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Impact of Assam petroleum crude oil on the germination of four crude oil resistant species

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ABSTRACT

The impact of Assam petroleum crude oil on the germination of Axonopus compressus (Sw) Beauv., Cynodon dactylon (L.) Pers., Cyperus brevifolius (L.) Mant. and Eclipta prostrata (Rottb.) Hassk were analysed. The parameter studied were percentage of germination, effect of time of storage period of seeds (unto 180 days after collection of seeds) on germination, percentage inhibition of germination and commencement of germination. Reduced germination percentage, increase inhibition percentage and delayed commencement of germination were observed in crude oil contaminated water and soil of oil fields and no germination was observed in fresh crude oil even 180 days after collection of seeds. In both the species, the maximal germination was found in 120 days stored seeds and minimal germination was found in 180 days stored seeds in all the treatment and fell significantly ($p < 0.001$) with controls. Seeds treated with fresh crude oil were found to be in viable in all the durations. Fresh crude is more toxic than weathered crude oil regarding germination of seeds.

Key words: Crude oil pollution, herbs and seed germination.

INTRODUCTION

Crude oil of different origins varies in physical properties and chemical compositions. Crude oil contains toxic substances such as polycyclic aromatic compounds which can combine with common environmental materials to form carcinogens [1]. The toxicity of different fraction of crude oil has been investigated to some extent. The most toxic crude oil have shown to be the aromatic hydrocarbon e.g. benzene, toluene and xylene and also phenolic substances e.g. napthenic acid [2]. Kuwati residue are found to be far less toxic than the fresh crude, indicating the high proportion of toxicity conferred by the volatile components of crude oil [3, 4].

The present work was undertaken to study the impact of different forms of Assam petroleum crude on the germination of four dominant (oil resistant or adapted) herbaceous species commonly growing in the crude oil spilled areas of Rudrasagar & Lakwa oil fields of upper Assam, India [5,6,7,8,9,10].

MATERIALS AND METHODS

During ecological survey of the oil fields flora of Rudrasagar and Lakwa, *Axonopus compressus* (Sw) Beauv., *Cynodon dactylon* (L.) Pers., *Cyperus brevifolius* (L.) Mant. and *Eclipta prostrata* (Rottb.) Hassk Mant. were found to be two dominant (oil resistant or adapted) herbs in the crude oil spilled areas were selected to know the effect of crude oil on its germination.

To know the effect of crude oil on germination, the seeds of four species were collected from the study area during the month of June, and the quality seeds were separated there from for further study. The seeds thus collected were packed into small cotton bags and stored at room temperature in the laboratory.

To know the effect of crude oil on the germination, the seeds of four species were subjects to the following experiments.

i. Seeds watered by double distilled water (control)
ii. Seeds treated with crude oil contaminated water of oil fields.
iii. Seeds treated with crude oil contaminated soil of oil fields.
iv. Seeds treated with fresh crude oil.

Crude oil contaminated waters and soils were collected from different crude oil spilled areas of Rudrasagar & Lakwa oil fields and fresh crude oils were collected from different oil collecting centre (OCS) and mixed all these samples separately in equal proportions to prepare three different stock samples as CW, CS and CO for crude oil contaminated water, crude oil contaminated soil fresh crude oil respectively.

Two sets of experiments were conducted in petridishes. Firstly, germination of stored seeds for 30, 60, 90, 120 and 180 days were observed in respective treatments (C, CW, CS, CO) in a periodical interval of 30 days. Secondly, the percentage inhibition of germination in respective treatments against control was also observed. All the germination tests were done in the petridishes. Each treatment comprised of five replicas and each replicate considering of 100 seeds. For each treatment, only one control experiment was run simultaneously. Before starting the experiments all the requirements were sterilized to prevent the entry of microorganisms during the experiments.

For control, seed were simply placed in the filter paper in the petridishes and added DDW to keep the filter paper moistened. Similarly, seed were placed in the petridishes and 10 ml. of crude oil contaminated water (CW) and fresh crude oil (CO) were applied at every alternative day from the stock solutions. For crude oil contaminated soil, soil was taken in the petridishes and seed were directly placed in the petridishes and the germination was observed. In CS and CO treated seeds, a little amount of DDW was provided to maintain its moisture favourable for

effective germination. In all the experiments five replicas were made for every lot and the data consisting of Highest percentage of germination (HPG) and Lowest percentage of germination (LPG) are expressed as mean ± SD (standard deviation) of five replicas. Student't' test was used to locate significant difference in treatment mean which have probability value (p) lower than 0.05 ($p< 0.05$) were considered as significant.

Only fresh crude oil (CO) treated seeds of both the species were removed at intervals and dissected to see the extent of crude oil penetration and damage. Tetrazolium tests for respiration/viability [11] were also performed.

RESULTS AND DISCUSSION

The percentage germination of both the species at different storage periods days after collection of seeds in control, CW, CS and CO are presented in the Table: 1. The results indicate that, the percentage of germination decreased significantly with the increase of storage period of seeds in all the parameters in all the experimental species. In *Axonopus compressus* (Sw) Beauv., the HPG in control is found to be 58.20 ± 3.12 in 90 days storage seeds ,whereas in CW and CS, it is found to be 19.20 ± 3.86 and 12.29 ± 0.74 respectively in the 90 and 120 days seeds. Similarly, the LPG in control is found to be 16.60 ± 2.06 in 180 days storage seeds ,whereas ,in CW and CS it is found to be 5.60 ± 1.01 and 2.80 ± 0.75 respectively in the 180 days seeds. In *Cynodon dactylon* (L) Pers., the HPG in control is found to be 52.00 ± 2.97 in 90 days storage seeds ,whereas in CW and CS, it is found to be 28.40 ± 1.72 and 12.40 ± 2.33 respectively in the 90 and 120 days storage seeds. Similarly, the LPG in control is found to be 19.20 ± 1.72 in 180 days storage seeds ,whereas ,in CW and CS it is found to be 7.00 ± 1.85 and 4.00 ± 1.41 respectively in the 180 days storage seeds .In *Cyperus Brevifolius* (L.) Mant. the HPG in control is found to be 57.02 ± 2.76 in 120days storage seeds, whereas in CW and CS, it is found to be 29.0 ± 1.41 and 16.04 ± 1.02 respectively in the 120 days storage seeds. Similarly, the LPG in control is found to be 24.06 ± 1.62 in 180 days storage seeds ,whereas ,in CW and CS it is found to be 9.60 ± 1.02 and 3.60 ± 1.01 respectively in the 180 days storage seeds . In *Eclipta prostrata* (Rottb) Hask., the HPG in control is found to be 87.6 ± 3.93 in 120 days storage seeds ,whereas in CW and CS, it is found to be 69.2 ± 2.13 and 57.00 ± 2.82 respectively in the 120 days storage seeds. Similarly, the LPG in control is found to be 16.60 ± 2.06 in 180 days storage seeds ,whereas ,in CW and CS it is found to be 5.60 ± 1.01 and 2.80 ± 0.75 respectively in the 180 days seeds

The percentage inhibition of germination for all the species by CW, CS & CO are summarized in the Table: 1 with compared and deducted from control data. As there is no germination of seeds of all the experimental species in the fresh crude oil throughout the experiment, hence significantly 100% inhibition in germination was observed. In *Axonopus compressus* (Sw) Beauv. , the highest percentage of inhibition in CW and CS is 77.48 and 84.10 respectively in 150 days storage seeds , whereas the lowest percentage of inhibition in CW and CS is 61.28 and 76.17 respectively in 120 days storage seeds. In., *Cynodon dactylon* (L) Pers. the highest percentage of inhibition in CW and CS is 62.57 (150 days storage seed) and 88.17 (30 days storage seed) respectively, whereas the lowest percentage of inhibition in CW and CS is 28.95 and 75.78 respectively in 60days storage seeds.

Table 1 Effect of Assam petroleum crude oil on the germination of four oil resistant herbs

Name of the species	Duration	Control	CW	CS	CO
Axonopus compressus	30 days	32.4 ± 2.72	11.8 ± 2.48 (63.58)***	6.2 ± 1.60 (80.64)***	0 (100)
	60 days	50.4 ± 2.87	14.4 ± 2.41 (71.43)***	10.8 ± 1.17 (78.57)***	0 (100)
	90 days	52.8 ± 3.12	19.2 ± 3.86 (63.64)***	9.6 ± 1.02 (81.81)***	0 (100)
	120 days	47.0 ± 3.85	18.2 ± 2.03 (61.28)***	11.2 ± 0.74 (76.14)***	0 (100)
	150 days	30.2 ± 2.04	6.8 ± 1.32 (77.48)***	4.8 ± 1.33 (84.10)***	0 (100)
	180 days	16.6 ± 2.06	5.6 ± 1.01 (66.26)***	2.8 ± 0.75 (83.13)***	0 (100)
Cynodon dactylon	30 days	37.2 ± 2.48	16.6 ± 2.15 (55.37)***	4.4 ± 1.74 (88.17)***	0 (100)
	60 days	38.0 ± 2.61	27.0 ± 2.28 (28.95)***	9.2 ± 1.72 (75.78)***	0 (100)
	90 days	49.8 ± 3.18	28.8 ± 1.72 (42.17)***	11.4 ± 2.40 (77.11)***	0 (100)
	120 days	52.0 ± 2.97	24.6 ± 2.42 (52.69)***	12.4 ± 2.33 (76.17)***	0 (100)
	150 days	37.4 ± 1.85	14.0 ± 1.40 (62.57)***	8.4 ± 1.35 (77.54)***	0 (100)
	180 days	19.2 ± 1.72	7 4 ± 2.48 (61.46)***	4.0 ± 1.41 (79.17)***	0 (100)
Cyperus brevifolius	30 days	41.6 ± 1.85	21.4 ± 1.36 (48.56)***	9.2 ± 0.75 (77.88)***	0 (100)
	60 days	48.0 ± 3.16	20.2 ± 0.74 (57.92)***	8.6 ± 0.80 (82.03)***	0 (100)
	90 days	52.8 ± 2.99	24.2 ± 1.47 (45.17)***	11.0 ± 1.09 (89.39)***	0 (100)
	120 days	57.0 ± 2.76	29.0 ± 1.41 (49.12)***	16.4 ± 1.02 (71.22)***	0 (100)
	150 days	31.2 ± 1.47	18.4 ± 2.58 (41.02)***	8.20 ± 0.74 (73.71)***	0 (100)
	180 days	24.6 ± 1.62	9.6 ± 1.02 (60.98)***	3.6 ± 1.01 (85.36)***	0 (100)
Eclipta prostrata	30 days	70.0 ± 1.41	38.6 ± 2.06 (44.86)***	10.0 ± 1.41 (85.71)***	0 (100)
	60 days	80.4 ± 3.01	58.0 ± 2.82 (27.86)***	29.6 ± 2.42 (63.18)***	0 (100)
	90 days	85.6 ± 2.41	66.4 ± 2.40 (22.43)***	48.4 ± 2.26 (43.46)***	0 (100)
	120 days	87.6 ± 3.93	69.2 ± 2.13 (21.00)***	57.0 ± 2.82 (34.93)***	0 (100)
	150 days	79.8 ± 2.71	63.4 ± 2.15 (20.55)***	48.8 ± 3.86 (38.85)***	0 (100)
	180 days	64.2 ± 3.31	36.4 ± 2.73 (43.30)***	37.6 ± 2.58 (41.59)***	0 (100)

( ) values in parentheses indicate percentage inhibition of germination against control.
**** significant treatment effect ($p < 0.001$).*

Table 2 Physico-Chemical Properties of Assam Petroleum Crudes

Properties		Rudrasagar Petroleum Crude	Lakwa Petroleum Crude
A. PHYSICAL PROPERTIES			
1.	Specific at 15^{o} c	0.9100 gm/ml	0.8946 gm/ml
2.	A.P.I. Gravity	23.80	26.60
3.	Water Content	0.40 %	0.60 %
4.	Basic Sediment and Water	0.60 %	0.80 %
5.	Salinity	0.04 kg/ton.	0.0098 kg/ton
6.	Pour Point	5^{o} c	33^{o} c
7.	Viscosity at 30^{o} c	8.5 cp	30 cp
8.	Surface Tension at 30^{o} c	26.91 dynes/cm.	22.66 dynes/cm.
9.	Electric Specific Conductivity at	0.26 x 10 -6	0.25 x 10 -6
10.	Dielectric Constant at	2.06	1.91
11.	API Gravity of Fraction from 250^{o}c-275^{o}c	42	42
B. CHEMICAL PROPERTIES			
1.	Oil Content	95.38 %	89.60 %
2.	Wax Content	04.20 %	10.50 %
3.	Resin Content	0.11 %	0.07 %
4.	Asphalting Content	0.25 %	0.17 %
5.	porphyrine content	Negligible	Negligible
6.	insoluble solids	0.06 %	0.10 %
7.	Carbonaceous residue unto 500^{o} c	2.30 %	2.25 %
8.	Carbon	85.00 %	87.20 %
9.	Hydrogen	10.18 %	10.06 %
10.	Nitrogen	Negligible	Negligible
11.	Sulphur	0.34 %	0.20 %
12.	Phosphorous	Negligible	Negligible
13.	Calcium	Negligible	Negligible
14.	Magnesium	Negligible	Negligible
15.	Potassium	Less than 6 ppm	Less than 5 ppm
16.	Iron	Less than 10 ppm	Less than 10 ppm
17.	Magnase	Negligible	Negligible
18.	Zinc	Negligible	Negligible
19.	Copper	Negligible	Negligible
20.	Boron	Negligible	Negligible
21.	Molybdenum	Negligible	Negligible
22.	Chlorine	Negligible	Negligible
23.	Vanadium	Negligible	Negligible
24.	Chromium	Negligible	Negligible
25.	Nickel	Negligible	Negligible
26.	Cobalt	Negligible	Negligible
27.	Tungsten	Negligible	Negligible
28.	Titanium	Negligible	Negligible
29.	Vanadium	Negligible	Negligible

Sources: Oil and Natural Gas Cooperation Limited (ONGCL) ,India & Sarma, 1979 [29]

In *Cyperus Brevifolius* (L.) Mant. the highest percentage of inhibition in CW and CS is 60.98 (180 days storage seed) and 89.39 (90 days storage seed) respectively, whereas the lowest percentage of inhibition in CW and CS is 41.02 (150 days storage seed) and 71.22 (120 days

storage seed) respectively .In *Eclipta prostrata* (Rottb) Hask the highest percentage of inhibition in CW and CS is 44.86 and 85.71 respectively in 30 days storage seeds , whereas , the lowest percentage of inhibition in CW and CS is 20.55 and 38.85 respectively in 150 days storage seeds. The germination percentage of all treated seeds of four species fell significantly ($p < 0.001$) in all the parameters when compared to their control values. Inhibition in germination was founding following trends Fresh crude oil (CO) > Crude oil contaminated water of oil fields (CW) > Crude oil contaminated soil of oil fields (CS).

Another interesting result is that seeds of all the experimental species which germinated in the crude oil contaminated water and soil differed from those of control seeds in their behaviour of germination. Oil treated seeds tended to have a longer lag phase preceding germination. The length of lag phase was found different in both CW and CS as seeds treated with CS showed more length of lag phase.

Seeds of four species those were dissected after treating with fresh crude oil showed penetration of crude oil in almost all seeds, through the region of grain stack, towards coleorhiza of the monocotyledonous seeds namely , *Axonopus compressus* (Sw) Beauv., *Cynodon dactylon* (L.) Pers., *Cyperus brevifolius* (L.) Mant. and towards the micropylar end of the dicotyledonous species *Ecliptta prostrata* (Rottb.) Hassk. Oil penetration was detected by the colouration imparted to the embryo. Upon the application of standard qualitative Tetrazolium test for radox reaction, almost all seeds of four species treated with fresh crude oil were found to be enviable in all the durations.

The findings indicate that seeds of these species are germinate at its maximum after a specific period, usually 120 days and it becomes decrease after a specific period., usually 180 days after collection of mature seeds. The lower percentage of germination in CW and CS and longer lag phase preceding germination were obviously due to inhibition of germination by crude oil which is mixed with water and soil. The inhibitory effect could be attributed principally to physical as well as biological harm on the seeds resulting from physical and chemical properties of crude oil (Physico-chemical properties of Assam petroleum crude oil are presented in in the Table:2).Observation also revels that in fresh crude oil no germination has taken place even 180 days after collection of seeds of all the species and this indicates that fresh crude oil directly damage all the seed typed that used in the experiment. This confirmation also got from standard Tetrazolium test, where almost all seeds of four species were found to be enviable after treating with fresh crude oil. Crude oil may enter through the region of grain stack, towards coleorhiza of the monocotyledonous seeds namely, *Axonopus compressus* (Sw) Beauv., *Cynodon dactylon* (L.) Pers., *Cyperus brevifolius* (L.) Mant and towards the micropylar end of the dicotyledonous species *Eclipta prostrata* (Rottb.) Hassk or simply through a creck, scar or injury. Whichever it takes place, penetration of crude oil would certainly endanger the life and growth activities of the embryo which is vital for germination. In the present investigation oil penetration was also detected by the colouration imparted to the embryo. In this experiment injury to embryo of the seeds may be fatal, particularly when treated with fresh crude oil which reflects to the failure of germination of seeds even after 180 days stored seeds as suggested by [12} or crude oil possibly killed the embryo[13] . The woks of [14, 15 and 16] have provided a basis for agreement on the toxicity of crude oil to the living tissues. Some other workers have provided evidence in support of the possible penetration of crude oil into plant tissue and cells [13, 17 and 18]. Studied on the

effect of crude oil pollution on the germination of *Zea mays* and *Capsicum frutescens* proved that crude oil directly inhibit the germination all the seed typed used and the rate of germination decreased significantly with increased in the length of period of pre-soaking in crude oil [19].

Another possible reason for inhibitory effect of crude oil on germination is its physical water repellent (hydrophobic) property. The persistent oil film around the seed may act as a physical barrier, prevent or reducing both water and oxygen uptake and thus adversely affecting gaseous exchange which causes inhibition of germination as well as delay in commencement germination(longer lag phase preceding germination) probably by checking the imbibitions of water and diffusion of gas into the seeds. This is in agreement with the earlier findings [3, 19 and 20]. But in case of seeds that treated with crude oil contaminated water (CW) and soil (CS) , though the oil layer surrounding the seed and prevent water to enter and thereby hampering germination in case of fresh crude oil treated seeds, the CW and CS in contrast to have some amount of water available for effective germination. Some seeds were able to swell in CW and CS clearly indicates that, though both are contaminated with crude oil a little amount of water and oxygen can enter the seeds to provide its germination and this effect could be due to the oil which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen as suggested by [21]. Oil surrounded seeds germinate after washing and this indicates that in some cases at least the effect is physical [22]. One of the most possible reasons for inhibition of germination in crude oil contaminated soil (CS) is due to unsatisfactory soil conditions because of insufficient aeration due to a decrease in air filled pore space and an increased demand of oxygen by oil decomposing microorganisms. Similar findings were also reported [23] , while studying the biological aspects of land rehabilitation following hydrocarbon contamination. Thus it may be argued that, the increased microbial activity around the surface of crude oil infested seeds leading to depletion of oxygen could have contributed to the inhibitory effect of crude oil on germination because developing embryo needs higher amount of oxygen and water. This factor appeared to have played a major role in delaying as well as reducing the germination of seeds in the present study since the microbial growth around the crude oil infested seeds was observable in these experiments. poor growth of some plants in polluted fields due to suffocation of the plants by exclusion of air and probably exhaustion of oxygen by microbial activities were well documented [24]. They also studied the effect of oil pollution of soils on germination of corn (*Zea mays*) and suggested that terminations and yields were drastically reduced as the level of pollution increased. Literature suggested that, the small amount of oil would delay germination and larger amount might even stops germination entirely [25]. Volatile fraction of oil had a high wetting capacity and high penetration power [12] and iIf contact with seeds, the oil would enter the seed coat and readily kill the embryo.

Present investigation also indicates that fresh Assam petroleum crude oil is more toxic to the embryo of used seeds than the weathered crude oil in the present experiment, some seeds of all the experimental species were found to be germinate in crude oil contaminated water (CW) and crude oil contaminated soil (CS) of oil fields, where crude oil is generally found in weathered form. This is in agreement with the earlier findings of [26,], that, not a single germination was found in *Festuca rubra* after 2 weeks, and wheat after four days incubation in fresh crude oil. Less toxicity of crude oil contaminated water (CW) than crude oil contaminated soil (CS) and fresh crude oil (CO) may be due to either solution of toxic water soluble fraction of crude oil or evaporation of some toxic lighter fraction of crude oil from contaminated water in the oil fields.

The fact that seeds of *Eclipta prostrata* (Rottb.) Hassk showed less inhibition of germination in CW and CS, indicates that the seed coat is highly resistant to penetration of crude oil and that an essential pre requisite to damage following from entry of oil is tissue penetration and establish itself as an oil tolerant (adapting) species. Though, the spilled crude oil destroy the seed bank and seeds of the other three species, these grass and sedge species can propagates very easily with their vegetative mode of propagation through their vegetative propagules specially the portion like creeping stem or rhizome (from where vegetative propagation occurs) which are resistant to crude oil as suggested earlier by [8, 9, 10, 27 and 28] and established themselves as dominant species in the crude oil spilled areas of oil fields of Assam, India.

CONCLUSION

It is concluded from the above study that crude oil certainly endanger for germination of seeds. Spilled crude oil not only destroy the seed bank through mixing in soil, seeds, inhibition in plant growth, lowering the species diversity and degraded the soil environment.. Therefore, it is essential that oil industry should take adequate measures to prevent pollution of environment with crude petroleum product. Flow pipes for transportation of crude oil from drilling site or wells to oil collecting station should be periodically checked and weak, damage, leaky once replaced. They should have functioning device such as straw for skimming and absorbing oils once there is any spillage in addition to use of chemical dispersants. They should also try to recover degraded soil environment (crude oil spilled areas) with the help of oil resistant (adapted) species. Bioremediation by applying nutrients along with the microbes well adapted to a particular environment should be considered as an effective `tool' for skirmishing oil spills. If not checked such pollutants i.e. crude oil may become responsible for complete annihilation of plant species from areas where such pollutants are allowed to spill off.

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