PERFORMANCE OF *Eleusine indica* TO ABIOTIC STRESS OCCASIONED BY POLLUTION OF THE PESTICIDE 2,2-DICHLOROVINYL DIMETHYLSULPHATE

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Abstract: The present study investigated growth performance of *Eleusine indica* to abiotic stress occasioned by pesticide pollution with a view to ascertaining its suitability for phytoremediation. Soil was spiked with 2,2-dichlorovinyl dimethyl phosphate (DDVP, 1000EC) in solution to obtain 6 different levels of pollution: 5.0, 3.75, 2.5, 0.5, 0.25, and 0.05 ml pesticide/kg of soil. The control was wetted with ordinary water. These treatments were replicated 3 times in completely randomized block design. The entire set up was left to naturally attenuate for 2 days before equal-lengthen 3-leaf tillers of *Eleusine indica*, the test plant, were transplanted from a nursery. Results showed that within 24 h, young tillers in the 5 ml/kg-soil treatment wilted entirely. Those in 2.5 – 5.0 ml/kg pesticide-impacted soil (PIS) also showed wilting signs, beginning first with folding of plant leaves. There was no evidence of wilting in plants sown in the 0.05 ml/kg PIS and the control. Total pesticide residual content after 11 weeks was 0.512 mg/kg in the 5 ml/kg-PIS (i.e. 10.24% reduction) that lacked plant presence (note, plants died during the first few days). However, Residual content ranged from 0.012 – 0.073 mg/kg in the treatments with plant presence, thus indicating the importance of plant presence. Remediation efficiency was 13.60 – 29.20% in treatments with surviving plant population. *Eleusine indica grass* showed great promise as a phytoremediator of pesticide. However, the fact that significant morphological changes occurred in the plant also goes to show the effects of increased concentrations of contaminants on the would-be phytoremediators.

Keywords: Pesticide residue, *Eleusine indica*, phytoremediation, DDVP, rhizosphere interaction

Introduction

In other to control or reduce the levels of insect pests attacking plants and animals with a view to reducing yield loss, pesticides are extensively required. However, risk is related with the use of pesticides and if not properly handled can be hazardous (Udoh *et al.*, 2011). Serious concerns about human health risk which result from pesticides residues found in food have raised a high alarm even though pesticides are produced under strict regulation processes. Olawale *et al*. (2011) suggested that pesticide residues, which remainin the soil or plant after the application and use, constitute a substantial health hazard in the current generation and future generation as a result of excessive use. This usually brings about the accumulation of great amount of the residues in the environment and this passes into the food chain and the drinking water. The persistence of pesticides in the environment is because of lack of the ability of most organisms to degrade this pesticide and also their physico-chemical properties.

A number of pesticides are available for use, including the organophosphates. These pesticides are composed of an active ingredient as an agricultural input and also of inert materials that are used in the control of pests (Lawal *et al.*, 2005). An example of such active ingredients is DDVP (2,2-dichlorovinyl dimethyl phosphate), 1000EC. These organophosphate pesticides don't stay long in the environment compared to organochlorine pesticide. DDVP is also known as Dichlorvos. Dichlorvos has an ADI which is estimated as 0 - 0.004 mg/kg bw (Desi and Nagymajtenyi, 1999; ATSDR, 1997; US EPA, 2006).

Dichlorvos is carcinogenic and also cause harm on plant and humans (Soares *et al*., 2003; Remor *et al*., 2009). In developing countries the misuse of pesticides is the major factor facing pesticide contamination or poisoning. The misuse of pesticide in different area of agriculture has been accompanied with health problem and contamination of the environment worldwide (Soares *et al*., 2003; Mancini *et al*., 2005; Remor *et al*., 2009). One of the major reasons for pesticide poisoning is the misuse of toxic chemicals without good regulation and guideline (Konradsen *et al*., 2003). Several reports have been stated during the last three decades which shows that global usage of pesticide has increased (Obida *et al*., 2012). In Nigeria, Borno State precisely, death of people has been recorded as a result of high pesticide residue on the food chain (Obida *et al.*, 2012).

Apart from the direct effects of pesticide use, a major concern arises from the indiscriminate disposal of pesticide containers (Tashkent, 1998). Pesticides can then accumulate in the soil or even can enter aquatic environment, where they cause harm to plants and animals (Ikpesu and Ariyo, 2013). The presence of pesticides and pesticide residues in soils can impose abiotic stress symptoms in plants. Plant abiotic stress involves changes in amino acid, carbohydrates and amine metabolic pathways. These changes are in most cases presented morphologically by the plants. Plants respond differently to abiotic stress factors. These responses are sometimes important in selecting stress-tolerant plants. Anoliefo *et al.* (2006, 2008) suggested that the abundance of a particular plant in a contaminated area indicates that such a plant may show tolerance to the contaminant, and therefore may be a likely candidate for phytoremediation of that contaminant.

In the present study, the growth response of *Eleusine indica* transplants in pesticide-polluted soil would be studied, as well as changes in pesticide residual components of the soil after transplantation of the test plant. *Eleusine indica* is a major problem in almost all forms of agriculture and can withstand trampling. The plant has been shown by a number of researchers to have the capability for remediation of hydrocarbon-polluted soils (Anoliefo *et al.*, 2006; Anoliefo *et al.*, 2008; Ikhajiagbe and Anoliefo, 2012).

Materials and Methods

Soil used in the present study was collectedrandomly from a marked plot area near the Botanic Garden, Department of Plant Biology and Biotechnology, University of Benin, Benin City. Thereafter, 20 kg of measured sun-dried soil was each placed in large bowls (28.6 cm height and diameter of 52.4 cm). Measured quantity of soil placed in each bowl occupied a dimension of 20.4 cm in depth and a radius of 23.2 cm. The surface area of top soil thus measured was 1691.6 cm^2.

Soil pollution using varying levels of pesticide in each bucket

The pesticide used for the present study was DDVP (2,2-dichlorovinyl dimethyl phosphate), 1000EC. It is an organophosphate pesticide. Soils in each bowl were wetted with the test pesticide. Having predetermined the water holding capacity of the soil to be 186.6 ml/kg soil, each bowl was initially wetted with 1000 ml of water initially and subsequently with another 1000 ml containing measured liquid quantities of the test pesticide. There were 6 different levels of pollution: 100, 75, 50, 25, 5, and 1 ml of the test pesticide mixed separately in 1000 ml of distilled water and used to evenly wet 20 kg soil. The concentrations amounted to 5, 3.75, 2.5. 0.5, 0.25, and 0.05 ml/kg of soil. The control was wetted with ordinary water. These treatments were replicated 3 times in completely randomized block design. The entire set up was left to naturally attenuate for 2 days before equal-lengthen 3-leaf tillers of *Eleusine indica*, the test plant, were transplanted from a nursery (2 weeks old).

Performance evaluation of transplants

The experimental set up, consisting of pesticide-polluted soils and the transplanted test plants, were left near the Botanic Garden for additional 3 months under prevailing weather (February through May, 2015). During this period, the following morphological parameters plant height, stem width, flag leave blade length, flag leave blade width, numbers of additional spike, leaves, tillers per plant, culm branching per plant length of longest spike, length of spikelet, depth of longest root, internode length, as well as plant dry weight, foliar and root morphology were determined.

Soil analyses

Soil pH was determined when 20 g of air-dried soil were sieved and 20 ml of distilled water was added to it and allowed to stand for 30 min. The mixture was stirred occasionally with a glass rod. The pH was determined by inserting the pH meter (Hanna Model) into the suspension. The meter was previously calibrated with pH buffer solution of 4.0, 7.0 and 9.0, rinsed severally with distilled water and was taken when the digital display was stable. The soil conductivity meter (Hanna Model) was used to determine soil conductivity. Soil Temperature was determined by using a thermometer. The mercury end of the thermometer was inserted into the soil to a depth of 10 cm above soil level.

Although, the soil was contaminated with DDVP, it was however necessary for analysis of organochlorine pesticide residues to be carried out. This was done following the standard methods of ASTM D6160 – 98 (2013). Isolation and characterization of bacterial and fungal isolates was carried out using the methods of Sabba (1995) and Cheesebrough (2001).Determination of whole plantcrude protein concentration plant sample was by the method of Bradford (1976).

Mean and statistical error of data was calculated. Analysis of variance in complete randomized design was done using the *SPSS-16* statistical software, and means were separated by using the Least Significant Difference (Ogbeibu, 2005).

Results and Discussion

The morphological parameters of tillers transplanted from the nursery have been presented on Table 1. Mean plant height was 15 cm, with an intermodal length of 4.3 cm. There were no evidences of chlorosis or necrosis in the young tillers prior to transplanting. Within 24 h of introducing young tillers into the 5 ml/kg-soil treatment, plants began to wilt. The soil seemed granular and less bristly to touch when compared with other treatment soils. On the other hand, plants in 2.5 – 5.0 ml/kg pesticide-impacted soil (PIS) also showed wilting signs, beginning first with folding of plant leaves. There was no evidence of wilting in plants sown in the 0.05 ml/kg PIS and the control.

Table 1: Morphological parameters of plants at eleven weeks after exposure to experimental conditions (Plants in 2.5 – 5.0 ml/kg-PIS were all dead before 11 weeks)

Plant parameters	Tiller from nursery	11 weeks after exposure			
		0.5 ml/kg	0.25 ml/kg	0.05 ml/kg	Control
Plant Height (cm)	15 ± 0.00	39.3 ± 5.7	45.7 ± 2.04	30.2 ± 4.0	46.7 ± 4.3
Stem Width (mm)	2.5 ± 0.00	5.1 ± 0.09	5.7 ± 0.43	6.7 ± 1.06	8.2 ± 0.79
Flag leave blade length (cm)	12 ± 0.00	19.5 ± 5.2	22.7 ± 9.3	24.5 ± 0.5	28.5 ± 0.5
Flag leave blade width (mm)	NA	4.5 ± 0.5	4.3 ± 0.6	5.1 ± 0.4	6.2 ± 0.4
Peduncle length	NA	4.75 ± 0.77	8.0 ± 1.00	6.2 ± 0.80	4.54 ± 0.80
Length of longest spike (cm)	NA	3.75 ± 3.76	4.68 ± 0.21	4.25 ± 0.25	4.68 ± 0.21
Length of spikelet (mm)	NA	0.15 ± 0.15	0.33 ± 0.12	0.31 ± 0.10	0.56± 0.05
Number of spikelets per plant	NA	84.96 ± 4.26	296.8 ± 6.20	223.5 ± 6.52	227.3 ± 5.24
Depth of longest root	5.1 ± 0.00	13.35 ± 3.40	26.5 ± 26.58	36 ± 3.01	37.5 ± 3.19
Internode length (cm)	4.3 ± 0.67	8.31 ± 0.51	8.29 ± 0.62	9.32 ± 1.20	9.63 ± 1.21
No. of Culm branching per plant	0	0	0	0	0
Plant moisture content (g)	NA	51.62 ± 2.14	65.21 ± 3.21	65.71 ± 2.09	43.51 ± 3.54
No. of tillers per plant	NA	5.5 ± 3.51	2.6 ± 0.19	3.0 ± 0.31	3.5 ± 0.50
Plant dry wt. (g)	NA	2.92 ± 0.19	2.63 ± 0.05	2.95 ± 0.15	3.62 ± 0.28
First day to flowering (DAT)	NA	39.5 ± 1.3	39.8 ± 2.1	31.6 ± 2.8	29.4 ± 2.0
No of leaves	4 ± 0.00	26 ± 9.03	18 ± 0.00	17 ± 1.00	40.5 ± 2.51
Number of spike	NA	0	6.5 ± 2.51	6 ± 1.00	0
Total No of primary root branches	18 ± 0.00	33.51 ± 4.04	26.5 ± 9.53	26.00 ± 0.00	23 ± 3.01
No of main root	NA	5.5 ± 3.51	2.5 ± 0.50	3 ± 0.00	3.5 ± 0.50
No of Branch root	NA	27.5 ± 1.53	24.2 ± 9.03	13.8 ± 0.00	19.5 ± 2.51

NA = not available

Thirty-six days after transplanting (36 DAT), there was folding or foliar twisting of more than 10% of the leaves of plants exposed to 3.75 – 2.50 ml pesticide/kg soil. Similarly, leaves of those plants exposed to lower concentrations of the pesticide in soil showed folding at 48 DAT. Folded leaves showed a particular pattern of folding; leaves, which were majorly chlorotic, were folded from foliar tip inwards. However, there were an insignificant number of twisted healthy leaves. In spite of the folding, folded leaves of plants sown in 0.05 ml/kg-treatment completely recovered at 53 DAT, whereas a third of the twisted leaves in 0.25 ml/kg-PIS regained their original shapes at 62 DAT. As at the period of conclusion of the study (12 weeks), the other leaves had not recovered from the twisted positions. Although the soils were adequately wetted to provide ample moisture for sown plants, the morphological presentations of the plants reported above were those similar to water-deprived plants, ultimately resulting in wilting as well as loss of leaves by the plants. Plants adjust to stress conditions by several mechanisms including by closure of their stomata, thus reducing the rate of water loss due to evapotranspiration, particularly under water stress. The closure of plants' stomata has a major consequence on the growth, development and metabolism of the plants, as carbon dioxide is entirely cut off from the plant, leading to decreased photosynthetic efficiency. This can result in plant death. In the present study, plants in the soils polluted with higher concentration of pesticide (2.5 – 5.0 ml/kg) had died before the 11th week (Table 1). Percentage wilting of *Eleusine indica* population in the pesticide-impacted was evident as from the 4th day after introduction of plants into polluted soils (Fig. 1). Percentage wilting in 5 ml/kg-PIS was 100%, compared with plants in 0.05 ml/kg-PISand control soils, which showed no evidence of wilting. This therefore underscores the significance of water, among other environmental factors, as sine qua non to plant growth and development (Roche *et al.*, 2009). It is suggested that perhaps the pesticide, when accumulated by the plants in higher concentration, simply disrupted the plant's innate capability to access water from the soil.

Plant height ranged from 51.5 – 67.0 cm in the 0.05 – 0.5 ml/kg PIS treatments at 10 weeks following transplanting of *Eleusine indica*, compared to 65.5 cm in the control. After 11 weeks, plant height in 0.5 ml/kg-PIS was 39.3cm, compared to 46.7 cm in the control (Table 1). Stem width of plants sown in the control was 8.2 mm whereas as plants in 0.5 ml/kg-PIS had stems that were 5.1 mm thick. No additional spikes were recorded in plants sown in both treatment and control soils. Owing to foliar necrosis, no foliar appendages were visible in plants exposed to 5.0 - 2.5 ml/kg-PIS (Table 1). As recorded in the present study, pesticide in soil hindered plant growth and morphological development particularly with increasing concentration (Abdul-Ghany *et al.*, 2003).

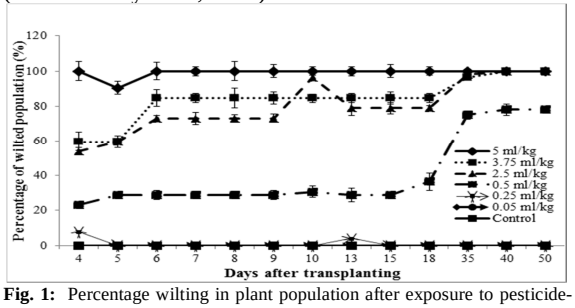

Fig. 1: Percentage wilting in plant population after exposure to pesticide-impacted soil

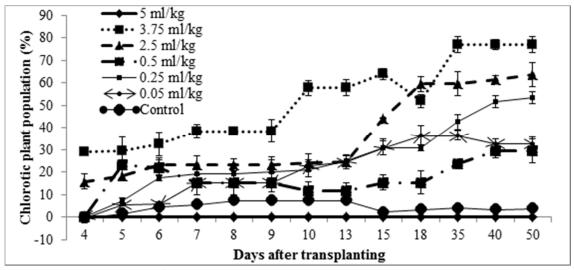

Fig. 2: Percentage of chlorotic plant population after exposure to pesticide-impacted soil

In this study, those plants that had >25% of its entire foliage being yellowish were defined as chlorotic (Fig. 2). Each plant was numbered as a chlorotic plant when >25% of its entire foliage turned yellow. Plants in 5 ml/kg-PIS did not show signs of chlorosis; they just simply wilted and dried without first turning yellow. There was increase in number of chlorotic plants from day 4 to 50. Highest percentage of chlorotic plant population was recorded in 3.75 ml/kg-PIS, whereas the control recorded the least. There was a slight decrease in percentage chlorotic plant population from day 13 to day 50 when plants where not exposed to pesticides (i.e. Control). Evidently, most of the leaves of affected plants recovered from foliar symptoms of chlorosis. Evidence of chlorosis possibly points at pesticide toxicity (Kubis *et al*., 2004). Chlorotic plants hardly photosynthesize, and thus may die unless the cause of lack of chlorophyll or possible degradation of chlorophyll is surmounted (Kubis *et al.*, 2004). This may have been recorded in treatments with a number of recovered leaves. This development eventually significantly distresses plant morphological improvement, as the metabolic energy required by plants for such development arises mostly from chlorophyll-related metabolism. According to Steve and Jack (2004), pesticide may cause chlorosis as observed in this study.

Whole plant crude protein content of *Eleusine indica* at 9 weeks after exposure to pesticide in soil have been presented in Fig. 3. Crude protein content was 6.27% in plants exposed to pesticide at concentrations of 2.5 ml/g-PIS. Plant protein content was lowest in control. According to Gygi *et al*. (2000) the exposure of plant to pesticide cause a reduction in plant protein, but Zhao *et al*. (2013) opined that proteins involved in signal perception was higher at the early stage of abiotic stress. Total protein in this study differed according to concentration of pesticide in soil. Although there were no significant differences in protein level of control plants and those sown in 2.5 ml/kg and 0.5 ml/kg-PIS. However, protein levels were significantly lower in plants exposed to lower pesticide concentrations (0.25 and 0.05 ml/kg-PIS).

The fact that significant morphological changes occurred in the plant goes to show the effects of increased concentrations of contaminants on the would-be phytoremediators. However, these plants, through physiological responses, adapt to their polluted environment before they stand the chance as remediators. One of such mechanisms by plants is the phytoaccumulation of stress proteins as a result of exposure to stress. In the present study, there was increase in protein content of whole plant. Since crude protein contain all amounts of proteins, it was deduced that the increase in protein content of pesticide-exposed plants compared to the control may have been due to production of stress proteins in response to the stress imposed by the pesticide.

It is noteworthy also that changes in protein levels with increased pesticide concentration in soil may be linked to plant water-deficit occasioned by the pesticide toxicity (Abdul-Ghany *et al*., 2003; Roche *et al*., 2009). Seki *et al.* (2007), Huang *et al.* (2008), Chaves *et al*. (2003) and Green (2011) reported that these responses were directly prompted by the fluctuating water status of the plant tissues. Wilting, as reported in the study may have resulted from root damage. Green and Capizzi (1990) reported that some toxic contact chemicals in the root zone, result in poor root development. Symptoms from root-contact chemicals are confined where the latter contacts the root, but eventually produce wide-ranging symptoms in the plant's shoot; these may show water and nutrient stress symptoms, including reduced growth and wilting as reported in the study. Roots are injured and root tips may be killed. This will result in a general stunting of the plant. Green (2011) also reported that in severe cases, wilting occurs even though the soil is wet.

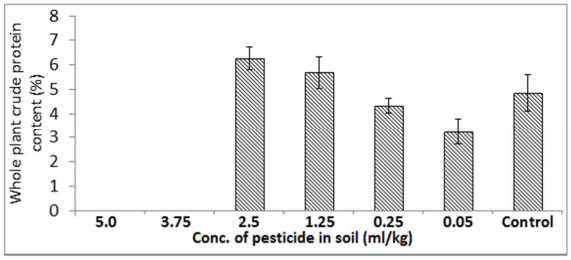

Fig. 3: Whole plant crude protein content of *E. indica* after exposure to pesticide in soil at 9 weeks after exposure

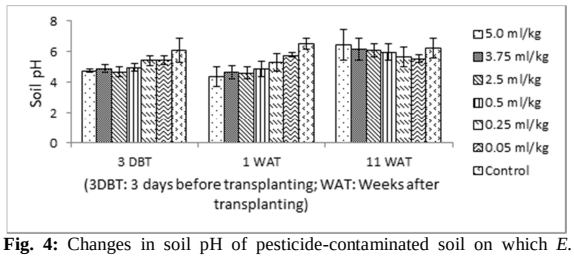

Fig. 4: Changes in soil pH of pesticide-contaminated soil on which *E. indica* was sown

Generally, soil was acidic (Fig. 4). pH of pesticide impacted soils ranged from 4.67–5.43 at 3 DBT, and from 5.53 – 6.43 at 11 WAT, thus indicating minimal increases with the introduction of test plant. Changes in pH of the pesticide-impacted soils showed low pH reading in 5ml/kg-PIS at 3 days before transplantation of test plant (3 DBT) (4.74) but a relatively higher pH 11 weeks after transplanting (11 WAT) (6.43). The pH of the control soil showed minimal changes throughout the experiment period (6.09–6.59). Conductivity of 5.0 ml/kg-PIS at 3 DBT was 30.5 µs/cm, compared to the control (36.6 µs/cm) (Fig. 5). These readings were lower compared to readings at 1 WAT (29 µs/cm and 19.64 µs/cm respectively), and at 11 WAT (34.6 µs/cm and 27.2 µs/cm, respectively). Changes in soil pH significantly disturb plant growth and development (Marschner, 2013). One of such ways is by affecting the accessibility of micronutrients and ions by plants. In present study, a pH range between 4 and 6 was obtained, which confirms that the soil was acidic. This has also been known to hamper plant development (Thompson *et al*., 2001). Notwithstanding, the pH range in the study; soils impacted with lower concentrations of the pesticides had lower pH values compared to higher pesticide concentrations at specific times. However, there was a general increase in soil pH as time of plant exposure increased. This study agrees with the report of Marscher (2013) that low performance growth is associated with gradual increase in soil pH values.

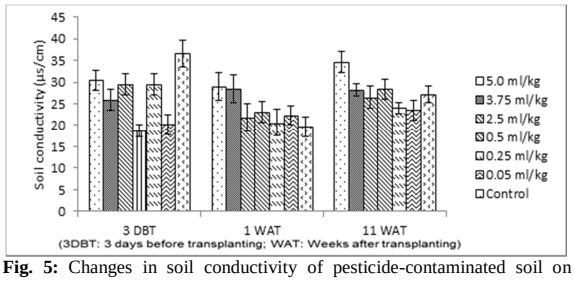

Fig. 5: Changes in soil conductivity of pesticide-contaminated soil on which *E. indica* was sown

Table 4: Microbial count (culturable) of soil three months after exposure to pesticide contaminant

Treatments	Bulk soil (x 10^2 cfu/g)		Rhizospheric soil (x 10^2 cfu/g)	
	Bacterial count	Fungal count	Bacterial count	Fungal count
5 ml/kg	2.4	2.0	1.9	5.6
3.75 ml/kg	1.4	2.3	2.5	5.9
2.5 ml/kg	1.1	7.6	1.5	5.2
0.5 ml/kg	4.3	7.1	2.1	4.6
0.25 ml/kg	3.1	7.7	3.2	8.6
0.05 ml/kg	4.1	9.4	4.1	8.1
Control	5.2	9.1	5.3	7.8

Table 4 shows total colony count of culturable bacteria and fungi in bulk and rhizospheric soils. Rhizospheric bacterial count varied from 0.15 x 10^3 cfu/g in 5 ml/kg-PIS to 0.53 x 10^3 cfu/g in the control; whereas fungal count ranged from 0.52 – 0.86 x 10^3cfu/g. Obviously, there were more bacteria than fungi. Microorganisms (Bacteria and fungi) throve well in the soil not impacted with pesticide. Microbial presence in the bulk soil was comparable with those in the plant's root zone. *Bacillus* species, Staphylococcus *aureus*, *Micrococcus* species, *Bacillus* sp. were prominent bacteria species (Table 5), whereas fungi species included *Aspergillus niger*, *Mucor* sp, *Trichordema* sp, and *Rhizopus* sp. The root zone is an area of rigorous interaction between the roots and soil. This is largely based on the composition, distribution and diversity of microorganisms. Although Karthikeyan *et al.* (2008) noted that microbial activity was better in the root zone than in the surrounding bulk soil, only minimal differences were recorded between total colony forming units in rhizospheric composition compared to those the bulk soil.

Total pesticide residual content after 11 weeks was 0.512 mg/kg in the 5 ml/kg-PIS treatment which lacked plant present due to plant death at the early week of plant exposure, indicating 10.24% reclamation (Fig. 6). Residual content ranged from 0.012 – 0.073 mg/kg in the treatments with plant presence, thus indicating the importance of plant presence. Remediation efficiency was also highest in the soil with the lowest concentration of pesticide in soil (>20% efficiency). This implies that the performance of the test plant as a phytoremediator may have been concentration-dependent. Similarly, percentage reclamation was 13.60–29.20% in treatments with surviving plant population. Plants adopt a number of mechanisms in the remediation of pesticide-impacted soils, including the dependence of their root structure for the accommodation of myriads of soil microorganisms which

directly utilize these compounds. *Eleusine indica* is one of many plants with enhanced fibrous rooting system. Similarly, when compared with the control, the roots of the test plant were more profuse when exposed to pesticide at low concentration (see Table 1). This may have also contributed to the >20% remediation of residual pesticide content of the soil. Yoshitomi and Shann (2001) suggested also that degradation of pesticide can take place in the rhizosphere of plants through the release of exudates from plants. Some of these exudates play a major role in plant-microbial interactions during pesticide utilization or degradation.

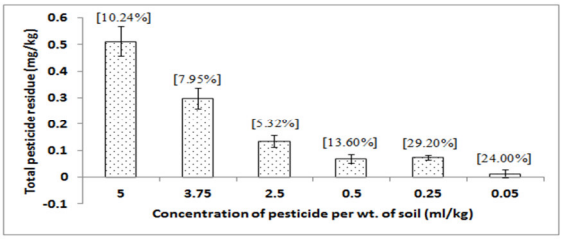

Fig. 6: Content of pesticide residue of soil at 11 weeks after exposure of plants to pesticide-impacted soils. Values in bracket represent percentage pesticide residue reclaimed.

During the degradation of target molecules like pesticides, soil-microorganism interactions lead to structural changes in the chemical constituents or total degradation. In most pesticide-polluted systems, bacteria and fungi co-exist to biotransform or degrade the pesticide (Briceño *et al.*, 2007). The fungi biotransform the pesticides by chemically inducing minor structural changes to the target molecule, thereby rendering it nontoxic, and then released into the environment. The former is further degraded by bacteria (Diez, 2010). The present study shows overabundance of fungi isolates compared to the bacteria (see Table 4), particularly in pesticide-impacted soils.

There was significant negative correlation between total fungal count of bulk soil and the total pesticide residual content of soil (-0.956). Similarly, highly negative correlation existed between soil pH and the total number of spikes produced per plant. There were no significant positive correlations among parameters compared.

Table 5: Bacterial and fungal isolates of rhizospheric and bulk soils three months after exposure to pesticide contaminant

Treatments	Bulk soil		Rhizospheric soil	
	Bacteria	**Fungi**	**Bacteria**	**Fungi**
5 ml/kg	*Staphylococcus aureus*, *Bacillus* sp., *Micrococcus* sp.	*Aspergillus niger*, *Trichoderma* sp., *A flavus*, *Penicillium* sp., *Mucor* sp.	*Bacillus* sp, *Staphylococcus aureus*	*A flavus*, *Mucor* sp.
3.75 ml/kg	*Staphylococcus* sp., *Bacillus* sp., *Micrococcus* sp.	*Penicillium* sp., *A. flavus* *Mucor* sp., *Aspergillus niger*, *Trichoderma* sp.	*Bacillus* sp., *Micrococcus* sp.	*Trichoderma* sp., *Aspergillus niger*, *Mucor* sp., *A flavus*
2.5 ml/kg	*Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp.	*Aspergillus niger*, *Mucor* sp., *Aspergillus niger*, *Penicillium* sp., *Mucor* sp.	*Bacillus* sp.	*Trichoderma* sp., *Fusarium* sp., *Mucor* sp., *Aspergillus niger*
0.5 ml/kg	*Bacillus* sp., *Micrococcus* sp.	*Microsporus* sp., *Trichoderma* sp., *A niger*, *Penicillium* sp., *Mucor* sp.	*Bacillus* sp.	*Trichoderma* sp., *Aspergillus niger*, *Mucor* sp., *A flavus*
0.25 ml/kg	*Bacillus sp*, *Staphylococcus* sp.	*Trichoderma* sp., *Microsporus* sp., *Mucor* sp., *A flavus*, *Penicillium* sp., *Aspergillus niger*	*Staphylococcus* sp., *Bacillus* sp., *Micrococcus* sp.	*Trichoderma* sp., *Penicillium* sp., *A flavus*, *Mucor* sp., *Aspergillus niger*
0.05 ml/kg	*Bacillus* sp., *Staphylococcus* sp.	*Aspergillus niger*, *Penicillium* sp., *Mucor* sp., *Trichoderma* sp., *Microsporus* sp.	*Bacillus* sp., *Micrococcus* sp.	*Aspergillus niger*, *Microsporus* sp., *Trichoderma* sp., *Penicillium* sp., *Rhizopus* sp, *Mucor sp.*
Control	*Bacillus* sp., *Micrococcus* sp.	*Aspergillus niger*, *Mucor* sp., *Penicillium* sp., *A flavus*, *Trichoderma* sp.	*Bacillus* sp., *Staphylococcus* sp.	*Trichoderma* sp., *Microsporus* sp. *Mucor* sp., *Aspergillus niger*

Table 6: Pearson Correlation of selected soil and plant parameters at 11 weeks after transplanting the test plant

Prot	1										
SoilpH	0.754	1									
BulkB	-0.798	-0.394	1								
BulkF	-0.518	-0.185	0.202	1							
RhizB	-0.664	-0.018	0.711	0.583	1						
RhizF	-0.536	0.076	0.713	0.655	0.842	1					
TPest	0.528	0.064	-0.266	-0.956*	-0.756	-0.709	1				
PlWt	-0.568	-0.067	0.781	0.007	0.803	0.518	-0.236	1			
RtBrNo	-0.156	-0.284	0.383	-0.754	-0.079	-0.315	0.653	0.518	1		
SpkNo	-0.827	-.0937*	0.361	0.414	0.234	0.033	-0.349	0.161	0.112	1	
PltHT	-0.369	-0.025	0.39	-0.095	0.615	0.128	-0.174	0.857	0.511	0.199	1
–	Prot	SoilpH	BulkB	BulkF	RhizB	RhizF	TPest	PltWt	RtBrNo	SpkNo	PltHT

*****Correlation is significant at the 0.05 level (2 tailed). Prot, Crude protein content of plant; SoilpH, Soil pH; BulkB, Total bacterial colony count of bulk soil; BulkF, Total fungal colony count of bulk soil; RhizB, Total rhizospheric bacterial colony count; RhizF, Total rhizospheric fungal colony count; TPest, Total residual pesticide conc. of soil; PlWt, Plant dry wt; RtBrNo, number of primary root branching; SpkNo number of spikes produced per plant; and PltHT, Plant height.

Conclusion

The widespread use of this pesticide over the years has resulted in problems by their interaction with the biological systems in the environment. Observing the effect of the toxicity, it is important to remove this pollutants from the environment. Biological removal using *Eleusine indica* grass (phytoremediation) becomes the choice of method since microorganisms can use the compounds for their growth and detoxify them. With excessive use of pesticide, environment hazards has led to problems such as deterioration of soil quality, leaching and reduced biodiversity.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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