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INFLUENCE OF PESTICIDE EFFLUENT ON *ALLIUM CEPA* L. (ONION) PLANTS

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Abstract

An investigation was made to find out the effect of different doses (25, 50, 75 and 100%) of pesticide effluents (Indian Pesticide Ltd) on plant growth and certain metabolic parameters (height, total chlorophyll, carotenoid, protein and sugar contents, MDA content, catalase and peroxidase activity) in *Allium cepa* L. plants. Plant growth in terms of its height, total chlorophyll, carotenoid, protein and sugar contents were found to be decreased at increasing doses of pesticide effluents. However, other activities of two antioxidative iron viz. catalase and peroxidase also the MDA (Malondialdehyde) content were found to be progressively stimulated at increasing doses of the effluent.

Key words: Pesticide effluent, *Allium cepa* L., total chlorophyll, carotenoid, proteins, sugars, antioxidative iron enzymes, MDA content.

Introduction

India is an agricultural based country and thus its economy is highly dependent on grain yields from agricultural crops. In order to save crops from attack of pests we have to use various types of chemical pesticides according to the nature of pest which attack that particular crop.

Ideally a pesticide must be lethal to the targeted pests but not to non-target species including man. Unfortunately, this is not the case so the controversy of use and abuse of pesticides has surfaced. A contradictory fact about pesticide is that often crops cannot be saved from pathogenic diseases until they are subjected to pesticide and on the other hand slightly more than required dose of pesticide used to have toxic effects on crops. So there is always a careful need to use the pesticide only in appropriate amounts otherwise it will lead to harmful effects.

In view of above, this study was made to find out the effect of different doses of pesticide on the growth and certain biochemical parameters (carotenoid, protein and sugar contents, MDA content, catalase and peroxidase activity) in *Allium cepa* L. plants.

Materials and Methods

In this study, assessment of certain metabolic parameters (height, total chlorophyll, carotenoid, protein and sugar contents, MDA content, catalase and peroxidase activity) at different doses of pesticide industry effluents were carried out in economically important plant *Allium cepa* L. Pesticide effluent was obtained from Indian Pesticide Ltd. (IPL) Located at Aishbagh, Lucknow. The specific pesticides formed in IPL were Captan, Foltet, Thiram etc. Liquid pesticide industry effluent was diluted to make its concentration in the % of 25, 50, 75 and the last dose of pure pesticide effluent i.e. of 100%. Physico-chemical analysis of effluent was carried out (APHA, 2005).

Physico-chemical characteristics of effluent of Indian Pesticide Ltd.:

Paramerters	Values
Colour	Light Brown
Odour	Pungent
Temperature	28.98±0.667
pH	6.5633±0.008
Sp.C in μmhos/cm	6175.333±1.5
Dissolved Oxygen in mgl^{-1}	5.36±0.544
Biological Oxygen Demand (BOD) in mgl^{-1}	35.667±0.333
Chemical Oxygen Demand (BOD) in mgl^{-1}	486.667±2.404
Total dissolved solid in mgl^{-1}	3805±2.88
Cl^- in mgl^{-1}	236±4.163
F^- in mgl^{-1}	0.62±0.07
Zn μgl^{-1}	110±1.1
Mn μgl^{-1}	95±1.5
Fe μgl^{-1}	180±1.8

Soil culture technique

Experiments were carried out in soil as pot culture under controlled glass house conditions. The soil samples for the experiment were collected from outskirts of Lucknow. These were allowed to dry in shade, and then powdered in agate mortar, finally sieved through a 1 mm sieve before analyzing the different chemical characteristics of the soil. characteristics of soil was as mentioned below (Sapna La' Verne, 2010):

S.N.	Properties	Values
Physical properties		
1.	pH (1:2 H_2O)	8.02
2.	EC (dsm^{-1})	0.23
3.	$CaCO_3$	1.0
4.	OC (%)	1.005
Nutrient concentration (ppm)		
1.	Zn	0.408
2.	Fe	6.064
3.	Mn	7.3
4.	Cu	0.256
5.	S	112

Medium sized (6-8 inched) earthen clay pots were used to carry out the experiments. The central drainage hole provided in these pots for leaching purpose was covered with an inverted watch glass over a pod of clean glass wool. The soil was thoroughly washed with water filled into these pots. Repeated flushing with distilled water was carried out (pH meter) to maintain the soil pH.

Nutrient solution

The composition of the nutrient solution used was based on the Long Ashton formula (Hewitt, 1966). Analytical reagents (AR) were used to prepare both the macro and micronutrient solutions. A concentrated stock solution of each macronutrient was prepared separately by dissolving AR grade salts directly in distilled water, which on 500 times dilution gave the required concentration of the nutrients in the medium.

Following metabolic parameters were observed at 8 weeks growth of each group of plants. Height of plant had been measured through scale in centimeters while fresh weight and dry weight have been measured by weighing balance. All parameters were carried out in the fresh leaf lamina which was finely chopped and 100 mg of the material for each parameter was taken and was ground in a clean pestle and mortar with 10 ml acetone.

Chlorophyll estimation: Chlorophyll was estimated following Arnon (1949) using Milton Roy spectronic 1201 Spectrophotometer. Carotenoid was recorded at 480 and 510 nm. and calculated by method by Duxbury and Yentsch, 1956.

Lipid peroxidation

The level of lipid peroxidation was measured in terms of malondialdehyde content (MDA) (a cytotoxic product of lipid peroxidation) by thiobarbituric acid (TBA) reaction. The plant samples were homogenized in 0.2% trichloroacetic acid (Health and Packer, 1968).

Protein content:

Protein concentration was estimated by the method of Lowry *et al.* (1951). The absorbance was recorded at 750 nm. Bovine serum albumin (sigma) was used as standard.

Sugar estimation: Sugar was estimated by the method of Dubias *et al.* (1956). Glucose curve is used as Standard.

Catalase

Catalase estimation was done by using the method off Euller and Josephson (1927).

Peroxidase

Peroxidase was assayed by the modified methods of Luck (1963). The optical density was read at 485 nm.

Statistical analysis

The experiments were conducted in three replicates (i.e. n=3) for each parameter. The data was subjected to test the significance of variance among the each parameter through two way ANOVA.

Analysis of variance (ANOVA)

The analysis of variance (two ways) was performed by Gomez and Gomez (1984) method as follows:

Results and Discussion

a. **Plant growth**

On increasing concentrations of pesticide industry effluents (25, 50 and 100%), plant height (growth rate) decreased significantly ($p<0.001$) at 28.46, 36.88 and 43.27% accordingly. It is maximum in the control viz. 49.923 cms and reduces to 28.323 cms in 100% effluent concentrations. Similarly, significant decrease was observed in the total fresh weight ($p<0.001$) and the total dry weight ($p<0.01$) with increasing effluent concentrations (Table 1).

Table 1: Effect of various concentrations of pesticide factory effluent on the plant height, total fresh and dry weight of *Allium cepa* L.

Concentrations (%)	Plant height (cm)	Total fresh weight (g)	Total dry weight (g)
Control	49.923±0.512	2.440±0.021	0.240±0.001
25	35.717**±0.361 (28.46%)	1.760**±0.000 (-27.87%)	0.174*±0.001 (-27.64%)
50	31.510**±0.289 (-36.88%)	1.580**±0.000 (-35.25%)	0.160*±0.001 (-33.33%)
100	28.323**±0.162 (-43.27%)	0.933**±0.000 (-61.76%)	0.036**±0.027 (-85.00%)

All values are mean of triplicates ±□S.E. ANOVA ($p<0.01$)

**Significant ($p<0.001$) compare to control, *Significant ($p<0.01$) compare to control.

Effluent of pesticide industry was acidic which might be due to the presence of a huge amount of acidic salts. Apart from this the effluent contained heavy metals Zn, Fe, Mn along with Cl^-, F^- ions. However, the availability of these nutrients decreases as per increase the acidic (4-5 pH) character of the soil. It may directly affect the vegetating growth of the crop plants; it gradually decreased as per increase in the effluent concentration (Charman and Murphy, 1991; Patterson *et al*., 2008). Finding of this study is in conformation with above result.

b. Metabolic activities

i. Chlorophyll

Total chlorophyll content in leaves of *Allium cepa* treated with pesticide factory effluent progressively decreased in dose dependent manner with respect to their control with increasing concentration of pesticide effluent (Table 2). Maximum reduction was by 27.45% at 100% whereas; least by 7.84% was noted for 25% pesticide effluent treated plants. At 50%, it showed 16.47% inhibition which is in between 25 and 100% effluent concentrations. Chlorophyll a and b both showed significant decrease with increasing pesticide concentrations ($p<0.01$). Chlorophyll a decreased by 9.47, 16.32 and 21.05% and chlorophyll b by 24.09, 25.74 and 47.19% at 25, 50 and 100% effluent concentrations, respectively (Table 2).

Table 2: Effect of various concentrations of pesticide factory effluent on pigment content in *Allium cepa* L.

Concentrations (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
Control	0.95±0.01	0.303± 0.008	1.275± 0.035	0.40± 0.344
25	0.860±0.0 (-9.47%)	0.23*±0.04 (-24.09%)	1.175±0.095 (-7.84%)	0.334±0.044 (-14.00%)
50	0.795*±0.045 (-16.32%)	0.225*±0.015 (-25.74%)	1.065*±0.055 (-16.47%)	0.295*±0.045 (-26.25%)
100	0.750**±0.05 (-21.05%)	0.16*±0.020 (-47.19%)	0.925*±0.055 (-27.45%)	0.274*±0.001 (-31.50%)

All values are mean of triplicates ±□S.E. ANOVA ($p<0.01$)

**Significant (p<0.001) compare to control, *Significant (p<0.01) compare to control.

A decline in chlorophyll content as observed in this study might be due to the lyses of cell wall and disruption of thylakoid membrane or inhibition of chlorophyll biosynthesis. Reduction in chlorophyll content induced by waste waters could be correlated with higher concentration of mineral ions. Accumulation of Cu, Zn and Cd would have inhibited the chlorophyll biosynthesis. According to Sharma and Chettri (2008) excess heavy metals accumulated in different plant tissues may influence in plant nutrition. Thus excess accumulation of heavy metals causes either deficiency or environment of other nutrients which has a direct effect on chlorophyll.

ii. Carotenoid

In control, the carotenoid content is 0.40 mg/g. It decreased to 0.344, 0.295 and 0.274 mg/g in 25, 50 and 100% pesticide industry effluent concentration. This decrease was significant at $p<0.001$ (Table 2). Presence of various toxic metal and organic pollutants in excess amount in pesticide pollutant might have resulted in lowering of carotenoid content in studied plants.

c. Protein content

It is found to be decreased for 25% effluent concentration, reported at 114.07 μg/g fw compared to that for the control at 119.607 μg/g fw. It decreased thereafter at a rate of 16.15% and 31.70% at 50 and 100% pesticide effluent concentration, respectively (Table 3). Protein reduction might have resulted due to certain toxic metals and pollutants which might have adversely affected the nitrogen metabolism of *Allium cepa* plant.

Table 3: Effect of various concentrations of pesticide factory effluent on the concentrations of sugar and protein of *Allium cepa* L.

Concentrations (%)	Sugar concentration (µg/g fw)	Protein concentration (µg/g fw)
Control	56.750± 1.838	119.607± 8.773
25	53.183± 1.545 (-6.29%)	114.07± 4.504 (-4.63%)
50	36.217**± 1.209 (-36.18%)	100.294± 0.958 (-16.15%)
100	26.483**± 0.659 (-53.33%)	81.691**± 3.141 (-31.70%)

All values are mean of triplicates ±□S.E. ANOVA ($p<0.01$)

**Significant ($p<0.001$) compare to control, *Significant ($p<0.01$) compare to control.

d. Sugar content

Sugar concentration decreased significantly ($p<0.001$). It is 56.750 µg/g fw for control and decreased by 53.33% at 26.483 µg/g fw for 100% effluent concentration (Table 3).

As reported earlier pesticide effluent might have contained excess amount of metals which might have replaced boron as essential element which is responsible for proper translocation of sugars in plant. Thus any disturbance in its translocation might have caused decrease in the sugar concentration.

e. Enzyme activity

i. Catalase: Activity is significantly ($p<0.001$) increased with increasing effluent concentrations. It became 110% of the control at 100%, 52% at 50% and 30% at 25% effluent concentrations, respectively (Table 4).

Table 4: Effect of various concentrations of pesticide factory effluent on different enzyme and MDA content of *Allium cepa* L.

Concentrations (%)	**Catalase activity (μ moles H_2O_2 decomposed/min/mg)**	**Peroxidase activity (ΔOD/mg protein)**	**MDA (mm/cm)**
Control	83.333±1.667	14.933±1.333	23.558±0.388
25	108.333*±3.333 (+30%)	21.933±1.933 (+46.88%)	38.957**±1.137 (+65.36%)
50	126.667**±8.333 (+52%)	28.800**±0.600 (+92.86%)	50.84±1.24 (+115.80%)
100	175.000**±0.000 (+110%)	34.067**±3.067 (+128.13%)	65.663**±0.885 (+178.73%)

All values are mean of triplicates ±□S.E. ANOVA ($p<0.01$)

**Significant ($p<0.001$) compare to control, *Significant ($p<0.01$) compare to control.

As already reported by Samual (2013), catalase activity enhancement might have resulted due to the presence of Fe in effluents as catalase is an Fe enzyme.

ii. Peroxidase

Peroxidase activity increased significantly ($p<0.001$) with increasing concentrations of pesticide effluent by 46.88, 92.86 and 128.13% at 25, 50 and 100% concentrations, respectively, as compared to the control (Table 4).

Similar to catalase, peroxidase is also an antioxidative iron enzyme whose activity might have favourably effected in *Allium* plants due to the presence of Fe in pesticide effluents.

iii. Lipid peroxidation

MDA content was measured in the plant with progressive increase in effluent concentrations. It increased by 65.36, 115.80 and 178.73% at 25, 50 and 100% concentration of the effluent, respectively (Table 4).

Sanwal (2013) has already reported that lipid peroxidation which is considered an indication of oxidative stress in plants can be induced via ROS that are generated as a result of heavy metal toxicity in plants.

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