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Effect of Diesel Fuel Contamination on Seed Germination and Growth of Four Agricultural Crops

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Abstract:

Plant toxicity bioassays through fast germinating agricultural crops can indicate the phytoremediation potential, effects on growth and survival and also assess extent of pollution. In the present study, the phytotoxic effect of diesel fuel contamination was studies on four agricultural crops namely *Zea mays, Vigna radiata, Sorghum vulgare* and *Pennisetum glaucum* at four levels of contamination. All the test plant species tolerated diesel fuel contamination at 2.5 - 5% levels and the total percent seed germination was between 43.7 to 86.7%. But fourth treatment level (7.5% diesel) significantly reduced the germination in *Zea mays* (74% decrease) followed by *Pennisetum glaucum* (67% decrease). Diesel fuel contamination also caused a reduction in the length of the radicle of the four crop plants studied. At 5% level of contamination, the longest radicle (1.92 cm) was recorded in *Vigna radiata* followed by *Zea mays* (1.36 cm). Also at 10% level of diesel contamination these two test species showed more radicle growth than *Sorghum vulgare* and *Pennisetum glaucum.* There was a reduction of radicle growth of all the species in subsequent treatment levels. Almost same trend was observed in plumule growth of all four species. Phytotoxicity bioassays results revealed that *Zea mays* and *Vigna radiata* species exhibited better growth and germination even at high concentration of diesel as compared to *Sorghum vulgare* and *Pennisetum glaucum*. Hence, these two species have higher potential for phytoremediation of diesel contaminated soils.

Keywords: Diesel, Phytotoxicity, Contamination, Germination, Bioassay, Phytoremediation

1.0 Introduction:

Contamination of soil by oil spills is a wide spread environmental problem that often requires cleaning up of the contaminated sites (Bundy *et al.,* 2002). These petroleum hydrocarbons adversely affect the germination and growth of plants in soils (Samina and Adams 2002). Oil spills affect plants by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them (Adam and Duncan, 2002). Phytoremediation is an alternative to more expensive remediation technologies because it is a feasible, effective and non-intrusive technology that utilizes natural plant processes to enhance degradation and removal of oil contaminants from the environment (Marmiroli and McCutcheon, 2003).

Phytotoxicity tests have been suggested as useful tools in assessing the risk of contaminated soil or to evaluate the efficacy of a remediation process (Haimi, 2000; Marwood *et al.,* 1998). Germination and root elongation are two critical stages in plant development that are sensitive to environmental contaminants (Baud-Grasset *et al.,* 1993). Plant height and shoot biomass are also good indicators of plant health and the sustenance of plant growth by the treated soil is an indication of enhanced bioremediation (Banks *et al.,* 2003). Many authors have reported a lower rate of germination in petroleum or its derivatives contaminated soil (Adam and Duncan, 2002; Vavrek and Campbell, 2002; Méndez-Natera *et al.,* 2004; Achuba, 2006; Smith *et al.,* 2006, Sharifi *et al.,* 2007; Korade and Fulekar, 2009; Ogbo, 2009). Petroleum hydrocarbons may form a film on the seed, preventing the entry of oxygen and water (Adam and Duncan, 2002) and toxic hydrocarbon molecules could inhibit the activities of amylase and starch phosphorylase and thereby affecting the assimilation of starch (Achuba, 2006). Henner *et al.* (1999) reported that petroleum hydrocarbons consisting of small molecules and those that are water soluble are more phytotoxic for the germination.

The most common and important symptoms observed in the plants contaminated with oil and its by products include the degradation of chlorophyll (Malallah *et al.,*1998), alterations in the stomatal mechanism and reduction in photosynthesis and respiration (Baker, 1970), increase in the production of stress-related phytohormones (Larcher, 2000), accumulation of toxic substances or their byproducts in vegetal tissue (Baker, 1970), decrease in size and less production of biomass (Brandt *et al.,* 2006; Daniel-Kalio and Pepple, 2006; Adenipekun *et al.,* 2008). The initial and main steps in the selection of the plants species for phytoremediation is the identification of species which are able to grow and develop in oil-contaminated soil, followed by an evaluation of the species role in the degradation of oil hydrocarbons and their by products (Merkl *et al.,* 2004). So, the objective of this study was to investigate the effect of soil contaminated with different concentrations of diesel oil on the germination and seedling growth of four agricultural plant species.

2.0 Materials and Methods:

2.1 Diesel Fuel and Test Crops:

Diesel fuel was purchased from a commercial filling station in Kurukshetra, Haryana and used for toxicity tests in hydroponic solution. The choice of the selected agricultural crops for the study was based on their universal acceptance as food and industrial raw materials. *Zea mays, Sorghum vulgare* and *Pennisetum glaucum* are important food, fodder and industrial crops (Food and Agricultural Organization, 2007; Rouanent, 1992), they are widely cultivated, consumed and service a number of industries as raw materials. *Vigna radiata* or mung bean or green gram has long been a food crop and is widely cultivated in Asia. It is also known as a useful green manure crop. Recently it has become of interest in many countries as a fodder crop.

2.2 Viability Test:

Seeds were tested for viability by the TZ test, which involves three steps: 1. Preconditioning (imbibition); 2. Preparation and staining (cutting the seed and then soaking the seed in a 2, 3, 5 triphenyl tetrazolium chloride solution) and 3. Evaluation (examining the seed for a color change in the embryo).

2.3 Experimental Design:

The experiment was a completely randomized block design with each treatment replicated three times. The experiment was conducted in glass petri dishes containing Whatman No.1 filter paper on the base of dishes. A modification of the methods described by Erute (2009) was adopted. Four different levels of diesel treatment were prepared with distilled water –2.5%, 5%, 7.5% and 10% diesel and distilled water was used as control. Ten seeds were placed in each Petri dish containing filter paper moisten with diesel contaminated water. Seeds were regarded germinated with the emergence of radical and plumule. Seed germination was monitored daily for cumulative percentage germination till there was no more seed germination (twenty days) and total germination (%) were recorded. Radicle and plumule length (cm) were recorded after twenty days when there was no more seed germination.

3.0 Results and Discussion:

The results obtained from present study are presented as follows.

3.1 Viability Test:

Hundred percent viability was obtained from the seeds using TZ test in case of *Zea mays, Vigna radiata, Sorghum vulgare* and *Pennisetum glaucum.*

3.2 Effect on Total Germination and Cumulative Germination:

The results of total seed germination at five treatment levels of diesel fuel contamination are showed in Figure 1 and of cumulative percent germination are shown in Figures 2a, 2b, 2c and 2d. In different levels of contamination, control had 100% seed germination (except *Sorghum vulgare* where germination was 96.4±5.8%), while a decrease in the percentage seed germination was observed, as the percentage of diesel fuel contamination increased because diesel fuel contamination has negative effect on the seed germination of agricultural crop. At 5% diesel treatment, almost 37% decrease in germination was observed in *Vigna radiata,* 47-57% in *Zea mays, Sorghum vulgare* and *Pennisetum glaucum.* But at 10% diesel treatment, *Sorghum vulgare* showed highest germination (33.4±5.8%) followed by *Vigna radiata* (26.7±5.8%) and *Zea mays* (23.4±5.8%).

The results of cumulative percent germination clearly showed that percentage of total germination and cumulative germination exhibited differences in germination patterns. At control, *Zea mays, Vigna radiata* and *Pennisetum glaucum* showed highest total germination (100%) but amongst these *Vigna radiata* exhibited fastest germination rate followed by *Zea mays* and *Pennisetum glaucum*. At 2.5% diesel contamination, *Vigna radiata* and *Zea mays* exhibited fastest germination rate followed by *Sorghum vulgare* and *Pennisetum glaucum* respectively. Same trend was observed in case of 5% diesel and 10% diesel, but at 7.5% diesel contamination, *Sorghum vulgare* showed exceptionally high germination equal to *Vigna radiata* and the germination rate was also comparable.

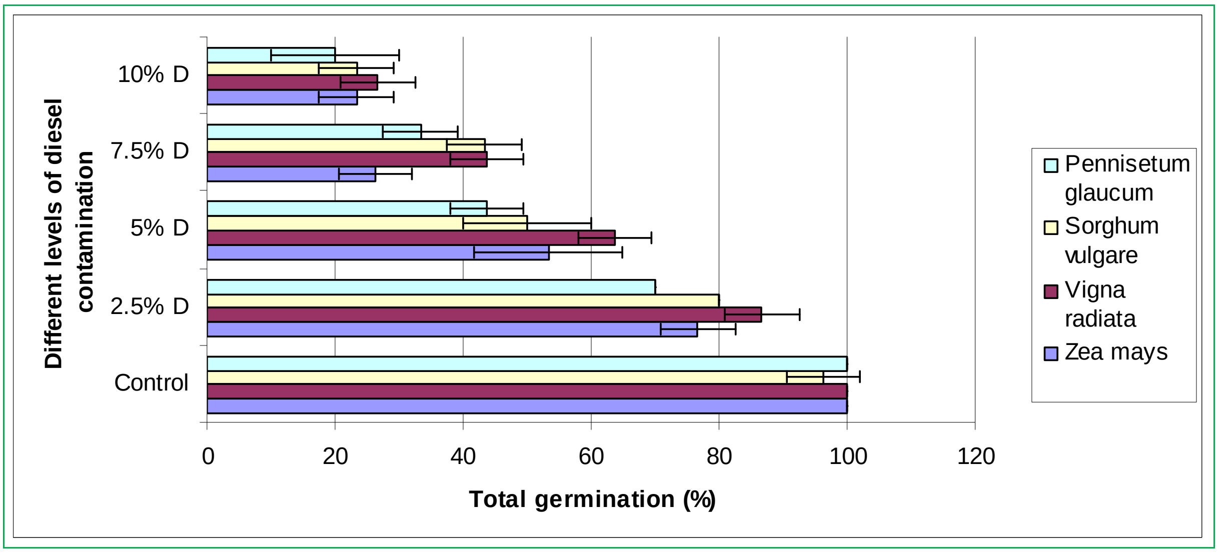

Fig.1: Total germination of the four plant species at different levels of diesel contamination

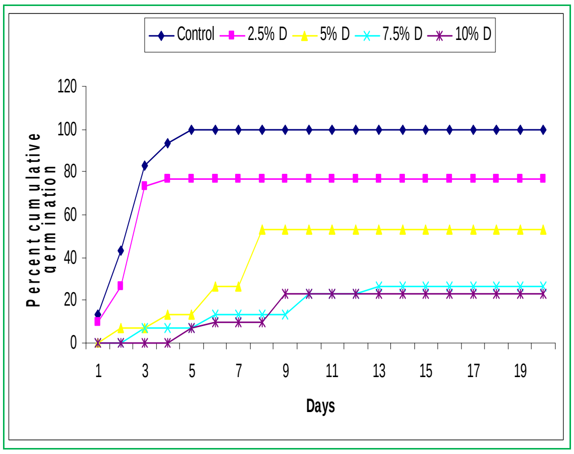

Fig.2(a): Percent Cumulative Germination of *Zea mays* seeds at different levels of Diesel contamination

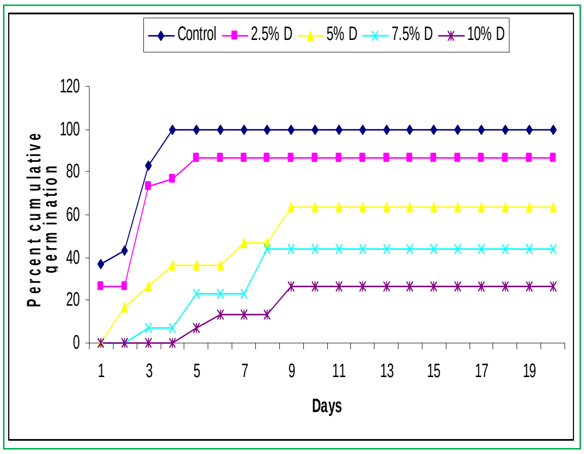

Fig.2(b): Percent Cumulative Germination of *Vigna radiata* seeds at different levels of Diesel contamination

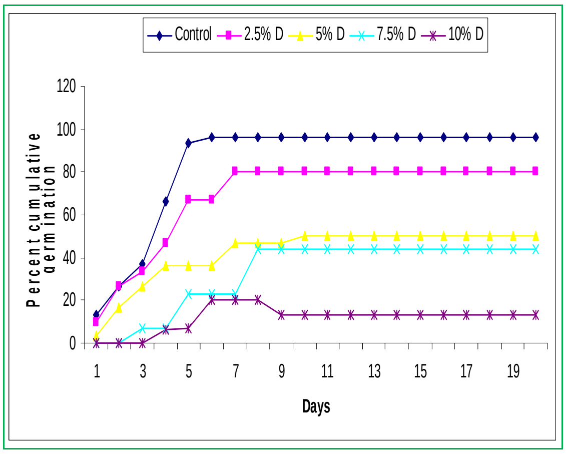

Fig.2(c): Percent Cumulative germination of *Sorghum vulgare* seeds at different levels of Diesel contamination

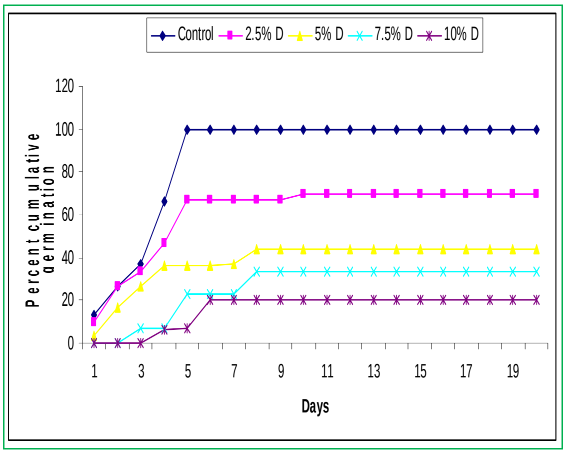

Fig.2(d): Percent Cumulative germination of *Pennisetum glaucum* seeds at different levels of Diesel contamination

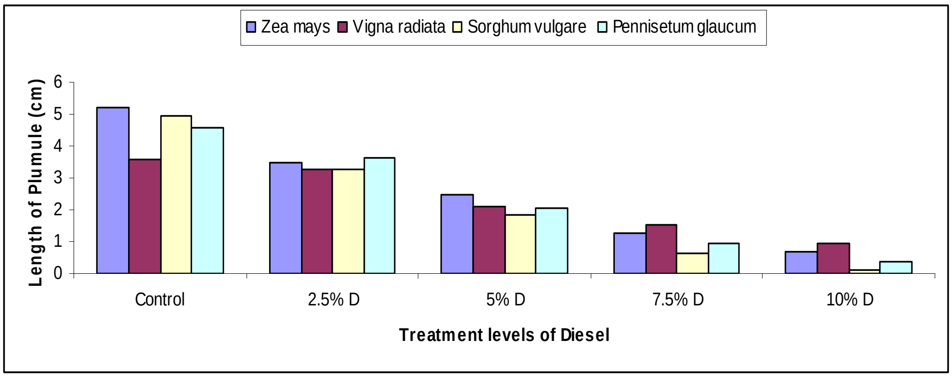

Fig.3: Length of plumule of four crop plants in response to different levels (%) of diesel oil contamination

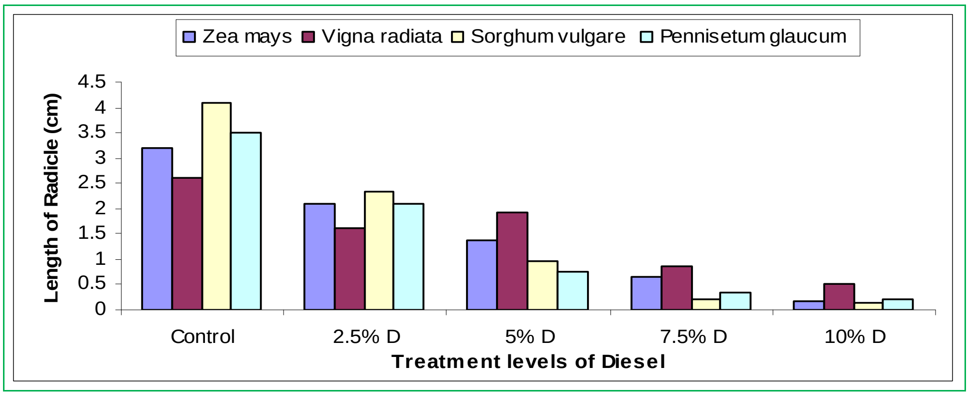

Fig.4: Length of radicle of four crop plants in response to different levels (%) of diesel oil contamination

3.3 Effect of Diesel Contamination on Plumule Growth:

The effect of different level of diesel contamination on plumule growth is presented in Figure 3. In control, *Zea mays Sorghum vulgare* and *Pennisetum glaucum* showed higher plumule growth than *Vigna radiata,* but at 2.5% level almost same plumule growth was observed in four test crop plants. In subsequent treatment levels up to highest diesel concentration, *Zea mays* and *Vigna radiata* maintained higher plumule growth than *Sorghum vulgare* and *Pennisetum glaucum.* These results indicate that the initial growth rate may be lower in *Zea may* and *Vigna radiata* but these species have tolerance to high diesel contaminations among all test species.

3.4 Effect of Diesel Contamination on Radicle Growth:

The results of effect of different level of diesel contamination are presented in Figure 4. In first and second treatment level, *Sorghum vulgare* and *Pennisetum glaucum* exhibited higher radicle growth than *Zea mays* and *Vigna radiata.* But in third, fourth and fifth level treatment, where diesel concentration increased, the radicle length was higher in *Vigna radiata* and *Zea mays.* The variations observed between species could possibly be because of their tolerance capacity and the role of genetic variation establishing individuality of species. However, significant differences exist between various levels of contamination.

Evidence from the results showed that diesel fuel contamination has negative effect on the four test agricultural crops under study. The results also indicated that the four crop plants can tolerate the contaminant by germinating successfully in it. The highest percentage germination was recorded in control (no contamination) for the four crop plants. All the test plants tolerated diesel fuel contamination at 2.5 - 5% levels as total percent seed germination was between 86.7±5.8 - 43.7±5.8 %. But third treatment level (7.5% diesel) significantly reduced the germination in *Zea mays* (74% decrease) followed by *Pennisetum glaucum* (67% decrease). At fourth level treatment, *Vigna radiata* showed highest germination followed by that *Zea mays* and *Pennisetum glaucum.* The cumulative percent germination also exhibits the higher germination rate and total germination by seeds of *Vigna radiata.* This confirmed the highest tolerance capacity of *Vigna radiata* among four test crops and after this *Zea mays* showed higher total germination and germination rate as compared to *Pennisetum glaucum* and *Sorghum vulgare.*

Kirk *et al.* (2002) have also recorded that four species of grasses germinated successfully in different levels of petroleum hydrocarbon contamination and on the basis of that, these species have recommended for phytoremediation of petroleum hydrocarbon contaminated soils. In this study also, a decrease in the percentage germination was recorded as the level of contamination increased. A similar effect was also observed by Adam and Duncan (2002) who reported reduction in germination rate caused by petroleum contaminants in several plant species, mainly in commercial crops. The process of phytoremediation is a complex one. The toxicity effect noticed in contaminated soils might not be just due to the contaminant concentration but also due to soil type and properties, hydrocarbon type, microbial community composition and plant species (Salanitro *et al.,* 1997).

The level of contamination determines the extent of damage and also inhibition. At high levels of contamination (5%-10% diesel) although there was germination in some crop plants, there was reduction in the seed germination of these crop plants and this affected the length of the radicle and plumule. This trend was also recorded for six agronomic plants by Issoufi *et al.* (2006). Similar results were also observed by other scientists i.e. Anoliefo and Vwioko (1995) and Anoliefo and Edegbai (2000), that petroleum hydrocarbons can inhibit the growth of plants. In their study, crop plants like *Abelmoschus esculentus* (okro), *Lycopersicon esculentum* (tomato), *Capsicum annuum* (pepper), *Solanum melongena* (egg plant) and *Solanum incanum* failed to germinate at 6% level of contamination, whereas in this study 4% was lethal to the germination of *Vigna unguiculata* and *Sorghum bicolor*. This indicates that the lethal concentrations of petroleum contaminants to different plants vary. The effect could also be as a result of formation of polar compounds dissolved in water that could penetrate the seed coat, exerting polar narcosis (Wang *et al.,* 2000; Adam and Duncan, 2002).

Diesel fuel contamination caused a reduction to varying degrees in the length of the radicle of the four crop plants studied. At 5% level of contamination, the longest radicle (1.92 cm) was recorded in *Vigna radiata* followed by *Zea mays* (1.36 cm). Also at 10% level of diesel contamination these two test species showed higher radicle growth than *Sorghum vulgare* and *Pennisetum glaucum.* There was a reduction of plumule growth of all the species in subsequent treatment levels. Almost same trend was observed in plumule growth of four species also. This may have been due to an inhibitory effect of some of the diesel's polycyclic aromatic hydrocarbon components which are more soluble than the aliphatic hydrocarbons (Wang and Bertha, 1990; Trapp *et al.,* 2001; Molina-Barahoma *et al.,* 2005). Therefore, *Zea mays* and *Vigna radiata* had higher total germination, germination rate, plumule and radicle length even at higher level of diesel contamination as compared to other two species studied and hence, had higher potential for phytoremediation. Issoufi *et al.* (2006) have also suggested that *Z. mays* can be used for phytoremediation of petroleum contaminated soils since, germination rates was higher in this species as compared to six other agronomic plants studied.

4.0 Conclusion:

The study reached the conclusion that higher level contamination by diesel fuel has negative effect on the all test agricultural crops under study. But, *Zea mays* and *Vigna radiata* species exhibited better growth and germination even at high concentration of diesel, so, these can be used for phytoremediation of diesel contaminated soils. On the other hand, bioassays using sensitive plants like *Sorghum vulgare* and *Pennisetum glaucum* can evaluate soil pollution and remediation because a plant's reactions can reflect the level of pollution in its environment. The results indicate a wider possibility for utilizing tolerant agricultural species in remediation on contaminants and sensitive species to monitor the level of polluted soil.

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