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Phytotoxicity Test to Check the Effectiveness of Mycoremediation to Support Seed Germination and Plant Growth in *Withania somnifera* (L.) Dunal

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Authors' contributions

This work was carried out in collaboration between both authors. Author KS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NSKH supervised and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

In the present study, the effect of different treatments on the growth parameters of *Withania somnifera* (L.) Dunal was evaluated. Soil samples were collected from the oil spill site by stratified random sampling method, i.e. near the well (NW), 20 metre distance, 40 metre distance, 60 metre distance and 80 metre distance from the oil spill well. Eight different treatments were applied to crude oil contaminated soil samples from five different distances. The treated soil samples were incubated for 14 days, and transferred to root trainers in a glass house and then the seeds of *Withania somnifera* (L.) Dunal were sown. It was kept for four months under a glass house and net house conditions. Growth parameters like collar diameter, shoot height, root length, fresh weight, dry weight and percentage germination of plants were measured in each treatment. Two-way analysis of variance was carried out to study the effect of treatments, distances and their interaction on growth parameters of *Withania somnifera* (L.) Dunal. Best values of the plant parameters (like

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collar diameter = 2.21 mm; shoot height = 3.200 cm; root length = 12.6 cm; fresh weight = 0.526 g; dry weight = 0.132 g) were obtained when the soil was sterilised and bioaugmented with yeast extract, molasses and *Aspergillus terreus*.

Keywords: Withania somnifera; soil; collar diameter; shoot height; root length; fresh weight; dry weight; percentage germination.

1. INTRODUCTION

The impacts of crude oil and other petroleum products on the ecosystem and various organisms have been reported by several authors [1,2,3,4,5,6]. Oil pollution in any form is toxic to plants and soil micro-organisms [7,8]. According to Wyszkowski et al. [9] because of the toxicity widespread presence and complex nature of petroleum, pollution due to petroleum has become a serious problem. In soil, petroleum hydrocarbons adversely affect the germination and growth of plants [10]. Oil spills affect plants adversely by creating anaerobic conditions which make essential nutrients like nitrogen and oxygen unavailable to plants.

The impacts of chemicals on the environment can be detected through toxicity testing. Toxicity tests have been suggested as useful tools in assessing the risk of contaminated soil or to evaluate the efficacy of a remediation process [11,12].

Phytotoxicity assays involve the use of plants to determine the toxicity of chemicals. It helps in selecting plant species that are able to withstand high levels of contaminants and screening out those, which are not able to establish themselves in such conditions, such as contaminated sites [13]. According to Omosun et al. [14], the responses of plants to pollutants provide a simple and cost effective method of environmental pollutants monitoring. Plant bioassays such as measurements of seed germination and early seedling growth have been used to monitor treatment effects of oil-contaminated sites [15]. This is because germination and root elongation are two critical stages in plant development that are sensitive to environmental contaminants [16]. Plant height and shoot biomass are also good indicators of plant health and the sustenance of plant growth by the treated substrates or soil is an indication of enhanced bioremediation [17].

Environmental pollutants and toxic substances are assessed with various organisms. Because of their stationary nature, plants are mostly preferred for toxicity tests. They eliminate the fear of source identification improvement improving and points of pollution mostly experienced with the use of animals. Plant bioassays such as measurements of seed germination and early seedling growth have been used to monitor treatment effects and restoration of oil contaminated sites [15].

For plant effect monitoring, pollution induced changes in individual parameters of *Withania somnifera* (L.) Dunal was quantified. Different accessions of *W. somnifera* (L.) Dunal were used to assess the toxicity of crude oil. It involved the study of different parameters like shoot height, root length, collar diameter, fresh weight, dry weight and percentage germination. The study is important because the results can help in the biological monitoring of soils to estimate the level of contamination. It is hoped that the data obtained will help to guide both the foresters on the accession that can be planted in soil with different levels of crude oil contamination and the environmentalists on the accession that can be tried for remediation of crude oil contaminated soils.

2. MATERIALS AND METHODS

2.1 Sample Collection

Crude oil contaminated soil samples were collected from the oil spill site of Oil and Natural Gas Corporation Limited, Gandhar Asset. GGS II site, Ankleshwar, Gujarat. As the spill occurred from the oil producing well and covered an area of 40 x 90 m (3,600 sqm) therefore, samples were collected at different distances from the well i.e. near the well (NW), at 20 m, at 40 m, at 60 m and at 80 m distances from the well. Distances were measured by using measuring tapes. Samples were collected in wide mouth glass jar with Teflon lined cap, brought to the laboratory and stored in a deep refrigerator at -20ºC as recommended by Weisman [18].

2.2 Soil Sterilisation

25 g of crude oil contaminated soil sample was taken in a 100 ml conical flask. It was sterilised in an autoclave at 121ºC, 15 psi for 20 min, which was then cooled down to 45-50ºC.

2.3 Fungus

Aspergillus terreus was isolated from crude oil contaminated soil sample by serial dilution technique. While, *Schizophyllum commune* Fr. was obtained from the National Type Culture Collection (NTCC), Forest Pathology Division, Forest Research Institute, Dehradun for this study. Fungal culture was maintained on potato dextrose agar slants and plates.

2.4 Amendments / Supplements

Molasses was obtained from Doon Valley Distilleries, Kuanwala, Dehradun. Yeast extract (RM 027) of Himedia Laboratories (India) was used as a nitrogen source in the present study. Molasses (1% w v^{-1}) and yeast extract (1% w v^{-1}) were prepared in distilled water and autoclaved at 121ºC, 15 psi for 20 min which was then cooled down to 45-50ºC.

Treatments applied were as follows:

T1 : Positive control (Unsterilised crude oil contaminated soil-25 g)- **US**
T2 : Unsterilised crude oil contaminated soil (25 g) + 1% Yeast extract (3 ml) + 1% Molasses (2 ml)- **USYM**
T3 : Unsterilised crude oil contaminated soil (25 g) + 1% Yeast extract (3 ml) + 1% Molasses (2 ml) + *Schizophyllum commune* (3 disc)- **USYMFSC**
T4 : Unsterilised crude oil contaminated soil (25 g) +1% Yeast extract (3 ml) + 1% Molasses (2 ml) + *Aspergillus terreus* (3 disc)- **USYMFAT**
T5 : Sterilised crude oil contaminated soil (25 g) + 1% Yeast extract (3 ml) + 1% Molasses (2 ml)- **SYM**
T6 : Sterilised crude oil contaminated soil (25 g) + 1% Yeast extract (3 ml) + 1% Molasses (2 ml) + *S. commune* (3 disc) - **SYMFSC**
T7 : Sterilised crude oil contaminated soil (25 g) + 1% Yeast extract (3 ml) + 1% Molasses (2 ml) + *A. terreus* (3 disc) - **SYMFAT**
T8 : Negative control - Untreated normal soil.

In the present study, BOD (biochemical oxygen demand) incubators were used for the growth and storage of crude oil contaminated soil samples along with the fungal cultures. These incubators are the most versatile and reliable low temperature incubators designed to provide a controlled and contaminant-free environment by regulating conditions of temperature, humidity and CO_2 (26°C temperature, 95% humidity and 5% CO_2 were set for this experiment). These soils were kept in the incubators for 14 days at 26±1°C, and then transferred to root trainers in a glass house and sowed with the seeds of *Withania somifera* (L.) Dunal. It was kept for four months under a glass house and net house condition. Growth parameters like collar diameter was measured using Vernier caliper in millimetre (mm), shoot height was measured using scale in centimetre (cm), root length was measured using scale in cm, fresh weight was measured on electronic weighing machine in grams (g), dry weight was measured on electronic weighing machine after drying the seedling in air incubator for 3 hours at 70°C and percentage germination of plants was measured [19,20,21].

Percentage germination of seeds was calculated with the following formula:

> Percentage germination = {(Number of seeds that germinated / Number of seeds sown) x 100}

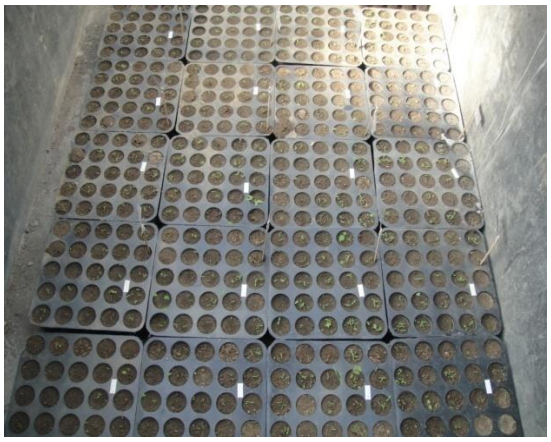
2.5 Data Analysis

Two-way analysis of variance (ANOVA) was carried out to study the effect of treatments, distances and their interaction on growth parameters of *Withania somnifera* (L.) Dunal and grouping was done based on Tukey's test (P < 0.01 at 1% level of significance).

3. RESULTS

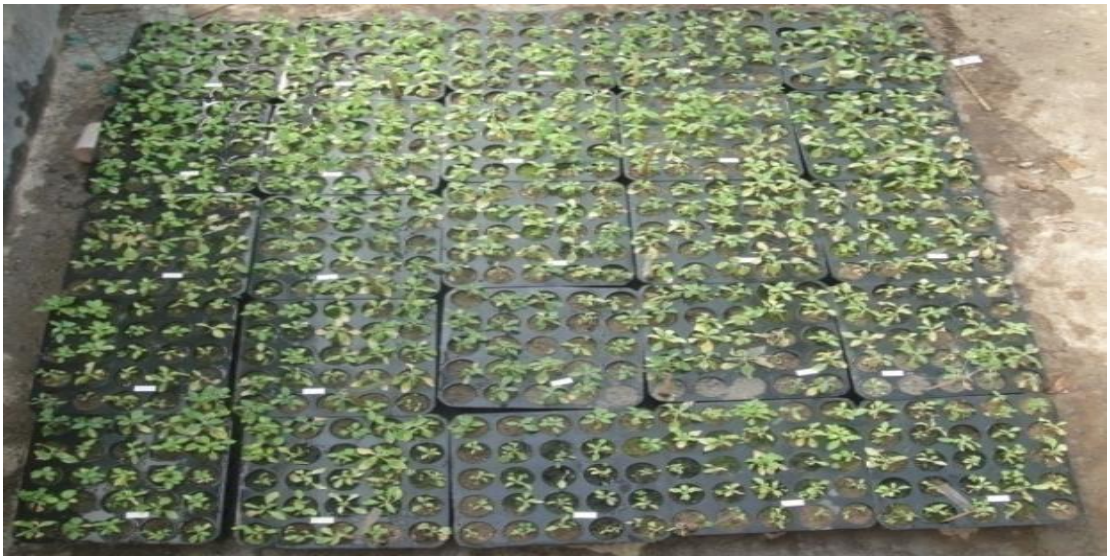
Seed germination percentage of *W. somnifera* (L.) Dunal in contaminated soil was 96.64%. Tables 1-3 show the outcomes of two-way ANOVA carried out to study the effect of treatments, distances and their interaction on growth parameters of *Withania somnifera* (L.) Dunal. The effect of treatments, distances and their interaction were significant and grouping was done based on Tukey's test (P < 0.01 at 1% level of significance). Table 1 shows that significant maximum value of 0.526 g fresh weight was observed at 80 m distance when the soil was sterilised, supplemented with yeast

***Withania somnifera* (L.) Dunal**



a) Experimental set up in Glass house

b) Seedlings after 120 days in net house



c) Seedlings of all treatments after 120 days

Fig. 1. Glass house experiment to check the ability of treated soil to support plant growth (*W. somnifera* (L.) Dunal)

extract and molasses and inoculated with *Aspergillus terreus*. It was followed by the fresh weight values (0.444 and 0.456g) which were not significantly different at 60 m and 80 m distances in sterilised soil enriched with nutrients and inoculated with *S. commune* (SYMFSC) treatment, fresh weight values (0.452 and 0.443) at 60m and 40 m distance in SYMFAT, and 0.431 g at 80 m distance in unsterilised soil, supplemented with nutrients and inoculated with *A. terreus* (USYMFAT) treatment. Minimum value (0.145 g) of fresh weight near the well was observed in treatment where the unsterilised crude oil contaminated soil (US) was used for seed germination. Maximum values of fresh weights in each treatment were observed at 80 m distance followed by 60 m, 40 m, 20 m and near well distances. Plants grown on normal uncontaminated soil showed maximum fresh weight value (0.586 g) as compared to the values of contaminated soil.

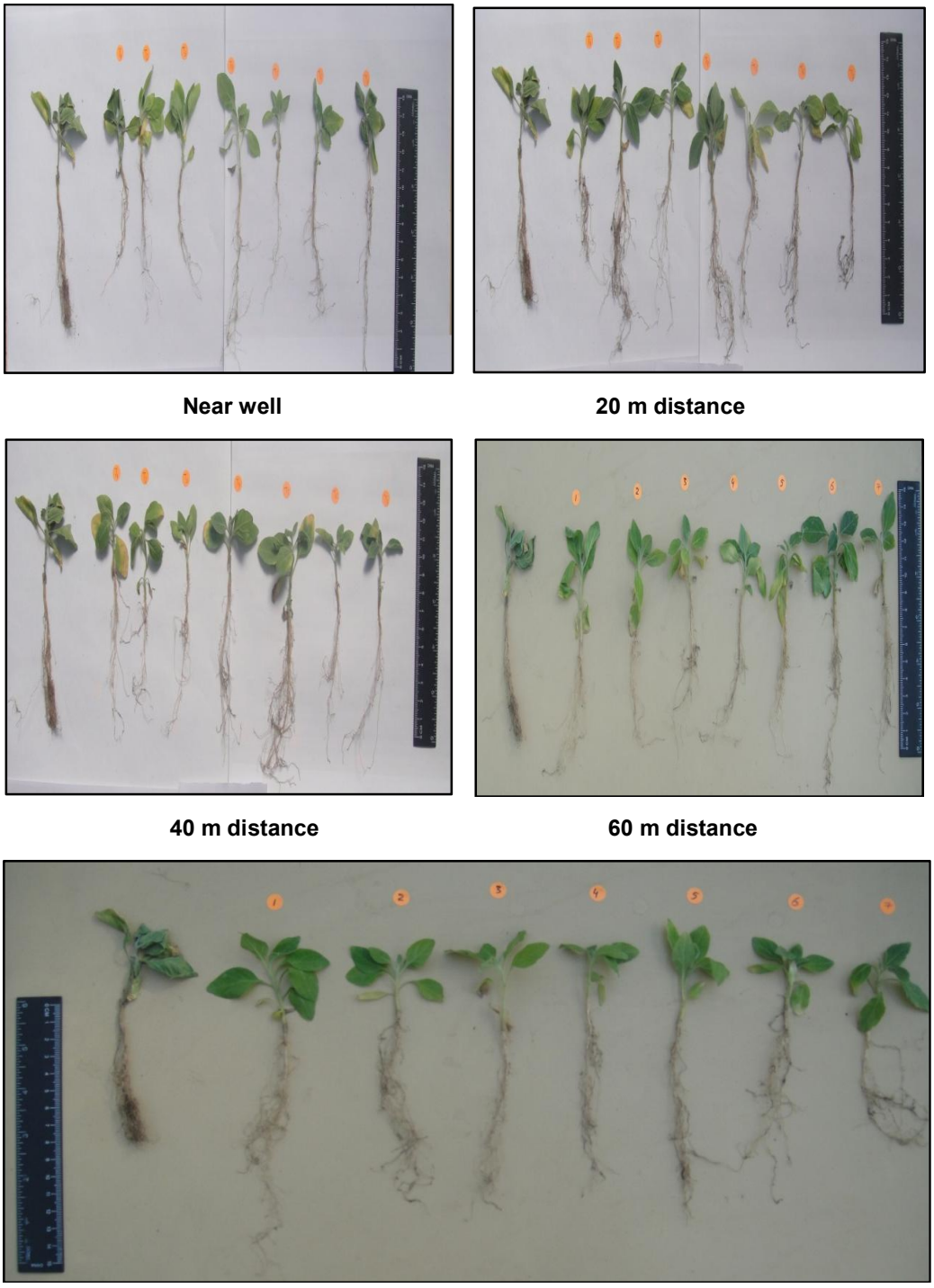


Fig. 2. Seedlings of *Withania somnifera* (L.) Dunal grown on different treatments

Where, numbers 1 = Unsterilised crude oil contaminated soil (Positive Control), 2 = Unsterilised crude oil contaminated soil with yeast extract and molasses, 3 = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, 4 = Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, 5 = Sterilised crude oil contaminated soil with yeast extract and molasses, 6 = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, 7 = Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 1. Effect of different treatments on the fresh weights (g) of *W. somnifera* (L.) Dunal [Values are means ± SE of 3 replicates]

Distance → Treatments ↓	Near Well	20m	40m	60m	80m	Mean
US	**0.145±0.004^{a}**	0.146±0.007^{a}	0.159±0.003^{a}	0.317±0.003^{efg}	**0.330±0.008^{fg}**	0.219±0.017
USYM	0.163±0.009^{a}	0.167±0.011^{ab}	0.238±0.018^{cd}	0.338±0.009^{g}	0.339±0.014^{g}	0.249±0.016
USYMFSC	0.175±0.011^{abc}	0.177±0.006^{abc}	0.260±0.017^{de}	0.351±0.009^{g}	0.354±0.009^{g}	0.263±0.016
USYMFAT	0.250±0.010^{d}	0.336±0.011^{g}	0.368±0.015^{gh}	0.375±0.014^{gh}	**0.431±0.010^{hi}**	0.352±0.013
SYM	0.227±0.010^{bcd}	0.264±0.009^{de}	0.269±0.011^{def}	0.362±0.016^{g}	0.363±0.012^{g}	0.297±0.012
SYMFSC	0.256±0.008^{de}	0.340±0.012^{g}	0.362±0.016^{g}	**0.444±0.015^{i}**	**0.456±0.012^{i}**	0.371±0.015
SYMFAT	0.332±0.008^{fg}	0.348±0.009^{g}	0.443±0.012^{i}	0.452±0.017^{i}	**0.526±0.015^{j}**	0.420±0.015
Mean	0.221±0.010	0.254±0.014	0.300±0.015	0.377±0.009	0.399±0.012	

Where, US = Unsterilised crude oil contaminated soil (Positive Control), USYM = Unsterilised crude oil contaminated soil with yeast extract and molasses, USYMFSC = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, USYMFAT= Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, SYM = Sterilised crude oil contaminated soil with yeast extract and molasses, SYMFSC = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, SYMFAT= Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 2. Effect of different treatments on the dry weights (g) of *W. somnifera* (L.) Dunal [Values are means ± SE of 3 replicates]

Distance → Treatments ↓	Near Well	20m	40m	60m	80m	Mean
US	**0.036±0.001^{a}**	0.044±0.001^{ab}	0.059±0.002^{bcd}	0.063±0.002^{bcde}	**0.085±0.001^{efgh}**	0.057±0.003
USYM	0.044±0.0008^{abc}	0.045±0.001^{abc}	0.066±0.0007^{cde}	0.074±0.001^{defg}	0.090±0.001^{fghi}	0.063±0.004
USYMFSC	0.044±0.001^{abc}	0.064±0.001^{bcde}	0.072±0.002^{def}	0.083±0.004^{efgh}	0.090±0.002^{fghi}	0.070±0.003
USYMFAT	0.056±0.002^{abcd}	0.083±0.0008^{efgh}	0.110±0.005^{ijkl}	0.114±0.004^{jkl}	**0.121±0.007^{klm}**	0.097±0.005
SYM	0.046±0.001^{abc}	0.065±0.001^{bcde}	0.095±0.001^{ghij}	0.099±0.002^{hij}	0.117±0.008^{jklm}	0.084±0.005
SYMFSC	0.066±0.001^{bcde}	0.085±0.001^{efgh}	0.115±0.004^{jkl}	0.122±0.007^{klm}	**0.132±0.006^{lm}**	0.103±0.005
SYMFAT	0.084±0.001^{efgh}	0.109±0.008^{ijk}	0.122±0.002^{klm}	0.126±0.011^{klm}	**0.137±0.01^{m}**	0.116±0.005

Where, US = Unsterilised crude oil contaminated soil (Positive Control), USYM = Unsterilised crude oil contaminated soil with yeast extract and molasses, USYMFSC = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, USYMFAT= Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, SYM = Sterilised crude oil contaminated soil with yeast extract and molasses, SYMFSC = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, SYMFAT= Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 3. Effect of different treatments on the collar diameters (mm) of *W. somnifera* (L.) Dunal [Values are means ± SE of 3 replicates]

Distance → Treatments ↓	Near Well	20m	40m	60m	80m	Mean
US	**1.060±0.02^{a}**	1.154±0.03^{abc}	1.158±0.02^{abc}	1.174±0.01^{abcd}	**1.320±0.016^{efg}**	1.173±0.019
USYM	1.112±0.04^{ab}	1.174±0.04^{abcd}	1.206±0.02^{bcde}	1.216±0.03^{bcde}	1.354±0.023^{fg}	1.212±0.020
USYMFSC	1.160±0.02^{abc}	1.236±0.02^{bcdef}	1.240±0.008^{bcdef}	1.298±0.02^{defg}	1.426±0.022^{g}	1.272±0.019
USYMFAT	1.276±0.04^{cdef}	1.282±0.04^{cdef}	1.320±0.02^{efg}	1.352±0.02^{fg}	**1.922±0.013^{J}**	1.430±0.051
SYM	1.240±0.01^{bcdef}	1.250±0.01^{cdef}	1.264±0.03^{cdef}	1.316±0.03^{efg}	1.916±0.021^{j}	1.397±0.054
SYMFSC	1.354±0.02^{fg}	1.362±0.02^{fg}	1.570±0.014^{h}	1.596±0.023^{h}	**2.178±0.052^{k}**	1.612±0.062
SYMFAT	1.426±0.01^{g}	1.646±0.01^{hi}	1.738±0.008^{i}	1.752±0.016^{i}	**2.212±0.036^{k}**	1.755±0.053
Mean	1.232±0.023	1.300±0.028	1.356±0.035	1.386±0.034	1.761±0.062	

Where, US = Unsterilised crude oil contaminated soil (Positive Control), USYM = Unsterilised crude oil contaminated soil with yeast extract and molasses, USYMFSC = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, USYMFAT= Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, SYM = Sterilised crude oil contaminated soil with yeast extract and molasses, SYMFSC = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, SYMFAT= Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 4. Effect of different treatments on the shoot heights (cm) of *W. somnifera* (L.) Dunal [Values are means ± SE of 3 replicates]

Distance→ Treatments↓	Near Well	20m	40m	60m	80m	Mean
US	**1.400±0.100^{a}**	1.520±0.020^{abc}	1.960±0.040^{abcdefgh}	2.000^{abcdefgh}	**2.200±0.122^{defghij}**	1.816±0.068
USYM	1.440±0.040^{ab}	1.660±0.213^{abcd}	2.000^{abcdefg}	2.100±0.100^{bcefghi}	**2.240±0.112^{defghij}**	1.888±0.077
USYMFSC	1.700±0.200^{abcde}	1.740±0.166^{abcdef}	2.100±0.100^{bcdefghi}	2.200±0.122^{defghij}	2.340±0.103^{efghij}	2.016±0.078
USYMFAT	1.900±0.100^{abcdefg}	2.200±0.122^{defghij}	2.300±0.122^{defghij}	2.480±0.020^{ghij}	**2.600±0.291^{hijk}**	2.296±0.081
SYM	1.840±0.160^{abcdefg}	1.900±0.100^{abcdefg}	2.140±0.140^{cdefghij}	2.400±0.100^{fghij}	2.500^{ghij}	2.156±0.070
SYMFSC	2.060±0.116^{abcdefghi}	2.260±0.112^{defghij}	2.400±0.170^{fghij}	2.500^{ghij}	**2.800±0.122^{jk}**	2.404±0.069
SYMFAT	2.240±0.150^{defghij}	2.400±0.100^{fghij}	2.500±0.055^{ghij}	2.700±0.122^{ijk}	**3.200±0.122^{k}**	2.608±0.082
Mean	1.797±0.067	1.954±0.069	2.200±0.048	2.340±0.049	2.554±0.075	

Where, US = Unsterilised crude oil contaminated soil (Positive Control), USYM = Unsterilised crude oil contaminated soil with yeast extract and molasses, USYMFSC = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, USYMFAT= Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, SYM = Sterilised crude oil contaminated soil with yeast extract and molasses, SYMFSC = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, SYMFAT= Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 5. Effect of different treatments on the root length (cm) of *W. somnifera* (L.) Dunal [Values are means ± SE of 3 replicates]

Distance → Treatments ↓	Near Well	20m	40m	60m	80m	Mean
US	**6.000^{a}**	8.100±0.100^{bc}	9.000^{cd}	9.100±0.100^{cde}	**9.300±0.300^{de}**	8.300±0.256
USYM	7.100±0.100^{ab}	9.600±0.187^{def}	9.700±0.200^{defg}	9.900±0.100^{defgh}	10.000^{defghi}	9.260±0.229
USYMFSC	7.200±0.200^{b}	9.700±0.122^{defg}	9.900±0.331^{defgh}	10.000^{defghi}	10.800±0.374^{ghijk}	9.520±0.268
USYMFAT	7.700±0.200^{b}	10.000^{defghi}	10.800±0.122^{ghijk}	11.000±0.447	**11.800±0.200^{klm}**	10.260±0.303
SYM	7.400±0.245^{b}	9.900±0.100^{defgh}	10.600±0.400^{fghij}	10.700±0.200^{fghijk}	10.900±0.400^{hijkl}	9.900±0.290
SYMFSC	7.800±0.200^{b}	10.100±0.100^{defghij}	10.900±0.100^{hijkl}	11.100±0.400^{ijkl}	**12.000^{lm}**	10.380±0.303
SYMFAT	8.000^{bc}	10.200±0.200^{efghij}	11.000^{hijkl}	11.200±0.200^{jkl}	**12.600±0.400^{m}**	10.600±0.321
Mean	7.314±0.119	9.657±0.122	10.271±0.141	10.428±0.152	11.057±0.209	

Where, US = Unsterilised crude oil contaminated soil (Positive Control), USYM = Unsterilised crude oil contaminated soil with yeast extract and molasses, USYMFSC = Unsterilised crude oil contaminated soil with yeast extract, molasses and S. commune, USYMFAT= Unsterilised crude oil contaminated soil with yeast extract, molasses and A. terreus, SYM = Sterilised crude oil contaminated soil with yeast extract and molasses, SYMFSC = Sterilised crude oil contaminated soil with yeast extract, molasses and S. commune, SYMFAT= Sterilised crude oil contaminated soil with yeast extract, molasses and A. terreus

Table 2 presents that maximum values of dry weights were observed at 80 m distance followed by 60 m, 40 m, 20 m and near well distances. Maximum value of dry weight (0.137 g) was observed with the sterilised soil supplemented with nutrients and inoculated with *A. terreus* (SYMFAT). This was not significantly different from the values (0.126 and 0.122 g) at 60 m and 40 m distance, values (0.132 and 0.122 g) observed at 80 m and 60 m distances, respectively with the sterilised soil supplemented with nutrients and inoculated with *S. commune* (SYMFSC) treatment. The minimum value of 0.036 g dry weight was observed at near the well when unsterilised crude oil contaminated soil (US) was used for the seed germination of *W. somnifera* (L.) Dunal. Plants grown on normal uncontaminated soil had maximum dry weight value (0.221 g) as compared to the values of contaminated soil.

Maximum values of collar diameters were observed (Table 3) at 80 m distance followed by 60 m, 40 m, 20 m and near well distance soil. The maximum value of collar diameter (2.212 mm) was observed with the sterilised soil, supplemented with nutrients and inoculated with *A. terreus* (SYMFAT) and the sterilised soil supplemented with nutrients and inoculated with *S. commune* SYMFSC (2.178 mm). The values of above mentioned treatments were not significantly different at 80 m distance. Minimum values were observed with unsterilised (US) treatment at all distances of contaminated soil as compared to the values of other treatments. Plants grown on normal uncontaminated soil showed maximum collar diameter (2.482 mm) as compared to the values of contaminated soil.

Tables 4 and 5 display the outcomes of two-way ANOVA carried out to study the effect of treatments, distances and their interaction on shoot heights and root lengths of *Withania somnifera* (L.) Dunal. The effect of treatments and distance was significant ($P < 0.01$) while their interaction effect was not significant ($P > 0.01$) for both the plant parameters. Grouping was done based on Tukey's test ($P < 0.01$ at 1% level of significance). Table 4 shows that maximum value (3.2 cm) of shoot height was observed at 80 m distance with the sterilised soil supplemented with nutrients and inoculated with *A. terreus* (SYMFAT) treated soil. This was not significantly different from the value 2.7 cm observed at 60 m distance and 2.8 cm of shoot height observed at 80 m distance with the sterilised soil supplemented with nutrients and inoculated with *S. commune* (SYMFSC) treatment. Minimum value was observed with unsterilised crude oil contaminated soil (1.4 cm) at near the well. Plants grown on normal uncontaminated soil showed maximum shoot height (3.9 cm) as compared to the values of contaminated soil.

Table 5 shows that the maximum value of root length (12.6 cm) observed at 80 m distance with sterilised soil supplemented with nutrients and inoculated with *A. terreus* were not significantly different from the value of 12.00 cm observed at 80 m distance with the sterilised soil supplemented with nutrients and inoculated with *S. commune*. The minimum value of 6.0 cm was observed in unsterilised soil (US) near the well. Plants grown on normal uncontaminated soil showed maximum root length value (13.2 cm) as compared to the values of contaminated soil. Therefore, with *Withania somnifera* (L.) Dunal, maximum values of each plant parameter were observed with the sterilised soil enriched with yeast extract and molasses and inoculated with *A. terreus* (SYMFAT) and *S. commune* (SYMFSC) treatment at 80 m distance, while the minimum value of each plant parameter was observed with unsterilised soil (US) collected from near the well.

4. DISCUSSION

Best values of all plant parameters with *Withania somnifera* (L.) Dunal were obtained with natural uncontaminated normal soil (used as a negative control) followed by the sterilised soil, supplemented with nutrients and treated with *A. terreus* and *S. commune* of 80 m distance soil. The minimum values were obtained when the seeds were sown on unsterilised crude oil contaminated soil. Mycoremediation degraded the oil and reduced the toxicity as observed with the work of [19] who investigated the ability of white rot fungus (*Pleurotus tuber-regium*) to ameliorate crude oil polluted soil and support seed germination, plant height, biomass accumulation and root elongation values of test plants and decrease in total hydrocarbon content, when seeds were planted 14 days post soil treatment.

The impact of chemicals on the environment can be detected through toxicity testing. In the present study, there was poor germination of seeds in contaminated soil while germination was better in soils which had been treated with fungi and nutrients. One of the possible reasons

of germination inhibition in crude oil contaminated soil may be unsatisfactory soil conditions because of insufficient aeration and a decrease in air filled space and increased demand of oxygen by oil decomposing microorganisms [22,23].

In the present study, crude oil contamination affected the growth indices of the plant negatively as shown by reduced biomass, plant height, collar diameter and root length as compared to the normal uncontaminated soil. Plants that are able to grow in contaminated sites take up long chain heavy alkanes into their roots rapidly and slowly translocate them into stems and leaves as a result of their low solubility in water [24].

In the present study, the uncontaminated soil had better biomass as compared to the contaminated soils. The reduction in the biomass is oil–concentration dependent, i.e. the leaf, stem and root biomass decreased with an increase in crude oil level in the soil. The results of the present study indicate a negative interaction between the soil crude oil content and biomass accumulation in *W. somnifera* (L.) Dunal which is in conformity with the reports of Agbogidi, Agbogidi and Eshegbeyi and Agbogidi et al. [25,26,27]. They noted that from oil polluted soil hydrocarbons accumulate in the chloroplasts of leaves, thereby reduce photosynthetic ability and affect translocation due to obstruction of the xylem and phloem vessels, hence reduction in photosynthate and biomass content occur.

In the present study, the reduction in plant height relative to control may have resulted from a systemic toxic effect of translocation of long chain alkanes to stems [28] or this could be due to the reduction of the mineral element with increasing oil concentration in the soil. This finding is similar to that of Odjegba and Sadiq [29], who also observed a significant effect of engine oil on the leafy vegetable *Amaranthus hydrides* L. This effect could also be as a result of reduced availability of mineral elements because according to Omosun et al. [30], plant nutrition is based not only on the presence of mineral elements in the soil but their availability.

Root length of seedlings of the species in contaminated soil was shorter as compared to the seedlings grown on uncontaminated normal soil. Baud-Grasset et al. [16] reported that a reduction in root length is a sensitive plant response to the exposure to chemical substances. Collar diameters of contaminated soil were lower than the values of collar diameters of uncontaminated natural soil. Presence of some toxic oil constituents which were absorbed by the roots and received by the mesophyll cells of the leaves could have inhibited the leaf growth [1] and its ability to function in photosynthesis and consequently the general plant growth including collar growth was retarded. Other explanation for this reduced growth can be the effect of small aliphatic, aromatic, napthalic and phenolic like compounds in crude oil that may reduce respiration, transpiration, photosynthesis II and hormonal stress response [1,31]. Diesel fuel is not a systemic killer, it kills plants cells on contact as contamination by diesel fuel can kill the roots, and this prevents the plant from taking up water and other nutrients. It can disrupt plant and water relationship in the soil [32].

5. CONCLUSION

The growth of *Withania somnifera* (L.) Dunal was found to be effective after treating the soil samples with different treatments. Best growth values in all plant parameters were obtained with *Aspergillus terreus* treated soil samples in unsterilised as well sterilised treatments followed by the soil samples which were treated with *Schizophyllum commune*. The oil degradation capacity of both the fungus increased as the distance increased from the well when the soil was treated for 14 days. Therefore, better growth results were obtained at 80 m distance soil sample followed by 60 m distance, 40 m distance, 20 m distance and near well distance soil sample.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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