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Research Article

EFFECT OF HYDROCARBON STRESS ON CROP PLANTS AND THEIR ALLEVIATION BY MICOBIOLOGICAL BIO-PREPARATION OF *RHODOCOCCUS ERYTHROPOLIS*

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ABSTRACT

The objective of the present study was to evaluate the effects of petroleum hydrocarbon contaminated soil on crop plants like, *Cyamopsis tetragonoloba*, Taub., *Vigna radiata*(L.) Wilczek. And *Vigna mungo* (L.) Hepper. The seedlings of *Cyamopsis tetragonoloba*, Taub., *Vigna radiata* (L.) Wilczek. And *Vigna mungo* (L.) Hepper were treated with various concentrations of petroleum (100µl, 200µl, 300µl, 400µl, 500µl) individually. After 15 days of treatment the plants were analysed. The morphometric characters such as seed germination, root length, shoot length, leaf area, fresh weight and dry weight were decreased with increasing the concentration of petroleum. In the present investigation, an attempt was also made to study the effect of microbiological bio-preparation made from *Rhodococcus* treated petroleum and its impact on crop plant was recorded. The intent of the present study is to present an overview of hydrocarbon stress and alleviation of hydrocarbon stress by microbiological bio-preparation on crop plants growth

INTRODUCTION

Petroleum based products are the major source of energy for industries and daily life. Today hydrocarbon contamination is one of the major environmental problem cause damages to the surrounding ecosystems. Release of hydrocarbons into the environment whether accidentally or due to anthropogenic activities is a main cause of water and soil pollution which leads to extensive damage to animals and plants (Bona *et al*., 2011) either directly or indirectly. Bioremediation technology is the most emerging technology for treatment of petroleum contaminated sites (Mirzakhani *et al*., 2016). Biological agents like microorganisms or plants transform the complex organic contaminants to other simpler organic compounds by owing to their diverse metabolic capabilities for the removal and degradation of many environmental pollutants. Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment. In the present study, we aimed to investigate the effect of petroleum hydrocarbon on morphometric characteristics of following crop plants such as *Cyamopsis tetragonoloba*, *Vigna radiata* (L.) Wilczek., *Vigna mungo* (L.) Hepper and in the same vein, we also investigated bioremediation properties of the varying amount of microbiological bio-preparations of *Rhodococcus erythropolis* culture to restore the reduced morphometric characteristics of crop plants.

MATERIAL AND METHODS

The experimental plant seeds were procured from Tamil Nadu Agricultural University Coimbatore. Various concentrations of petroleum (100µl, 200µl, 300µl, 400µl, 500µl) and microbiological bio-preparations of petroleum hydrocarbons treated with *Rhodococcus erythropolis* (100ml, 200ml, 300ml, 400ml, 500ml) were prepared. Both control and experimental plants were allowed to grow in soil mixture of red, black and garden soil in the ratio of 1:1:1. The soil was sterilized and seeds were surface sterilized. The pot sown with the seeds of *Cyamopsis tetragonoloba*, *Vigna radiata* and *Vigna mungo* separately and were inoculated with petroleum hydrocarbon as well as microbial treated petroleum. The pots were watered regularly. Various morphometric characters such as seed germination, root length, shoot length, leaf area, fresh weight and dry weight were recorded on the 15th day of treated and

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control plants. To measure the root length and shoot length, the plants were uprooted carefully from soil, washed with water and their lengths were measured. The fresh weights of the samples were recorded after uprooting the plants carefully from soil and washed thoroughly with water and weighed using balance. After drying the samples in hot air oven, the dry weight of the samples were recorded.

Statistics Analysis

The growth parameters were determined with ten independent replicates and the average was considered. The data reported as mean ± SE and with figures in parentheses represent the percent activity. Statistical analysis (One way ANOVA - Tukey test) was applied using the statistical package, SPSS 16.0.

RESULT AND DISCUSSION

The seedlings of *Cyamopsis tetragonoloba* Taub., *Vigna radiate* (L.) Wilczek., *Vigna mungo* (L.) Hepper grown in different concentration of petroleum hydrocarbon exhibited inhibition in seed germination, root and shoot length, leaf area, fresh weight, dry weight, with shoot being affected more than the root (Table 1-3). After 15 days of treatment, a significant reduction of 85%, 67%, 80%; 85%, 67%, 63% and 85%, 65%, 67% and in seed germination, root growth and shoot growth respectively because of petroleum hydrocarbon was noticed under 500µl concentration (Table 1).

delayed seed germination, which was due to poor moisture availability and aeration of the soil. Amakin and Onofeghara (1978) also reported that, the effect of crude oil on *Zea mays* and *Capsicum fruitescens* recorded a significant decrease in the rate of germination, In the present study, we also noticed the near inhibition of germination of experimental seeds at 500µl concentration due to the absorption of oil by the seeds, which caused them to be swollen and slimy as was observed when compared to control seeds. Similarly, Graj *et al*., (2013) reported that poor seed germination in Indian mustard plant under diesel oil contaminated soil. Bona *et al* (2011) reported that diesel oil significantly affected the seedling growth of *Schinus terebinthifolius.* Similar observation was reported by Al-Yemeni *et al*., (2010) and Nwoko *et al*., (2007) in *Phaseolus vulgaris* grown on petroleum polluted soil and spent oil polluted soil respectively. According to Sharifi *et al*., (2007), who reported that, the reduction of germination rate due to the formation of biofilm over entire seed surface which altered the physiological process inside the seed.

We also recorded that, leaf area, fresh weight and dry weight also significantly decreased to the tune of 72%, 66%, 68%; 80%, 70%, 76% and 82%, 80%, 78% respectively in *Cyamopsis tetragonoloba*, *Vigna radiate* and *Vigna mungo* at 500µl concentration of petroleum hydrocarbon.

Table 1 Effect of various concentration of petroleum hydrocarbon on the morphometric characteristics of *Cyamopsis tetragonoloba*, Taub

Growth Parameters	Control	100µl	200 µl	300 µl	400 µl	500 µl
Seed germination	15.00±0.113 (100)	7.00±0.007 (50) a*	5.00±0.213 (36) a*	4.00±0.057 (29) a*	3.00±0.213 (22) a*	2.00±0.021 (16) a*
Root Length (cm)	9.52±0.061 (100)	8.20±0.057 (84) a*	7.33±0.088 (73) a*	5.54±0.013 (52) a*	4.37±0.072 (45) a*	3.03±0.026 (31) a*
Shoot Length (cm)	12.13±0.088 (100)	10.61±0.057 (87) a*	8.63±0.085 (71) a*	7.30±0.059 (59) a*	4.90±0.081 (40) a*	3.76±0.013 (31) a*
Leaf Area (cm^2)	4.53±0.145 (100)	3.80±0.033 (83) a*	3.13±0.183 (69) a*	2.20±0.057 (48) a*	1.53±0.043 (33) a*	1.30±0.032 (28) a*
Fresh Weight (gm)	2.13±0.008 (100)	1.86±0.011 (87) a*	1.67±0.046 (78) a*	1.26±0.025 (58) a*	0.75±0.014 (44) a*	0.43±0.062 (20) a*
Dry Weight (gm)	0.94±0.060 (100)	0.82±0.017 (86) a*	0.68±0.013 (73) a*	0.44±0.012 (54) a*	0.30±0.003 (33) a*	0.17±0.018 (18) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant.

Table 2 Effect of various concentration of petroleum hydrocarbon on the morphometric characteristics of *Vigna radiata* (L.) Wilczek

Growth Parameters	Control	100µl	200 µl	300 µl	400 µl	500 µl
Seed germination	15.00±0.113 (100)	8.00±0.162 (56) a*	6.00±0.163 (43) a*	4.00±0.112 (29) a*	3.00±0.135 (22) a*	2.00±0.097 (16) a*
Root Length (cm)	10.23±0.035 (100)	9.10±0.057 (87) a*	7.56±0.129 (73) a*	5.45±0.120 (56) a*	4.56±0.152 (44) a*	3.66±0.061 (33) a*
Shoot Length (cm)	14.23±0.033 (100)	12.43±0.051 (87) a*	10.90±0.028 (76) a*	7.34±0.035 (52) a*	5.26±0.055 (37) a*	4.33±0.032 (35) a*
Leaf Area (cm^2)	5.43±0.156 (100)	4.63±0.152 (85) a*	4.06±0.053 (74) a*	3.03±0.023 (55) a*	2.43±0.015 (41) a*	1.86±0.017 (32) a*
Fresh Weight (gm)	3.10±0.028 (100)	2.73±0.037 (88) a*	2.30±0.067 (74) a*	1.75±0.025 (56) a*	1.18±0.022 (38) a*	0.95±0.023 (24) a*
Dry Weight (gm)	1.13±0.033 (100)	0.95±0.003 (84) a*	0.81±0.008 (71) a*	0.57±0.023 (52) a*	0.37±0.103 (31) a*	0.23±0.011 (20) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant.

The plants grown in our experiment on the soil contaminated with petroleum hydrocarbons recorded a lower mass of shoots than those in control experiment plants. Similar results were obtained in studies on the effect of crude oil on the growth of chilli and tomato plants (Anoliefo and Vwioko, 1995). They also recorded the oil contaminated soil generally causes

Table 3 Effect of various concentration of petroleum hydrocarbon on the morphometric characteristics of *Vigna mungo* (L.) Hepper

Growth Parameters	Control	100µl	200 µl	300 µl	400 µl	500 µl
Seed germination	15.00±0.113 (100)	8.00±0.124 (77) a*	6.00±0.213 (56) a*	5.00±0.145 (36) a*	4.00±0.125 (29) a*	3.00±0.136 (22) a*
Root Length (cm)	11.40±0.115 (100)	10.03±0.034 (88) a*	8.66±0.051 (77) a*	6.43±0.033 (58) a*	5.43±0.121 (48) a*	3.81±0.116 (37) a*
Shoot Length (cm)	15.26±0.012 (100)	13.36±0.013 (87) a*	11.20±0.016 (73) a*	8.33±0.032 (54) a*	6.90±0.081 (44) a*	4.93±0.031 (36) a*
Leaf Area (cm^2)	5.63±0.145 (100)	4.76±0.057 (85) a*	4.11±0.088 (72) a*	3.23±0.081 (57) a*	2.46±0.023 (43) a*	1.83±0.125 (34) a*
Fresh Weight (gm)	3.56±0.012 (100)	3.13±0.214 (87) a*	2.76±0.032 (77) a*	1.93±0.005 (54) a*	1.18±0.015 (33) a*	0.86±0.026 (29) a*
Dry Weight (gm)	1.70±0.067 (100)	1.46±0.023 (85) a*	1.30±0.017 (76) a*	1.01±0.005 (57) a*	0.56±0.120 (33) a*	0.39±0.126 (22) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant.

Table 4 Effect of various concentration of petroleum hydrocarbon and *Rhodococcus erytheopolis* on the morphometric characteristics of *Cyamopsis tetragonoloba*, Taub.

Growth Parameters	Control	100µl+100 ml RE	200 µl+200 ml RE	300 µl+300 ml RE	400 µl+400 ml RE	500 µl+500 ml RE
Seed germination	15.00±0.113 (100)	8.00±0.079 (56) a*	10.00±0.068 (70) a*	11.00±0.056 (77) a*	12.00±0.171 (84) a*	13.00±0.012 (90) a*
Root Length (cm)	9.52±0.061 (100)	9.70±0.167 (104) a*	10.20±0.033 (107) a*	11.41±0.023 (119) a*	12.23±0.179 (128) a*	12.88±0.065 (137) a*
Shoot Length (cm)	12.13±0.088 (100)	13.33±0.055 (109) a*	14.23±0.210 (117) a*	14.53±0.169 (119) a*	16.06±0.035 (132) a*	17.86±0.134 (147) a*
Leaf Area (cm^2)	4.53±0.145 (100)	4.83±0.028 (106) a*	5.46±0.133 (120) a*	6.36±0.024 (140) a*	6.86±0.024 (151) a*	7.40±0.046 (163) a*
Fresh Weight (gm)	2.13±0.008 (100)	2.70±0.038 (126) a*	3.31±0.057 (154) a*	3.80±0.037 (177) a*	4.26±0.214 (199) a*	4.70±0.005 (220) a*
Dry Weight (gm)	0.94±0.060 (100)	1.24±0.017 (132) a*	1.53±0.013 (164) a*	2.03±0.012 (216) a*	2.30.±0.003 (244) a*	2.70±0.018 (287) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant. RE-*Rhodococcus erythropolis*

Table 5 Effect of various concentration of petroleum hydrocarbon and *Rhodococcus erythropolis* on the morphometric characteristics of *Vigna radiata* L

Growth Parameters	Control	100µl+100 ml RE	200 µl+200 ml RE	300 µl+300 ml RE	400 µl+400 ml RE	500 µl+500 ml RE
Seed germination	15.00±0.113 (100)	9.00±0.168 (63) a*	10.00±0.181 (70) a*	11.00±0.189 (77) a*	12.00±0.191 (84) a*	13.00±0.142 (90) a*
Root Length (cm)	10.23±0.035 (100)	10.86±0.120 (105) a*	11.16±0.115 (108) a*	11.66±0.152 (112) a*	12.16±0.033 (117) a*	12.83±0.056 (124) a*
Shoot Length (cm)	14.23±0.033 (100)	14.70±0.120 (103) a*	15.26±0.218 (107) a*	15.83±0.127 (111) a*	16.23±0.163 (114) a*	16.83±0.072 (43) a*
Leaf Area (cm^2)	5.43±0.156 (100)	5.86±0.088 (108) a*	6.20±0.120 (114) a*	6.86±0.214 (126) a*	7.20±0.218 (132) a*	7.73±0.152 (142) a*
Fresh Weight (gm)	3.10±0.028 (100)	3.60±0.031 (116) a*	3.86±0.208 (124) a*	4.03±0.039 (130) a*	4.33±0.129 (139) a*	4.86±0.037 (157) a*
Dry Weight (gm)	1.13±0.033 (100)	1.63±0.126 (144) a*	1.93±0.055 (170) a*	2.20±0.116 (194) a*	2.33±0.211 (205) a*	2.76±0.170 (244) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant. RE-*Rhodococcus erythropolis*

Table 6 Effect of various concentration of petroleum hydrocarbon and *Rhodococcus erythropolis* on the morphometric characteristics of *Vigna mungo* (L.) Hepper

Growth Parameters	Control	100µl+100 ml RE	200 µl+200 ml RE	300 µl+300 ml RE	400 µl+400 ml RE	500 µl+500 ml RE
Seed germination	15.00±0.113 (100)	9.00±0.168 (63) a*	11.00±0.181 (77) a*	12.00±0.138 (84) a*	13.00±0.031 (90) a*	14.00±0.161 (97) a*
Root Length (cm)	11.40±0.115 (100)	11.60±0.045 (101) a*	12.30±0.133 (107) a*	12.86±0.208 (112) a*	13.23±0.124 (116) a*	13.86±0.234 (121) a*
Shoot Length (cm)	15.26±0.012 (100)	15.73±0.036 (103) a*	16.26±0.039 (106) a*	16.83±0.173 (110) a*	17.20±0.366 (112) a*	18.03±0.086 (118) a*
Leaf Area (cm^2)	5.63±0.145 (100)	5.93±0.218 (105) a*	6.23±0.057 (110) a*	6.83±0.214 (121) a*	7.26±0.146 (129) a*	7.83±0.315 (139) a*
Fresh Weight (gm)	3.56±0.012 (100)	3.93±0.120 (110) a*	4.23±0.033 (118) a*	4.63±0.117 (129) a*	4.93±0.029 (138) a*	5.06±0.135 (142) a*
Dry Weight (gm)	1.70±0.067 (100)	1.93±0.066 (113) a*	2.10±0.175 (123) a*	2.53±0.068 (149) a*	2.80±0.208 (164) a*	2.93±0.236 (172) a*

Values in parenthesis indicate percent activity Values are an average of five observations. Values in parentheses are percentage activity with respect to control. Mean ± SE. a* – 100µl to 500µl concentrations compared with control, * Significance at P < 0.05 level. a# – refers to non-significant. RE-*Rhodococcus erythropolis*

The findings of the present study agreed with the findings of Iqbal *et al*., (2016) who reported that the reduction in root length and shoot length, total dry weight in *Parkinsonia aculeata* L. in oil polluted soil. In the same vein, Al-Moaikal *et al*., (2012) also noticed similar observation in *Simmodsia chinensis* due to crude oil stress. It was also reported that the reduction in seed germination, root length and leaf area in *Festuca arundinacea* grown on diesel fuel contaminated soil (Zarinkamar *et al*., 2013).

In the present study, the petroleum hydrocarbons significantly dropped the leaf area in all our experimental plants *Cyamopsis tetragonoloba*, *Vigna radiata* and *Vigna mungo* at increasing concentrations when compared to control plants. Adenipekun *et al*., (2009) observed that, the *Corchorus olitorius* seedlings in oil contaminated soil had a lower number of the leaves and reduced leaf area compared to those in uncontaminated soil. Nitrogen, carbon, and sulphur are the most important organogenic compounds that form the principal mass of plants. These elements are principal factors for the normal growth and development of plants. It is clear that the plants react in different ways to contaminants and that the seedlings are more susceptible to the contamination. The decrease in the size of leaves is likely a result of reduced absorption of the nutrients and water, affecting the development of the above ground system because such absorption is essential for the growth of the plant (Proffitt *et al*., 1995 and Adenipekun *et al*., 2009). Agbogidi *et al*., (2006) suggested that the reduced number of leaves and leaf area reported in *Dennettia tripetala* growing in the contaminated soil was caused by the water stress resulting from the immobilization of the nutrients and by the changes in the activity of some metabolites within the plant.

During our investigation, *Cyamopsis tetragonoloba*, *Vigna radiate* and *Vigna mungo* seeds when cultivated alone were seen sensitive to petroleum hydrocarbons with decreased growth. But the same plants, when sown with the microbiological biopreparation of *Rhodococcus erythropolis* exhibited no toxicity symptoms and reduction of growth. Bioremediation studies showed an significant increase in morphometric characteristics such as seed germination, root length, shoot length, leaf area fresh weight and dry weight after the applications of *Rhodococcus erythropolis* (Table 4-6). According to Rojo (2009) microorganisms have the ability to metabolize many organic contaminants, using them as an energy source or converting them to non-toxic products (carbon dioxide, water and biomass). The *Rhodococcus erythropolis* used in the present study to remove the large quantity of petroleum from contaminated soil. The results of the present study were confirmed by Mirzakhani *et al*., (2016) who also reported that plants and their associated microorganisms presented a potential for removal of petroleum hydrocarbons in contaminated soils. Results of previous study by Ivanova *et al*., (2015) on degradation of petroleum hydrocarbon by microorganisms such as *Rhodococcus erythropolis*, *Acinetobacter baumannii* and *Pseudomonas putida* in oil contaminated soil support our results.

CONCLUSION

In recent years, a number of diverse studies have been conducted on the effects of petroleum-derived compounds on crop plants. In the present investigation, the plants treated with hydrocarbons alone recorded a declining trend in the growth with the increase in hydrocarbon concentrations. In general, the hydrocarbon stress declined the shoot length, root length, seed germination, leaf area fresh weight and dry weight compared to control plants, while hydrocarbons treated with microbial inoculum alleviated the stress by sustaining the growth when compared to control plants. The microbiological bio preparation used in the experiment presented in this paper had a beneficial effect on the analyzed morphological parameters of plants. It is clear that, adding micorbiological bio preparation to the soil contaminated with petroleum-derived compounds decreased the amount of total petroleum hydrocarbons in the soil. Result of the present study clearly suggest that the *Rhodococcus erythropolis* can efficiently remove the hydrocarbon toxins from contaminated soil. Hence we strongly suggest that *Rhodococcus erythropolis* can be used as a bioremediation to degrade the petroleum hydrocarbon from the polluted environment for sustainable agriculture.

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