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# ENHANCEMENT IN SEED GERMINATION THROUGH SIMULTANEOUS DEGRADATION OF ORGANOCHLORINE PESTICIDES (LINDANE AND DDT) BY A NOVEL MICROBIAL CONSORTIUM

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**ABSTRACT**

*The study involved testing the effects of mixture of Organochlorine pesticides (Lindane and DDT) on the germination of seeds and simultaneous bioremediation of these pesticidesby a novel microbial consortium. These two pesticides (Lindane and DDT) were selected as they are Persistent Organic Pollutants (POP), widely used in the developing economies for agricultural usage and in particular DDT which is used for mass healthcare programs thereby contributing to environmental pollution. The study was done using green gram seeds which is one of the commonly consumed legumes in India. The seed growth in presence of these pesticides exhibited a noticeable reduction in vigour index that significantly reduced withincreasing concentrations of organochlorine mixture, Lindane and DDT, at 10 ppm, 20 ppm and 30 ppm concentrations. The germination of green gram seeds was repressed upto a viguor index of 21.1on moist filter paper at 30 ppm concentrations which was evident in reduction of amylase and protease enzyme activity that showed a concomitant reduction with increased pesticide concentrations.*

*Bioremediation of Lindane and DDT contaminated water through a novel microbial consortium showed enhanced seed germination and reduction of the adverse effects of the pesticides. The vigour indices of bioremediated water grown seeds were found to improve drastically from 54.8 to 75.7 in 10 ppm, 23.5 to 72.2 in 20 ppm and 21.1 to 74 in 30 ppmand activities of the enzymes, amylase and protease, that were comparable or betterin bioremediated water.*

***Keywords: amylase, bioremediation, ddt, dichlorodiphenyltrichloroethane, hch, hexachlorocyclohexane, lindane, microbial consortium,protease, vigour index.***

## I. INTRODUCTION

Chemicals have been used to control pests for centuries but have come into widespread use only within the past century, with the development of a variety of synthetic pesticides mainly for agriculture usage [1]. Among all classes of pesticides, organochlorines (e.g. DDT, HCH, Endosulfanetc) have found extensive use in the infestation and pest management during the last few decades. The broad spectrum, high persistence and low cost of these

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compounds made them the best opted for and indiscriminately used ones, both in agriculture and health management practices [2].

DDT (dichlorodiphenyltrichloroethane) and Lindane were the major OCP (organochlorine pesticides) that have been extensively used by many countries for pest management. Production of organochlorine pesticides excluding endosulfan is proscribed in several parts of developed nations; however they are ubiquitously used in developing nations.Lindane also known as gamma –HCH (γ-HCH), is made up of at least 99 % of the gamma isomer of hexachlorocyclohexane (HCH). While DDT is in restricted use since July, 1989, HCH is banned in April, 1997[3].

Organochlorine pesticides are a group of recalcitrant molecules and thereby these compounds can get accumulated through food chains and produce a significant magnification at each tropic level. The major sink for persistent organic pollutants discharged into environment is the water ecosystem i.e. rivers and lake beds. Several persistent organochlorine pesticides were detected in rivers where higher concentrations of Endosulfan sulfate and DDT were detected [4] and even found their presence in drinking and bottled water [5]. Hence, it becomes imperative to remove these pollutants from the environment, from the sinks primarily soil and water ecosystems to finally eliminate their residues. Microorganisms are found to be potential degradersof organochlorine compounds, notably soil habitants belonging to genera *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Micrococcus* were found to be effective bio-degraders [6].

In this paper we present the effects of DDT-Lindane mixture on the germination of green gram, application of a novel microbial consortium to bioremediate the DDT-Lindane mixture and post remedial effects on the germination.

## II. MATERIALS AND METHODS

### 2.1. Chemicals

Lindane γ-HCH (insecticidal isomer) was of 97% purity and obtained from Sigma- Aldrich, USA. DDT, 99.4% pure, was donated by Hindustan Insecticides Ltd, India. All other chemicals and reagents used in the study were of analytical grade and were purchased from standard manufacturers.

### 2.2. Seeds, waster and microbial consortium

Seeds of green gram used for the study werepurchased from local vendor.The water used for the waster was sterile mineral water procured form standard companies. The microbial consortium used was developed indigenously over a period of 12 months using contaminated soil and water systems enriched by sole carbon source from DDT and Lindane.

### 2.3 Seed germination test by filter paper method

The seed germination test was carried employing filter paper method [7,8]. Seeds of green gram were first surface sterilized using 2% mercuric chloride and were then placed on sterile Whatman No. 1 filter paper discs kept in petri plate (sterile) and was moistened with sterile water containing different concentrations of Lindane and DDT (10ppm, 20ppm and 30ppm).In each petri plate, 10 seeds were placed and germinated at room temperature with 12h-12h cycles of light and darkness. For each concentration of Lindane and DDT, 9 replicates of 10 seeds were taken. Controls were maintained on the filter paper moistened with sterile water. 1ml of sterile distilled water was added

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every day to maintain the moisture level in the petri plates. The seedlings were evaluated for germination after 7 days.

### 2.4 Bioremediation of Lindane and DDT contaminated water

Three sterile Erlenmeyer flasks were taken and 100ml sterile water was added in it. The water was spiked with different concentrations of Lindane and DDT (10ppm, 20ppm and 30ppm). 2ml of previously induced microbial consortium was added to each flask. The contents in the flasks were mixed well and were then placed in a rotary shaker for 3 days maintained at room temperature and 150 rpm. After three days, the contents of all the flasks were centrifuged separately at 4˚C and 10000 rpm for 20 min to separate the cells and the supernatant. The supernatants were collected in separate flasks (sterile). 100ml of each of these supernatants was taken further used for seed germination and enzyme activity studies.

### 2.5 Germination of green gram seeds on bioremediated water

Sterile Whatman No.1 filter paper discs were placed on sterile petri plates. The filter paper discs were moistened with sterile water. 1ml of previously bio-remediated water was added in each petri plate and seeds of green gram were placed on it. The experiment was performed in the replicates of nine with 10 seeds in each plate. The plates were incubated at 28-30ºC temperature with 12h-12h cycles of light and darkness. 1ml of sterile distilled water was added every alternate day to maintain the moisture level in the plates.

### 2.6 Enzyme activities of green gram seeds grown in bioremediated water

Bioremediated water with pesticides(Lindane + DDT) at concentrations (10ppm, 20ppm and 30ppm) and 1000 seeds of green gram were added in three sterile 250 ml Erlenmeyer flasks. The amylase and protease enzyme activities of seeds in each flask were assayed after 0hr, 24hr, 48hr, 72hr and 96hr.

## III. RESULTS AND DISCUSSION

### 3.1 Effect of Lindane and DDT on seeds of green gram on filter paper

Effect of different concentrations of Lindane and DDT on the germination of green gram seeds was studied. The seeds were found to be affected by the addition of pesticide (Lindane and DDT) [9, 10]**.** The seeds of green gram (*Vigna radiate*) found to be very susceptible to the presence of these applied pesticides(**Figs 1 and 2)**.

**TABLE 1**reports the results obtained from green gram seeds in presence of various concentrations of Lindane and DDT.**TABLE 2**represents the outcome when seeds were grown in bioremediated water.

**Table 1:Average values of effect of Lindane and DDT on the germination of green gram seeds tested by filter paper method**

| Sample | Shoot length (cm) | Root length (cm) | Number of secondary roots | Number of leaves | Length of leaves (cm) | Width of leaves (cm) | Percent germination (%) | Vigour Index |
|---|---|---|---|---|---|---|---|---|
| **Control** | 3.04 | 0.5 | 1.4 | 1.6 | 1.06 | 0.4 | 100 | **40.07** |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **10ppm** | 4.2 | 0.7 | 1.9 | 1.3 | 1.01 | 0.3 | 98.89 | **54.7951** |
| **20ppm** | 1.6 | 0.6 | 0.9 | 0.8 | 0.7 | 0.2 | 93.33 | **23.4859** |
| **30ppm** | 1.8 | 0.3 | 0.6 | 0.8 | 0.6 | 0.2 | 86.67 | **21.15382** |

**Table 2: Average values of the germination of green gram seeds post bioremediation tested by filter paper method**

| Sample | Shoot length (cm) | Root length (cm) | Number of secondary roots | Number of leaves | Length of leaves (cm) | Width of leaves (cm) | Percent germination (%) | Vigour Index |
|---|---|---|---|---|---|---|---|---|
| **10ppm** | 5.6 | 1.1 | 5.06 | 1.7 | 1.01 | 0.2 | 98.89 | **75.73699** |
| **20ppm** | 5.4 | 1.08 | 3.8 | 1.9 | 0.9 | 0.2 | 100 | **72.24691** |
| **30ppm** | 5.7 | 1.07 | 4.3 | 1.8 | 0.9 | 0.2 | 97.78 | **74.00967** |


**Fig.1. Seed germination on filter paper observed after 7 days at different concentrations of Lindane and DDT**


**Fig.2. Seeds germinated at different concentrations of Lindane and DDT**

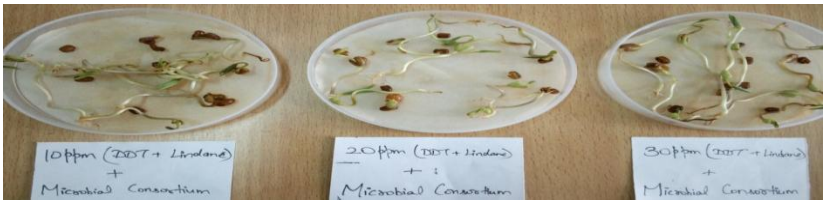


**Fig.3. Seeds germinated in bio-remediated waster**

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The inhibitory effects towards germination increased with increase in concentration of Lindane and DDT mixtures. A low percentage of germination was observed at 30ppm Lindane and DDT level (**TABLE 1**). But the percent germinationin the treated (bio-remediated waster) increased (**TABLE 2**) indicating degradation of pesticide mixture by microbial consortium, thereby eliminating the toxic effects of this pesticide mixture. Root and shoot lengths were also observed to be much better as compared to the controls (**Fig 3**). This indicates that the consortium not only simultaneously degraded the pesticides, but also enhanced the growth of the seedlings.

### 3.2 Effect of Lindane-DDT mixtures on the enzyme activity of green gram seeds

The effect of pesticides was also observed by soaking the seeds in water spiked with Lindane and DDT and assaying the amylase and protease enzyme activities [11,12]. All the tests were done in triplicates and the results presented here (**TABLES 3, 4, 5 and 6**) are the average of triplicates.

**Table 3: Amylases Enzyme Activity (Contaminated with Lindane+DDT)**

| S.No | Test Sample (ppm) | Enzyme activity (U/ml) | | | |
|---|---|---|---|---|---|
| | | Control | 10 ppm | 20 ppm | 30 ppm |
| 1. | 24 h | 0.029315 | 0.016946 | 0.010296 | 0.000358 |
| 2. | 48 h | 0.011011 | 0.014229 | 0.018815 | 0.010941 |
| 3. | 72 h | 0.026956 | 0.019182 | 0.011861 | 0.002861 |
| 4. | 96 h | 0.020878 | 0.011583 | 0.009581 | 0.009438 |

**Table 4: Amylases Enzyme Activity (bioremediated water grown seeds)**

| S.No | Test Sample (ppm) | Enzyme activity (U/ml) | | | |
|---|---|---|---|---|---|
| | | Control | 10 ppm | 20 ppm | 30 ppm |
| 1. | 24 h | 0.029315 | 0.023524 | 0.023585 | 0.00200 |
| 2. | 48 h | 0.011011 | 0.019591 | 0.01666 | 0.01951 |
| 3. | 72 h | 0.026956 | 0.038825 | 0.02095 | 0.02867 |
| 4. | 96 h | 0.020878 | 0.028243 | 0.027385 | 0.02958 |

**Table 5: Proteases Enzyme Activity (Contaminated with Lindane+DDT)**

| S. No | Test Sample (ppm) | Enzyme activity (U/ml) | | | |
|---|---|---|---|---|---|
| | | Control | 10 ppm | 20 ppm | 30 ppm |
| 1. | 24 h | 18.70690 | 18.32759 | 17.84483 | 16.17241 |
| 2. | 48 h | 18.12069 | 17.51724 | 16.34483 | 15.65517 |
| 3. | 72 h | 21.79310 | 20.39655 | 20.2069 | 19.50981 |
| 4. | 96 h | 15.94828 | 13.68966 | 12.60345 | 12.06897 |

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**Table 6: Proteases Enzyme Activity ((bioremediated water grown seeds)**

| S.No | Test Sample (ppm) | Enzyme activity (U/ml) | | | |
|---|---|---|---|---|---|
| | | Control | 10 ppm | 20 ppm | 30 ppm |
| 1. | 24 h | 18.70690 | 19.79312 | 19.20691 | 18.22414 |
| 2. | 48 h | 18.12069 | 19.7069 | 19.12069 | 18.72414 |
| 3. | 72 h | 21.79310 | 23.2069 | 22.2069 | 22.55172 |
| 4. | 96 h | 15.94828 | 20.46552 | 20.48276 | 21.18966 |

The results indicate a reduction in Amylase and Protease activity when the seeds are subjected to contaminated pesticide water and the enzyme activity increased in bioremediated water.

## IV. CONCLUSION

The study demonstrated reduced growth of green gram seeds in pesticide waster, a noticeable reduction in seed vigour indexupon increasing concentrations of organochlorine mixture, Lindane and DDT, at 10 ppm, 20 ppm and 30 ppm concentrations. Bioremediation of Lindane and DDT waster through a novel microbial consortium showed enhanced seed germination thereby eliminating the deleterious adverse effects of these pesticides and provided a pragmatic evidence for simultaneous degradation of Lindane+DDT, organochlorine mixture by novel microbial consortium. The vigour indices of bio-remediated water grown seeds showed an enhancement of seed germination and viguor index increased from 54.8 to 75.7 in 10 ppm, 23.5 to 72.2 in 20 ppm and 21.1 to 74 in 30 ppm under laboratory conditions. Although research has been carried out using single strain and single compound of organochlorine [13, 14, 15] the current study data provides a promising solution for bioremediation of organochlorine mixtures using a microbial consortium.

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