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An Investigation on Antigenotoxic Effect of Vermiwash against Industrial Effluent on *Pisum sativum*

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Abstract

The present investigation was directed to study the antigenotoxic impact of vermiwash in *P. sativum* root meristem cells on industrial effluent mediated seed treatment with the aim to understand effects of vermiwash on reducing the percentage of structural chromosomal anomalies. For the experiment 30 physiological and morphological uniform seeds of the experimental plant system where treated for the duration of 2 hrs with 35%, 45% and 55% concentration of the effluent released from plastic and dye industries. The effluent contents many toxic elements like chlorinated solvents, nonylephenol ethoxylate, heavy metals (antimony, mercury, cadmium and lead), chlorobenzene and formaldehyde which are harmful to living beings, effecting their genetic, physiology and metabolic. Therefore, initially the frequency of karyo morphological anomalies were determined and the materials are retreated with vermiwash to evaluate its effect on chromosomes to determine its antigenotoxic impact. The root tips of treated materials were fixed in acetic alcohol for screening of mitotic aberration and MI. The decrease in MI was noted in treated material from 2.86 to 3.16 against 4.86 in control, indicating mitotic depressive activities. This impact was associated with the induction of structural chromosomal aberration from 2.33 to 7.33. The aberration scored were sticky chromosomes, fragmentation and chromosomal bridges. The frequency of sticky chromosomes was high in higher concentration of (55%) effluent. The anomalies were considered the marker of cyto and genotoxicity. The antigenotoxic strains was carries out in similar way except that the effluent treated seed sample where again subjected to the treatment of vermiwash solution for 1hrs.The distilled water was used as negative control. Then the material was subjected to the mitotic screening. The result suggested that there was a marked variation in MI compared to effluent treatment. In vermiwash treatment MI was recovered 3.90-5.90. Similarly, the frequency of chromosomal aberration also was reduced in vermiwash treated sample, it was in the rage of 1.60-3.00 against 2.3-7.3 in effluent treated samples. The result found in present work indicated recovery in the MI and chromosomal aberration in vermiwash treatment. There by suggesting that vermiwash treatment contributed to minimize the genotoxic effects caused by effluent.

Keywords: *Pisum sativum;* Plastic industrial effluent; Vermiwash; Anti-mutagenicity; Cytotoxicity; Chromosomal aberration

Abbreviations:

MI: Mitotic Index; VW: Vermiwash; IE: Industrial effluent.

Introduction

Industries take part in the accelerating process of economic development in the world. It elevates prosperity amongst citizens and supplies material goods they required for survival. But industries are also the core cause of pollution and major consumer of natural resources. The relative contribution to the total pollution load is obviously higher to the industry related pollutants.

Crops, animals and human health is affected by industrial pollutants, such as toxic metals and chemicals used for manufacturing of the product. Plants take up those harmful toxins easily and the cytological and genotoxic effects can be studied easily and conveniently. It is well recognized that pollution lowers the quality of life in various aspects and affects health and life span [1]. Other than health effects, danger of pollutants lies in the fact that they may be mutagenic or toxic. Grover and Kaur, Lah et al., Abdel-Migid et al. and Junior et al. [1-4] have demonstrated the existence of genotoxic activity in wastewater extracts of both industrial and urban origin. The ecological monitoring uses different methods for establishing the degree of pollution and its effects [5]. Different plant test systems are useful for the studying of the cytotoxicity and genotoxicity of industrial effluent. Chemically grown foods have adversely affected human health all over the world. It has been established that *Pisum sativum* test systems are useful tools for detection of potentially genotoxic substances in water screening programs [6,7]. Vermiwash or also called as worm tea, a brownish leachate is produced during the vermicomposting process. Vermiwash is also rich in the macro and micronutrients of a fertilizer. Vermiwash (VW), generally used as a foliar spray, is a liquid bio fertilizer collected by the passage of water through a column of worm activation. There have been several reports on the use of vermiwash and its growth promoting effects. This vermiwash would have enzymes, which would stimulate the growth and yield of crops and even develop resistance in crops, such preparation would certainly have the soluble plant nutrients apart from some organic acids mucus of earthworm and microbes [8]. Studies have found that earthworms effectively bio accumulate or biodegrade several organic and inorganic chemicals including 'heavy metals', 'organochlorides pesticide' and 'polycyclic aromatic hydrocarbons' residues in which it inhabits [9-11]. Composting is an environmentally acceptable method for treating wastes and its product furnishes various benefits when compared with raw wastes: (i) pathogens are destroyed, (ii) nitrogen is converted into stable organic forms, (iii) the volume of wastes is decreased, and (iv) the general physical-chemical characteristics of residues are improved (Imbeah). To minimize the pollution of soils by heavy metals, Jordao et al. [12] added vermicompost to tropical soils with the intention of decreasing the mobility of Cd^{2+} and Cu^{2+}. The work shows that vermicompost is able to bioremediate soils containing metallic species and this ability is also extended to other organic substrates. By Park et al. [13], bioremediation of metals by organic substrates is a consequence of the following mechanisms: (1) Immobilization, (2) reduction, (3) volatilization, and (4) modification of the rhizosphere. The extremity of phytotoxicity linked with the high use of vermicompost in various soils is still an unsure aspect and needs to be investigated more closely [14].

The present work was carried out to evaluate the anti-mutagenic property of vermiwash by considering germination and suppression percentage of chromosomal aberration against industrial effluent induced aberration in the *Pisum sativum* root meristem cell, yet no report exists on the anti-mutagenic effects of vermiwash in plant model. The present paper is designed for the treatment of secondary industrial effluent with the involvement of vermiculture biotechnology.

Materials and Methods

Effluent sample was collected from the drainage where the chemical effluents from various factories are dumped. The effluent was collected in plastic container and stored in the refrigerator at 4°C [15,16]. Before each test, the effluent was equilibrate to room temperature and diluted with tap water to produce the series of dilutions investigated (35%, 45%, 55%). Vermiwash was purchased from Parvathi krishi sanvardan Kendra, Thane, Maharashtra, which was prepared by locally available earthworm species, *Eisenia fetida*.

Pisum sativum were used as experimental model in which the chromosomal aberration induced by industrial effluent and anti-mutagenic property of vermiwash was studied.

Pisum sativum root tip assay: Pre-Treatment with industrial effluent

Uniform and healthy seeds of *Pisum sativum* were used to study genotoxic effect of industrial effluent in the present study. The two batches of 100 pre-conditioned seeds were treated with selected dilution of industrial effluent (35%, 45% and 55%) along with control for 1 hr. On completion of treatment period the seeds were washed with distilled water and placed in Petri dish on wet blotting paper. The seeds were allowed to germinate in Petri plates overnight under laboratory conditions.

Root harvest and slide preparation

The treated seeds were germinated in sterilized petri plates for mitotic screening. Total of 100 seeds per treatment were used. The root tips were cut and fixed in ethanol: glacial acetic acid (3:1) washed in running tap water and preserved in 70% alcohol. The root tips were hydrolysed in 1N HCl at 60°C and stained with 2% acetocarmine. The dividing cells were observed and recorded. Mitotic index and frequency of chromosomal aberration were calculated using the following formula:

$$MI\% = \frac{divided\ cell\ number}{total\ cell\ number} \times 100$$

$$Frequency\ of\ CA\ (\%) = \frac{Average\ number\ of\ aberrant\ cells}{Total\ number\ of\ cells\ counted} \times 100$$

Mitotic aberration was determined together with MI study.

Microscopic parameter

Slides were coded and evaluated blind. The mitotic index (MI) was determined by the investigating of 100 cells per slide. Characterization of mitosis and chromosomal aberrations were recorded in 100 cells per slide. The mitotic cell number, the % of abnormal cells, total anomaly cells and types of aberrations for each concentration were counted in microscopic analysis. Olympus light microscope with digital camera was used to get the clear image of the chromosome anomalies.

Pre-treatment with specific dilution concentration of industrial effluent and post-treatment with vermiwash

In the initial step of the experiment the percentage of effluent dilution and the level of chromosomal aberration was analysed and recorded. This step help in the post treatment of the effluent treated *Pisum sativum* meristematic root tip with respective amount of vermiwash of same dilution.

In the second step of the experiment the initial pre-treatment step is repeated for 1 hr. Then the post-treatment of root tip with selected percentage of vermiwash dilution is carried out. This will lead the present study of anti-mutagenic property of the vermiwash to its final level.

Microscopic parameters

Root harvest and slide preparation step is repeated. The dividing cells were observed and recorded. Mitotic index is determined. Mitotic aberration was determined together with MI study. Slides (2 per concentration) were observed for different stages of mitosis and chromosomal aberrations under Olympus light microscope with digital camera. All slides were coded and examined blind. The mitotic index (MI) was determined by the examination of 100 cells per slide. Characterization of mitosis and chromosomal aberrations were scored in 100 cells per slide. Number of dividing cells as well as chromosomal aberrations in counted cells on a slide was recorded.

The suppression percentage (SP) of vermiwash on chromosomal aberrations of industrial effluent is calculated as Shukla et al. [17].

Antimutagenic evaluation

The activity of the vermiwash in suppressing the mutagenic action of polluted industrial water on the chromosomes of *Pisum sativum* was tested in the similar way to the mutagenic activity in this study. Percentage of mitotic index (MI) and frequency of chromosomal aberrations (CA), recorded at each concentration were calculated. Percentage reduction of industrial pollutant induced chromosomal aberrations at different concentrations of vermiwash was calculated as stated further:

$$\%R\ of\ CA = \frac{A-B}{A-C} \times 100$$

Where '% R' is the percentage reduction of chromosomal aberration(CA); 'A' is the frequency of CA in industrial effluent alone; 'B' is the frequency of CA induced by mixture of industrial effluent and

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vermiwash and 'C' is the frequency of the CA induced by tap water alone.

Results

Antigenotoxic effect of vermiwash against industrial effluent

The decrease in MI was noted in treated material from 2.86 to 3.16 against 4.86 in control, indicating mitotic depressive activities. This impact was associated with the induction of structural chromosomal aberration from 2.33 to 7.33. The aberration scored were sticky chromosomes, fragmentation and chromosomal bridges (Figure 1). The frequency of sticky chromosomes was high in higher concentration of (55%) effluent. The anomalies were considered as the marker of cyto and genotoxicity. Then the material was subjected to the mitotic screening. The result suggested that there was a marked variation in MI compared to effluent treatment. In vermiwash treatment MI was retrieved 3.90-5.90. Similarly, the frequency of chromosomal aberration also was reduced in vermiwash treated sample, it was in the rage of 1.60-3.00 against 2.3-7.3 in effluent treated samples (Table 1). The result found in present work indicated recovery in the MI and chromosomal aberration in vermiwash treated samples, particularly 45% conc. of VW in IE showed 75% reduction in chromosomal aberration compared with 35% and 55% of vermiwash concentration (Table 2).

$$\%R\ of\ CA = \frac{A-B}{A-C} \times 100$$

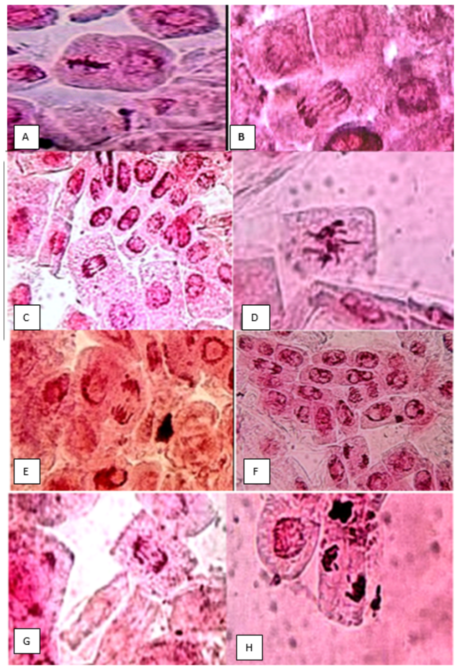

Figure 1: *Pisum sativum* cells showing A) Clumped metaphase B) Broken chromosomal bridge in Anaphase C) Vacuolated nucleus with sticky Anaphase D) Normal Metaphase E) Broken chromosomal fragments in late Anaphase F) Normal anaphase G) Broken bridge and laggard in Anaphase H) Clumped chromosome in Anaphase with fragments.

Treatment	MI	Disturbed spindles	Chromosomal bridge	Chromosomal break	Sticky Chromosome	Total	Frequency in CA
Untreated control	4.86	2	1	-		3	1.0
IE							
35%	2.86	4	-	3		7	2.33
45%	3.40	2	-	5	4	11	3.66
55%	3.16	8	1	8	5	22	7.33
IE+VW							
35%	5.90	7	-	-	-	7	2.33
45%	3.90	2	-	1	2	5	1.66
55%	5.26	5	-	2	2	9	3

Table 1: Different types of chromosomal aberrations in 200 cells analyzed and mitotic index observed in *Pisum sativum* after treatment with IE and IE+VW. CA-chromosomal aberration, MI-mitotic index, IE-industrial effluent and VW-vermiwash.

Treatment /IE+VW	Mitotic Index	Frequency of CA	% Reduction in CA
35%	5.90	2.33	00.0
45%	3.90	1.66	75.0
55%	5.26	3.00	68.4

Table 2: Percentage reduction in chromosomal aberration in *Pisum sativum* after treatment with VW.

Discussion

The diverse effect of industrial effluent in living organisms are studied widely using plants at morphological and genetic level. Various morphological impacts of pollutants in plants are reported as low germination capacity of seeds and discoloration of leafs including stunted growth. Genetic impacts are reported in the form of chromosomal aberration which causes gene mutation [1-4].

Vermiwash is an eco-friendly brown liquid fertilizer produce by passing water through worm culture column. It consists of worm excretory matters, microbes, enzymes and micronutrients from the soil. It helps in elevating nutritive value of the soil and helps in plants growth. The vermin cast has been reported as nontoxic and heavy metal adsorbing agent in various literatures. It is used as waste water decontaminant [18]. Vermiwash contain the entire nutrient present in vermicompost in liquid form used as fungicide and insecticide. It was observed that vermiwash increase the germination percentage in *Pisum sativum* and act as antigenotoxic agent in lowering the chromosomal aberrations. The work shows that vermiwash is able to bioremediation soils containing metallic species and this ability is also extended to other organic substrates.

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