EFFECTS ON MORPHOLOGY AND CYTOLOGY OF GREEN VEGETABLES GROWN IN CONTROLLED VERSUS POLLUTED WATER

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Abstract

Seed germination was carried out through Roll-paper- towel method (Anonymous 1990). 100 certified seeds each of brinjal *(S. melongena* var *esculentum)* and radish *(R. sativus* var. daikon, *Japani white)* per replicate were taken for the experiment. Seeds were soaked overnight in tap water and considered as control ones. Seeds were soaked overnight in industrial effluent polluted water of Nalla. 100 seeds per replicate and three replicates for each species were used. After soaking, they were placed on the blotter sheets for germination and data for seed germination % and seedling morphological characteristics were gathered after 6th day

Seed germination % and seedling morphological characteristics include comparative study of parameters like number of days of initiation of germination, type of germination, day of maximum germination, germination percentage, length of root, length of shoot, root-shoot ratio, length of cotyledon, number of cotyledon, colour and nature of cotyledon, total length of seedling, seed vigour index and seedling abnormalities. These parameters were studied in both radish and brinjal after the treatments of tap water and Nalla water with industrial effluents.

The data of comparative study of seedling morphological characters are summarized in Table-1 and Table-2 and Fig.1 to 6. Similarly the effects and changes were also analyzed in mature plants grown in different water conditions in different agricultural sites of sanganer region.

Keywords: Seeds, Industrial effluent, tap water, polluted water, comparative study

Introduction

Green Vegetables constitute essential components of the diet by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply (Thompson and Kelly, 1990). However, these plants contain both essential and toxic elements over a wide range of concentrations.

Several studies have indicated that vegetables, particularly leafy crops, grown in heavy metals contaminated soils have higher concentrations of heavy metals than those grown in uncontaminated soil (Guttormsen *et al*., 1995). A major pathway of soil contamination is through atmospheric deposition of heavy metals from point sources such as metaliferous mining, smelting, agricultural and industrial activities (Singh *et al.*, 1995). In addition, foliar uptake of atmospheric heavy metals emissions has also been identified as an important pathway of metal contamination in vegetable crops (Salim *et al.*, 1992).

Some members of the Brassicaceae family have been shown to accumulate from moderate to high levels of Pb, Cr, Cd, Ni, Zn and Cu (Ebbs *et al.*, 1997). Carbonell-Barrachina *et al.*, 1999 reported that radish plants grown on higher soil concentrations of As accumulated high As concentration in roots and shoots. Cytological abnormalities were investigated by Zeerak, N. A., 1991in brinjal .

Wang *et al*., (2009) carried out a study on detrimental concentration of Zinc (Zn) in rapeseed seedlings. To investigate Zn toxicity, rapeseed (Brassica napus) seedlings were treated with 0.07-1.12 mM Zn for 7d. Inhibition of plant growth along with root damage, chlorosis and decreased chlorophyll (a and b) content in newly expanded leaves (the second and third leaves formed following cotyledons) were found under Zn stress.

Ch. Chandra Shekhar *et al*., (2011) investigated the seeds of (Lycopersicon esculentum Mill.) variety Pusa Ruby to evaluate as to what effect this heavy metal mercury would create on the vegetable crop plant at different stages of its growth and development. Biswas *et al*., (2012) examined total arsenic concentrations in 32 types of vegetables and 7 types of pulses. Range of total arsenic concentration in edible parts of vegetables collected from grown fields was 0.114-0.910 mg/kg. Highest arsenic values were

in spinach 0.910 mg/kg. Vegetable samples were grouped into leafy, non-leafy-fruity, root-tubers.

Effects of Mythos SC 300 (300 g/l pyrimethanil), a fungicide on pollen meiosis of tomato plants were assessed by Teoman and Lkay (2007). They found that the fungicide caused various anomalies in pollen meiosis, decrease in pollen yield and consequently influenced future seed formation.

Yekeen et al., (2011) investigated nutritional qualities of vegetables, Vernonia amygdalina; Amaranthus caudatum and Telfairia occidentalis, along with the cytotoxic effects of their aqueous extracts to ascertain the potential risk that may be associated with the consumption of vegetables from unknown sources. Ramesh and Murthy (2012) analyzed randomly collected waste water, soil and green leafy vegetable samples from the five stations of Bangalore Urban district. Their study explains the extent of heavy metal contamination in two leafy vegetables viz., palak (Beta vulgaris) and coriander (Coriandrum sativum). Cytological studies indicated the formation of Anaphase Bridge, irregular metaphase, chromosome stickiness, precocious chromosome at anaphase and vagrant chromosomes.

Materials and methods

The control sites were Department of Biosciences, Mody University (Site-I) and Shikarpura, Sanganer (Site - II) and the polluted sites were Govindpura (Jotadawala), Sanganer (Site - III) and near Shikarpura Flyover, Sanganer (Site-IV) of Amanishah Nalla (Dravyawati river).

Effect on morphology and Cytology of plants

Seed germination and seedling morphology

The data on seed germination percentage seedling morphology were taken through Roll paper towel method (Anonymous, 1990).

External morphology

Various parameters were studied and data were taken from seedling as well as mature plants of selected crops rose in control and polluted waters respectively.

Cytological Studies

For mitotic studies, the root tips were collected from the seeds of radish and brinjal raised in normal and polluted waters. They were fixed in Carnoy's fluid I (1:3 glacial acetic acid: absolute alcohol) for 24 hrs at room temperature and later on stored in 70% alcohol. They were boiled in testtube containing 1% acetocarmine and 1NHCl (9:1) for 15 to 20 minutes. These tips were stained in 1% acetocarmine. Pressing, heating and staining were repeated for better cytological preparations. Data on mitosis and microphotographs were taken from temporary prepared slides.

$$\text{Mitotic Index (\%)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells}} \times 100$$

For meiotic studies, the floral buds were collected between 6 and 7 AM from radish and brinjal plants irrigated by normal and polluted waters. They were fixed in Carnoy's fluid I (1:3 glacial acetic acid: absolute alcohol) for 24 hrs at room temperature and later on stored in 70% alcohol. Squashes were prepared in 1% acetocarmine. The data on meiosis and microphotographs were taken from temporary prepared slides.

$$\text{Mitotic Index (\%)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells}} \times 100$$

Other morphological parameters were compared like height, shape, color, length and width of leaves as well as fruits of seedlings and mature plants both. Cytological characteristics were compared on the basis of shape and types of cells microscopically.

Results and Discussions

Seedling Morphological characteristics in Brinjal

Germination of seeds began on 2nd day after seed wetting in all the types of seed treatments. Germination of remaining seeds continued up to 8th day after the initiation of germination.

Germination percentage was found to be 92% at control site (Site I) which becomes reduced at Nalla water treated site (Site III -75 % and Site IV-63%). Seeds showed epigeal type of germination.

Seed Vigour Index (SVI) was found to be 603.60 in control site (Site I), 520.75 in site III and 539.02in site

IV (Table - 1).

The average **length of root** in control seedlings at Site I was found 5.19 cm. In Nalla water - treated seedlings, the length was found 5.45cmat site III whereas it was reduced (4.76 cm) at site IV (Fig. l).

The average **length of shoot** of seedlings was found 3.87 cm at site III, 4.12 cm (maximum) in control at Site I and 2.87cmat site IV (Fig. 1).

In control seedlings at Site I, **root-shoot ratio** was found 1:1.26; while at site III, it was1:1.41 and at Site IV was1:1.69.

The average **length of cotyledons** in control seedlings at Site I was found 0.83 cm, 0.61 cm at site III and 0.72cmat site IV (Fig. 1).

4.2% of seedlings at Site IV were found of tricotyledonous type (Table 1). Generally seedlings were dicotyledonous and smooth in nature. This may be due to presence of higher concentration of organic pollutants and industrial effluents of Nalla water.

The **percentage of seedlings** with green cotyledons was found 74%, 78.3 % and 94 % at site IV, site III and site I respectively (Table 1). Seedlings with pale yellow cotyledons were found21.7%, 16% and 6 % at site III, site IV and site I respectively (Table 1).

The **total length of seedlings** was found 6.55cm, 6.93cm and 8.54 cm at site I, site III and at site IV respectively. (Fig. l).

Seedlings were broadly categorized as **normal** and **modified** types. The latter type was further categorized as stunted seedlings, seedling with lateral roots and twisted seedlings (Fig. 5).

The maximum percentage (49.64%) of **twisted seedlings** was observed at site IV, followed by site III (45.23%) and control site I (20.29%). Seedlings with **lateral roots** were found 15.64%, 3.45% and 1.4 % at site IV, site III and at control site I respectively. 3.61 %, 12.95 % and 20.40 % seedlings were **stunted** at Site I, Site III and Site IV respectively (Fig. 2) (Table. 1).

Seedling Morphological Characteristics in Radish

The germination began on 4th day after seed wetting in all the three types of seedling treatments. Germination in remaining seeds continued up to 6th day after radicle emergence. Maximum germination occurred on the 3rd day after radicle emergence.

Germination percentage was found to be 93% at control site (Site I) which become reduced at Nalla water treated site (Site IV -85 % and Site III-82 %). Germination was epigeal.

Seed Vigour Index (SVI) was found714.52 at control site (Site I), 591.58 at site III and 510.34 at site IV.

The average **length of root** in seedlings at Site IV was found5.09 cm. In Nalla water-treated seedlings, the length was found6.03 cm at site III whereas it was found6.34 cm in control at site I (Fig. 3).

The average **length of shoot** of seedlings was found 3.15 cm at site III and 4.17 cm (maximum) in control at Site I and 3.88cm at site IV (Fig. 3).

In control seedlings at Site I, **root-shoot ratio** was found 1:1.534; while at site III, it was1:1.925 and at Site IV was1:1.42 (Table-2).

The average **length of cotyledons** in control seedlings at Site I was found 0.63 cm, 0.72 cm at site III and 0.71cm at site IV (Fig. 3).

2.4% of seedlings at Site IV were tricotyledonous type (Table 2). Generally seedlings were dicotyledonous and smooth in nature. This may be due to presence of higher concentration of organic pollutants and industrial effluents in Site IV of Nalla water.

The **percentage of seedlings** with green cotyledons was found 58%, 79 % and 96 % at site IV, site III and site I respectively (Table 2). Seedlings with pale yellow cotyledons were found 21%, 42% and 4 % at site III, site IV and site I respectively (Table 2).

The **total length of seedlings** was found 7.64cm, 7.19cm and 5.98 cm at site I, site III and at site IV respectively (Fig. 3).

Seedlings were broadly categorized as **normal** and **modified** types. Modified types of seedlings were further categorized as stunted seedlings, seedling with lateral roots and twisted seedlings (Fig. 6).

The maximum percentage (15.44%) of **twisted seedlings** was observed at site IV, followed by site

III (7.34%) and control site I (2.3%).Seedlings with **lateral roots** were found 23.00 %, 9.67 % and 2.73 % at site IV, site III and at control site I respectively. 9.12 %, 18.61 % and 27.10 % seedlings were **stunted** at Site I, Site III and Site IV respectively (Fig. 4) (Table 2).

External morphological characteristics of brinjal and radish seeds were shown and discussed in Fig. 7 and Table 5.

Table 1: Seedling Morphology of Brinjal after treatment with tapwater and Nalla water

Characters	**Site-I (Control)**	**Site-III**	**Site-IV**
1) Seed germination %	92%	75%	63%
2) Seed Vigour Index (SVI)	603.6	520.75	539.02
3) Length of root (cm)	5.19	5.45	4.76
4) Length of shoot (cm)	4.12	3.87	2.87
5) Ratio of R/S	1:1.26	1:1.41	1:1.69
6) Length of cotyledon (cm)	0.83	0.61	0.72
7) Number of cotyledon (%)	2	2	3(4.2%)
8) Colour of cotyledon	Green (94%), Pale yellow (6%)	Green (78.3%), Pale yellow (21.7%)	Green (74%), Pale yellow (16%)
9) Total length of seedling (cm)	6.55	6.93	8.54
10) Modified seedlings(Abnormality)			
(A) Twisted seedling	20.29%	45.23%	49.64%
(B) Seedling with lateral roots	1.4%	3.45%	15.64%
(C) Stunted seedling	3.61%	12.95%	20.4%

Table 2: Seedling Morphology of Radish after treatment with tapwater and Nalla water

Characters	**Control**	**Site-III**	**Site-IV**

	(Site-I)		
1) Seed germination %	93%	82%	85%
2) Seed Vigour Index (SVI)	714.52	591.58	510.34
3) Length of root (cm)	6.34	6.03	5.09
4) Length of shoot (cm)	4.17	3.15	3.88
5) Ratio of R/S	1:1.534	1:1.925	1:1.42
6) Length of cotyledon (cm)	0.63	0.72	0.71
7) Number of cotyledon (%)	2	2	3(2.4%)
8) Colour of cotyledon	Green (96%), Pale yellow (4%)	Green (79%), Pale yellow (21%)	Green (58%), Pale yellow (42%)
9) Total length of seedling (cm)	7.64	7.19	5.98
10) Modified seedlings (Abnormality)			
(A) Twisted seedling	2.3%	7.34%	15.44%
(B) Seedling with lateral roots	2.73%	9.67%	23%
(C) Stunted seedling	9.12%	18.61%	27.1%

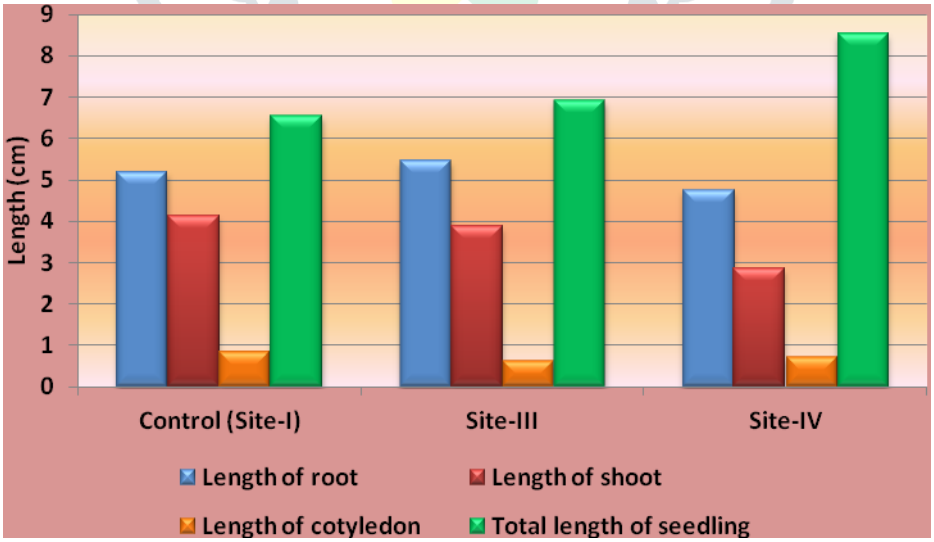

Fig. 1: Comparative seedling morphology in Brinjal seedlings

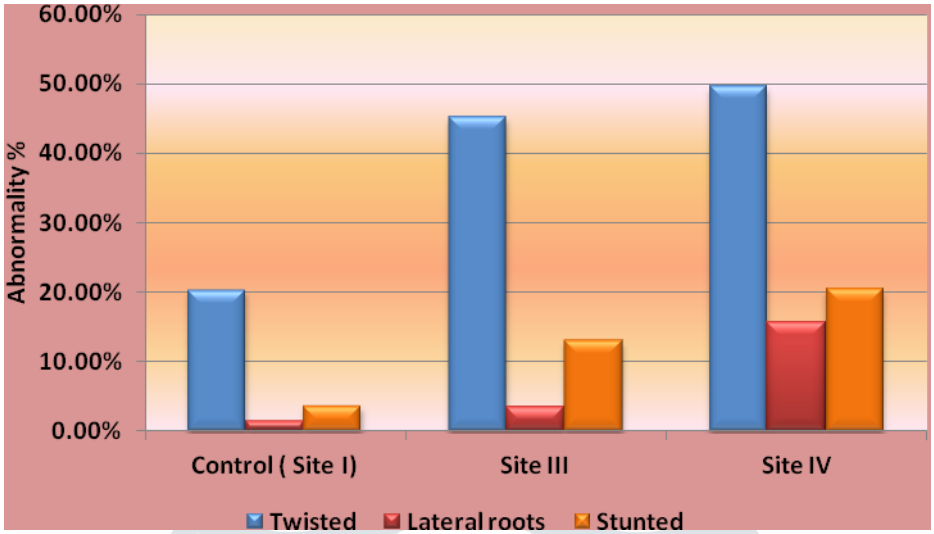

Fig. 2: Different type of seedling abnormalities (%) in Brinjal

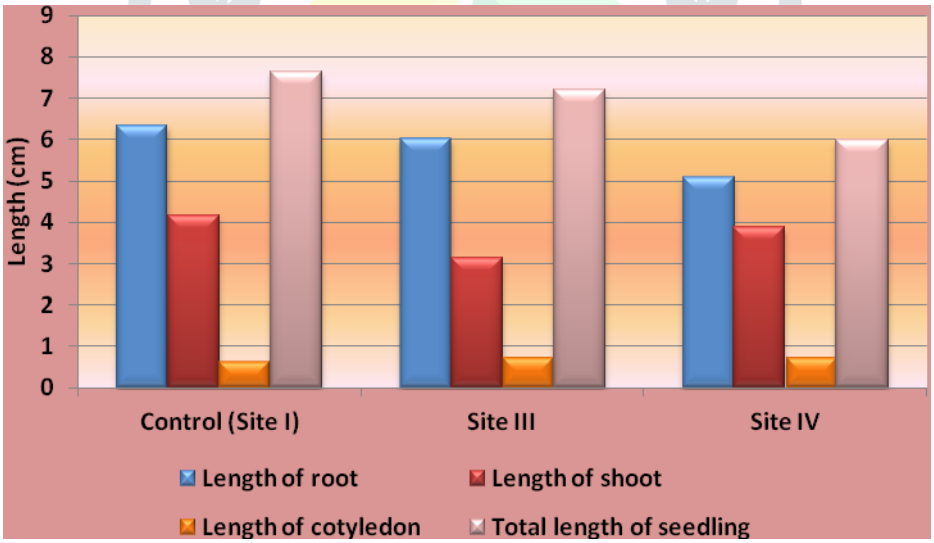

Fig. 3: Comparative seedling morphology in Radish seedlings

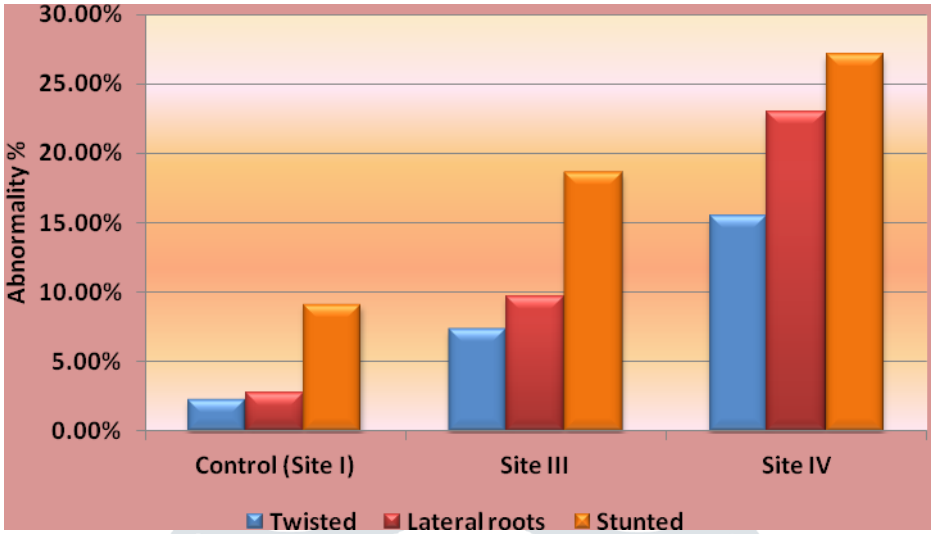

Fig. 4: Different type of seedling abnormalities (%) in Radish

External Morphological Studies of Mature Plants

Brinjal and Radish crops were studied at control and polluted sites. The data was taken evaluating average values as given in the Table 3 -.5.

Comparative morphology of Control and Polluted Brinjal plants

Various details and data on comparative morphology of Control and Polluted Brinjal plants are summarized in Table - 3 - 5 and Fig. 8 -22.

The average plant height in control and polluted plants was 61.6 cm and 68.1 cm respectively (Fig. 8). The average plant height differed significantly in polluted plants over controls.

It shows the nursery bed of seedlings of brinjal as well as magnified view of seedlings.

Polluted plants showed increased average length of flower (1.9cm) in comparison to controls (1.82cm).While no remarkable difference was found in the breadth of flowers in controls (0.64cm) and polluted plants (0.65cm).

In control brinjals, the size (circumference) of fruit was 7.65cm. The average size of fruit increased in polluted plants (8.5cm) (Table. 4) (Fig. 10). Significant difference was found in the size of fruit in polluted plants over controls. In polluted plants, the colour of brinjals were brownish purple having blackish tinge as compared to control ones (purple).

Brinjal seeds were of light brown colour and hairy, hard texture. The average length of control and polluted

seeds were 3.1mm and 2.85 mm respectively. The breadth of control and polluted seeds were found to be of exactly same size i.e. 2.7mm (Table 5) (Fig. 7).

In some polluted sites, it was observed that brinjals generally begin rotting after 3 - 4 days of picking from the plants. Majority of the sites was having high incidences (30 - 40 %) of virus infections. Brinjal fruits were purple brownish coloured with blackish tinge.

Comparative Morphology of Control and Polluted Radish Plants

The data and details of morphological characters of control and polluted radishes are described in Table - 3 to 5; Fig. 8-10.

Observations showed that polluted water of Nalla enhances the plant height. Average plant height increased in polluted plants (88.4 cm) in comparison to control plants (83.2 cm) (Fig. 8). It shows the nursery bed of seedlings as well as magnified view of radish seedlings. It shows the mature fruits of Radish. The deformed fruits of polluted radish were compared with control ones.

The average size of control and polluted radish fruits were found to be of length 4.65 cm and 5.23 cm respectively (Table. 4) (Fig. 10).

Radish seeds were of yellow colour and had smooth, hard texture. The average length of seed in both control and polluted seeds were approximately 4.9 mm. No major variation found in the breadth of seeds of control (3.25 mm) and polluted seeds (3.18mm). (Table - 5)(Fig. 7)

Just like brinjals, it was observed that many plants being damaged by various pests, resulting into poor yield at some polluted sites particularly in summers. 60% of leaves showed black patches on the surface, besides curling and blackish tinge along with dark white colour in March 2012.

The average length and breadth of black patches was 4.91cm x 2.56cm. They mostly appeared on the tip of the leaf, in most cases (~50 %) they were scattered throughout the dorsal surface but interestingly in some leaves they developed parallel throughout the midrib. As per field observation 60% plants leaves were affected. These leaves were usually short as compared to control leaves.

Table 3: Comparative Morphology of Brinjal and Radish

Characters	Control Brinjal	Polluted Brinjal	Control Radish	Polluted Radish
Plant height(cm)	61.6	68.1	83.2	88.4
STEM				
i. Number of Nodes/branch	6.7	8.16	-	-
ii. Length of internode(cm)	2.45	3.83	-	-
LEAF				
i. Length of petiole (cm)	1.59	1.59	5.2	3.1
ii. Number of Leaflets/branch or leaf branch(cm)	6.66	5.6	11.8	12.3
iii. Length of odd leaflet or leaf(cm)	8.66	7.15	10.5	7.25
iv. Breadth of odd leaflet or leaf(cm)	6.38	4.55	6.34	3.75
INFLORESENCE				
i. No. of flowers/inflorescence	5.33	6.2	3	3
ii. Total number of flowers/branch	14.5	15.7	7.2	10.5
FLOWER				
i. Length of Flower(cm)	1.82	1.9	1.63	1.99
ii. Breadth of Flower(cm)	0.64	0.65	1.18	1.14

Table 4: Comparative Morphology of fruits of Brinjal and Radish

Characters	Control Radish	Polluted Radish	Control Brinjal	Polluted Brinjal
1. Size of fruit(cm)	4.65	5.23	7.65 x 6.5	8.5 x 7.24
2.Shape of fruit	Long. Spiny end siliques or Pods	Long, spiny end siliques or Pods	Round/egg shaped	Round/egg shaped
3.Fruit Pulp	White Spongy	Yellowish Spongy	White/greenish and gelly like	Greenish Spongy
4.Colour of fruit	Deep White	Deep white with blackish tinge	Purple	Brownish purple
5.Number of affected	1	2-3	1	2

fruits/plant				
6. Size of affected area(LxB) cm^2	1.9 X0.6	3.1X1	3.05X1.8	4.25 X2.15

Table 5: Comparative morphology of seeds of radish and brinjal

Characterts	Control radish	Polluted radish	Control brinjal	Polluted brinjal
1.Colour of seed	Light Yellow	Light Yellow	Light Brown	Light Brown
2.Length of seed(mm)	4.9	4.85	3.1	2.85
3.Breadth of seed(mm)	3.25	3.18	2.7	2.7
4.Nature of seed	Smooth	Smooth	Hairy	Hairy

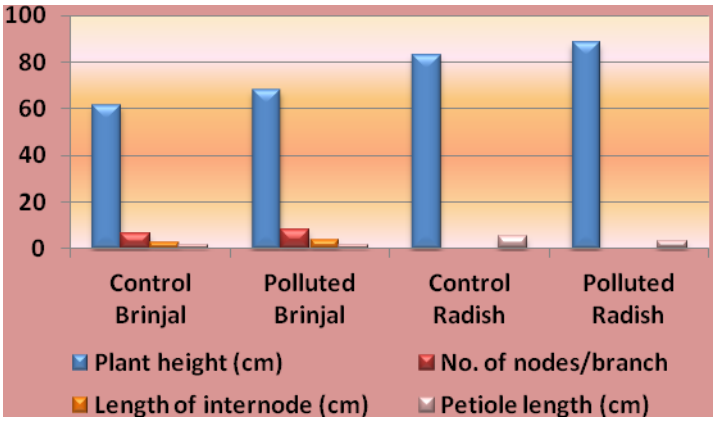

Fig. 8. Some morphological characters in normal and polluted Brinjal and Radish

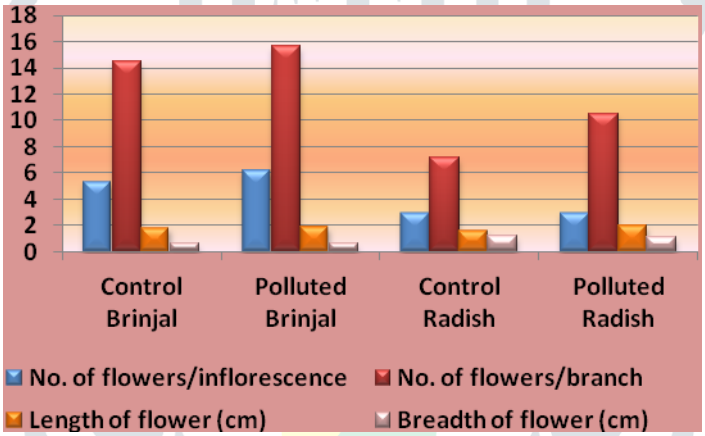

Fig. 9. Size and number of flowers in normal and polluted Brinjal and Radish

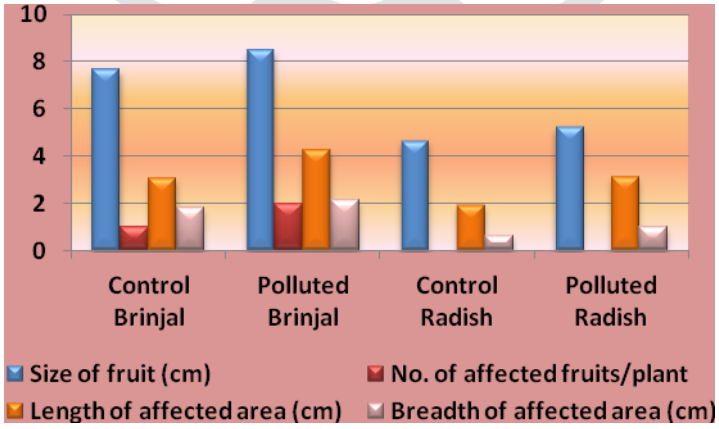

Fig. 10. Comparative fruit morphological features

Cytological Studies

The cytological effects of water pollutants containing heavy metals on the somatic as well as

gametic cells of brinjal and radish were compared. Thus mitotic and meiotic conditions were found similar in both the cases. For both plants in control and polluted conditions the cell shape and nucleus shape was found rectangular and oval respectively.

Table 6: General cytological studies in the root tip cells of control and polluted brinjal and radish

Characters	Brinjal		Radish	
	Control	Polluted	Control	Polluted
1. Cell shape	Rectangular	Rectangular	Rectangular	Rectangular
2. Nucleus shape	Oval	Oval	Oval	Oval

Conclusion

The results have clearly shown that there is agreat adverse effect on mprhology of plants grown in polluted water as compared to control water. There was no significant difference found in the normal and polluted cytological aspects.

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