Effect of different dilutions of (heavy) crude oil on the physiological responses of *Amaranthus hybridus* L. (Never-fading flower) species

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Abstract:
The remediation of oil contaminated soils has been a major problem in oil producing countries. This study evaluated the effect of petroleum crude oil contaminated soil on the plant Amaranths hybridus L. Soil polluted by crude oil have been found to inhibit plant growth. Changes in soil properties can lead to water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus. Oil pollution has damaging effects by rising in the toxic levels of certain elements Germination of seeds in soil polluted with crude oil may be significantly reduced the seeds germination. The effected of crude oil (heavy) was used on different concentrations (0.0 V/V, 0.5 V/V, 1.0 V/V, 2.0 V/V, 4.0 V/V, 6.0 V/V, 8.0 V/V, 10.0 V/V) examined for seed germination and seedling performance the Amaranths hybridus L . showed promoted heavy crude oil of Amaranths hybridus L performance was not affected by heavy oil applicationsand enhancement under heavy crude oil of seed germination. This might be due to that heavy oil is more viscous and less soluble in water.

Keywords: The contaminated ; Amaranths hybridus L ; Heavy crude oil

1.1. Introduction:

Germination of seeds in soil polluted with crude oil may significantly reduce the seed germination (Ekundayo *et al*., 2001; Achuba & Peretimo, 2007). Apparently, the hydrocarbon film created on the seed surface prevents the imbibitions of water and oxygen to the developing embryo. The inhibition of seed germination in oil contaminated soil also can be directly or indirectly related to the presence of oil in germination media, furthermore Anolifefo *et al*., (2010) and Edema (2012) poor germination of seed is attributed to the penetrating power of the volatile fractions of oil. In contact with a seed, oil would enter the seed coat and readily kill the embryo (Bhat *et al.*, 2008 & Njoku *et al*., 2009). The seed germination also can be indirectly prevented by unfavorable soil conditions such as surface crusts and drought conditions related to soil being contaminated with crude oil residues (Adam & Duncan, 2002; Achuba, 2006). Sharifi *et al*. (2007) have reported that the crude oil soaked cotyledons or embryo lead to poor germination of seeds.
Many studies of the effects of crude oil in maize (corn) have examined the effects of crude on physiological parameters such as percentages of seed germination (Ekundayo *et al.*, 2001).

Seed germination of okra (Adenipekun *et al.*, 2009), soybeans (Agbogidi *et al*., 2006) and *Amaranths hybridus* under the effect of oil (Odjegba & Sadiq, 2002; Agbogidi & Edema, 2011) plant species were delayed in due to the soil polluted with crude oil. Generally, petroleum hydrocarbon contamination causes retardation of seed germination (Pezeshki *et al.*, 2000; Omosun *et al*., 2008) and resulted in adverse

biological effects (Edema, 2012). Some researchers consider naphthalene as a more important source of crude oil toxicity than low molecular weight aromatics. Gasoline on the other hand is a complex mixture of organic compounds and it also has been shown to be toxic to plants and delayed the seed germination (Trapp *et al*., 2001; Anon, 2003). Germination and root elongation are two critical stages in plant development that are sensitive to environmental contaminants. Plants that are able to germinate successfully and tolerate the contaminant are considered tolerant plants (Ogbo, 2009). The effects of oil pollution vary according to the type, time and age of the plant species (Edema, 2012). Oil polluted soils are not well ventilated which in turn decrease plant growth and development (Victor & Sadiq, 2002). Oil pollutions reduce some plant growth parameters such as plant height, leaf number, leaf surface, plant fresh and dry weight and biomass (Omosun *et al.*, 2008), photosynthetic pigments and also nutrient absorption (Rosso *et al.*, 2005). Plant responses to oil pollution are different and depend on plant species, type of oil, amount and concentration, exposure times and environmental conditions (Pezeshki *et al*., 2000; Spiares *et al*., 2001; Zangh *et al.*, 2007; Besalatpoor *et al*., 2008). Although the effects of the individual petroleum products on plants have been evaluated by many reports (Victor & Sadiq, 2002; Omosun *et al.*, 2008). Environmental pollution of crude oil has been shown to have adverse effects on plant growth (Opeolu, 2000). Toxicity symptoms observed in plants exposed to oil pollution include chlorosis, necrosis, stunted growth, suppression of leaves, and reduction in biomass, and large stomatal abnormalities. When oil coats plant leaves, it can block stomata and reducing the phototsynthesis of plants (Adenipekun *et al.*, 2008). The presence of petroleum hydrocarbons in soils has a negative impact on plant growth and development (Das & Mukherjee, 2007). Their harmful effects include inhibition of plant growth, reduction of photosynthetic pigments; slow down of nutrient assimilation and reduction in the growth of roots and aerial organs. It is also postulated that some fractions of petroleum can dissolve biological membranes and as a consequence, disrupt the plant root structure (Peng *et al*., 2009). Generally, crude oil contamination causes reduction of plant growth as it interferes with the uptake of nutrients by plants. It also causes competition for the little nutrient available in the polluted soils between plants and soil microbes and ultimately suppresses the growth of plants in such soil (Johnson *et al.*, 2005). Cell membranes are damaged by penetration of hydrocarbon molecules, leading to leakage of cell contents (Agbogidi & Eshegbeyi, 2006. Grasses are sometimes used to remediate chemically contaminated soils, because their root systems are extensive and fibrous (Ogbo, 2009). *Amaranths hybridus* L. is defined as a "never-fading flower" in Greek. Its habitat is waste ground, cultivated fields, disturbed sites, roadsides, railroads. *Amaranths hybridus* is an edible plant which is best eaten when young. Grain of amaranth has higher protein contents than other cereal grains and has significantly higher lysine content. It has been shown that amaranth leaves are an excellent source of protein, with its maximal accumulation of (17.2 – 32.6 %) of the dry weight (Kadoshnikov Sergey *et al.*, 2005) and high lipid fraction (Pogojeva *et al.*, 2006). Azizi and Fuji (2006) reported that germination of *Amaranthus hybridus* seeds was significantly affected under spent engine oil-polluted soil. While growth of seedlings, chlorophyll and protein contents were seriously affected (Odjegba & Sadiq, 2002. Germination of *Amaranths hybridus* seeds were significantly affected in the soil polluted by oil, few seeds of *Amaranths hybridus* sown in the soil contaminated with crude oil, germinated but very slowly. Oil contaminated soils generally causes delayed seed emergence (Merkl *et al*., 2005c). Reduction in the general growth parameters of plants grown on contaminated sites have been attributed to insufficient aeration of crude oil

contaminated soils (Njoku *et al*., 2008a) and limited water supply to the plants (Agbogidi *et al*. ,2007). The reduction in root length limits the quantity of nutrients the plant is able to absorb. Also, the reduction in leaf number and leaf area reduces the rate of photosynthesis, these effects together lead to the reduction of plant growth (Anoliefo *et al*., 2003; Omosun *et al*., 2008).

2.1. Material and Methods:

Viable seeds of *Amaranthus hybridus* L. (Never-fading flower) family Amaranthaceae.
Chemicals: Formaldehyde, distilled water (DW), the crude oil used was (From AL-Breiga port, field Alamal) heavy and light 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).

2.2. Germination test : Seed Preparation prior to germination: All 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 five seeds. Six replicates were used for each treatment 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% = number of seeds that germinated / Number of seeds sown * 100
Germination rate (GR)= N / D
(N) number of emerged seeds in day (D) is day after planting (Rastegar. *et al.*, 2011)
Germination index (GI) =GS / GC * LS /LC * 100. (Abdul-Baki and Anderson, 1973)
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.
Number of non-germinated seeds was calculated daily to determine daily inhibition of germination percentages by using the following formula:
Coefficient of germination velocity (CGV) = A_1+ A_2 + A_3…+ A_n / A_1T_1 + A_2T_2 +…..
Where (A) is the numbers of seeds germinating and (T) is the number of days taken to germinate, was calculated. (Maguire, 1962).
Mean germination time (MGT) = n1 x d1 + n2 x d2 + n3 x d3 + -------- / Total number of days .
Where, n is the number of germinated seed d is the number of days. Ellis and Roberts, 1981)
Mean daily germination (MDG) = Final GP / number of days to final GP.
(Czabator, F. J. (1962). Scott SJ, etal (1984)

2.3. Early seedling development:

For determining the effect of these compounds on the seedling growth, germinated seeds were allowed to develop in to seedling for another two weeks for the following

different parameters. In the case of weeds, the length was measured as whole seedlings due to their smaller size.
Relative water content was estimated **by** (Gairola. *et al*., 2011). formula as follow:
Different parameters were measured for the determination of seedling growth**: -** these measurements include:
Root and shoot length (cm) for Amaranthus by using a ruler.
RWC (%) = Fresh weight – dry weight / Fresh weight *100
Percentage seedling emergence= Number of seedling that emerged / Number of seeds sown * 100. (Agbogidi, 2011b).
Inhibition percentage of fresh and dry parameters of seedlings in relation to control treatment was calculated by a given formula:
Seedling vigor index (SVI) is calculated using the following modified formula:
SVI = Seedling length (cm) * final germination percentages.
(Kaydan and Yagmur, 2008; Mut *et al.,* 2010). Abdul-Baki and Anderson (1973): Hangarter RP.,1997
Tolerance index (TI) is calculated using the following modified formula:
TI = Length of seedling in treatment / Length of seedling in control (Royo, *et al*., 2006; Maiti,etal 1996).

2.4. Statistical analysis

The data of all experiments will be statistically analyzed using a computer program of Minitab (Version 13). One – way analysis of variance will be used for the determination of the significance within treatment, and Tukey's pariwise comparisons test to determine the significant differences between the means. Test for data normality and transformation will be also carried out (Verdeguer *et al*, 2009).

3. Result:

3.1. *Amaranthus hybridus* L.

3.1.1. Seed germination

Germination percentages (%) of *Amaranthus hybridus* were calculated under different dilutions of heavy oil are shown in (Table 3.1). (GP %) were not significant differences in days (1, 2, and 3). Differences in GP % of days 4 and 5 were significant (F = 4.86, $P< 0.01$, F = 4.97, $P< 0.01$) respectively within heavy oil treatments where they had been increased by concentrations of heavy crude oil. Tukey's pairwise comparison test reveals significant differences of *Amaranthus hybridus* between untreated (control) and dilutions 1.0, 10.0 (% v/ v) of same oil. During the sixth and final days of germination percentages were significant differences (F = 2.66, $P< 0.05$, F = 3.36, $P< 0.05$) respectively. The results shown increased under all crude oil concentrations, but it was reduced under control treatment. Tukey's pairwise comparison test reveals significant differences of germination percentages between control and concentration of 1.0 (% v / v) (Table. 3.1 and Fig. 3.1) for days fourth and seventh day. Germination rate (GR) of *Amaranthus hybridus* was estimated under different dilutions of heavy crude oil and is shown in table 3.2. There were significant differences (F = 3.36, $P< 0.05$) within treatments of this measure. It is shown to be increased under concentrations of heavy crude oil. Tukey's pairwise comparison test reveals significant differences of this parameter between untreated (control) and concentration 1.0(v/v)

Table 3.1. Effect of different dilutions of heavy crude oil on daily germination percentages (%) of *Amaranthus hybridus* L (Never-fading flower) seeds.

+ = Not significant. * = Significant at $P< 0.05$. ** = Significant at $P< 0.01$ ± = SEMean. Similar letters = not significan. 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	+ 23.3 ± 3.3	+ 23.3 ± 3.3	** 26.7^{a} ± 3.3	** 26.7^{a} ± 3.3	* 26.7^{a} ± 3.3	* 33.3^{a} ± 3.3
0.5	0.00 ± 0.00	10.0 ± 5.8	16.7 ± 3.3	30.0^{a} ± 5.8	33.3^{ab} ± 3.3	40.0^{ac} ± 5.8	50.0^{ab} ± 5.8
1.0	0.00 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	63.3^{b} ± 12.0	63.3^{b} ± 12.0	66.7^{bc} ± 13.3	80.0^{b} ± 0.0
2.0	0.00 ± 0.00	6.70 ± 6.7	6.70 ± 6.7	26.7^{a} ± 3.3	26.7^{a} ± 3.3	30.0^{a} ± 5.8	36.7^{a} ± 12.0
4.0	0.00 ± 0.00	10.0± 0.00	10.0± 0.00	43.3^{ab} ± 6.7	43.3^{ab} ± 6.7	46.7^{a} ± 8.8	56.7^{ab} ± 12.0
6.0	0.00 ± 0.00	6.70 ± 3.3	6.70 ± 3.3	30.0^{a}± 10.0	30.0^{a}± 10.0	46.7^{abc} ±1 3.3	56.7^{ab} ± 8.8
8.0	0.00 ± 0.00	3.33 ± 3.3	3.33 ± 3.3	40.0^{ab} ± 5.8	40.0^{ab} ± 5.8	43.3^{abc} ± 8.8	63.3^{ab} ± 12.0
10.0	0.00 ± 0.00	20.0 ± 11.5	20.0 ± 11.5	63.3^{b} ± 3.3	63.3^{b} ± 3.3	63.3^{bc} ± 3.33	66.7^{ab} ± 3.3

Parameters of mean daily germination (MDG), mean germination time (MGT), of never-fading flower (Table 3.2). Were differences in (MDG), (MGT) significantly (F = 3.36, $P< 0.05$, F = 3.59, $P< 0.05$) respectively within treatments. Was most affected by different dilutions of heavy crude oil 1.0 up to 10.0 (% v / v) increased. The mean daily germination and mean germination time were significantly different between control and concentration of 1.0 (% v / v). Parameters of coefficient of germination velocity (CGV) of same plant were shown under in (Fig. 3.2). Was significant (F = 5.29, $P< 0.01$), within treatments. Tukey's pairwise comparisons test showed that the significant differences were found between control and other treatments means of heavy crude oil. Furthermore, the germination index was not affected by different dilutions of heavy crude oil(Table 3.2).

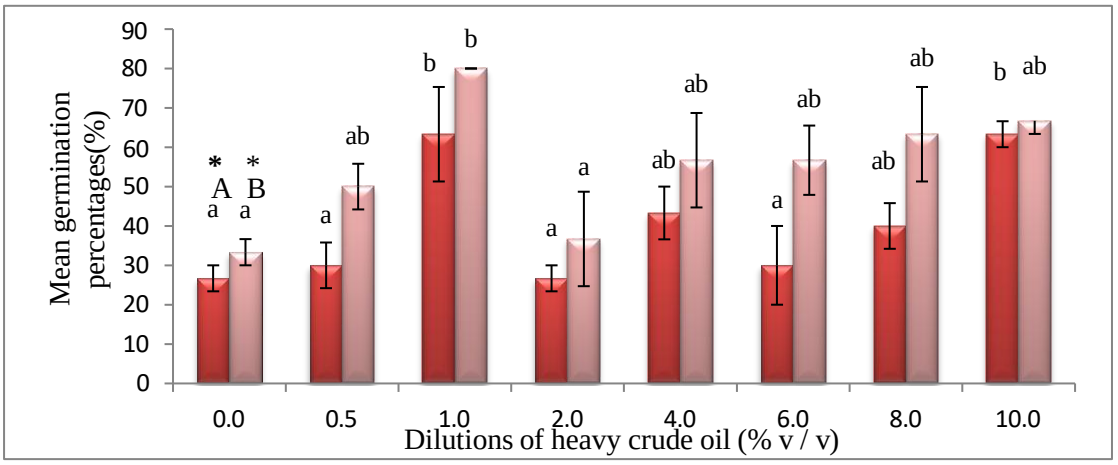

Fig. 3.1. Effect of different dilutions of heavy crude oil on daily germination percentage (%) during thefourth day (A) and the seventh day (B) of *Amaranthus hybridus* L. (Never-fading flower) seeds.

* = Significant at *P*< 0.05. ** = Significant at *P*< 0.01. Bars = SEMean.
Similar letters = Not significant. Different letters = Significant. .

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 *Amaranthus hybridus* L. (Never-fading flower) seeds.

+ = Not significant. * = Significant at *P*< 0.05. ± = SEMean. Similar letters = not significant. Different letters = significant.

Treatment (%)	GR	MDG	MGT	GI
0.0	* $0.50^{a} \pm 0.05$	* $4.8^{a} \pm 0.50$	* $10.7^{a} \pm 1.00$	+ 100.0 ± 0.00
0.5	$0.70^{ab} \pm 0.08$	$7.10^{ab} \pm 0.80$	$13.5^{ab} \pm 1.30$	244.8 ± 59.9
1.0	$1.10^{b} \pm 0.00$	$11.40^{b} \pm 0.00$	$22.6^{b} \pm 2.70$	604.7 ± 80.4
2.0	$0.52^{a} \pm 0.20$	$5.20^{a} \pm 1.70$	$10.10^{a} \pm 2.50$	135.9 ± 72.7
4.0	$0.80^{ab} \pm 0.20$	$8.10^{ab} \pm 1.70$	$15.95^{a} \pm 2.70$	329 ± 129
6.0	$0.80^{ab} \pm 0.10$	$8.10^{ab} \pm 1.30$	$14.0^{a} \pm 3.50$	290 ± 105
8.0	$0.90^{ab} \pm 0.20$	$9.05^{ab} \pm 1.70$	$15.4^{a} \pm 2.90$	446 ± 202
10.0	$0.95^{ab} \pm 0.05$	$9.50^{ab} \pm 0.50$	$21.7^{a} \pm 1.70$	378 ± 82.6

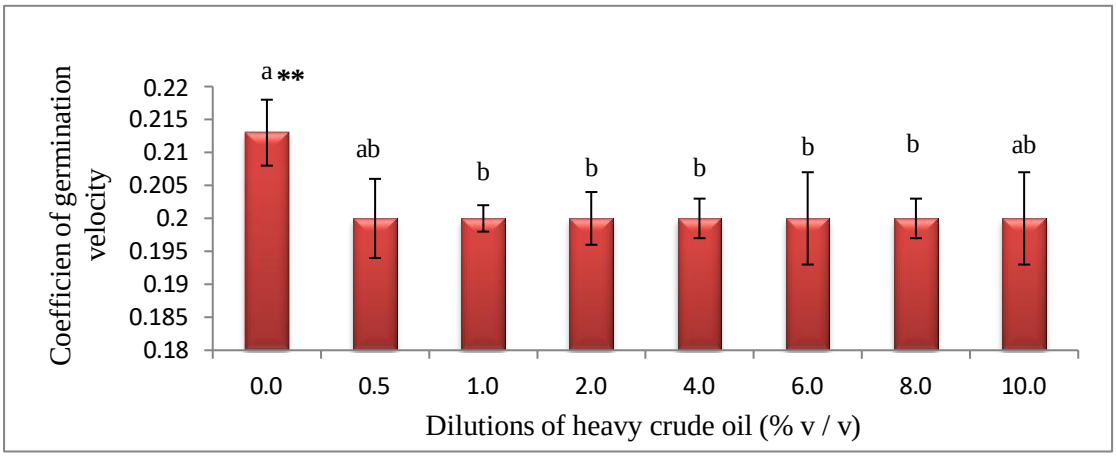

Fig. 3.2. Effect of different dilutions of heavy crude oil on coefficient of germination velocity (CGV) of *Amaranthus hybridus* L. (Never-fading flower) seeds.

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

3.1.2. Seedling growth

Length seedling and fresh weight of *Amaranthus hybridus* were tested under different dilutions of heavy crude oil (Table 3.3). Results showed that different concentrations of heavy crude oil caused significantly (F = 3.88, *P*< 0.05, F = 2.97, *P*< 0.05) respectively within treatments reduced of length seedling, fresh weight of same plant in control and concentration of 2.0 (% v / v), but increased in other concentrations of heavy crude oil.

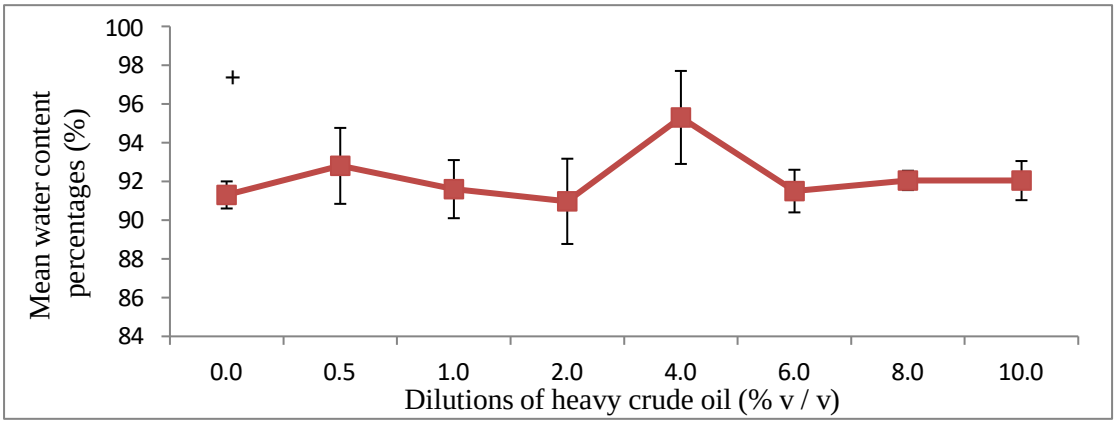

Fig. 3.3. Effect of different dilutions of heavy crude oil on relative water content percentages (%) of *Amaranthus hybridus* L. (Never-fading flower) seedlings.
+ = Not significant. Bars = SEMean.

Tukey's pairwise comparisons test showed that significant differences were found between untreated (controls) and concentration of 1.0 (% v / v). Parameters of dry weigh table3.3 and relative water content percentages (RWC %) (Fig. 3.3). Seedling emergence percentages (SE %), seedling vigor (SVI) and tolerance indexes (TI) of *Amaranthus hybridus* (Table 3.4). Were found significantly (F =3.36, *P*< 0.05, F = 3.88, *P*< 0.05, F = 2.95, *P*< 0.05) respectively within treatments increased of concentration above 1.0 (% v / v) in all parameters. Tukey's pairwise comparisons test

showed that the significant differences were found between control and concentration of 1.0 (% v / v) (Fig 3.4, 3. 5 and 3.6).

Table 3.3. Effect of different dilutions of heavy crude oil on seedling length (cm), fresh and dry weight (g) seedling and relative water content percentages (RWC %) of *Amaranthus hybridus* L. (Never-fading flower) seedlings.

+ = Not significant. * = Significant at P< 0.05. ± = SEMean.

Similar letters = not significant. Different letters = significant.

Treatment (%)	Mean values			
	Length (cm)	Fresh weight (g)	Dry weight (g)	Relative water content percentages
0.0	* 13.4a ± 1.20	* 0.013^{a} ± 0.001	+ 0.001 ± 0.0001	+ 91.3 ± 0.70
0.5	21.0ab ± 2.70	0.022^{ab} ± 0.004	0.002 ± 0.0005	92.8 ± 1.96
1.0	32.7b ± 1.20	0.032^{b} ± 0.002	0.003 ± 0.0004	91.6 ± 1.50
2.0	13.1ab ± 3.20	0.014^{ab} ± 0.003	0.001 ± 0.0005	90.97 ± 2.20
4.0	22.5ab ± 3.60	0.030^{ab} ± 0.005	0.001 ± 0.0007	95.3 ± 2.40
6.0	19.4ab ± 3.60	0.022^{ab} ± 0.004	0.002 ± 0.0004	91.5 ± 1.10
8.0	27.1ab ± 6.30	0.030^{ab} ± 0.006	0.002 ± 0.0004	92.05 ± 0.50
10.0	23.7ab ± 1.50	0.030^{ab} ± 0.003	0.002 ± 0.0004	92.04 ± 1.01

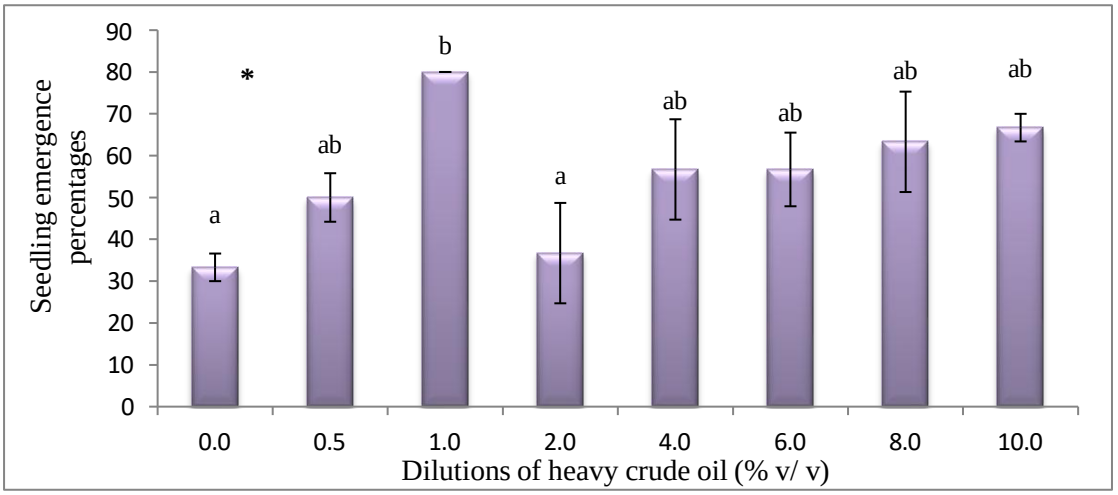

Fig. 3.4. Effect of different dilutions of heavy crude oil on seedling emergence percentages (%) of *Amaranthus hybridus* L. (Never-fading flower) seedlings.

* = Significant at *P*< 0.05. Similar letters = Not significant.
Different letters = Significant. Bars =SEMean.

Table 3.4. Effect of different dilutions of heavy crude oil on seedling emergence percentages (SE %), seedling vigor (SVI) and tolerance indices (TI) of *Amaranthus hybridus* L. (Never-fading flower) seedlings.

* = significant at $P< 0.05$. ± = SEMean.
Similar letters = not significant. Different letters = significant.

Treatment (%)	Mean values		
	SE %	SVI	TI
0.0	* 33.3^{a} ± 3.3	* 453.7^{a} ± 81.2	* 1.0^{a} ± 0.00
0.5	50.0^{ab} ± 5.8	1083^{ab} ± 256	1.6^{ab} ± 0.13
1.0	80.0^{b} ± 0.0	2613.3^{b} ± 97.0	2.5^{b} ± 0.20
2.0	36.7^{a} ± 12.0	558^{a} ± 302	0.99^{a} ± 0.24
4.0	56.7^{ab} ± 12.0	1363^{ab} ± 507	1.70^{ab} ± 0.30
6.0	56.7^{ab} ± 8.8	1161^{ab} ± 359	1.50^{ab} ± 0.34
8.0	63.3^{ab} ± 12.0	1867^{ab} ± 658	2.09^{ab} ± 0.60
10.0	66.7^{ab} ± 3.3	1584^{ab} ± 162	1.80^{ab} ± 0.20

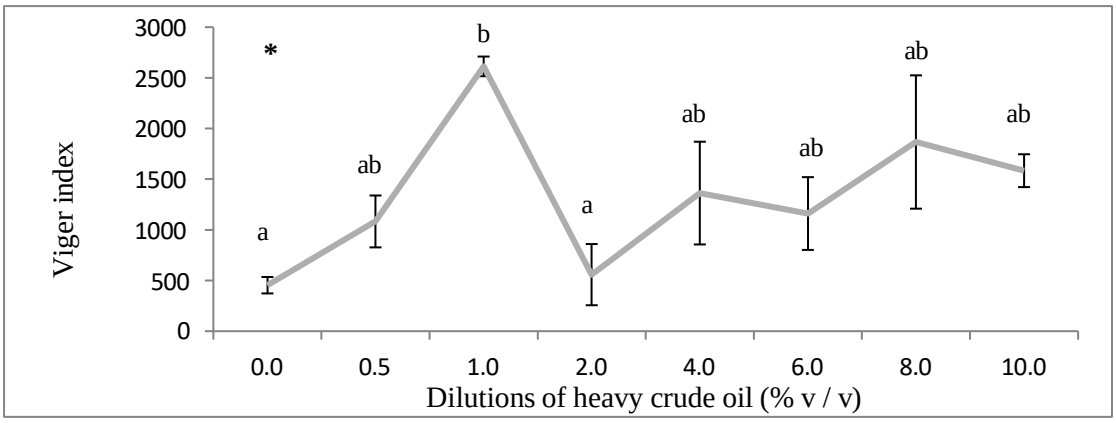

Fig. 3.5. Effect of different dilutions of heavy crude oil on seedling vigor index (SVI) of *Amaranthus hybridus* L. (Never-fading flower) seedlings.

* = Significant at $P< 0.05$. Similar letters = Not significant.
Different letters = Significant. Bars =SEMean.

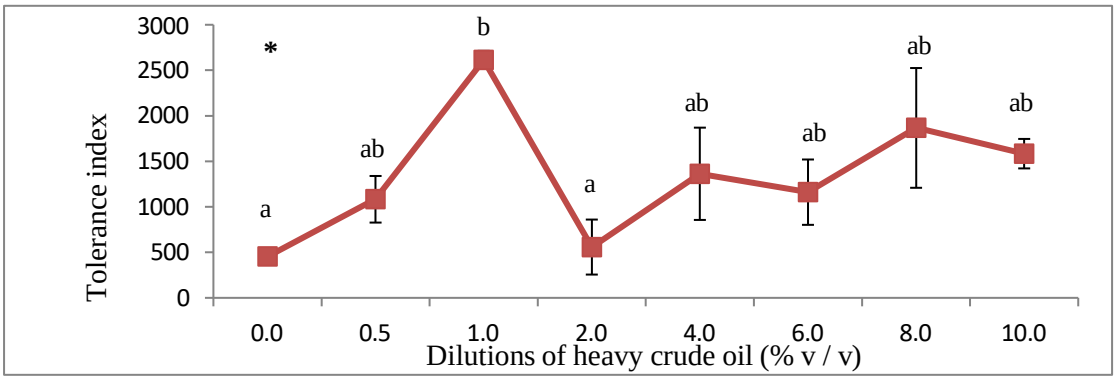

Fig. 3.6. Effect of different dilutions of heavy crude oil on tolerance index of *Amaranthus hybridus* L. (Never-fading flower) seedlings.

* = Significant at $P< 0.05$. Similar letters = Not significant.
Different letters = Significant. Bars =SEMean.

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 were investigated for some seed parameters of some weeds which include Solanum nigrum, Amaranthus hybridus and some cultivated plants such as Triticum aestivum and Zea mays seeds. the Amaranthus hybridus responded differently to the effect of various kinds of oil residues. This study had revealed that introduction of Amaranthus hybridus into light residue of crude oil adversely and severely inhibits their seed germination and seed parameters of these species were significantly reduced with no seed germination under the highest application of light crude oil in the case of Amaranthus hybridus. 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 formation 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). Reported that growth parameters in Amaranthus hybridus decreased as the concentration of crude oil contamination increased. But seed measures of Amaranthus hybridus plant species were promoted by the application of different dilutions of heavy residue of petroleum oil. Whereas, coefficient of germination velocity of plant species showed similar patterns of response under light and heavy levels of oil residues. In general, seed germination used in this work was enhanced under the stimulation of heavy crude oil. It had been found that exposure of plants into petroleum oil caused an increase in the production of stress related phytohormones such as ethylene and abscisic acid (Njoku

et al., 2012). Most studies of the effects of crude oil have examined the effect of petroleum residues on physiological parameters such as percentages of seed germination of corn (Ekundayo et al., 2001: Kyung et al., 2004), Okra (Adenipekun et al, 2009), soybeans (Agbogidi et al., 2006) and Amaranths hybridus (Odjegba & Sadiq, 2002) where their germination was delayed in the soil polluted by crude oil. Generally, contamination by petroleum hydrocarbon causes retardation of seed germination (Pezeshki et al., 2000; Omosun et al., 2008) that led to adverse biological effects of some plant species (Edema, 2012). Shoot growth retardation in plants due to petroleum pollution as observed in this work had been reported by different workers on related studies (Adoki & Orugbani, 2007; Lin & Mendelsshohn, 2009; Bamidele, 2010; Debojit et al., 2011 and Bamidele & Igiri, 2011). Adoki & Orugbani (2007) during their study with three vascular plants (fluted pumpkin, maize and okra) reported retardation in their shoot growth as a result of crude oil contamination. This is in agreement with the result obtained by (Adenipekun, et al., 2009) concentration affects the growth of okra adversely and severely. In the case of Amaranthus hybridus seedling devolvement their response is similar to the pattern followed by their seed germination processes, where their seedling performance was reduced under concentrations of tested type of light oil 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). 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. But seedlings measured of never-fading flower were promoted under the effect of heavy crude oil. . 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). The highly depression in seedling mergence, seedling vigor index and tolerance of Amaranthus hybridus and Zea mays treated with different light crude oil concentration is as a result of changes in the growing media. Accumulation of toxic substances resulted in a decrease in size and biomass production of maize plant species (Brandt et al., 2006; Daniel- Kalio and Pepple, 2006; Adenipekun et al., 2009). Spiares et al., 2001of the reduction in the growth of seedlings which growing in the media polluted by petroleum oil residues probably due to the poisoning effects of the crude oil on the plant development. 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). Who also observed significant effect of engine oil on leaf vegetable (Amaranthus hydrides L.). Noticed a significant reduction in height of seedlings, of treatment relative to the control. Different plants can tolerate different levels of petroleum hydrocarbons. Hydrocarbon contamination of soil reduced plant growth but increased microbial activity (Xu and Johnson, 1995). Whether the effect of the contaminant is beneficial or adverse depends, to a certain degree, on the concentration of the contaminant and type of plant species. It seems to the present study that the adverse effect was noticed on the treated never-fading flower plants may be due to unfavorable germination conditions created by toxic substances contained in the crude oil. Growth reduction in crude oil polluted media as observed in this study for some plant species may also be attributed to a disruption in aeration. This observation is in line with the findings of Bamidele & Agbogidi (2000), and Odjegba & Sadiq (2002) and Agbogidi et al. (2007)

reported delayed in germination and reduction in grain yield of maize in crude oil polluted soil.

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