Physiological responses of Solanum nigrum L. Species to the heavy crude oil

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Abstract

The remediation of oil contaminated soils has been a major problem in oil producing countries. Recently use of phytoremediation to clean such polluted sites has been on investigated. In order to identify plants that can enhance the remediation, *Solanum nigrum* L. (black nightshade) was used on different concentrations (0.0,0.5,1.0,2.0,4.0,6.0,8.0,10.0 V/V) for seed germination and seedling growth. The results showed that seed germination and seedling performance were enhanced under heavy crude oil compound. This study indicates that black nightshade have more potential for resistance to crude oil concentrations and that can be used as promising tools for phytoremediation technology.

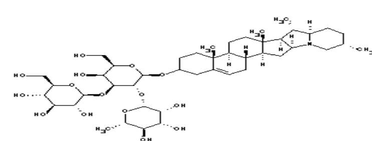
Key words: *Solanum nigrum* L. black nightshade, heavy crude oil, phytoremediation.

1. Introduction

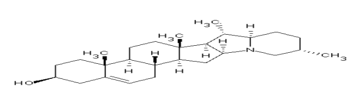
The word petroleum means "rocky oil" or "oil from the earth". Although exactly how crude oil originated is not established, it is generally agreed that crude oil is derived from the remains of animals and plants that lived in marine water millions of years ago, types of crude oil**:** Based on the density crude oil is divided in to the following groups: Light crude oil and heavy crude oil

1.1. Effects of heavy crude oil on tested plant *Solanum nigrum* L.

Commonly known as (Black nightshade) is a dicot weed in the Solanaceae family, annual herb, all green and unripe parts contain steroid glycosides, in form of steroid glycoalkaloids. In the genus Solanum they are important, and are widely regarded as defensive allelochemicals of the plants against microorganisms and herbivores. The main steroid alkaloids are solasonine and solanine, and are called solatrioses (Fig. 1. 1).



Solanine



Solasodine

Fig. 1.1. Structure of alkaloids in *Solanum nigrum* weed.

Diesel oil pollution is harmful on the chlorophyll and protein contents of the black nightshade. It inhibited the growth of plants causing reduction of both chlorophyll and water contents (Seklemora et al., 2001). Pollution-induced degradation in photosynthetic pigments were recorded by a number of researches (Puckett et al., 2003; Mut et al., 2010). Diesel oil was found to inhibit the metabolic and physiological processes including photosynthesis and transportation. The photosynthetic pigments are the most likely to be damaged by diesel pollution. Chlorophyll pigments under stress may undergo several photochemical reactions such as oxidation. Hence any alteration in chlorophyll concentration may change the morphological, physiological and biochemical behaviour of the plant. Plant responses to oil pollution are different and depend on plant species, oil kind, amount and concentration, exposure times and environmental condition (Pezeshki et al., 2000; Spiares et al., 2001; Zangh et al., 2007; Besalatpoor et al., 2008). Soil polluted by crude oil have been found to inhibit plant growth (Agbogidi, 2011) resulting in hypoxic state of the soil the displacement of air from the soil pore spaces by the crude oil. Soil polluted with petroleum (Adedokun & Ataga, 2007; Besaltpour et al., 2008). Changes in soil properties due to contamination with petroleum derived substances can lead to water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus (Njoku, 2008). Cell membranes are damaged by penetration of hydrocarbon molecules, leading to leakage of cell contents. Oils reduce transpiration rate, probably by blocking stomata and intercellular spaces. This may also be the reason for the reduction of photosynthesis. Many researches showed that the presence of the oil resides in the soil has negative effects on the plant metabolism and protein synthesis (Ekpo & Nwaankpa, 2005; Richard et al., 2007; Okpokwasili & Odokuma, 2007; Besaltpour et al., 2008; Teng et al., 2010; Bamidele & Igiri, 2011). Oil pollutions reduce some plant growth parameters such as: plant height, leaf number, leaf surface, plant fresh and dry weight, biomass (Omosun et al., 2008), photosynthetic pigments and also nutrient absorption (Rosso et al., 2005). Crude oil induced environmental stress up on the plant seedlings. Therefore, the overall objective of thin research in to investigate the response of *Solanum nigrum* L weed plant to the effect of heavy crude oil.

2. Material and Methods

2.1. Plant seed:

seeds of *Solanum nigrum* L. (Black nightshade) family Solanaceae. seeds (weeds) were collected from Hei Alsalam area and were stored at room temperature ranges from 25 to 30°C.

2.2. Chemicals:

Formaldehyde, distilled water (DW), the crude oil used was (From AL-Breiga port, field Alamal) heavy crude oil, with the following concentrations of each type of oil. (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 (% v/v).

2.3 Germination test

Seed Preparation prior to germination: The seeds to be used in this work were surface sterilized by washing with 10 % formaldehyde and rinsed three times with sterile water for 10 minutes (Wood et al., 2006).

Sterilized glass petri dishes (9.0cm) lined with double layers of Watmann No.1.filter paper was used. Glass petri dishes were cleaned and sterilized in an oven at 180°C for 2 hours. Seeds were placed in the petri dishes each contains ten. Six replicates were used for each treatment of different kinds of crude oil.

The filter paper was watered by adding 3 ml of distilled water or solution to be tested. All petridishes were in incubated in an incubator of (Gallerkamp) at temperature of 20°C for one week. Distilled water was or tested solution was added to the petridishes whenever it was needed to all replicates at the same time. Germinated seeds were counted daily and germination percentage was calculated at the end of the germination period for each treatment as following: Germination percentage =

$$\frac{\text{Number of seed that germinated}}{\text{Number of seeds sown}}$$

(Yang et al., 2005).

Germination rate (GR) = $\frac{N}{D}$

(N) number of emerged seeds in day (D) is day after planting (Rastegar. et al., 2011).

Mean germination time (MGT) =

$$\frac{n_1*d_1+n_2*d_2+n_3*d_3 \ldots\ldots\ldots\ldots}{\text{Total number of days}}$$

(Gairola et al., 2011).

Where, n = number of germinated seed, d = number of days

Daily and final germination percentages (%) were calculated for the determination of some of the following parameters.

Mean daily germination is an index of daily germination rate

Mean daily germination (MDG) = $\frac{FGP}{D}$

FGP is final germination percent, (D) is day of maximum germination (experiment period) (Rastegar et al., 2011). Germination index (GI) =

$$\frac{GS}{GC} * \frac{LC}{LC} *100\ \%$$

(Gairola. et al., 2011)

Where (Gs) and (Gc) are number of seeds germinated in the sample and control, respectively, whereas Ls and Lc are the radicle length in the sample and control, respectively.

In the case of weeds, the length was measured as whole seedlings due to their smaller size. Ten seedlings of each replicated of each treatment were weighed together due to the small size of weed the seedlings. Relative water contents (%) = fresh weight - Dry weight * 100

(Gairola. et al., 2011).

Percentages of seedling emergence =

$$\frac{\text{Number of seedling that emerged}}{\text{Number of seeds sown}} *100$$

(Agbogidi, 2011b).

Seedling vigor index (SVI) is calculated using the following modified formula: SVI = Seedling length (cm) * final germination percentages.

(Mut et al., 2010).

Tolerance index (TI) is calculated using the following modified formula:

TI = Length of seedling in treatment

Length of seedling in control

(Verdeguer et al., 2009).

3. RESULTS:

Table.3.1 showed the effect of heavy crude oil on the mean values of daily seed germination percentages of S*olanum nigrum* L. (black nightshade). The results indicated no significant differences of seed germination percentages (%), i.e. no seed germination had occurred during the first four days of germination period, under all different dilutions of heavy crude oil including the control treatment . During the fifth day, only seeds treated with distilled water (control) were germinated ($F = 4.00$, $P < 0.01$). Tukey's pariwise comparisons test reveals significant differences between control and other treatment means of heavy crude oil, but daily germination percentages were found to be higher during the last days of germination time Figure 3.1. The Effect of different dilutions of heavy crude oil on the means of germination rate (GR), mean daily germination (MDG) and mean germination time (MGT) of *Solanum nigrum* are represented in Table 3.2. Results indicated that, all the above mentioned measures of this seed were not significantly affected by exogenous application of different dilutions of heavy crude oil. Whereas, the germination index (GI) of same plant was significant ($F=3.81$, $P < 0.05$) within treatments which was increased under some treatments and reduced by higher dilutions of heavy oil. Tukey's pariwise comparisons test reveals significant differences between control and dilutions of 4.0, 8.0 10.0 (% v / v). Seedling length (cm) of *Solanum nigrum* measured under different dilutions of heavy crude oil Table 3.3. Was significant ($F = 10.30$, $P < 0.001$), within different treatments. Different dilutions of lower concentration of same oil had increased the length of black nightshade seedlings with increasing concentrations of heavy crude oil above 8.0 (% v / v). Tukey's pariwise comparisons test reveals significant differences in seedling length of *Solanum nigrum* under the control in comparison to other different treatment means Table 3.3. Seedling fresh weight (g) parameter of *Solanum nigrum* was not affected by different concentrations of heavy crude oil. But seedling's dry weight of the same plant species was significantly increased under higher concentrations of this oil ($F = 3.73$, $P < 0.01$). Tukey's pairwise comparison test reveals significant differences in seedling dry weight (g) of *Solanum nigrum* between lower concentration including control and the highest concentration 10.0 (% v / v) of heavy crude oil Table 3.3. Relative water content percentages (RWC %) of black nightshade are shown in Figure 3.2. This parameter was significantly reduced under higher dilutions of heavy oil ($F = 5.87$, $P < 0.01$). Tukey's pairwise comparison test reveals significant differences in relative water content percentages of same plant between lower concentration including control and highest concentration 10.0 (% v / v) of heavy crude oil. Seedling emergence percentages (%) were not affected by the same oil Figure 3.3. There were small redactions in seedling vigor index and tolerance index of the same target plant species. Using one way analysis various, results showed significant differences in these parameters within different dilutions of crude oil ($F = 2.90$, $P <$

0.05) and (F = 7.64, $P< 0.001$) respectively. Tukey's pairwise comparison test reveals significant differences between different concentrations of heavy crude oil in (SVI). While in the case of (TI) differences were found in the seedlings developed under untreated (control) in compared to the different treatments means of the oil Table .3.4.

4. Discussion

Oil pollution in whatever form is toxic to some plant species and their environment has been observed by many researcher workers (Opeolu, 2000; Adenipekun & Kassim, 2006; Adenipekun et al., 2009; Kelechi *et al*., 2012) that crude oil affects soil properties and this in turn affects the physiological, anatomical and development of plants grown on such soils. The germination process is a very extremely sensitive phase in plant growth and development, being indicative to any type of environmental contaminants. The effect of heavy crude oil residues was investigated for some seed parameters of some weeds which include *Solanum nigrum*. These parameters of *Solanum nigrum* was promoted by different dilutions of heavy crude oil. These results are agreed with the findings reported by Objegda & Atebe (2007) who found that germination index of Indian mustard was not affect with diesel oil contaminated soil**.** Kirk et al*.*, 2002 of grasses germinated successfully in different levels of petroleum hydrocarbon contamination. The effect of phenol and naphthol compounds, as water soluble fractions of crude oil, on the germination and seedling development was investigated for seeds of some crops cultivated in Libya. The obtained results showed that, low concentrations of both phenol and naphthol caused an increase of germination percentages of seeds of tested plant. This is probably due to that, low dilutions of these compounds may act as signal for α- amylase production in the seeds (Edema, 2012). These results agreed with those obtained by (El-Barghathi, 1985) who found that low dilutions of naphtol had a promoting effect on rate and final germination of oat seeds. This was probably caused by strong resistant qualities of the black nightshade seeds. This high quality of resistance marks the foregoing species to be considered as promising candidates for the phytoremediation of sites crude polluted with petroleum oil. The study underscores the need for the use of cheap, available, and environmental friendly technology as a remedy for the harmful effects of petroleum contaminants in the environment. Coating the seeds with oily substances prevent water and air movement in to the seed and directly causes toxic actions. One of the most possible reasons for seed germination inhibitory effects in crude oil contaminated sites is due to insufficient aeration of hypoxic or anoxic (having little or no oxygen, respectively), conditions. The embryo of seeds could have been injured or killed if it comes in contact with the oil. This effect could also be as a result of format-

Table 3.1. Effect of different dilutions of heavy crude oil on daily germination percentages (%) of *Solanum nigrum* L. (black nightshade) seeds.

+ = Not significant. ** = Significant at P< 0.01. ± = SEMean.

Similar letters = not significant. Different letters = significant.

Treatment (%)	Germination percentages (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.0	+ 0.00 ± 0.00	+ 0.00 ± 0.00	+ 0.00 ± 0.00	+ 0.00 ± 0.00	** 6.67^{a} ± 3.3	+ 96.8 ± 3.3	+ 96.8 ± 3.3
0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	86.8 ± 6.7	86.8 ± 6.7
1.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	93.3 ± 3.3	93.3 ± 3.3
2.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	86.7 ± 8.8	90.0 ± 10.0
4.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	80.0 ± 0.0	86.7 ± 6.7
6.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	93.3 ± 6.7	100.0 ± 0.0
8.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	80.0 ± 5.8	83.3 ± 6.7
10.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00^{b} ± 0.0	83.3 ± 8.8	83.3 ± 8.8

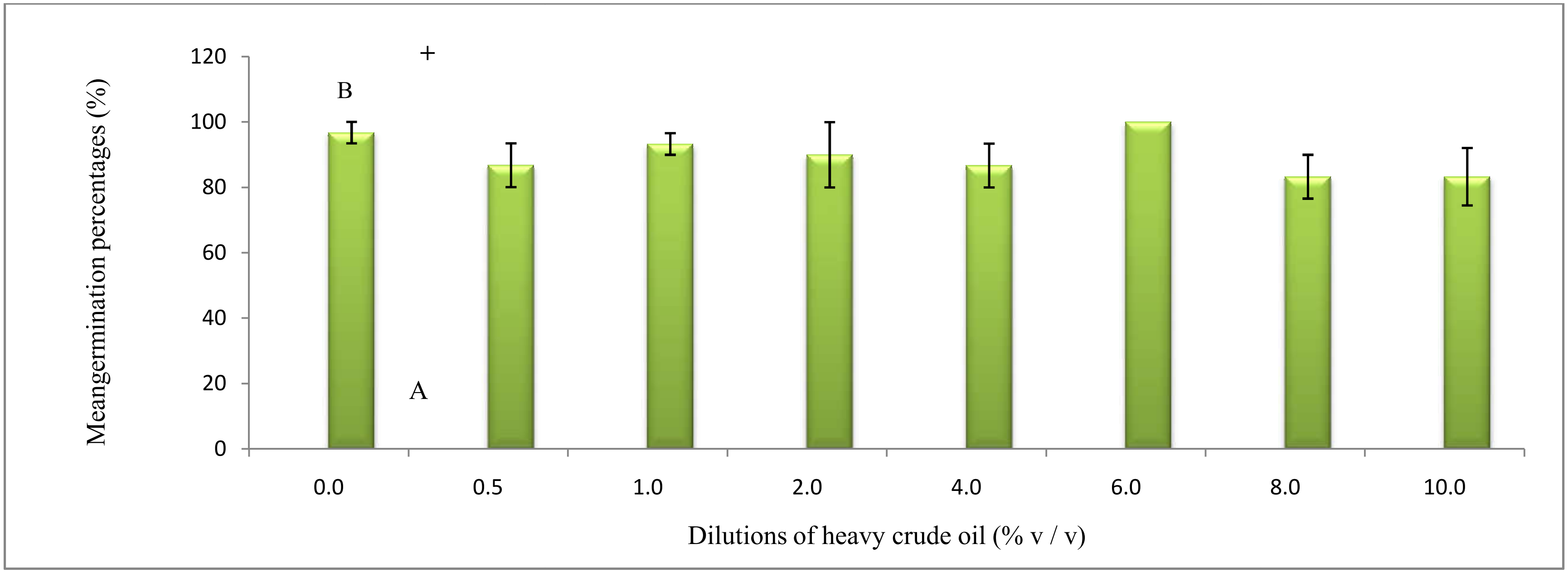


Fig. 3. 1. Effect of different dilutions of heavy crude oil on daily germination percentages (%) during the fifth day (A) and the seventh day (B) of *Solanum nigrum* L. (black nightshade) seeds.

+ = Not significant. Bars = SEMean.

Table 3.2. Effect different dilutions of heavy crude oil on the means of germination rate (GR), mean daily germination (MDG), mean germination time (MGT) and germination index (GI) of *Solanum nigrum* L. (black nightshade) seeds.

+ = Not significant. * = significant at $P< 0.001$. ± = SEMean.
Similar letters = not significant. Different letters = significant.

Treatment (%)	GR	MDG	MGT	GI
0.0	+ 1.4 ± 0.05	+ 13.8 ± 0.5	+ 18.4 ± 0.9	* 100.0^{a} ± 0.00
0.5	1.2 ± 0.09	12.4 ± 0.95	16.1 ± 1.2	81.0^{ab} ± 9.0
1.0	1.3 ± 0.05	13.3 ± 0.5	17.3 ± 0.62	76.95^{a} ± 4.9
2.0	1.3 ± 0.14	12.9 ± 1.4	16.4 ± 1.7	71.4^{ab} ± 8.8
4.0	1.2 ± 0.09	12.4 ± 0.95	15.5 ± 0.7	67.9^{b} ± 5.6
6.0	1.4 ± 0.00	14.3 ± 0.00	18.0 ± 0.6	81.9^{ab} ± 2.9
8.0	1.2 ± 0.09	11.9 ± 0.95	15.2 ± 1.1	64.4^{b} ± 5.0
10.0	1.2 ± 0.10	11.9 ± 1.3	15.5 ± 1.6	64.7^{b} ± 6.9

Table 3.3. Effect of different dilutions of heavy crude oil on seedling length (cm), fresh and dry weight (g) of *Solanum nigrum* L. (black nightshade) seedlings.

+ = Not significant. ** = Significant at *P*< 0.01. * ** = significant at *P*< 0.001. ± = SEMean.
Similar letters = not significant. Different letters = significant.

Treatment (%)	Mean values		
	Length (cm)	Fresh weight (g)	Dry weight (g)
0.0	*** $89.9^{a} \pm 2.0$	+ 0.01 ± 0.002	** $0.003^{a} \pm 0.0002$
0.5	$81.0^{ab} \pm 4.8$	0.10 ± 0.010	$0.003^{a} \pm 0.0002$
1.0	$71.5^{b} \pm 3.2$	0.01 ± 0.010	$0.003^{a} \pm 0.0004$
2.0	$68.8^{b} \pm 2.9$	0.01 ± 0.009	$0.003^{ab} \pm 0.0003$
4.0	$67.97^{bc} \pm 1.6$	0.01 ± 0.007	$0.003^{ab} \pm 0.00006$
6.0	$70.97^{b} \pm 1.2$	0.01 ± 0.006	$0.003^{ab} \pm 0.0002$
8.0	$67.10^{bc} \pm 0.8$	0.01 ± 0.006	$0.004^{ab} \pm 0.0002$
10.0	$67.40^{bc} \pm 1.2$	0.01 ± 0.007	$0.004^{b} \pm 0.0006$

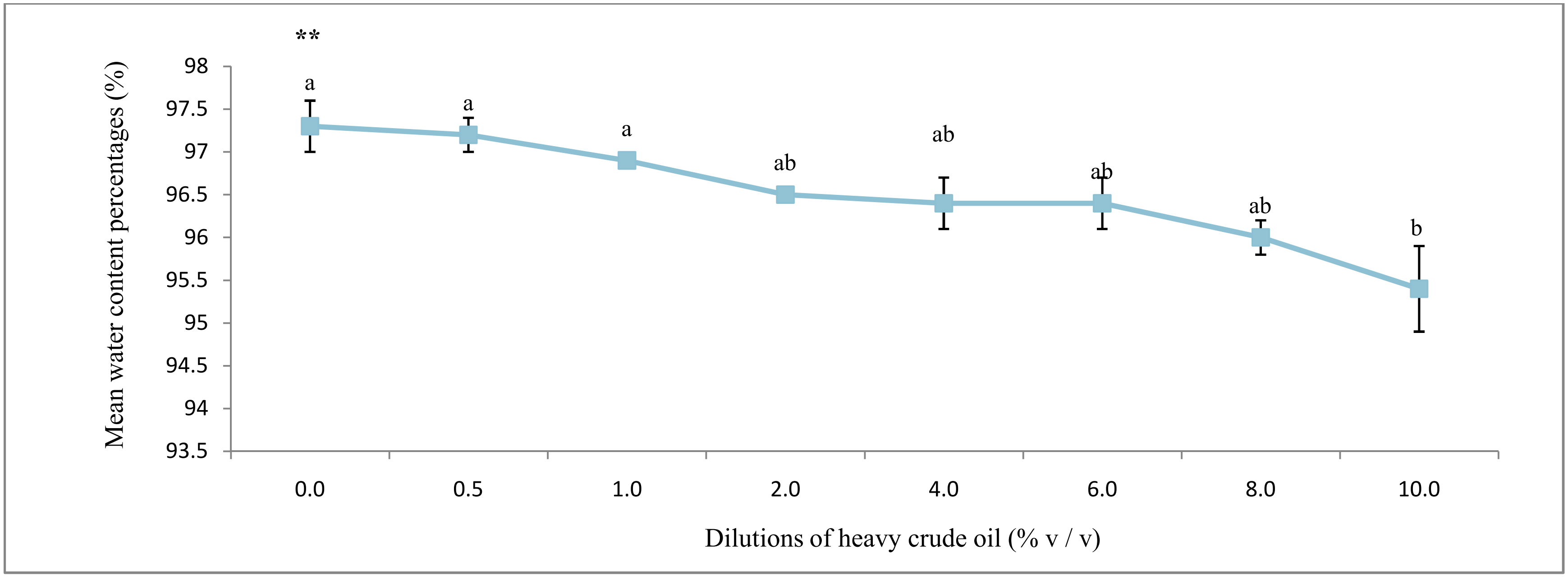


Fig. 3.2. Effect of different dilutions of heavy crude oil on relative water content percentages (%) of *Solanum nigrum* L. (black nightshade) seedlings.

** = Significant at $P < 0.01$. Similar letters = Not significant. Different letters = Significant. Bars = SEMean.

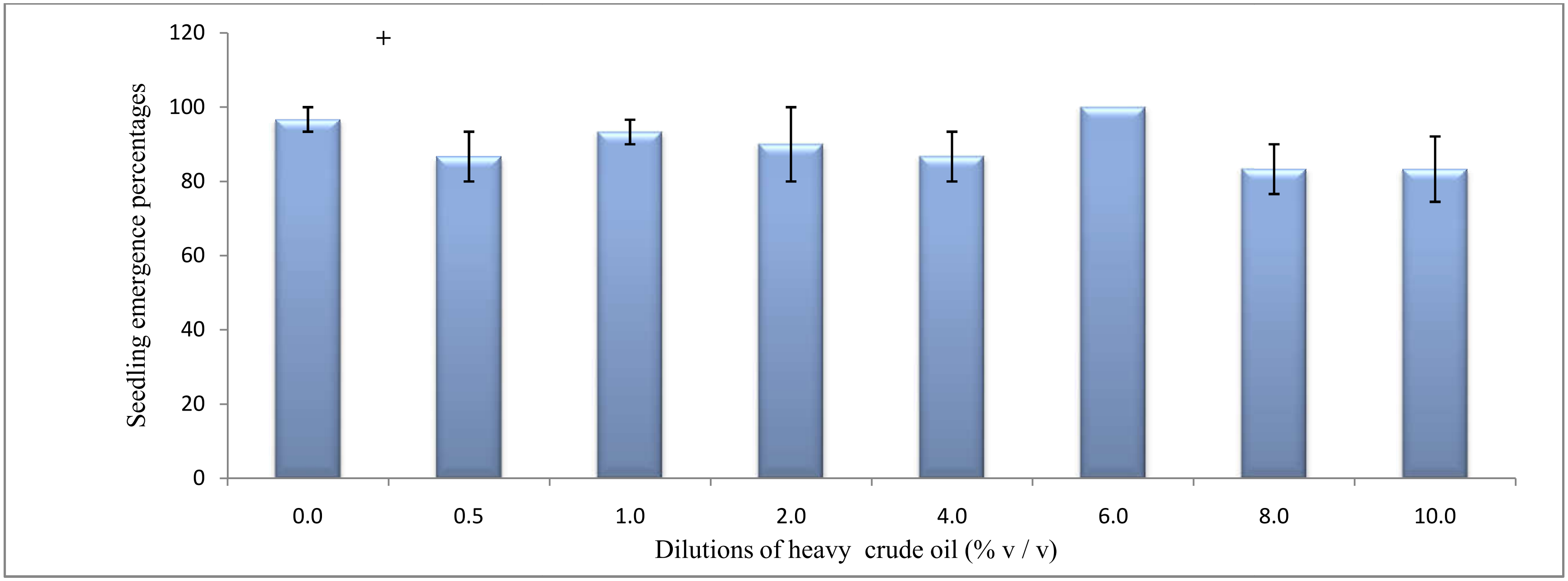


Fig. 3.3. Effect of different dilutions of heavy crude oil on seedling emergence percentages (%) of *Solanum nigrum* L. (black nightshade) seedlings.

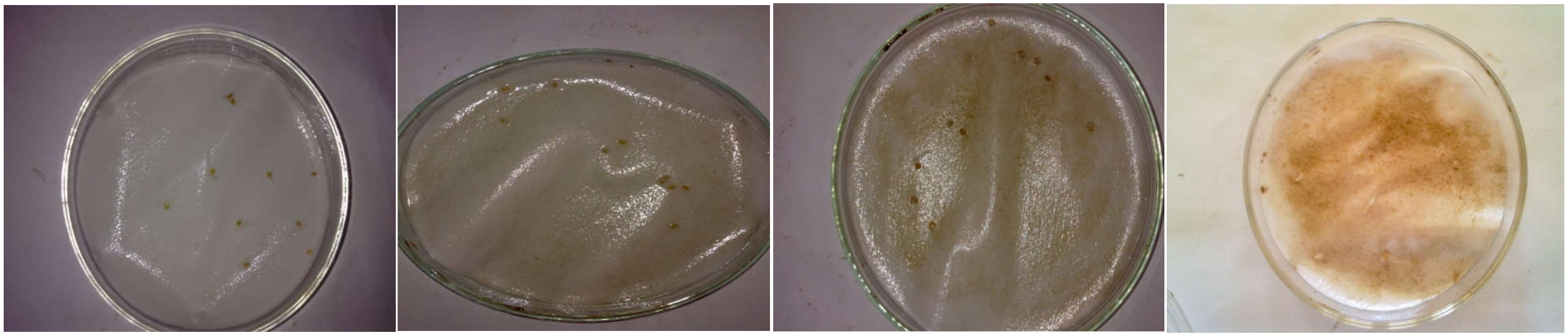
+ = Not significant. Bars = SEMean.

Table 3.4. Effect of different dilutions of heavy crude oil on seedling vigor index (SVI) and tolerance index (TI) of *Solanum nigrum* L. (black nightshade) seedlings.

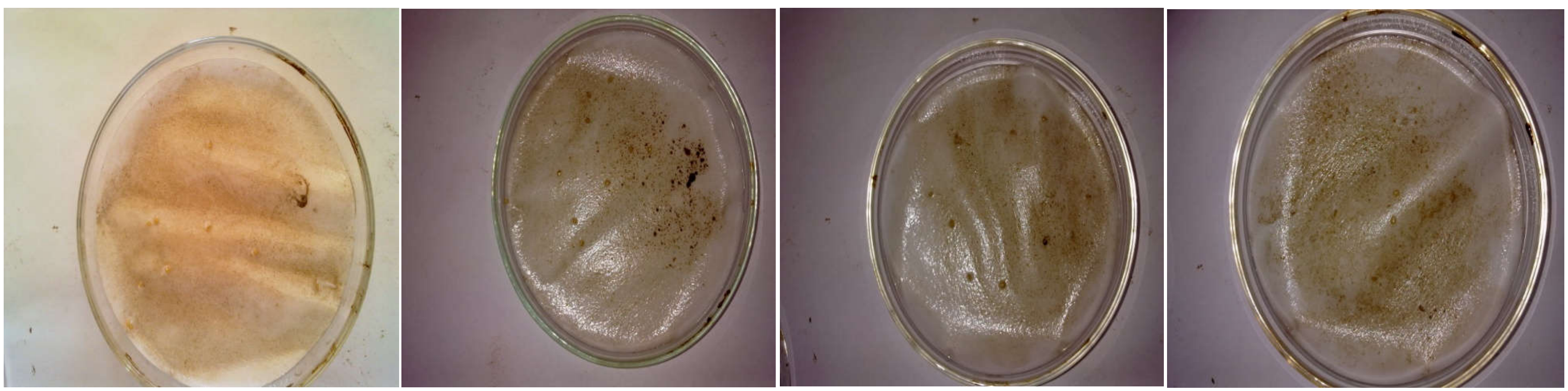
* = significant at *P*< 0.001. * ** = significant at *P*< 0.001. ± = SEMean.
Similar letters = not significant. Different letters = significant.

Treatment (%)	Mean values	
	SVI	TI
0.0	* $8682^{a} \pm 164$	*** $1.0^{a} \pm 0.00$
0.5	$7070^{ab} \pm 922$	$0.9^{ab} \pm 0.07$
1.0	$6694^{ab} \pm 543$	$0.8^{b} \pm 0.04$
2.0	$6227^{ab} \pm 867$	$0.8^{b} \pm 0.04$
4.0	$5909^{ab} \pm 588$	$0.8^{b} \pm 0.02$
6.0	$7097^{ab} \pm 118$	$0.8^{b} \pm 0.013$
8.0	$5586^{b} \pm 416$	$0.8^{b} \pm 0.02$
10.0	$5631^{b} \pm 676$	$0.8^{b} \pm 0.009$

0.0 0.5 1.0 2.0



4.0 6.0 8.0 10.0



Shows the effect of different dilutions (% v / v) of heavy crude oil on the germination of *Solanum nigrum* L. (black nightshade) seeds.

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-ion of polar compounds dissolved in water that could penetrate the seed coat and prevent the germination process (Wang et al., 2000; Adam & Duncan, 2002). The cessation of seed germination by crude oil is in line with previous research reports (Anoliefo &Vwioko 2001; Trapp et al., 2001 ; Anon, 2003; Sharafi et al., 2007; Malek- Hossein & Gholamreza, 2007; Omosun et al., 2008 ; Bamidele & Igiri, 2011 and Debojit et al., 2011; Sheta Omar et al., 2013; Agbogidi & April, 2013). In general, seed germination of species used in this work was enhanced under the stimulation of heavy crude oil. This is might be due to the hydrophopicity of heavy oil which possesses less solubility in water and therefore causes phytotoxicity. There is however, lack of information on the effects of crude oil on some biochemical processes such as oxidative stress parameters in plant species used in study.

Seedling performance of plants used in this study was measured under different dilutions of different of oil compound. Seedling growth of *Solanum nigrum.* Plants that are able to germinate successfully and tolerate the contaminant and show root elongation are tolerant plants (Ogbo, 2009 & Obj et al., 2008). But different seedling parameters in terms of fresh and dry measures were increased under different dilutions of the used oils. The high survival rate of these seedlings due to their tolerance to the high levels of oil compounds (Anoliefo & Edegbai, 2001). This stress condition may interfered with water absorption and gaseous exchange and led to reduction in seedling growth which apparent in the decrease of growth seedling parameters in poorly aerated environment (Quinones-Aquilar et al., 2003; Bamidele JF. 2010). This can be attributed to the decrease in relative water content plant dry weight and plant fresh weight of corn seedlings as the crude oil concentrations increased. These results revealed that both black nightshade and wheat showed good performance under both types of oil used in the study. Impact of stressful conditions of crude oil pollution has been shown to have adverse effects on plant growth and these may range from morphological aberrations, reduction in biomass to stomatal abnormalities (Victor & Sadiq, 2002). Growth reduction could also be explained as being due to harmful effects of oil. Growth reductions following oil pollution of soil have been reported by same authors such as Anoliefo & Edegbai (2000), of (Odjegba and Sadiq, 2002; Baran et al., 2002; Ikhajiagbe and Anoliefo, 2011). Different plants can tolerate different levels of petroleum hydrocarbons. Hydrocarbon contamination of soil reduced plant growth but increased microbial activity (Xu and Johnson, 1995; wioko & Fashemi, 2005). This study has demonstrated that crude oil contamination of soil has a highly significant effect of a reducing the biomass accumulation in *Jatropha curcas* seedlings. This study has implication on sustainability of using *Jatropha curcas* as a biodiesel species. Crude oil and petroleum products vary considerably in their toxicity, and the sensitivity to petroleum varies according to plant species. The toxicity of crude oil can be interpreted as the toxicity of a complex mixture of inorganic and organic, chemicals. The observed negative in the germination percentages, rate of germination as well as, the growth parameters (seedling length , fresh weight and biomass production) measured could be attributed to the numerous hydrocarbons and related compounds which are toxic to living organisms including plants. Generally, the highest performance in terms of percentage emergence and seedling development was recorded in black nightshade This study indicates that both black nightshade and wheat have more potential for resistance of crude oil concentrations and they can be used as promising tools for phytoremediation technology. Plant that tolerates higher concentrations of crude oil includes *Solanum nigrum* L. (black nightshade) weed plant species. This type of plants are recommended to be used as phytoremedation models especially the weed *Solanum nigarum* for cleaning up areas polluted with heavy crude oil residues this is propably due to its toxic contents of secandry metabolites that may counteats that hanful effects of these compounds used in the presend work. The influence of heavy crude oil upon different target plant species used in this research was not clearly pronounced (different) ie all plant species were not affected by the applications of heavy crude oil residues. This might be due to that heavy oil is more viscous and less soluble in water.

Summary and conclusions

The effected of crude oil (heavy) was examined for seed germination and seedling performance in the case of seed measures of *Solanum nigrum* L. (black nightshade). Was enhanced under of heavy crude oil compound. Furthermore, seedling performance was noticed to be good in residues of *Solanum nigrum* under heavy crude oil compound. Based on the obtained results, it is indicated that black nightshade have more potential for resistance to heavy crude oil concentrations and can be used as promising tools for phytoremediation technology. *Solanum nigrum* L. (black nightshade) as a weed plant species. Is recommended to be used as phytoremedation model for cleaning up areas polluted with crude oil.

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