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Response of Tomato Infected with *Ralstonia solanacearum* in Soil Contaminated with Crude Oil

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ABSTRACT
The response of tomato (*Solanum lycopersicum* L) infected with *Ralstonia solanacearum* (Smith) to soil contaminated with crude oil was investigated in a series of pot experiments. Tomato variety (Ibadan) was sowed on *R. solanacearum*-infested soil contaminated with 0, 1, 2 and 4% of crude oil after two weeks of contamination. Soils containing 0% and 1% oil levels had significant higher seedling emergence than soils with 2 and 4% oil levels. Tomato seedlings that survived in all treatments had severe stunting with maximum height of 2.7 cm suggesting that oil contaminated soils cannot be used in the nursery. Furthermore, seedlings inoculated with the bacterium transplanted to sterile soil contaminated with different levels of crude oil (1, 2 and 4%) had significantly lower plant height, number of leaves and number of flower per plant than the control (0%). Wilted tomato plants were observed only on seedlings inoculated with bacterium transplanted on soil not contaminated with crude oil. The control and treatment containing 1% oil level had statistically comparable fruit weights but higher than weights recorded for other treatments. Thus, low oil contaminated soil reduced the pathogenic potential of *R. solanacearum* on tomato, and with little adverse effect on growth of tomato.

Keywords *Solanum lycopersicum, Ralstonia solanacearum,* Response, Crude oil, Bacterial wilt, Nigeria

INTRODUCTION

The Nigerian economy depends largely on crude oil, but its extraction can have serious adverse effects on the environment. Kauss *et al*., (1976) showed that the soluble fraction of crude oil has components that are fully or slightly soluble in water and contains dispersed particulate oil, soluble metallic ions and dissolved hydrocarbons. Oil spillage is inevitable, and is one problem for which no effective and final solution has been found anywhere in the world in spite of efforts to prevent it. The Niger Delta area where most of the oil is produced has been subjected to frequent oil spills involving blow-outs and leakages since the first well came on line. Severe ecological damage has occurred in the Niger Delta (Benka-Coker and Ekundayo, 1995; Ogri 2001). Studies estimated that as much as 1,843,047 barrels of crude oil was spilled in the region between 1976 and 1980 (Awobajo, 1981). Oil spills render the soil unproductive as substances in spills deplete nutrients for germination and growth of plants; the amount of land available for agricultural activity is reduced leading to food insufficiency. What develops is the over-dependence on food imports for a product that could be produced locally. Due to the impact of spillages on the ecosystem it is necessary to gather information about the biological depletion and to find ways of rehabilitating and managing oil polluted lands. The nature and chemical composition of the crude oil, crop type and age, topography, soil type, drainage characteristics and climatic factors influence the recovery rate of land (Hutchinson and Freeman, 1978). The level of oil spilled is a factor exerting a large influence on agricultural lands (Isirimah *et al.* 1989). Isirimah *et al*. (1989) further reported that plants exposed to light oil pollution suffer from leaf yellowing and leaf drop; complete shedding of leaves follows heavy contamination. Rowel (1977) and Odu (1981) observed possible recovery of plants if the soil is not heavily polluted. Soil oil concentrations of 0.5% and 1% (Isirimah *et al.*, 1989; Odu, 1981) has been reported to be beneficial to crops.

Tomato (*Solanum lycopersicum* L.) is an important vegetable. The major constraints to production are pests and diseases. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) is a major disease of tomato in the tropics and subtropics including Nigeria. Disease incidences of 35 to 70% have been observed causing heavy loss of revenue (Adebayo and Ekpo, 2005). The pathogen is soilborne and has an extremely wide host range (Hayward, 1991). Various strategies have been developed for control of bacterial wilt of tomato but are limited in application. Planting resistant cultivars is the most effective means of disease control, but this is also limited because of location specificity of resistance which is related to environmental conditions, especially temperature (Prior *et al*., 1990).

Adverse effects on the environment occur due to oil spillage (Awobajo, 1981; Odu, 1981; Burger, 1993; Ekundayo and Obuekwe, 2000; Wells, 2001). There is paucity of information on the effect of oil pollution on specific crop diseases in relation to crop performance. This study was carried out to investigate the effect of crude oil on soil, and the pathogenicity of *R. solanacearum* on the performance of tomato.

MATERIALS AND METHODS

Sources of Materials

The experiment was conducted in the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. Tomato seeds of variety Ibadan local (small), *R. solanacearum* biovar 3, race 1 (Adebayo and Ekpo, 2005) and naturally infested soils were obtained from National Horticultural Research Institute, Ibadan, Nigeria. Crude oil (light) used had a specific gravity of 0.7454 $g \cdot cc^{-1}$ was collected from Shell Oil Company, Port Harcourt, Rivers State, Nigeria. Topsoil was collected at the experimental garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria and was autoclaved at 1.05 kg/cm^3 (121°C) for 25 min.

Naturally infested soil

Naturally infested soil (4,000 g) was weighed and placed in pots (24 cm deep and 27 cm wide). Two thousand-g of soil was removed and contaminated with crude oil at 0, 1, 2 and 4% (based on weight of oil/weight of soil), thoroughly mixed and returned to the pots (Isirimah *et al*., 1989). Two weeks after treatment, 20 seed of the tomato variety were sown per pot and watered with a watering can every other day. The inoculum concentration in the soil was 6×10^7 $cfu \cdot mL^{-1}$ determined by serial dilution through the use of counter. The experiment was arranged in a completely randomized design with four replicates per treatment. The treatments were: (a) naturally infested soil, no crude oil, 0% (N); (b) naturally infested soil, with 1% crude oil, (NL); (c) naturally infested soil, with 2% crude oil, (NM), and (d) naturally infested soil, with 4% crude oil, (NH). Data collected included seedling emergence, seedling mortality, plant height, and number of leaves per plant. Seedling emergence was determined 7, 10, and 13 days after sowing (DAS). Plant height was measured from the soil surface to the tip of the plant and number of leaves counted 14 and 28 DAS. Plants were visually examined for foliar chlorosis and incidence of wilt. Percentage seedling emergence and mortality were determined.

Artificially inoculated soil and seedlings

In order to determine the effect of *R. solanacearum*, the soil was treated with different levels of crude oil as described above. Inoculum was prepared on triphenyl tetrazolium chloride agar (Kelman, 1954) at 30° C. After 48 hrs a suspension of the bacterium was prepared in sterile distilled water and adjusted to optical density at 600 nm (OD_{600}) = 0.3 (approximately 6×10^8 $CFU \cdot mL^{-1}$) using a Yenway 6010 photocolorimeter. Tomato seedlings at the fourth to fifth true-leaf stages were inoculated by puncturing the basal part of the stem with a needle dipped in the inoculum suspension (Winstead and Kelman, 1952) and 10 mL of the inoculum was poured around the base of each seedling (Adebayo and Ekpo, 2005). Six seedlings were transplanted to pots per replicate and watered every other day if it did not rain. The experiment was arranged in a completely randomized design with four replicates per treatment. The seedlings were planted in: (i) soil without crude oil (0%), soil not-inoculated with bacterium (SS); (ii) soil not contaminated with crude oil, but with inoculation with bacterium, (SSB); (iii) soil contaminated with 1% oil and with inoculation with bacterium (SLSB); (iv) soil contaminated with 2% oil and seedling inoculated with bacterium (SMSB); (v) soil contaminated with 4% oil and seedling inoculated with bacterium (SHSB).

Data collected included seedling mortality, plant height, number of leaves per plant, number of flowers per plant, number of fruits per plant, fruit weight, and seedling abnormalities. Plant heights were measured as before and numbers of leaves counted at 21, 35, 48 days after inoculation (DAI). Number of fruits per plant was determined based on all surviving plants in each replicate. Plants were visually examined for foliar chlorosis and incidence of wilt associated with treatment. Fruit yields per plant were obtained by weighing all fruit from each replicate and dividing by the number of plants. Soil samples were collected for analysis 15 and 53 days after contamination (DAC) at the Soil Chemistry Laboratory of the Institute of Agricultural Research and Training (IAR&T),

Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.
Data were subjected to analysis of variance (ANOVA) using the general linear model of SAS (ver. 9.1; SAS, Institute, Cary, N.C.). Means separation was done by using LSD (0.005) and the standard error.

Results

Naturally infected soil

Crude oil level affected seedling emergence and seedling mortality in naturally infested soils (Table 1). At 7, 10, and 13 DAS, there were significant differences (P=0.05) in seedling emergence. Seven DAS the percent seedling emergence associated with naturally infested soil, with 1% crude oil, (NL) was similar to that of naturally infested soil, no crude oil, 0% (N). The naturally infested soil, with 2% crude oil, (NM) and naturally infested soil, with 4% crude oil, (NH) treatments had lower percent seedling emergence when compared to N. Similar results occurred at 10 DAS. However, at 13 DAS, no further seedling emergence occurred for N and NL, but there were slight increases in emergence in treatments NM and NH. Seedling mortality for all treatments was not different at 13 DAS. The percent seedling mortality associated with N was significantly lower than recorded for NL, NM, and NH, which were similar at 18 DAS.
Fourteen days after planting, there were significant differences (P=0.05) in plant heights and number of leaves among treatments, but no significant difference was observed 28 DAS. At 14 DAS, plant height associated with N was higher than those for NL, NM and NH. Plant heights for NL and NM were similar but higher than for NH. At 28 DAS plant heights were similar. The shortest and tallest plants were associated with NH and N respectively (Fig. 1). Numbers of leaves, at 14 DAS, for N were higher than for NH, but similar to NL and NM. Similar trends were observed at 28 days after sowing (Fig. 2).

Table 1. Effect of crude oil on seedling emergence and seedling mortality for seed sown in naturally infested soil

	% Seedling emergence			% Seedling mortality	
Treatment (% oil)[a]	7DAS[b]	10DAS	13DAS	13DAS	18DAS
N	55.0	58.8	58.8	10.3	14.8
NL	52.5	57.5	56.2	13.3	60.0
NM	34.0	40.0	45.0	28.0	67.0
NH	6.3	13.8	17.5	64.0	64.0

[a] N = seed sown in naturally infested soil, not contaminated with crude oil (0%); NL = seed sown in naturally infested soil, with 1% crude oil; NM = seed sown in naturally infested soil, with 2% crude oil; and NH = seed sown in naturally infested soil, with 4% crude oil

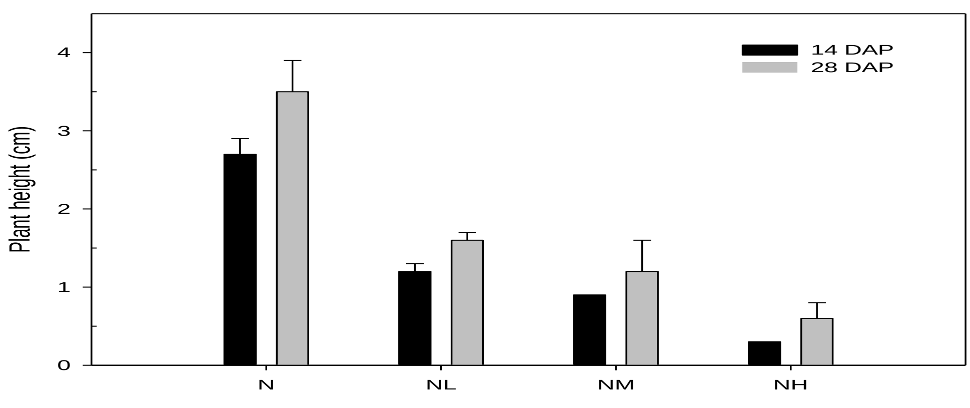

Figure 1: Effect of crude oil on tomato plant height in naturally *Ralstonia solanacearum* infested soil. N = seed sown in naturally infested soil, not contaminated with crude oil (0%); NL = seed sown in naturally infested soil, with 1% crude oil; NM = seed sown in naturally infested soil, with 2% crude oil; and NH = seed sown in naturally infested soil, with 4% crude oil

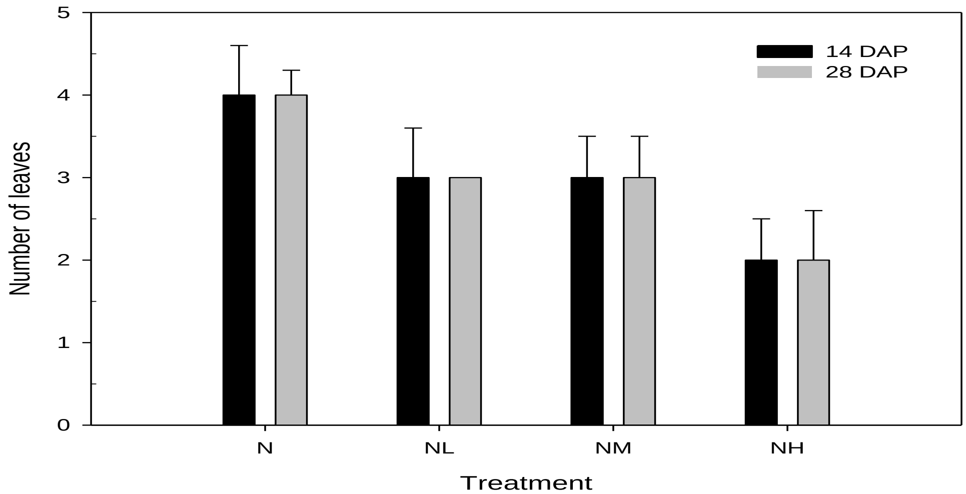

Figure 2. Effect of crude oil on tomato number of leaves in naturally *Ralstonia solanacearum* infested soil. N = seed sown in naturally infested soil, not contaminated with crude oil (0%); NL = seed sown in naturally infested soil, with 1% crude oil; NM = seed sown in naturally infested soil, with 2% crude oil; and NH = seed sown in naturally infested soil, with 4% crude oil

Artificially inoculated soil and plants

Crude oil level affected plant height and numbers of leaves of plants inoculated with *R. solanacearum* (Fig. 3, 4). At 21, 35 and 48 DAI, there were significant differences (P=0.05) in plant height and numbers of leaves due to treatment. At 21 DAI, plant height associated with SS was greater than those recorded for SSB, SLSB, SMSB and SHSB. Plant height associated with SSB was greater than those associated with treatments SLSB, SMSB, and SHSB, while plant height in SLSB and SMSB were similar (Fig. 3). A similar trend was recorded for number of leaves at 21 DAI; SS was significantly (P=0.05) higher than for all other treatments, with SHSB having the fewest leaves (Fig. 4).

At 35 DAI, plant height associated with SS was greater than those recorded for other treatments. Plant heights associated with SSB and SMSB were similar. Plant height associated with SLSB was greater than for SSB, SMSB and SHSB (Fig. 3). For number of leaves, SS had more than other treatments, with number of leaves associated with SSB, SLSB, and SMSB being similar, but higher than that associated with SHSB (Fig. 4).

At 48 DAI, plant height for SS was higher than for other treatments. Plant heights in SLSB, SSB and SMSB were similar, but higher than that associated with SHSB (Fig. 3). Treatment did not affect numbers of leaves at 48 DAI, average 6.5 (Fig. 4).

Crude oil affected numbers of flowers per plant and fruit weight in plants inoculated with *R. solanacearum* (Fig. 5). Average numbers of flowers per plant ranged from 1.1 to 4.7. However, numbers of flowers associated with SHSB and SLSB were lower than for SS and SLSB. For SSB numbers of flowers were similar to SMSB and SHSB (Fig. 5). Fruit weight per plant for SS and SLSB were similar, but significantly (P=0.05) higher than for SS, SMSB and SHSB (Fig.6).

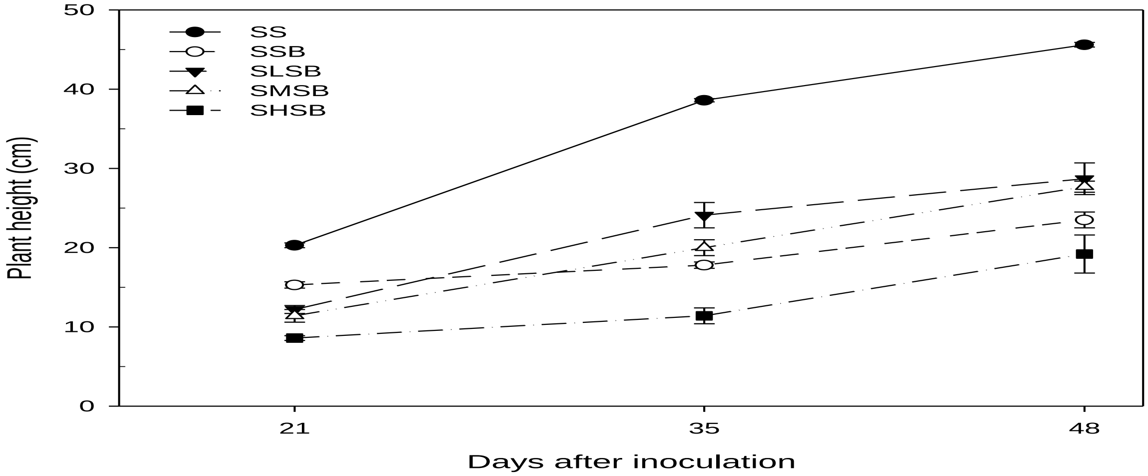

Figure 3: Effect of crude oil on tomato plant height soil artificially inoculated with *Ralstonia solanacearum*. SS = Sterile soil not contaminated with crude oil, seedling not inoculated with bacterium; SSB = Sterile soil not contaminated with crude oil, seedling inoculated with bacterium; SLSB = Sterile soil contaminated with low level (1%) of crude oil, seedling inoculated with bacterium; SMSB = Sterile soil contaminated with medium level (2%) of crude oil, seedling inoculated with bacterium; SHSB = Sterile soil contaminated with high level (4%) of crude oil, seedling inoculated with bacterium

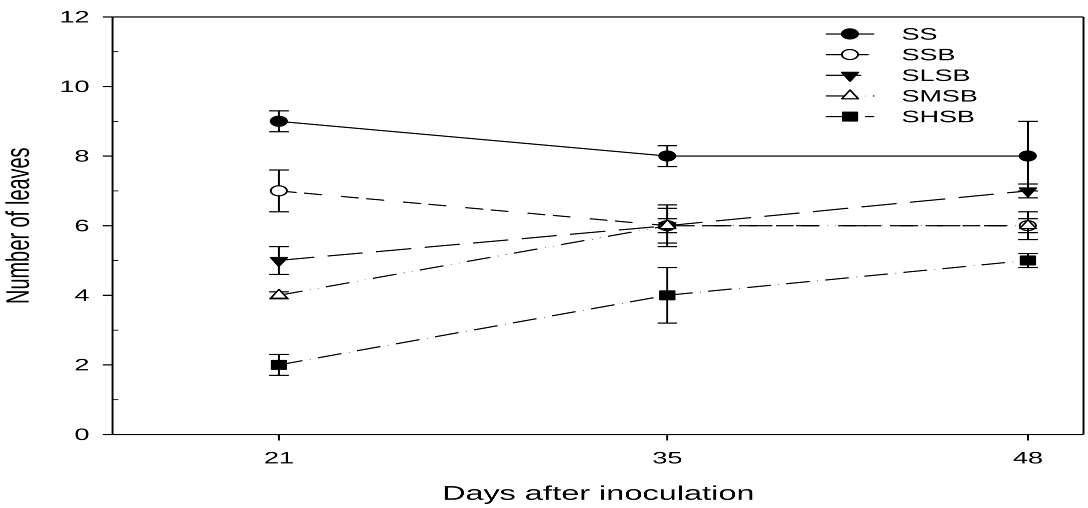

Figure 4: Effect of crude oil on tomato number of leaves soil artificially inoculated with *Ralstonia solanacearum*. SS = Sterile soil not contaminated with crude oil, seedling not inoculated with bacterium; SSB = Sterile soil not contaminated with crude oil, seedling inoculated with bacterium; SLSB = Sterile soil contaminated with low level (1%) of crude oil, seedling inoculated with bacterium; SMSB = Sterile soil contaminated with medium level (2%) of crude oil, seedling inoculated with bacterium; SHSB = Sterile soil contaminated with high level (4%) of crude oil, seedling inoculated with bacterium

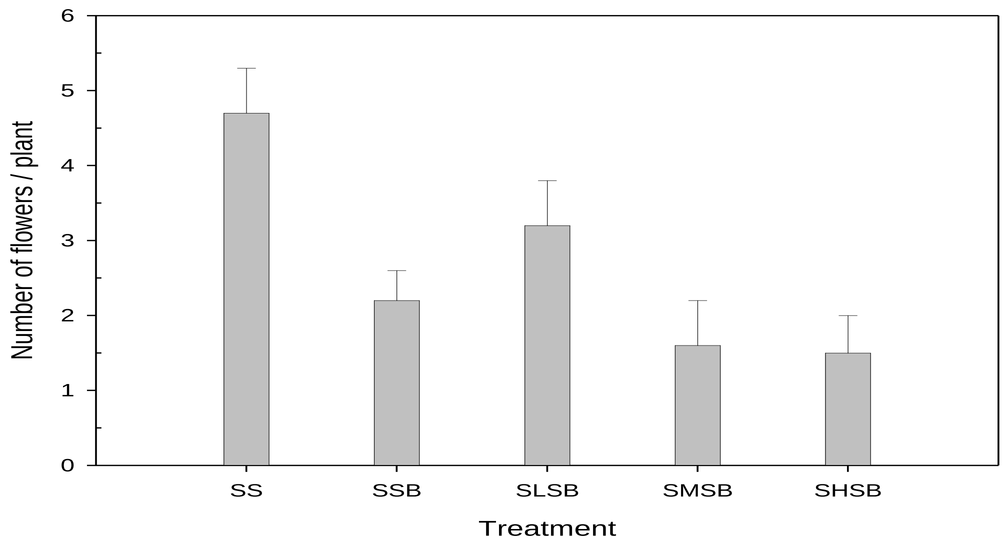

Figure 5: Effect of crude oil on tomato number of flowers/plant in soil artificially inoculated with *Ralstonia solanacearum*. SS = Sterile soil not contaminated with crude oil, seedling not inoculated with bacterium; SSB = Sterile soil not contaminated with crude oil, seedling inoculated with bacterium; SLSB = Sterile soil contaminated with low level (1%) of crude oil, seedling inoculated with bacterium; SMSB = Sterile soil contaminated with medium level (2%) of crude oil, seedling inoculated with bacterium; SHSB = Sterile soil contaminated with high level (4%) of crude oil, seedling inoculated with bacterium

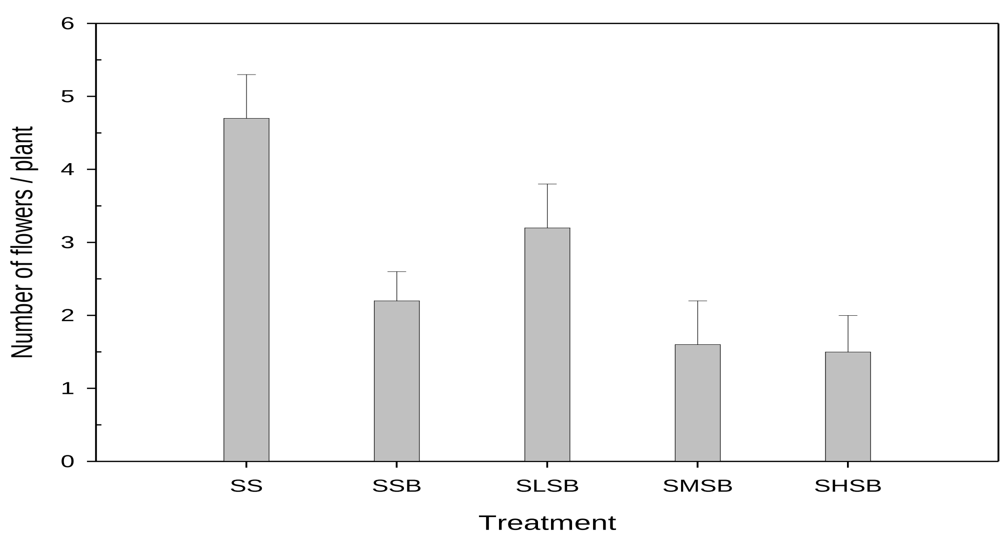

Figure 6: Effect of crude oil on tomato fruit weight (g) soil artificially inoculated with *Ralstonia solanacearum*. SS = Sterile soil not contaminated with crude oil, seedling not inoculated with bacterium; SSB = Sterile soil not contaminated with crude oil, seedling inoculated with bacterium; SLSB = Sterile soil contaminated with low level (1%) of crude oil, seedling inoculated with bacterium; SMSB = Sterile soil contaminated with medium level (2%) of crude oil, seedling inoculated with bacterium; SHSB = Sterile soil contaminated with high level (4%) of crude oil, seedling inoculated with bacterium

Soil properties

Fifteen days after oil contamination, the pH level, percent carbon and percent nitrogen of soil increased as oil level increased. However, available phosphorus decreased as oil level increased (Table 2). After 53 DAC, soil pH decreased as oil level increased; percent carbon and percent nitrogen increased with increase in oil levels. The available phosphorus increased as soil level increased to SMSB and then decreased with higher oil level, SHSB. There was a decrease in pH from 15 to 53 DAC, while there was an increase in percent carbon, nitrogen and available phosphorus in the same period (Table 2).

Table 2. Effect of crude oil on soil pH, percentage carbon, percentage nitrogen and available phosphorus of soil under tomato crop

Treatments[a]	pH		% Carbon		% Nitrogen		Available Phosphorus (ppm)	
	15DAC[b]	53DAC	15DAC	53DAC	15DAC	53DAC	15DAC	53DAC
SS	7.10	6.3	2.50	2.24	0.25	0.21	15.09	17.22
SSB	6.95	6.1	2.73	3.07	0.27	0.30	19.03	11.32
SLSB	7.15	6.2	3.19	3.45	0.32	0.31	11.79	15.76
SMSB	7.20	6.1	3.25	4.26	0.33	0.40	10.85	15.23
SHSB	7.30	6.1	4.02	4.49	0.40	0.45	06.60	9.35

[a] SS = Sterile soil not contaminated with crude oil, seedling not inoculated with bacterium; SSB = Sterile soil not contaminated with crude oil, seedling inoculated with bacterium; SLSB = Sterile soil contaminated with low level (1%) of crude oil, seedling inoculated with bacterium; SMSB = Sterile soil contaminated with medium level (2%) of crude oil, seedling inoculated with bacterium; SHSB = Sterile soil contaminated with high level (4%) of crude oil, seedling inoculated with bacterium

[b] DAC = Days after contamination

Discussion

This is the first report of the effects of crude oil on tomato infected with *Ralstonia solanacearum.* Treatments affected seedling emergence. Non-contaminated soil (0%) and 1% oil had higher seedling emergence than those with 2 and 4% oil. This suggests that there was growth inhibition as a result of inadequate air created by oil contamination despite the fact that the soil was allowed to stabilize for two weeks before sowing. This is similar to Isirimah *et al.,* (1989) findings that the low percent of seedling emergence associated with 2 and 4% oil is likely due to poor wettability and aeration of soil. This result shows that soil contaminated with oil should be avoided while growing tomato seedlings.

Plants in soil contaminated with medium and high levels (2 and 4%) of oil developed yellowing shedding of leaves and retardation of growth and similar to that reported by Isirimah et al. (1989). Toogood and Rowell (1977) and Odu (1981) reported retardation of growth at high levels of oil. Tomato plants in soil contaminated with 1% oil displayed slight yellowing and leaf drop. Plants in non-contaminated soil did not exhibit yellowing of leaves, but growth was retarded and showed stunting was severe at 28 DAS. The retarded growth associated with soil contaminated with 1% oil might be due to leaf yellowing and reduced leaf area which adversely affected photosynthesis rate. It was also an evidence of retrogressive changes in soil-plant water relation.

Inoculated tomato plants in soil contaminated with 2 and 4% oil developed yellowing, and exhibited heavy leaf drop which agrees with Isirimah *et al.*, (1989) and both treatments had the highest seedling mortality. The few seedlings that survived had remission of chlorosis. Plant height and leaf production in soils contaminated with high crude oil level were lower than controls.

Number of flowers for plants grown in soil with 1% oil was less than the control, and produced fewer but bigger fruits, than the control which had more vegetative growth and smaller fruit, although fruit weights were not different. This suggests that 1% oil was not deleterious to plants. This finding corresponds with the report of Odu, 1981; Isirimah *et al.*, 1989; Lum and Ekpo, 2005) that low oil concentration on soil surface was not detrimental to plants' growth. The non incidence of tomato mortality on 1% oil concentration suggests that there are some active ingredients in the crude oil that suppresses the activities of the bacterium. Odu (1977) confirmed that 1% oil contamination suppresses the activities of microorganism, while Lum and Ekpo, (2005) reported that 1 and 3% oil contamination significantly reduced the pathogenic potential of *Slerotium rolfsii* on cowpea. In non-contaminated soil, seedling inoculated with *R. solanacearum* had a high incidence of mortality and there was no significant difference in fruit weight when compared to those in soil contaminated with 2 and 4% oil.

Soil contaminated with crude oil significantly reduced the *R. solanacearum* pathogenic potential as evidence by the fruit weight in the presence of 1% oil, total absence of wilt incidence and on the stands of infected tomato plants on both media and high level of oil contamination. This suggests that crude oil, and most oil fractions, are to some extent toxic to soil organisms and confirms Odu (1977) report that oil contamination leads to an initial depression in numbers of microorganisms.

Tomato grows well over a range of levels of nutrients. Manipulation of the nutrient supply is essential to achieving high yields of good quality fruit. Plant vigor generally increased with nitrogen level. Previous reports suggest that plant height, leaf area and the number of flowers produced increased in response to nitrogen level but the response depends on the initial soil nitrogen content and immobilization or mineralization of nitrogen or de-nitrification during cropping (Adams, 1986). The pH of the soil increased with increased oil level and decrease with time. This is similar to the findings of Isirimah *et al*., (1989). Percent carbon and percent nitrogen increased as oil level increased and over time. Available phosphorus decreased as oil level increased and with time. This is also similar to the findings of Isirimah *et al*., (1989) who reported that, increasing pH increase phosphorus availability up to a pH of about 5.5 – 6.0., thereafter phosphorus availability starts to decrease. While an inadequate level of phosphorus may restrict both growth and development, reduce numbers of flower buds formed and delay anthesis (Menary and Van Staden, 1976), a high level of phosphorus can increase the proportion of unevenly ripened fruit, and depress yield depending on soil and medium pH (Adams, 1986). In this study, the fruit weight observed for low oil contamination compared to the control despite bacterium infection might be attributed to an increase in the available phosphorus to the plants. This suggests that the low oil level not only reduced the pathogenic potential of the bacterium but also supports the growth of the crop.

In conclusion, although not measured directly there appeared to be decreased weed populations as oil level increased as a result of the lethal effects of the oil on the weeds. The recovery of plants on 1% oil level and subsequent good yield suggests that residual oil was gradually removed by rainfall and water application during the growing period. This reassures peasant tomato growers that low oil contaminated fields should not be totally abandoned but could still be used for agricultural activities and serve as way of reducing the menace of tomato wilt. Furthermore, for the land to be useful, it should be left fallow for few months for the oil level to be reduced by rainfall or tidal actions. Further studies are however required to elicit the active hydrocarbons in the oil that inhibit the bacterium virulence.

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