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Effect of *Sclerotium rolfsii* on the Performance of Cowpea in Engine Oil- and Spent Engine Oil-Contaminated Soil

Lum, A.F. and Ekpo, E.J.A.
Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.

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ABSTRACT: The pathogenicity of *Sclerotium rolfsii* and performance of cowpea were studied in soil contaminated with engine oil and spent engine oil. Topsoil (250g) from each pot (24cm deep and 27cm wide) containing 2000g of moistened sterile soil was mixed with 3 plates of 12-day-old sclerotia-forming cultures. Four days after soil inoculation, engine oil and spent engine oil were applied at 0.0, 1.0 (low) and 3.0% (high) levels using a hand sprayer. Cowpea seeds of Ife Brown variety were planted 7 days after oil application.

Plants in soil not infested with the fungus but contaminated with 3.0% level of engine oil (HE) had significantly ($P \leq 0.05$) lower percentage seedling emergence than all other treatments. Plants on soil contaminated with 1.0% level of engine oil or spent engine oil but not infested with the fungus, and those on non-contaminated, non-infested soil (NS) had similar and higher percentages of seedling emergence than all other treatments. The incidence of wilt was high in uncontaminated soil infested with *Sclerotium rolfsii*, low in infested soils at 1.0% level, and absent in infested soils at 3.0% level. HE was associated with significantly ($P \leq 0.05$) shorter plants than all other treatments. Grain yield per plant was similar for all treatments (3.2–5.6g). Hundred seed weight associated with NS (16.6g) was significantly ($P \leq 0.05$) higher than that associated with all other treatments (12.4–16.1g).

In conclusion, soil contamination with either engine oil or spent engine oil at 1.0% and 3.0% levels significantly ($P \leq 0.05$) reduced the pathogenic potential of *Sclerotium rolfsii*.

Key words: Engine oil, pathogenicity, *Sclerotium rolfsii*, spent engine oil.

INTRODUCTION

In the oil industry the possibility of oil spillage is either through leaks or damages to oil pipelines, or from wagons or tankers used in transporting oil (Odu, 1972). Crank case oil from mechanics' workshops is also disposed of in the environment (Aina, 1992). Between 1976 and 1980, 1,843,047 barrels of crude oil were reported spilled in the Niger Delta area of Nigeria with over 16 spills having more than 100 barrels per spill (Awobajo, 1981).

As the population and demand for arable land for food production both increase, it is necessary to investigate the effects of oil spillage on agricultural lands, and the potential for the restoration of oil polluted lands for use in agriculture. The level of oil spillage is a factor which exerts a very high influence on agricultural lands (Isirimah *et al.*, 1989). Plants exposed to light oil pollution have been reported to suffer from leaf yellowing and leaf drop, while complete shedding of leaves follows heavy contamination (Isirimah *et al.*, 1989). Odu (1981) however reported that light oil contamination of about 1% is beneficial to crops.

Cowpea (*Vigna unguiculata* (L.) Walp) is a grain legume that is commonly utilised in the tropics and subtropics. In the developing countries especially, it is important as part of the human diet, being high in protein as well as other essential nutrients (Rachie, 1985; Quin, 1997). It is also an important component of intercropping as it contributes to improve soil fertility (Duke, 1990; Jain and Mehra, 1980; Quin, 1997). However, like most crops with a long history of cultivation, cowpea is subject to substantial annual losses as a result of pests and diseases (IITA, 1986). The majority of cowpea pathogens are viruses and fungi (Singh and Rachie, 1985). Prominent among these is *Sclerotium rolfsii* Sacc which causes stem rot disease (Okonkwo, 1989) leading to plant wilt (Raychaudhuri and Verma, 1987).

Although there are reports on the adverse effects of oil pollution on the environment (Awobajo, 1981; Burger, 1993; Ekundayo and Obuekwe, 2000; Odu, 1981; Wells, 2001), there is little or no information on the effect of oil spillage on specific diseases of crops in relation to crop performance. The objectives of this study were to investigate the effect of engine oil and spent engine oil on the pathogenicity of *S. rolfsii* and the performance of cowpea.

It is hoped that the results obtained will establish the impact of oil spillage on the incidence of *Sclerotium* stem rot, and the performance of cowpea in engine oil- and spent engine oil-contaminated soils.

MATERIALS AND METHODS

The experiment was carried out in 1998 on the rooftop garden of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Ibadan, Nigeria. Topsoil was collected from the CPEB experimental garden and autoclaved at 1.05 kg/cm^2 (121 °C) for 25 minutes. Ninety plastic pots (24 cm deep and 27 cm wide) were filled with 2000g of the sterile soil and watered. Next, 250g of the soil was removed from each pot and mixed with three potato dextrose agar plates of 12-day-old sclerotia-forming cultures of *S. rolfsii* using a shovel. The infested soil was returned to the pots and watered. Four days after soil inoculation, engine oil (E) and spent engine oil (C) were separately added to the soil at three levels, 0.0, 1.0 (low) and 3.0% (high), based on weight of oil / weight of soil. The oil was applied evenly using a hand sprayer and properly worked into the soil to a depth of about 10 cm. Cowpea seeds of Ife Brown variety were planted 7 days after oil application at the rate of 30 seeds per pot and watered every other day. At 14 days after planting (DAP), the plants were thinned to 5 plants per pot. The experiment was a randomised complete block design with 3 replicates each of 3 pots per treatment. The treatments were: seeds planted in soil contaminated with a low level of engine oil (LE) or spent engine oil (LC); seeds planted in soil contaminated with a high level of engine oil (HE) or spent engine oil (HC); seeds planted in non-polluted soil (NS); seeds planted in non-polluted soil containing the fungus (NSF); seeds planted in soil contaminated with low level of engine oil or spent engine oil and containing the fungus (LEF and LCF); and seeds planted in soil contaminated with high level of engine oil or spent engine oil and containing the fungus (HEF and HCF). The engine oil was purchased from Elf Petrol Station, Ojoo, Ibadan and was labelled: 'Super heavy duty +, SAE 40, API Service SD/CC, MIL – L – 2104B', while spent engine oil was obtained from the University of Ibadan mechanics' workshop, Ibadan.

Data collected were: seedling emergence, plant height, number of leaves, leaf area, number of flowers per plant, grain yield per plant, and 100-seed weight. Seedling emergence was recorded for all pots at 3, 7, and 14 DAP. Plant heights and number of trifoliate leaves were determined at 14, 28, and 42 DAP from 5 randomly selected plants per replicate. Leaf area was determined at 63 DAP from 3 trifoliate leaves selected at random per replicate, using graph sheets. The number of flowers per plant was determined from daily counts recorded from the date of first flowering, from 9 pre-tagged plants selected at random in each replicate. All plants were visually examined for foliar chlorosis and incidence of wilt associated with the treatments. At maturity, all pods were harvested from surviving plants and threshed manually for grain yield determination. Grain yield per plant was obtained by weighing all the seeds from each replicate and dividing by the number of plants. One hundred seed weight was based on 100 seeds selected at random from each replicate. All data were subjected to analysis of variance using PROC GLM from SAS (SAS, 1995) and where tests were significant, treatment means were separated using the least significant difference (LSD) test at $P \leq 0.05$.

RESULTS

Effect of *Sclerotium rolfsii* on cowpea seedling emergence in engine oil- or spent engine oil-contaminated soil.

The effect of *S. rolfsii* on percentage seedling emergence in engine oil- or spent engine oil-contaminated soil is shown in Fig 1. At 3, 7, and 14 DAP, there were significant ($P \leq 0.05$) differences in percentage seedling emergence among the treatments.

At 3 DAP, percentages of seedling emergence associated with treatments LC (74.5%), LE (58.9%), and LEF (62.2%) were similar and statistically comparable to that associated with NS (85.6%). All other treatments resulted in significantly ($P \leq 0.05$) lower percentage seedling emergence (1.1–48.9). The lowest percentage seedling emergence of 1.1% was recorded for HE treatment.

At 7 DAP, percentages of seedling emergence recorded for LC, NS, LE, LEF, and HCF were statistically similar and significantly ($P \leq 0.05$) higher than those recorded for all other treatments. The lowest percentage seedling emergence was recorded for HE.

At 14 DAP, the trend in percentage seedling emergence was similar to that at 7 DAP. However, slight increases (1.1–2.3%) in percentage seedling emergence were recorded for LE and LC.

Effect of *S. rolfsii* on cowpea plant height and number of leaves in engine oil- or spent engine oil-contaminated soil

The effects of *S. rolfsii* on plant height and number of leaves of cowpea in engine oil- or spent engine oil-contaminated soil are presented in Table 1. There were significant ($P \leq 0.05$) treatment differences in plant height at 14, 28, and 42 DAP.

At 14 DAP, plant heights associated with treatments LC (18.1 cm), LE (17.0 cm), NSF (16.7 cm), LCF (16.4 cm), and LEF (17.1 cm) were similar and statistically comparable to that associated with NS (19.0 cm). All other treatments resulted in significantly ($P \leq 0.05$) lower plant heights (8.1–14.1 cm). The lowest plant height was recorded for HE, corresponding to 57.4% reduction in plant height.

At 28 DAP, the tallest plants were recorded for NS (30.0 cm), and this was similar to that recorded for all treatments (25.9–28.0 cm) except HC (23.8 cm) and HE (18.7 cm). HE gave the shortest plants.

At 42 DAP, plant heights in all treatments (29.6–34.8 cm) other than HE (24.9 cm) were similar to that of NS (32.5 cm). A slight increase of 0.6–2.3 cm in plant height was associated with LC, LCF, LEF, and HCF when compared to plant height in NS.

The treatments had no significant effect on the number of leaves per plant, 14 DAP (Table 1). At 28 DAP, the number of leaves per plant varied significantly ($P \leq 0.05$) among the treatments. All treatments other than HC and HE had numbers of leaves similar to NS.

Overall, the number of leaves per plant was lowest in HE (4.0).
At 42 DAP, LC, LCF, LEF, and NSF caused an increase of 4.6–22.8% in the number of leaves per plant when compared to NS.

Effect of *Sclerotium rolfsii* on cowpea leaf area, number of flowers/plant, grain yield/ plant and 100-seed weight in engine oil- or spent engine oil-contaminated soil

Leaf areas varied significantly ($P \leq 0.05$) among the treatments. Leaf areas (45–54.6 cm^2) associated with treatments LE, LC, HC, and HEF were similar to that (56.1 cm^2) associated with NS (Table 2). All other treatments caused significantly ($P<0.01$) lower leaf areas (22.8–41.8 cm^2) when compared to NS. The lowest leaf area of 22.8 cm^2 was recorded for HE.
There were no significant differences in the numbers of flowers (10.0–15.0) associated with all the treatments (Table 2). However, the numbers of flowers associated with LCF (14.0) and LEF (15.0) were slightly higher than that (13.0) associated with NS.
The grain yield per plant recorded for NS (5.0g) was similar to those (3.7–5.6 g) recorded for all other treatments (Table 2). However, grain yield per plant recorded for LEF (5.6g) was slightly higher than that recorded for NS, while the yield for each of the other treatments was slightly lower than that recorded for NS.
The 100-seed weight varied significantly ($P \leq 0.05$) among the treatments. The 100-seed weight associated with NS (16.6 g) was significantly ($P<0.01$) higher than those (12.4–16.1 g) associated with all other treatments. The lowest 100-seed weight was recorded for LCF.

Visual examination of cowpea in engine oil- or spent engine oil-contaminated soil infested with *S. rolfsii*

Plants grown in soil contaminated with high levels (3%) of engine oil or spent engine oil developed yellowing and heavy shedding of leaves between seedling emergence and 42 DAP. By 53 DAP, there was a remission of the yellowing symptoms earlier observed on leaves of affected plants. Plants grown in soil contaminated with a low level of oil developed slight foliar chlorosis compared to plants in non-contaminated soils. Some plants grown in soil not contaminated with oil but infested with *S. rolfsii* (NSF) wilted and died prematurely (data not shown). Wilting was also associated with some plants grown in soil infested with *S. rolfsii* and contaminated with a low level of spent engine oil (LCF). No wilt symptoms were observed in plants grown in heavily contaminated soil infested *with S. rolfsii*.
Cowpea roots from soil contaminated with a low level of oil were denser than those from a high level of oil (data not shown). Those from soil contaminated with a low level of oil infested with *S. rolfsii* were less dense than those from soil contaminated with a high level of oil, infested with *S. rolfsii*. In general, roots from non-contaminated, non-infested soil (NS) were denser than those from all other treatments.

DISCUSSION

Percentage seedling emergence recorded for treatment NSF was significantly ($P \leq 0.05$) lower than that associated with treatment NS, thus indicating that Ife Brown is highly susceptible to pre-emergence mortality caused by *S. rolfsii*. The low level of seedling emergence observed in soil containing a high level of oil (HC and HE) is similar to the report of Isirimah *et al.* (1989) and is attributable to poor wettability and aeration of soil (Strafford, 1973).
In this study, plants in soil contaminated with a high level (3%) of oil developed yellowing associated with heavy shedding of leaves. This observation is similar to the report of Isirimah *et al.* (1989) that maize plants exposed to heavy crude oil contamination suffered yellowing and complete shedding of leaves. Fewer leaves with a significantly ($P \leq 0.05$) smaller leaf area were associated with HE, compared to the higher number of leaves and larger leaf area of plants grown in non-contaminated soil and soil contaminated with a low level of engine oil or spent engine oil. Leaf yellowing and reduction in leaf area might have adversely affected the rate of photosynthesis leading to poor growth as evidenced by stunting, delayed flowering, and a slight reduction in grain yield. Retardation of crop growth at high levels of crude oil treatments has also been observed by Toogood and Rowell (1977) and Odu (1981).
Plants in soil contaminated with a low level (1%) of oil suffered from slight yellowing and leaf drop. The plants, however, recovered and became dark green in colour, similar to those in non-contaminated soil. They flowered at 37 DAP like the control. Plant height and leaf production were not different from the control. Furthermore, the number of flowers and leaf area of plants in soil with light contamination were similar to those of plants in non-contaminated soil. This suggests that the low level (1%) of both engine oil and spent engine oil were not deleterious to the plant. This finding is similar to the report of Odu (1981), that crude oil concentration of about 1% is beneficial to crops.
The height of Ife Brown in non-contaminated soil was comparable to plant height in soil contaminated with a low (1%) level of oil. However significant ($P \leq 0.05$) stunting was associated with plants gown in soil contaminated with a high (3%) level of oil at the three assessment periods (14, 28, and 42 DAP). Plant height

ᶻDAP=days after planting

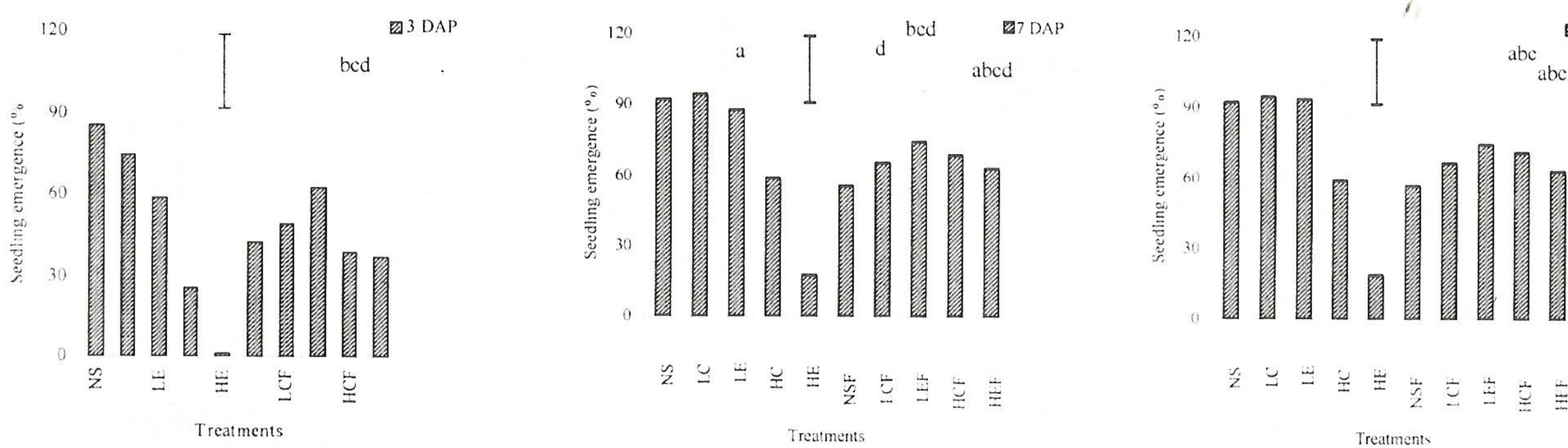

Fig. 1. Effect of *Sclerotium rolfsii* on cowpea percentage seedling emergence at 3, 7 and 14 DAP in engine
oil- and spent engine oil-contaminated soil. Bars represent LSD (least significant difference) values.
*Means of 3 replicates each of 90 seeds planted. Means in each column with the same letter, are not significantly different at 5% level.
Treatments: NS=non-contaminated, non-infested soil; LC=soil contaminated with low level of spent engine oil; LE=soil contaminated with low level of engine oil; HC= soil contaminated with high level of spent engine oil; HE= soil contaminated with high level of engine oil; NSF=non-contaminated soil containing the fungus; LCF=soil
contaminated with low level of spent engine oil and containing the fungus; LEF= soil contaminated with low level of engine oil and containing the fungus; HCF=soil contaminated with high level of spent engine oil and containing the fungus; HEF=soil contaminated with high level of engine oil and containing the fungus.

Table 1: Effect of *Sclerotium rolfsii* on cowpea plant height and number of leaves in engine oil- and spent engine oil-contaminated soil.

	Plant height per plant (cm)*			Number of trifoliate leaves per plant*		
Treatments[x]	14 DAP[z]	28 DAP	42 DAP	14 DAP	28 DAP	42 DAP
NS	19.0a	30.3a	32.5ab	2.0a	7.0abc	7.0ab
LC	18.1ab	27.9ab	34.8a	1.0a	6.0abcd	8.0a
LE	17.0ab	26.4ab	32.4ab	2.0a	5.0cde	7.0ab
HC	12.5c	23.8b	29.6b	2.0a	5.0de	6.0ab
HE	8.1d	18.7c	24.9c	2.0a	4.0e	5.0b
NSF	16.7ab	27.6ab	31.2ab	2.0a	7.0a	8.0a
LCF	16.4abc	27.1ab	34.2a	2.0a	6.0bcd	9.0a
LEF	17.1ab	28.0ab	33.1ab	2.0a	7.0ab	8.0a
HCF	14.1bc	25.9ab	34.6a	2.0a	6.0bcd	7.0ab
HEF	14.1bc	26.2ab	32.5ab	2.0a	6.0abcd	7.0ab
LSD[y]	4.0	4.5	4.1	0.5	1.4	2.4
CV	15.2	10.0	7.5	17.6	14.2	19.0

*Means of 3 replicates each of 5 plants. Means in each column with the same letter, are not significantly different at 5% level.

[x]Treatments: NS=non-contaminated, non-infested soil; LC=soil contaminated with low level of spent engine oil; LE=soil contaminated with low level of engine oil; HC= soil contaminated with high level of spent engine oil; HE= soil contaminated with high level of engine oil; NSF=non-contaminated soil containing the fungus; LCF=soil contaminated with low level of spent engine oil and containing the fungus; LEF= soil contaminated with low level of engine oil and containing the fungus; HCF=soil contaminated with high level of spent engine oil and containing the fungus; HEF=soil contaminated with high level of engine oil and containing the fungus.

[y]LSD=least significant difference.

[z]DAP=days after planting.

Table 2: Effect of *Sclerotium rolfsii* on cowpea leaf area, number of flowers/plant, grain yield/plant and 100-seed weight in engine oil- and spent engine oil-contaminated soil.

Treatments*	Leaf area[y] (cm^2)	Flowers/plant[z]	Grain yield/ plant (g)	100-seed wt (g)
NS	56.1a	13.0a	5.0ab	16.6a
LC	45.7abc	13.0a	4.7ab	15.2cd
LE	53.6ab	11.0a	4.0ab	14.1e
HC	48.1abc	10.0a	3.2b	14.3e
HE	22.8e	10.0a	3.7ab	13.4f
NSF	37.9cd	13.0a	4.6ab	16.0b
CF	39.5cd	14.0a	4.8ab	12.4g
LEF	29.7de	15.0a	5.6a	16.1b
HCF	41.8bc	10.0a	4.1ab	15.6c
HEF	54.6a	12.0a	4.5ab	15.1d
LSD[y]	12.0	5.3	2.0	0.4
CV	16.3	25.8	26.6	1.6

[y]Means of 3 trifoliate leaves per replicate.

[z]Means of 9 plants per replicate.

Means in each column with the same letter, are not significantly different at 5% level.

*Treatments: NS=non-contaminated, non-infested soil; LC=soil contaminated with low level of spent engine oil; LE=soil contaminated with low level of engine oil; HC= soil contaminated with high level of spent engine oil; HE= soil contaminated with high level of engine oil; NSF=non-contaminated soil containing the fungus; LCF=soil contaminated with low level of spent engine oil and containing the fungus; LEF= soil contaminated with low level of engine oil and containing the fungus; HCF=soil contaminated with high level of spent engine oil and containing the fungus; HEF=soil contaminated with high level of engine oil and containing the fungus.

[y]LSD=least significant difference.

in soil infested with *S. rolfsii* was similar to heights recorded for plants grown in soil infested with the fungus and contaminated with a low (1%) level of both oils. However, the severe stunting associated with soil contaminated with a high (3%) level of engine oil was significantly ($P \leq 0.05$) reduced in the presence of *S.*

rolfsii indicating that the engine oil was probably degraded to a less toxic compound that had little effect on plant growth. The stunting associated with plants in soils contaminated with a high level of spent engine oil was only significantly reduced at 42 DAP.Soil contamination with oil alone or in combination with *S. rolfsii* had little or no effect on the number of leaves per plant. However, all treatments except soil contamination with a low (1%) level of oil and a high level of engine oil in the presence of *S. rolfsii* significantly ($P \leq 0.05$) reduced the leaf area of Ife Brown. Also *S. rolfsii*, oil at low or high levels and oil in the presence of *S. rolfsii* caused significant reductions in 100-seed weight.

Overall, this study showed that a high level of soil contamination with either engine oil or spent engine oil adversely affected Ife Brown seedling emergence, caused stunted growth and led to reduction in leaf area and 100-seed weight. Soil contamination with both oils significantly reduced the pathogenic potential of *S. rolfsii* as evidenced by lower pre-emergence mortality (higher seedling emergence) in oil-contaminated soil infested with the fungus, and the total absence of wilt incidence in oil-contaminated soil infested with *S. rolfsii* compared to a high incidence of wilt observed in uncontaminated soil infested with the fungus. For profitable cultivation of Ife Brown, farmers are advised to avoid soils contaminated with petroleum related products such as engine oil and soils infested with *S. rolfsii*, the causal agent of pre- and post-emergence mortality in many arable crops.

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