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EFFECTS OF CRUDE OIL POLLUTED SOIL ON THE SEEDLING GROWTH OF *PENNISETUM GLAUCUM* (L.) R. BR.

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Abstract: Pollution by crude oil is an important environmental issue all around the world. Increase in oil pollution level in the environment produce toxic effects on flora and fauna of the region. The effects of different levels (0%, 5%, 10%, 15%, and 20%) of crude oil polluted soil on the growth of pearl millet (*Pennisetum glaucum*) were studied. The polluted soil affected the root, shoot length, seedling size, number of leaves and leaf area of *P. glaucum*. The significant ($p<0.05$) effects of polluted soil on fresh and dry weight of root, stem, leaves, and seedling of *P. glaucum* were also recorded. Leaf area, leaf number and total seedling dry weight were noticeably reduced in 10 and 15% polluted soil than control soil treatment. Principally, 20% crude oil polluted soil treatment exhibited highest percentage of decrease in most of the seedling growth parameters of *P. glaucum* than control. Hence, the effects on seedling growth parameters were increased with increasing levels of polluted soil. For most of the growth parameters, the mean values obtained were found higher for the control soil and progressively decreased from 5-20% crude oil polluted soils. The seedlings of *P. glaucum* were also tested for tolerance to polluted soil treatment. The results showed that the seedlings of *P. glaucum* showed high percentage of tolerance to low concentration (5%) of polluted soil treatment as compared to control soil treatment (0%).

Keywords: Contamination, crude oil, growth, *Pennisetum glaucum*, soil.

Introduction

Crude oil contains a wide variety of elements combined in various forms [ABB, 1997]. The petroleum products are strongly enriched with hydrocarbons, leaving most crude based inorganic materials and other types of organic compounds [POTTER & SIMMONS, 1998]. The effect of crude oil pollution from an accidental blowout of an oil well on soil pH, temperature, crude oil content and its flora was studied [DEBOJIT, 2006]. The oil pollution significantly affected the soil environment and reduced the number of plant species and vegetation productivity. Perennials are less affected then the annuals. About 84% of the plant species were reported wiped out in the highly oil contaminated site. Crude oil is a naturally occurring hydrocarbon compound used by humans in a variety of ways: fuelling of cars, lorries and trucks; heating of homes, cooking and other fractions utilized in the manufacture of synthetic products [EDEMA & al. 2009].

The industrial revolution of the past century has resulted in significant damage to environmental resources such as air, water and soil [ABEDI-KOUPA & al. 2007]. Soil, which is the result of interaction between climate, organisms and mother rock and formed under specific topographic conditions within a certain time, is an essential physical component that

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covers extensive areas above the lithosphere. The conservation of soil which is the base of plant growth became a national demand. Soil profiles are subjected to a continuous exhaustion in several forms, such as: soil erosion, depletion of nutrients and pollution [ODAT & ALSHAMMARI, 2011]. Petroleum and its products are of specific concern in pollution studies due to their structural complexity, slow biodegradability, bio-magnification potential and above all the serious health hazards associated with their release into the environment and their transport across the world frequently result in oil spillage, contaminating the soil and water alike [KATHI & KHAN, 2011]. Spent engine oil is the hydrocarbon product of crude oil and it is unsatisfactory for growth of *Azadirachta indica* A. Juss due to insufficient reaction of the soil and the microbes because of the displacement of air from spaces between soil particles [ALAMU, 2012]. It has been observed by various researchers also that oil spills have significant effects on agricultural lands, crop, trees, forest, and their seedlings [UDO & FAYEMI, 1975; BARTHA, 1977; UDO & OPARA, 1984]. Seed germination, percentage of seedling emergence, number of root nodules/plant, total soluble sugars, total soluble proteins, free amino acids, total chlorophyll and carotenoids and nucleic acids of the leaves of *Vigna mungo* (L.) Hepper grown in the oil polluted soil decreased significantly due to persistence of hydrocarbon [ILANGOVAN & VIVEKANANDAN, 1992]. The study of plant behavior in petroleum contaminated soils allows the identification and selection of oil pollution indicating species [MARANHO & al. 2009].

The continuous growth of environmental pollution and anthropological disturbances to ecosystems has made the study of abiotic stress responses in plants [ALKIO & al. 2005]. Effects of waste engine oil pollution on physical and chemical properties of soil have been observed [ATUANYA, 1987; EKUNDAYO & OBUEKWE, 1994; BENKA-COKER & EKUNDAYO, 1995]. Effects of crude oil pollution on the growth of some plant species viz. *Zea mays, Abelmoschus esculentus, Capsicum frutescens, Capsicum annuum, Lycopersicon esculentum* were reported by AMAKITRI & ONAFEGHARA, 1983; ANOLIEFO & VWIOKO, 1994. The effects of crude oil contaminated soils on seedling growth of six agronomic crop species observed and concluded that *Zea mays* and *Glycine max* seedlings show the greatest potential to enhance remediation compared to *Medicago sativa*, *Lolium perenne*, *Triticum aestivum* and *Vicia villosa* [ISSOUFI & al. 2006].

Oil pollution is an important problem in many parts of the world. The disposal of used lubricating oil into the immediate environment is an important environmental issue affecting on plant growth. Therefore, the present study was carried out with the aim to study the effects of crude oil polluted soil on the growth of an important annual grass pearl millet (*Pennisetum glaucum* (L.) R. Br.).

Materials and methods

This study was conducted at the Department of Botany, University of Karachi, Pakistan. Seeds of *Pennisetum glaucum* were obtained from the local market. Spent crude oil was obtained from Motor Transport Workshop located at University of Karachi Campus. Spent oil was used rather than fresh one because the former would more closely mimic in nature. Seeds of *P. glaucum* were sown in garden soil at 1 cm depth in large earthen pots placed in natural environmental conditions of Department of Botany, University of Karachi. The pot was kept moist by adding water when necessary. Initially the experiment was conducted with high level of polluted soil but all the plants died. Later 20% crude oil polluted soil was chosen as the highest level of polluted soil other levels were 5%, 10%, 15% polluted oil soil. The experiment was lasted for 35 days. Seedlings were allowed to grow in the pots for 7-8 days to enable a reasonable height of 3 cm. Seedlings of equal height were selected and transplanted into small pots of 7.3 cm in diameter and 9.6 cm in depth having different ratios of crude oil contaminated soil (5% crude oil contaminated soil + 95% garden soil; 10% crude oil contaminated soil + 90% garden soil; 15% crude oil contaminated soil + 85% garden soil; 20% crude oil contaminated soil + 80% garden soil) and garden soil without crude oil contamination used as a control. One seedling of *P. glaucum* was planted in a small pot representing each treatment and this was replicated five times and the experiment was completely randomized. Normal water was added to all the samples when necessary in order to keep the soil moist and no nutrient solution was provided. The height of the plants was measured from the soil level to the terminal bud using a steel scale. This was done at a regular interval of seven days. The numbers of leaves were counted as the plant grew. This was done by visual counting of the leaves at regular intervals of seven days. The plants were carefully uprooted after 35 days and the root part rinsed with clean water. The fresh weight of the root, stem and leaves was then determined separately and kept separated part of the plant in marked paper envelope and finally place in an oven at 80 degree centigrade for 24 hours to obtain the dry weight. Fresh and oven dried weights for roots, shoot, leaves and total plant weight was recorded. Leaf area were determined by multiplying length and breadth and multiply 2/3. While the tolerance indices was determined by the following formulae:

Mean root length in oil polluted soil concentration / Mean root length in without oil polluted soil concentration x 100

The means as well as standard errors were calculated. Data collected were subject to one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) using personal computer software packages COSTAT version 3.00. Level of significance for these tests was at P <0.05.

Results

The effect of different levels (0%, 5%, 10%, 15%, 20%) of crude oil polluted soil on the seedling growth performances of pearl millet *Pennisetum glaucum* was recorded (Figs. 1-3). The oil pollution contamination affected root, shoot length, seedling size, number of leaves and leaf area significantly (p<0.05) as compared to control (Fig. 1). The mean root, shoot, seedling length, number of leaves and leaf area of *P. glaucum* was found high in control soil treatment. The treatment of 5% and 10% oil polluted soil decreased root, shoot and seedling length of *P. glaucum* as compared to control soil. Number of leaves and leaf area also decreased at similar contamination of crude oil. The mean values obtained for root length (11.74 cm), shoot length (9.10 cm), seedling length (20.84 cm), number of leaves (7.40), leaf area (3.71 sq. cm) were highest in control soil. The seedling length (18.10 cm), root length (10.04 cm), number of leaves (6.80), leaf area (3.77 sq. cm) were found significantly low when treated with 05% crude oil soils. Further increase in treatment of crude oil polluted soil treatment at 10% significantly reduced seedling growth (17.74 cm), root length (9.48 cm), number of leaves (5.80) and leaf area (2.87 sq. cm) of *P. glaucum* as compared to control. The lower mean values obtained for seedling length (13.86 cm), root length (6.28 cm), number of leaves (3.40) and leaf area (1.95 sq. cm) were found for 15% crude oil treated soils. A slight increase in seedling length (14.32 cm) and root length (7.90 cm) were found for the 20% crude oil treated soils as compared to 15% oil polluted soil treatment. The significant effects of crude oil polluted soil on root, shoot, leaves and seedling fresh weight were observed (Fig. 2). The fresh biomass production of *P. glaucum* in terms of total seedling fresh weight (1.09 g), root weight (0.38 g), shoot weight (0.389 g) was recorded in control soil. The lowest seedling fresh weight (0.14 g), root weight (0.03 g) and leaf weight (0.05 g) was recorded in 20% polluted soil treatment. The effects of oil polluted soil on root, shoot, leaves and seedling dry weight were also observed (Fig. 3). A significant ($p<0.05$) relationship was found to exist between the inhibitory effects of oil polluted soil on seedling dry weight with the increase in polluted soil treatment concentrations as compared to control. 20% crude oil polluted soil exhibited more reduction in seedling dry weight of *P. glaucum* as compared to control. Similarly biomass production of *P. glaucum* in terms of total seedling dry weight (0.06 g), root dry weight (0.01 g), shoot dry weight (0.02 g) and leaf dry weight (0.02 g) was recorded in 20% oil polluted soil as compared to control soil treatment.

Oil polluted soil treatment at different concentration (5, 10, 15, 20%) decreased high percentage of root, shoot, seedling length, number of leaves, leaf area and seedling fresh and dry weight of *P. glaucum* as compared to control treatment (Tab. 1). Oil polluted soil treatment of 5% concentration was found responsible for high percentage of decrease in seedling length (13.14%), root length (10.87%), shoot length (11.42%), number of leaves (8.10%) and leaf area (0.27%) of *P. glaucum* as compared to control, while polluted soil at 10% and 15% concentration was found responsible for further decrease in seedling length, root length, number of leaves and leaf area *P. glaucum* as compared to control. A slight increase in root and seedling enhanced at 20% concentration of oil polluted soil as compared to 15% polluted oil soil. Similarly, oil polluted soil treatment at 5-20% concentration was found responsible for decrease in seedling fresh and dry weight of *P. glaucum* as compared to control (Tab. 1).

The seedlings of *P. glaucum* were also tested for percentage of tolerance to polluted oil soil treatment as compared to control (Tab. 2). The results showed that seedlings of *P. glaucum* have greater tolerance to low concentration of oil polluted soil treatment as compared to control. Increase in oil polluted soil treatment decreased the low percentage of tolerance indices in seedlings of *P. glaucum* as compared to control. According to tolerance test it was observed that seedlings of *P. glaucum* showed high percentage of tolerance (85.51%) with the treatment of 5% crude oil polluted soil as compared to control soil treatment. The treatment of 10% crude oil contaminated soil showed low percentage of tolerance (80.74%) in seedlings of *P. glaucum* as compared to garden soil treatment. Similarly, seedling growth of *P. glaucum* showed lowest percentage of tolerance (53.49%) in 15 % crude oil contaminated soil treatment.

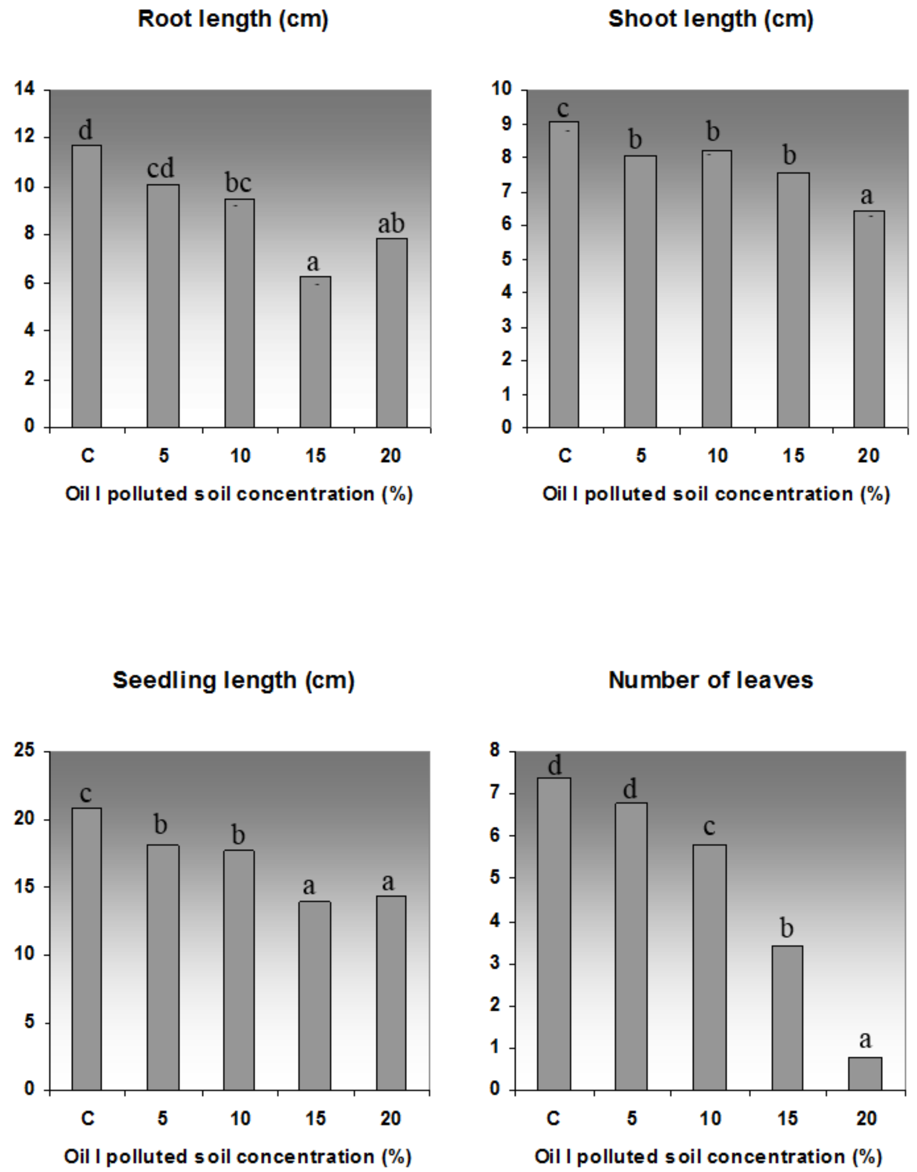

Fig. 1. Effects of different concentration of polluted soil on root, shoot, seedling length (cm) and number of leaves of P. *glaucum* as compared to control.

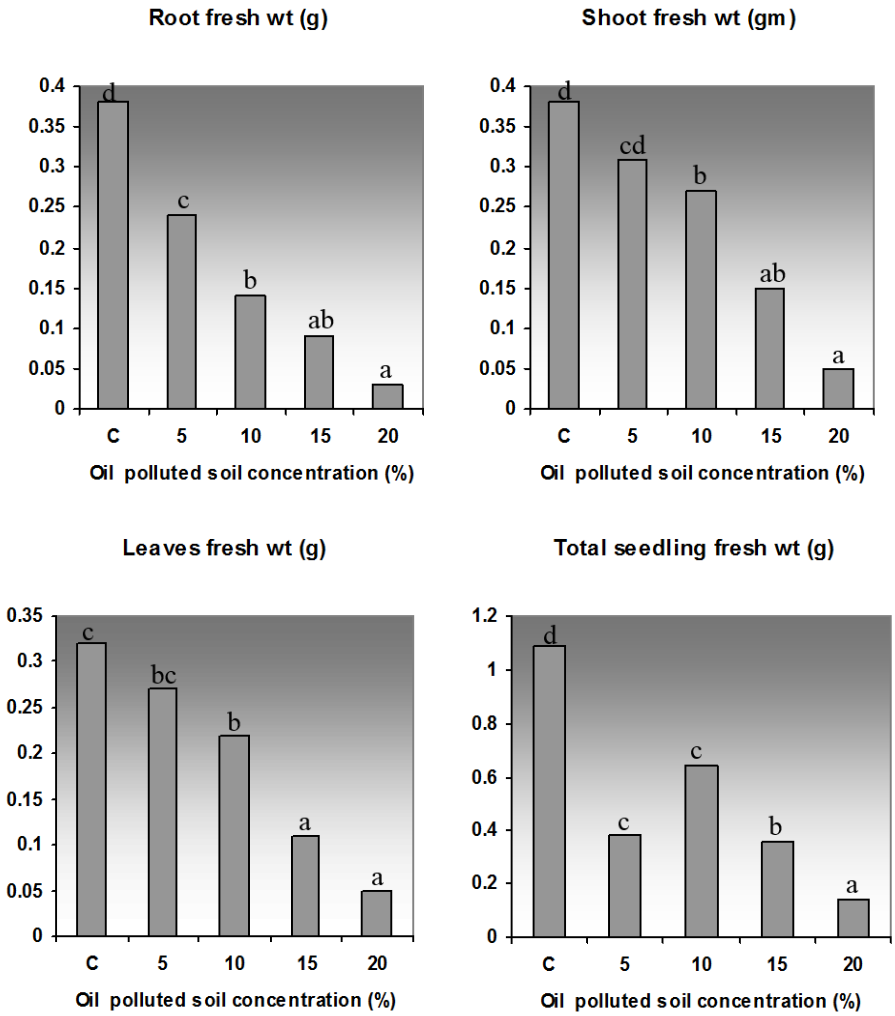

Fig. 2. Effects of different concentration of polluted soil on root, shoot, leaves and total seedling fresh weight (g) of P. *glaucum* as compared to control.

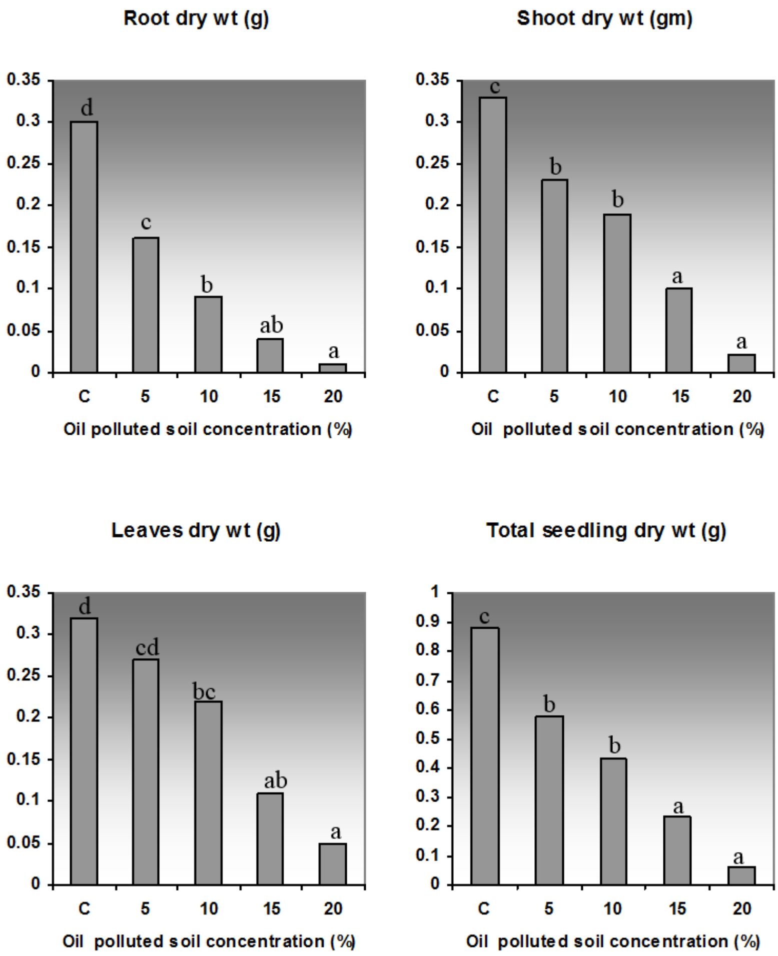

Fig. 3. Effects of different concentration of polluted soil on root, shoot, leaves and total seedling dry weight (g) of P. *glaucum* as compared to control.

Tab. 1. Percentage decrease in seedling growth parameter and biomass production of *Pennisetum glaucum* under different concentration (05, 10, 15, 20%) of crude oil polluted soil as compared to control soil (0%).

Growth parameters	Soil concentration (%)			
	05	**10**	**15**	**20**
Seedling length	13.14	15.06	33.49	31.28
Root length	14.48	19.25	46.50	32.70
Shoot length	11.42	9.89	16.70	29.45
Number of leaves	8.10	21.62	54.05	89.18
Leaf area	0.27	22.64	47.43	59.56
Root fresh weight	36.84	63.15	76.31	92.10
Shoot fresh weight	20.51	64.10	76.92	92.30
Leaf fresh weight	12.82	31.25	65.62	77.14
Seedling fresh weight	65.13	41.28	66.97	87.15
Root dry weight	46.66	53.84	86.66	96.66
Shoot dry weight	30.30	42.42	69.69	93.93
Leaf dry weight	20.83	41.66	66.66	91.66
Seedling dry weight	34.09	51.13	73.86	93.18

Tab. 2. Percentage of tolerance in seedlings of *P. glaucum* against different (05, 10, 15, 20%) concentrations of crude oil polluted soil treatment as compared to control.

	Oil polluted soil concentration (%)			
	5	**10**	**15**	**20**
Tolerance indices	85.51	80.74	53.49	67.29

Discussion

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention [MILLIOLI & al. 2009]. The results of the present study showed variability in seedling growth performance of *Pennisetum glaucum* as compared to control soil treatment. In the present studies the treatment of different level of oil polluted soil showed a clear variation in seedling growth performance of *P. glaucum* as compared to control soil treatment. Seedling length of *P. glaucum* was variable and appeared to be driven mostly by the treatment of different level of oil polluted soil treatment. The toxic effects of spent engine oil on chlorophyll and protein levels of *Amaranthus hybridus* and germination of perennial rye grass and maize growth performance was reported by some workers [ISIRIMAH & al. 1989; ODJEGBA & SADIQ, 2002; SIDDIQUE & ADAMS, 2002]. This study demonstrated that crude oil application at high concentration in soil has significant effect on the seedling growth performance of *P. glaucum.* These results are in conformity with the findings of other researcher's recording the effects of crude oil on the growth of few plant species [ANOLIEFO & al. 2003; VWIOKO & FASHEMI, 2005]. Reduction in the number of leaves of *P. glaucum* recorded when treated with different level of crude oil soil as compared to control. Similar trend of decline in leaf growth was recorded by ANOLIEFO & EDEGBAI (2001). The negative effects of oil contamination on the reduction of the total biomass and the length of the roots in *Avena sativa*, *Secale cereale* and *Hordeum vulgare* was recorded and suggested that these plants could be used as test organisms in analyzing the toxicity of pollutants in soil and water [PETUKHOV & al. 2000]. In another investigation, MARANHO & al. (2006) investigated the effect of petroleum pollution on the leaf structure of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae), and concluded that the leaf anatomy revealed a large variability related to pollution. Our data also showed the negative influence of oil polluted soil on leaf area of *P. glaucum* as compared to control soil treatment. The relatively low leaf area was observed in oil polluted soil at the level of 5, 10, 15 and 20% polluted soil. The availability of crude oil in soil make unsuitable environment for the development of root development for *P. glaucum*. Crude oil is phytotoxic because it creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to inhibited aeration of the soil [EDEMA & al. 2009].

Crude oil contamination at higher level (10-20%) affected root growth performance of *P. glaucum* due to development of unsuitable growth condition by oil pollution. Oil in soil creates unsatisfactory conditions for plant growth [DE JONG, 1980] probably due to insufficient aeration of the soil [ROWELL, 1977]. The contamination with petroleum affects the development of plants due to different physical effects. The oil film that covers the roots, modifying water absorption and nutrients considered as the main physical effect [XU & JOHNSON, 1995; HESTER & MENDELSSOHN, 2000; PEZESHKI & al. 2000]. According to BONA & SANTOS (2003) oil diminishes the soil capacity for retaining water, thus interfering with plant growth. The seedlings biomass of *P. glaucum* was least productive in oil contaminated soil. The continuous decrease in the seedling growth of growth parameter of *P. glaucum* in this study revealed that it is due to abiotic stress. Increase in the crude oil contamination which contains a wide variety of elements such as carbon, hydrogen, sulphur, nitrogen and oxygen [ABB, 1997; POTTER & SIMMONS, 1998; MARANHO & al. 2009] could be an important cause of decline in average shoot length of *P. glaucum*. There was a similar trend of negative effects on the productivity of *P. glaucum* recorded. The addition of different level of crude oil can leads to some physical and chemical changes in the soil resulting reduction in seedling growth performance of *P. glaucum*. The presence of the toxic pollutants from the crude oil in soil can be an important cause of decrease in seedling growth and ultimately resulted in reduction of biomass production performance for *P. glaucum*. Crude oil contains components that are toxic to plants [JESSUP & LEIGHTON, 1996].

Conclusions

It is concluded that the significant reduction in the seedling growth and production of *P. glaucum* grown in the crude oil contaminated soil can be served as good pollutant indicator of crude oil pollution. The results also showed that seedlings growth performance of *P. glaucum* decreased with the increase in oil polluted soil treatment as compared to control. Similar types of studies are suggested for other plant species to ascertain their possible use of plantation in oil polluted areas.

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