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GROWTH AND MITOTIC CHROMOSOMES OF SOYBEAN (*Glycine max* L. MERRIL) PLANTS AS AFFECTED BY SPENT ENGINE OIL IN SANDY-LOAM SOILS

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ABSTRACT
Soybean (Glycine max L. Merril) is one of the most nutritionally valuable legumes in Africa. However, spent automobile engine oil is always disposed indiscriminately on lands and water bodies in Nigeria, which pollutes both terrestrial and aquatic ecosystems, with adverse effects on crop growth and productivity. This study investigated the effects of spent engine oil (SEO) polluted soils on growth parameters and mitotic chromosomes of soybean. Two soybean varieties (TGX-1448-2E and local) were planted in pots filled with sandy-loam soil treated with 0, 0.1, 0.4 and 0.7% v/w SEO concentrations, in 2 × 4 factorial experiment laid in a completely randomized design with six replications. Data were collected at weekly intervals for four weeks on growth parameters (plant height, number of leaves, leaf width and leaf length) and chromosomal aberration. Data were subjected to ANOVA and means separated with LSD. Among the treatments, seed germination rate was significantly higher in TGX-1448-2E (95.0 ± 0.0-96.0 ± 0.7%) than local variety (70.0 ± 0.7-71.0 ± 0.7%). Both 0.4 and 0.7% SEO caused significant (p ≤ 0.01) reductions in plant height, number of leaves, leaf length and leaf width whereas, 0.1% did not reduce growth parameters. Treatments 0.4 and 0.7% SEO caused high chromosomal aberrations in soybean as they reduced chromosome numbers (from 40.0 to 23%), cell division, mitotic index (70.0 to 20.8%) and produced 31.7% abnormal cells. Abnormalities such as bridges, laggards and chromosome stickiness occurred. This study shows that 0.4-0.7% SEO polluted soils is phytotoxic to soybeans.

Key words: chromosomal aberrations, growth parameters, mitotic index, oil pollution, soybean varieties

INTRODUCTION
Nigeria is one of the major petroleum producing countries in the world, having oil as the mainstay of its economy (Adenipekun *et al.,* 2008). Over the years, exploration and exploitation of petroleum in Nigeria has brought so much pollution to the environments. Petroleum-based industries including oil refineries are parts of the major sectors that generate tremendous amount of pollutants into the environment which pollutes both terrestrial and aquatic ecosystems (Badrul, 2015). Pollution emanating from spent engine lubricating oil has been reported to be more widespread and prevalent than that of petroleum (Odjegba and Sadiq, 2002). Spent auto-engine oil is a waste produced from automobile workshops, mechanical and electrical engine repairer's shops after servicing vehicles, power generating sets and other types of engines (Okonokhua *et al.,* 2007). It includes mono and multi-grade lubricating oils from petrol and diesel engines, together with gear oils and transmission fluids. Lubricating engine oil contains chemical additives including amines, phenols, benzenes, calcium, zinc, barium, magnesium, phosphorus, sulphur and lead (Kirk *et al.,* 2005; Lale *et al.,* 2014).

Spent engine oil (SEO) is produced during routine maintenance of automobile and power generating engines and often indiscriminately disposed into gutters, municipal drainage systems, open vacant plots roadsides and farmlands in Nigeria by auto-technicians and allied artisans (Anoliefo and Vwioko, 2005). Ahamefule *et al.* (2017) reported that crude oil (petroleum) in soils has deleterious effects on biological, chemical and physical properties of the soil depending on the type and dose of the oil and other factors. Such effects on soil physicochemical properties may, over time, cease to be an ecological problem (Umoren *et al.*, 2019), but may remain an agronomic problem. Spent auto-engine oil can contain higher levels of heavy metals compared to the unused oil, depending on the process of generating the waste (Okonokhua *et al.,* 2007). Agbodigi (2010) reported poor germination of cowpea with increasing dose of SEO. Vwioko and Fashemi (2005) observed stimulation of germination at 1% w/w SEO in soil for castor bean (*Ricinus communis*) seedlings whereas, germination was reduced at higher concentration (2, 3, 4, 5 and 6% v/w). Some growth inhibitory effects of SEO have also been reported on soybeans (Ahamefule *et al.*, 2017).

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Soybean (*Glycine max* L. Merril) is one of the most valuable, versatile, and nutritionally important legumes globally. It can be grown under a wide range of climatic conditions, using a variety of management practices, and for diverse purposes (Shea *et al*., 2020). Nigeria is the second largest producer of soybean in Africa, after South Africa, with annual production of about 600,000 tonnes (FAOSTAT, 2020). Soybean is increasingly cultivated in Nigeria due to its high nutritive and diverse domestic and industrial importance (Qiu and Chang, 2010; Obalum *et al*., 2011). The key advantages of soybean are its high protein content and insoluble fiber and a good source of several vitamins and minerals, including vitamin K, folate, thiamine, copper, manganese and phosphorus (Fabiyi, 2006; Riaz, 2006). Soybean products include: soy flour, soy protein, soy sauce, soybean vegetable oil, livestock feeds, natto, tempeh, tofu, soy milk, soy cheese and curds, most of which contain antioxidants and phytonutrients that have been linked to various health benefits (Fabiyi, 2006). The various uses of soybeans can be constrained because planting on SEO contaminated soils results in chromosomal abnormality and growth reduction.

The capacity of SEO to inhibit cell division, reduce mitotic index, induce other chromosomal aberrations and reduce growth has been reported in crops (Obute *et al*., 2016; Ahamefule *et al*., 2017). Chromosomal aberration includes changes either in chromosome structure or number. Alterations in the chromosomal structure can be caused by breaks of DNA, DNA synthesis inhibition and modification in the DNA replication (Leme and Marin-Morales, 2009). In the absence of telomeres, chromosomes turn out to be sticky which may join to other fragmented chromosomal ends. Stickiness is caused by either increased contraction or condensation of chromosomes or DNA depolymerization and partial dissolution of nucleoproteins. Sticky chromosomes produce toxic effects which are usually irreversible and might lead to cellular deaths. Chromosomal bridges result when chromosomes become sticky and their separation is delayed; chromosomes remain connected by bridges that move freely, while lagging chromosomes arise when chromosomes fail to remain connected with spindle fibre, which may move to either of poles (Leme and Marin-Morales, 2009). The anti-mitotic influence of chemical oil treatments is amplified in the chromosomal aberrations they induced. Bellani *et al.* (2000) demonstrated that such aberrations monitored by *Allium* test could be translated to toxicity and teratogenicity in animals and humans. Even at low concentrations, spent engine oil has genotoxic effects on DNA of plants and could be aggravated with increase in exposure time (Olorunfemi *et al*., 2011).

Oil contaminated land can invariably change the physical and chemical properties of soil. It can destroy soil rhizosphere system including microbial populations, hamper soil aeration, soil water and fertility, leading to adverse effects on seed germination, plant growth and productivity (Baruah and Sarma, 2002; Tanti *et al*., 2009). Tanti *et al*. (2009) and Obute *et al*. (2016) have shown that heavy metals in SEO have strong toxic effects on soybean plants. Studies have shown that 1% v/w SEO contaminated soil significantly increased leaf area, dry matter content and grain yield, whereas above 1% v/w hindered soil water movement and subsequently reduced growth and yield of soybean (Ahamefule *et al*., 2017). Therefore, the purpose of this study was to assess the effect of SEO polluted soils on growth parameters and chromosomal aberrations of soybean seedlings.

MATERIALS AND METHODS

Sources of Planting Materials and Site Description

Two soybean varieties used in this study were TGX-1448-2E (improved variety) obtained from the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Oyo State, Nigeria and a local variety obtained from farmers in Abule Egba, Lagos State, Nigeria. The study was carried out at the experimental plot of the Department of Biological Sciences, Bells University of Technology, Ota, Ogun, State Nigeria. Ota city falls in geographical coordinates 6.69° N, 3.23° E and possesses climate of tropical rainforest with two main climatic conditions *viz* the rainy season which last for between seven and eight months from April to October with an interruption in August, and the dry season running through November till February (Ufoegbune *et al*., 2016; Ogunsola and Igun, 2019). The geology of the study area is chiefly on the Nigerian Dahomey basin (Usikalu *et al*., 2018).

Soil Test, Treatments, Planting and Seedling Maintenance

Sandy loam soil samples were collected at 15 cm depth (topsoil) from four locations on fallow land in Bells University of Technology, Ota, Ogun State. Soil samples were bulked for the experiment, air-dried, ground and passed through a 2-mm sieve (Ogunsola and Adetunji, 2016). The soil was tested free of SEO. The SEO used was obtained from vehicles undergoing service maintenance at two automobile workshops in Ota, Ogun State. Oil polluted soil was simulated in pot experiment. About 5 kg of the soil was weighed into plastic pots and four soil treatments: 0.0, 0.1, 0.4 and 0.7% v/w of SEO were prepared comprising of 0, 5, 20 and 35 ml SEO concentrations per 5 kg soil. The treated soils were left for 24 h before sowing to ensure oil penetration into the soil. Five seeds were sowed per pot and seedlings were thinned down to two on the 9th day. There were 24 pots per variety, making a total of 48 pots arranged in a two (varieties) by four (SEO treatments) factorial experiment laid-out in a completely randomized design with six replications. The study was conducted between January and April, 2020. The potted plants were maintained by daily watering and weed control was carried out manually. Insect pests were managed with cypermethrin insecticide (cyperforce, 10% EC) at 4 ml per litre of water sprayed at two-week interval, starting from four weeks after sowing (WAS). Growth parameters (plant height, number of leaves, leaf width and leaf length) were recorded weekly from one WAS.

Cytological Assessment of the Root Tips of Soybeans

At 4 WAS, seedlings were uprooted at 10 a.m. and the root tips of the treated and untreated plants were excised and washed with sterile distilled water. The root tips samples were directly fixed in 3:1 ethanol (96%) to acetic acid for 24 h to arrest cell in metaphase without any pre-treatment. The fixed root tips were hydrolyzed in 18% HCL for 5 min. in order to degrade cell wall. The root cells were prepared for the chromosome counting using squash method (Ostergren and Heneen, 1962). The cells were stained with Flp Orcein solution for 2 h to allow enough dye accumulation. The prepared samples were mounted between slides and coverslip and observed under 40× and captured using a compound light microscope for 100×, for each soil treatments. About seven individual cells on different slides at metaphase stage were prepared from the plants and used to count the number of chromosomes and the average used to determine the number of chromosomes. Another prepared samples, mounted between slides and cover slip were observed for total cells, including dividing cells and number of aberrated cells, under 100× magnification for different phases of cell divisions and the chromosomal aberrations using the trinocular microscope (Euromax Microscopen, Spain) and cytological observed photos taken from mounted digital camera. Data were collected on seed germination rate at 7 days after sowing (DAS) and growth parameters: plant height (cm), number of leaves, leaf breadth (cm) and leaf length (cm). Data were also taken on chromosome number at metaphase stage, number of cells, number of dividing cells and number of aberrated cells under 40× and captured at 100× using a compound light microscope as earlier mentioned.

Data Analysis

Seed germination rate was determined by the ratio of the number of germinated seeds to the total number of seed sown, expressed in percentage (Ahamefule *et al*., 2017) with the data transformed using square root transformation method. Data on growth parameters and germination percentage were subjected to analyses of variance (ANOVA) using the PROC GLM statement of Statistical Analysis System, version 9.2 (SAS, 2008) and means separated using least significant difference ($p \leq 0.01$). A total of 1,000 cells were counted at 100× for different phases of cell divisions and checked for chromosomal aberrations. Data on effects of the SEO on cell division and chromosome number of soybean seedlings were estimated using the mitotic index (MI) and the chromosomal aberrations calculated as described by Bakare *et al.* (2000) as follows:

$$\text{Mitotic index (MI \%)} = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100 \text{ .. (1);}$$

$$\text{\% aberrated cells} = \frac{\text{Number of aberrated cells}}{\text{Total number of cells in division}} \times 100 \text{ ... (2).}$$

RESULTS AND DISCUSSION

Effect of Oil Polluted Soils on Seed Germination

The growth parameters of soybean seedlings (TGX-1448-2E and local varieties) in the untreated and treated soils varied significantly ($p < 0.01$). Seed germination was observed from four to seven DAS, which was earlier (4 to 5 days) in the 0.0, 0.1, 0.4% treatments but delayed (6-7 days) in 0.7% v/w SEO treated soils. Percentage seed germination of TGX-1448-2E in the four treatments which ranged from 95-96% were significantly higher ($p < 0.01$) than those of the local variety (70-71%) (see Table 1). This is contrary to the report of Katsivela *et al.* (2005) on seed germination caused by SEO polluted soils. Inhibitory effect of SEO on germination percentage has been reported to be dose dependent (Agbogidi and Ilondu, 2013). Spent engine oil concentrations evaluated in this study were lower than 1% (Agbogidi, 2010) and 3% v/w (Ahamefule *et al.*, 2017) previously reported to have inhibited seed germination. The result of this study shows that seed germination of the local variety obtained from the farmer was lower than the improved variety. This agrees with an earlier study by Grau *et al*. (2004).

Growth Inhibition of Soybean by Spent Engine Oil Polluted Soil

The reduction in plant height, number of leaves, leaf breadth and leaf length of soybean were observed on seedlings imposed with different levels of SEO. The trend of growth from the first to the fourth WAS (Figure 1 (a-d) showed taller plant and higher number of leaves from untreated (control) than soils treated with SEO in both varieties. Plant height and number of leaves reduced with increased doses; 0.7% SEO produced the least. The result showed that 0.1% SEO did not show a reduction in number of leaves, whereas 0.7% reduced the number of leaves of local variety and TGX-1448-2E from 2 and 3 WAS, respectively.

Significant differences ($p \leq 0.01$) were observed in plant height, number of leaves, leaf width and leaf length of the two varieties. TGX-1448-2E produced taller soybean plants of 24.90 ± 0.90 cm in untreated

Table 1: Percentage germination of soybean seeds planted in spent engine oil (SEO) contaminated soil

Variety	SEO concentration (%)	Seed germination (%)
TGX-1448-2E	0.00	96.00[a]
	0.10	96.00[a]
	0.40	95.00[a]
	0.70	95.00[a]
Local variety	0.00	71.00[b]
	0.10	70.00[b]
	0.40	70.00[b]
	0.70	70.00[b]

Means with the same letter are not significantly ($p \leq 0.01$) different.

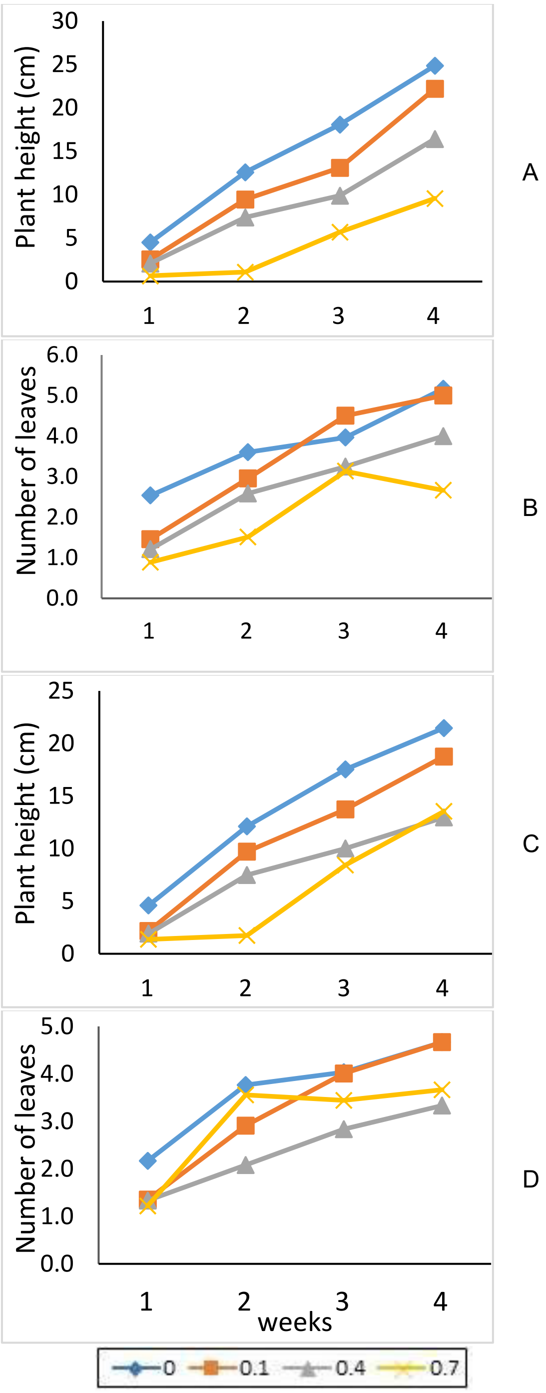

Figure 1: Effect of spent engine oil treated soils on plant height and number of leaves of soybean varieties for four weeks. A and B are plant height and number of leaves of TGX-1448-2E, C and D are of local variety.

soil (control), whereas 0.7% SEO produced the least (9.60 ± 8.00 cm), other growth parameters followed the same trend (Table 2). However, no significant difference in the growth parameters were produced by the untreated (control) soil and 0.1% SEO in both soybean varieties with the exception of plant height of the local variety. This implies that 0.1% SEO did not reduce growth parameters of soybean significantly ($p \leq 0.01$) unlike the two higher concentrations (0.4 and 0.7% v/w SEO) (see Table 2).

Inhibitory effects of SEO on crop growth have been previously reported (Agbogidi and Ejemete, 2005; Kayode *et al.,* 2009; Ekpo *et al.*, 2012). The lower growth parameters might be due to a reduction in mineral absorption with increasing oil concentration in the soil (Odjegba and Atebe, 2007). For instance, a study on maize *(Zea mays*) has shown that oil pollution makes nutrient elements in the soil unavailable to plants (Udebuani *et al.*, 2017). In addition, inhibition of plant growth by SEO could be due to increased acidity in the soil (Achuba and Peretiemo-Clark, 2007) due to microbial metabolism of the substrate (Udebuani *et al.*, 2017). The presence of some heavy metals in the SEO soil might have also contributed to the observed stunted growth of soybeans seedlings (Okonokhua *et al.,* 2007). High concentration of cadmium as well as nickel, lead, copper and zinc which are toxic to plant have been reported in oil polluted soil (Udebuani *et al*., 2017; Umoren *et al*., 2019). The SEO produces adverse effects on the physical, chemical, and biological properties of the soil (Agbogidi and Ejemete, 2005; Ahamefule *et al.,* 2017). When present in the soil, crude or spent engine oil occupies the macrospores and coats macro aggregates, thereby reducing the water film thickness around macro aggregates of the soil (Adewole and Moyinoluwa, 2012; Badrul, 2015; Umoren *et al*., 2019). The poor performance of soybean in the oil-polluted soil has demanded for bioremediation and/or phytoremediation measures on such soils before use for agronomic production (Ezenne *et al.,* 2014; Sanderson *et al*., 2018).

Cytotoxic Effects: Chromosomal Aberrations, Aberrated Cells and Mitotic Index

The number of chromosomes, number of cells and aberrant cells and mitotic index of the two soybean varieties varied when imposed with SEO treatment. The number of chromosomes counted at the metaphase stage of the soybean cells decreased with increase in concentration of SEO (Table 3). Number of chromosomes were higher (36 to 40) for plants from untreated soil in both soybean varieties which is within the normal chromosome number ($2n$ = 40) of soybean (Hailemariam and Tesfaye, 2018) than from 0.4% (24-31) and 0.7% SEO treated soil (21-27). This implies that 0.4 and 0.7% SEO treatments were cytotoxic to soybean plants, producing aberrant chromosomes.

Table 2: Effects of spent engine oil (SEO) on growth parameters of soybeans at week four

Variety	SEO concentration (%)	Plant height (cm)	Number of leaves	Leaf width (cm)	Leaf length (cm)
TGX-1448-2E	0.00 (control)	24.90 ± 0.90^{a}	5.20 ± 0.50^{a}	2.90 ± 0.20^{a}	7.60 ± 0.70^{a}
	0.10	22.20 ± 0.60^{ab}	5.00 ± 0.50^{a}	2.80 ± 0.30^{ab}	5.70 ± 1.40^{ab}
	0.40	16.40 ± 1.00^{bc}	4.00 ± 0.50^{ab}	2.50 ± 0.30^{ab}	5.80 ± 1.60^{ab}
	0.70	9.60 ± 8.00^{c}	2.70 ± 2.30^{b}	1.50 ± 1.40^{b}	3.40 ± 2.90^{b}
Local variety	0.00 (control)	21.50 ± 0.50^{a}	4.60 ± 0.30^{a}	2.70 ± 0.20^{a}	5.90 ± 0.10^{a}
	0.10	18.80 ± 1.10^{b}	4.70 ± 0.80^{a}	2.70 ± 0.30^{a}	5.70 ± 0.90^{a}
	0.40	12.90 ± 0.30^{c}	3.30 ± 0.30^{b}	2.30 ± 0.30^{ab}	4.50 ± 0.40^{b}
	0.70	13.50 ± 0.40^{c}	3.70 ± 0.30^{b}	2.10 ± 0.10^{b}	3.40 ± 0.20^{c}

Means with the same letter within column per variety are not significantly different by LSD ($p \leq 0.01$).

Table 3: Chromosome counts of soybean (2n = 40) root cells at metaphase stage in soils inoculated with spent engine oil (SEO)

Variety	SEO concentration	Number of chromosomes from seven cells at metaphase							
	(%)	1	2	3	4	5	6	7	Mean
TGX-1448-2E	0.00 (control)	38.00	40.00	37.00	40.00	38.00	39.00	40.00	39.00
	0.10	34.00	32.00	32.00	35.00	32.00	29.00	37.00	33.00
	0.40	25.00	25.00	33.00	24.00	27.00	24.00	30.00	27.00
	0.70	22.00	23.00	26.00	22.00	23.00	27.00	21.00	23.00
Local variety	0.00 (control)	38.00	39.00	36.00	40.00	40.00	36.00	37.00	38.00
	0.10	29.00	30.00	33.00	29.00	36.00	31.00	32.00	31.00
	0.40	24.00	29.00	25.00	23.00	26.00	27.00	31.00	26.00
	0.70	22.00	21.00	20.00	26.00	24.00	23.00	25.00	23.00

Cytological observation of root tip cells of soybean raised in SEO polluted soil showed various types of cellular and chromosomal aberrations: bridge and laggard chromosome, sticky chromosome, dislocation of poles, chromosome bridge and disturbance in metaphasic spindle, and destruction of nuclei (Figure 2). Although 0.1, 0.4, and 0.7% SEO treatment showed chromosomal aberrations in soybean seedlings, the effect was greater with higher concentrations (0.4 and 0.7% SEO) than lower dose (0.1%) (see Table 4).

Aberrant cells percentage abnormality (percent aberrant cells) was highest at the highest SEO concentration whereas no aberrant cell was detected from untreated control soil (Table 5). The mitotic index (MI) also decreased with increase in SEO treatments. The highest values of 70.00 and 68.50 were obtained from untreated plants (control) whereas the least (22.60 and 20.80) were observed from 0.7% v/w SEO treatment level for TGX-1448-2E and local varieties, respectively. The decreased mitotic index also indicates a strong trend of inhibition

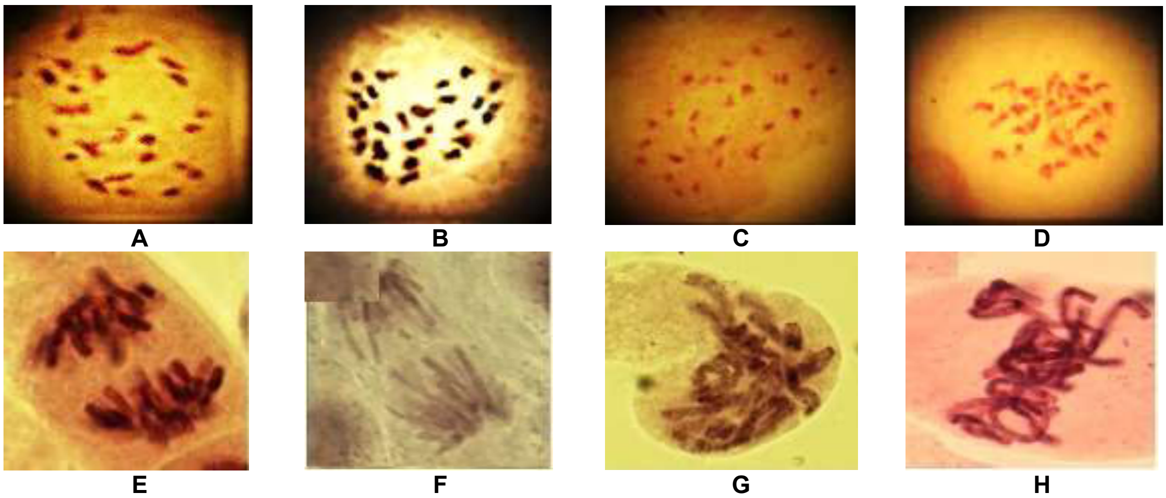

Figure 2: Aberrations in mitotic cell division in soybean induced by spent engine oil-polluted soil. A and B are mitotic cell divisions; C is disturbed metaphase; D is stickiness at metaphase, E is stickiness with chromosome fragments, F is bridge and laggard chromosome, G is chromosome bridge and disturbance in metaphasic spindle, H is vagrant chromosomes and c-mitosis.

Table 4: Cytotoxic effects of varied concentrations of spent engine oil (SEO) treated soil on soybean

Types of cytological effects observed in both varieties	Mean frequency of cell and chromosomal aberrations SEO concentration (%)			
	0.00 (control)	0.10	0.40	0.70
Bridge and laggard chromosomes	0.00	6.00	13.00	20.00
Sticky chromosomes	0.00	10.00	20.00	32.00
Prolonged prophase and abnormal cytokinesis	0.00	15.00	28.00	34.00
Dislocation of poles	0.00	10.00	18.00	30.00
Chromosome bridge and disturbance in metaphasic spindle	0.00	2.00	2.00	9.00
Destruction of nuclei	0.00	9.00	20.00	2.00
Total	0.00	52.00	101.00	127.00

Table 5: Mitotic index and percentage aberrated cells of soybean sown in soil treated with spent engine oil (SEO)

Variety	SEO concentration (%)	Total cells observed	Total cells in division	Total aberrated cells	Aberration (%)	Mitotic index (%)
TGX-1448-2E	0.00 (control)	1,000.00	700.00	0.00	0.00	70.00
	0.10	1,000.00	560.00	38.00	6.80	56.00
	0.40	1,000.00	355.00	49.00	13.80	35.50
	0.70	1,000.00	226.00	70.00	30.90	22.60
Local variety	0.00 (control)	1,000.00	685.00	0.00	0.00	68.50
	0.10	1,000.00	515.00	36.00	6.90	51.50
	0.40	1,000.00	368.00	53.00	14.40	36.80
	0.70	1,000.00	208.00	66.00	31.70	20.80

of cell division in soybean by oil-treated soils. The observed reduction in the frequency of dividing cells (cell division) might be due to lack of spindle fibres formation (Timothy *et al*., 2014). Borah and Talukdar (2002) reported that the frequency of cells and chromosomal aberrations could be associated with the toxicity of the spent engine oil. Udebuani *et al*. (2017) reported that polluted soils with SEO produce abnormal increase in all size (hypertrophy) in maize plant which later ruptures as the cells grow older. Also, reports have shown that exposure of plant cells to SEO can produce cells showing nuclei disintegration or destruction (Tanti *et al.,* 2009). In this study, number of cells having c-mitosis and few vagrant chromosomes were also observed and the failure of the spindle apparatus organization and normal function could be the cause of c-mitosis and vagrant chromosome movement ahead of its associated chromosomal group toward the poles, which usually leads to the unequal separation of chromosomes in the daughter cells (Leme and Marin-Morales, 2009). However, studies have indicated cytotoxicity of plants at 1% of SEO concentration and above (Agbogidi, 2010; Ahamefule *et al.*, 2017) although 1% concentration has been shown to stimulate seed germination and crop grain yields in other studies (Vwioko and Fashemi, 2005; Ahamefule *et al.*, 2017).

CONCLUSION

Discharge of SEO on lands has inhibitory and detrimental effects on growth parameters of soybean plants. Low spent oil pollution of 0.4 and 0.7% v/w in the soil is phytotoxic to soybean plants resulting in significant reduction in their growth parameters and chromosomal aberrations such as reduction in chromosome numbers and mitotic index, sticky chromosomes and bridge and laggard chromosomes. These cytotoxic effects of SEO necessitate the need of soil test for the determination of oil concentration before soybean cultivation, especially in areas with close proximity to auto-mechanic workshops and oil wells. Phytotoxicity at a low concentration calls for a public health awareness of the danger of indiscriminate discharge of oil or oil spillage, while a possible regulation and environment-friendly solution by the Government to check disposal of SEO may be helpful.

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