831 | 4 |
| 100 ppm $HgCl_2$ | 29.95± 0387 | 27.45±0.585 | 1 | 25.85±0.039 | 5 | 249.95±1.464 | 39.84±2.731 | 4 |
| 200 ppm $HgCl_2$ | 29.90±0.387 | 27.15±0.318 | 1 | 19.50±0.348 | 5 | 248.00±1.678 | 39.50±3.401 | 4 |
| 150 ppm IAA + 100 ppm $HgCl_2$ | 30.50±0.334 | 27.25±0.434 | 1 | 20.25±0.046 | 5 | 250.50±0.223 | 40.00±0.336 | 4 |
| 150 ppm IAA+ 200 ppm $HgCl_2$ | 30.25±0.844 | 27.00±0.379 | 1 | 20.20±0.046 | 5 | 250.15±0.313 | 39.90±0.148 | 4 |
| 300 ppm IAA+ 150 ppm $HgCl_2$ | 31.80±0.413 | 28.00±0.121 | 1 | 23.25±0.666 | 5 | 253.50±0.875 | 42.85±0.321 | 4 |
| 300 ppmIAA+ 200 ppm $HgCl_2$ | 31.89±0.299 | 28.50±0.346 | 1 | 23.00±0.421 | 5 | 253.25±0.062 | 42.75±0.921 | 4 |

negligible increase (Table 4). The cortical region was more responsive to the treatments i.e., IAA doses showed marked increase in width, whereas $HgCl_2$ treatments showed some inhibition. In the mixed doses, increase was observed when compared with control, thus showing the dominant effect of IAA. The endodermis cells revealed insignificant increase (Table 4). The metaxylem elements of root showed marked increase with IAA e.g., 300 ppm IAA registered 26.21% increase when compared with control. The $HgCl_2$ treatments showed inhibition. In the mixed doses the antagonistic effect of IAA and $HgCl_2$ was observed (Table 4). Likewise, the pith cells also responded well to IAA doses. The $HgCl_2$ treatments revealed some expansion.

**Stem:** The epidermal cells were less responsive both to IAA and $HgCl_2$. The cortical region showed expansion to applied IAA whereas $HgCl_2$ more or less showed no effect. Likewise the mixed doses showed some expansion (Table 5). In applied IAA vascular bundles showed increase when compared with control (Fig. 3 and 4). One significant observation was decrease in number when $HgCl_2$ was applied. The cambial layers showed increase with IAA (Fig. 4). However applied $HgCl_2$ showed no response when compared with control (Table 5). The metaxylem elements showed expansion with the application of IAA as compared to control. Similarly $HgCl_2$ showed no inhibition (Table 5). The pith cells showed no significant response (Fig. 5).

**Leaf:** The adaxial and abaxial epidermis showed expansion with IAA, contrary to this some inhibition was observed with extraneous $HgCl_2$. In the mixed doses i.e., 150 ppm IAA + 200 ppm $HgCl_2$ inhibition was recorded whereas the high dose of mixed IAA showed expansion (Fig. 6). The number of layers remained totally unchanged in both palisade and spongy mesophyll (Table 6). The cells showed significant expansion with IAA and inhibition with $HgCl_2$. Moreover the number of xylem strands in midvein remained totally undisturbed when compared with control (Table 6).

## DISCUSSION

**Root:** Plant hormones influence the growth and development of plants, however extension growth was hindered[12] with IAA and expansion was registered, moreover increase was also observed in the number of

lateral roots (Table 1). Similar results have been obtained by Carlos *et al.*[13] and Wang *et al.*[14]. The $HgCl_2$ treatments revealed inhibition in length and reduction in the number of lateral roots, however diameter showed increase. $HgCl_2$ treatments had inhibitory effects on roots has been further confirmed by Mortens and Boyd[15].

In the internal morphology the epidermis of root was unresponsive. The cortical region showed expansion with IAA and no significant effect of $HgCl_2$. The radial expansion of cells has been observed with IAA treatments by Alsono *et al.*[16]. Similarly the xylary region responded very well with extraneous IAA. Secondary xylem development is stimulated with IAA application[17,18]. $HgCl_2$ showed no significant effect.

**Stem:** The epidermal cells more or less revealed no effect (Table 5). The cortical region registered expansion with IAA. Contrary to this $HgCl_2$ treatments showed inhibition. The inhibitory effects of heavy metals were registered as reported by Tuominen *et al.*[19]. The xylary region showed well marked results i.e., when IAA was applied the number of vascular bundles showed increase thus revealing the effect of IAA on xylem differentiation[20]. $HgCl_2$ registered inhibition in number (Table 5). Many workers have observed similar results[10]. The pith cells also responded well with IAA (Table 5).

**Leaf:** As aforementioned the adaxial and abaxial epidermis showed no significant result. The palisade mesophyll has a single layer (Fig. 6) which remained constant with all treatments. Similarly the layers of spongy mesophyll remained the same when compared with control (Table 6). However the ratio of expansion/inhibition was the same as observed in the root and stem.

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