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Impact of gasoline fuel emissions on *Rhizopus stolonifer* (Ehrenb.) Vuill. and *Fusarium oxysporum* (Schlecht.)

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Abstract

Background: Alteration in the normal composition of gasses in the atmosphere referred to as air pollution can occur as a result of different processes, including emissions from vehicles and power generators. Gasses affect living things directly and indirectly by playing roles in respiration, membrane function, synthesis, and growth. The gasses contained in fumes emitted from vehicles and generators may likely have effect on microorganisms in the environment including microfungi. Two microfungi were selected to study the effect of generator emissions on their growth.

Results: The gaseous emissions from power generators fueled by gasoline caused reduction in spore germination and germ tube lengths of *R. stolonifer* and *F. oxysporum*. For the former, spore germination decreased with length of exposure after an initial increase by ~ 100%. The fungus exhibited a single major germination peak at 10 min and a minor one at 30–35 min exposures. Germ tube length of the fungus also decreased with increased exposure. *F. oxysporum* too showed reduced spore germination and germ tube length with exposure but the fungus seemed to adjust better to the unfavorable environment created by emitted gasses showing multiple peaks of reduced heights as time progressed, though another rise that could reach a peak appeared at the 45-min maximum exposure for germ tube length. The peaks were however more broad for spore germination experiments indicating more stability in adjustment than observed for germ tube length. Greatest reduction in spore germination was by 25% in *R. stolonifer* and 71% in *F. oxysporum* Germ tube length reduction for *R. stolonifer* was by 24–76%, the greatest occurring at 35-min exposure, while for *F. oxysporum* it was 5–83%, the greatest occurring at 40 min exposure.

Conclusions: These observations reveal the toxicity of the gasses emitted to the growth of the two filamentous fungi and the potential harmful effect to other fungi which might be useful in the ecosystem as decomposers and to those that may be pathogenic to higher plants.

Keywords: Fungi, Spore germination, Germ tube, Growth, Air pollutant, Gasoline emissions

Background

Fossil fuel emissions are a combination of hydrocarbons, oxides of carbon, nitrogen, and sulfur with particulate matter (Tavares et al. 2010). Emissions are released from wood and refuse burning, motor vehicles on roads, and power generators in homes, offices, small businesses, and big factories (Aliyu et al. 2019; Marais et al. 2014; Fagbeja et al. 2008; Osuji and Avwiri 2005). The major types of fuel used by vehicles and power generating sets are gasoline and diesel. The concentrations of the various gasses emitted during gasoline combustion depend partly on the condition of the engine. Water vapor is also produced when the fuel is efficiently combusted. All the gasses and droplets released into the atmosphere affect not only higher animals including man, but also

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plants (Darrall 2006; Winner and Greitner 2000) and most likely microorganisms in the environment. Many filamentous fungi are present in the environment as spores and hyphal fragments which can grow actively being involved in many processes affecting other organisms living or dead. *Rhizopus stolonifer* and *Fusarium oxysporum* are filamentous fungi found everywhere in air and soils and on plants causing wilts on stems, and rots on fruits and vegetables. The atmospheric compositions of gasses have direct and indirect effects on fungal metabolism and hence on their ability to grow.

Since the atmospheric composition of gasses is significantly altered by fuel combustion in cities partly as a result of pollution from motor vehicles and power generators (Marais et al. 2014), it is conceivable that this might also affect certain aspects of fungal physiology. It is therefore the reason that the effect of fuel emissions is presently being studied in relation to ability of some fungi to grow. Vehicular emissions cause more air pollution than generators (Aliyu et al. 2019) but for practical ease of testing microorganisms, the fumes from a generator will be employed to simulate the gasses from vehicle exhausts. Gas pollutants from both sources are similar (Marais et al. 2014) though their concentrations differ. Specifically, the spores of *R. stolonifer* and *F. oxysporum* both of which live saprophytically and pathogenically (Bautista-Banos et al. 2014; Ignjatov et al. 2012; Jin-Hyeuk et al*.* 2001) are being studied in this work. Power generators are increasingly being used in homes, offices, and production factories in Nigeria and the air in the surrounding environment is polluted by the fumes discharged from their exhausts.

Methods

The investigation was carried out in vitro on *R. stolonifer* and *F. oxysporum* isolated from diseased tomato fruits. Spore germination and germ tube growth of both fungi were studied. Suspensions of the spores of the fungi were prepared from 10-day-old cultures on malt extract agar, separately in sterile water. Two drops of spore suspension were placed on each of several microscope slides. The slides were separately exposed to emissions in a closed wooden chamber for 5–45 min at 5-min intervals. The emissions were fumes from the exhaust of a power generator fed into the box through a rubber tube fitted tightly into a hole of 2.5-cm diameter at the base of the box. The slides were thereafter incubated in a humid chamber at 28 °C. Incubation was for 16 and 24 h, respectively, for *R. stolonifer* and *F. oxysporum* after which percentage germination was determined in ten different microscope fields on each slide under × 40 objective of the light microscope. Spore germination was taken as protrusion of the cell wall beyond 1 μm. Mean percentage germination in the ten fields was calculated. The slides were further incubated for 12 h and their total germ tube lengths were measured with ocular micrometer after calibration with stage micrometer under × 40 objective of the microscope.

Kane portable auto 4-1 gas analyzer (01ML class 1 Pro kit—1SSWWW) was used to gage the amounts of carbon monoxide (CO), carbon dioxide (CO_2), oxygen, and hydrocarbons in the exhaust fumes. This was done by inserting the probe of the analyzer into the generator exhaust pipe for 15- and 30-min periods while the generating set was running. The quality of emitted gasses was noted on the meter of the equipment. The emission applied had equivalent quantities of the gasses on Table 1.

Results

Exposure of spores to emissions initially resulted into increased germination percentage in *R. stolonifer* but as length of exposure increased, significant decrease in germination occurred up to 25 min then, a small insignificant increase occurred from 30 to 35 min (Fig. 1). After that, there was consistent reduction till 45 min. There were two germination peaks observed, a distinct high one at 10 min and a less conspicuous small one at 30–35 min. Reduction in spore germination was by 5–25% in *R. stolonifer* while enhancement was by ~ 100%. Germ tube length of the fungus however initially reduced by ~ 38% at 5 min exposure, it then increased by the 10th minute exposure, before a consistently significant reduction up till 25 min. After that, the length fluctuated statistically insignificantly, though still significantly less than that of control. All exposure periods were statistically significantly different from the control being reduced by 24–76% (Fig. 2).

For *F. oxysporum* spores, germination percentage reduced at 5 min exposure by 31% then increased at 10 min before consistently reducing till the 25th minute. It then increased at 30 min by 10%, before reduction again up to 40 min (Fig. 1). Results also showed two germination peaks at 10 and 30 min. At 40-min exposure, germination was significantly different from that of control while other exposures were only slightly significantly different from control and not different from each other. Reduction was by 5–71%. Germ tube length followed a similar trend as germination with length of exposure,

Table 1 Composition of gasses detected in gasoline emissions from power generator

Type of gas	Min of running generator/concentration of gas	
	15	30
Carbon monoxide (%)	3.55	3.90
Carbon dioxide (%)	4.40	5.20
Oxygen (%)	0.00	0.00
Hydrocarbons (ppm)	0.2649	0.2499

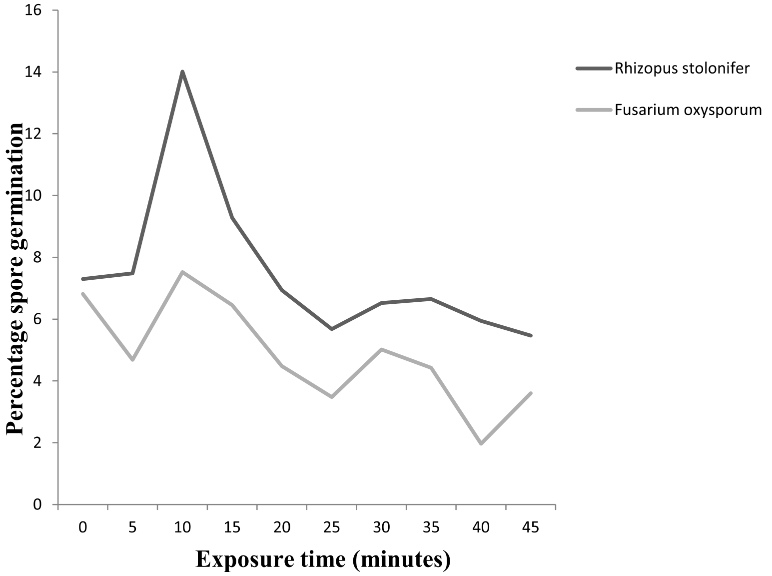

Fig. 1 Percentage germination of *Rhizopus stolonifer* and *Fusarium oxysporum* spores after exposure to gasoline emission and incubation at 28 °C

but there was a prominent peak at 30 min exposure which was less and not significantly different from the control (Fig. 2). There were also multiple peaks, a lower one at 10–20 min and a higher one at 30 min exposure but all lengths were less than that of the control though some were not significant. Reduction was by 5–83%. At 40-min exposure, greatest reduction in germ tube length occurred and was significantly different from control (Fig.2).

The fumes from the generator had 0% oxygen, increase in carbon monoxide concentration from 3.55 to 3.90%, and carbon dioxide from 4.4 to 5.2%, while hydrocarbons content reduced from 0.2649 to 0.2499 ppm at the 15- and 30-min testing periods (Table 1).

Discussion

Results from this study revealed that gasoline emissions lacking oxygen, low in hydrocarbons but high in carbon monoxide and carbon dioxide, negatively influenced growth of *R. stolonifer* and *F. oxysporum* although there was an initial stimulation of spore germination in *R. stolonifer*. Generally however, spore germination and germ tube lengths of *R. stolonifer* decreased with exposure. The greatest inhibitory effect of emissions observed at

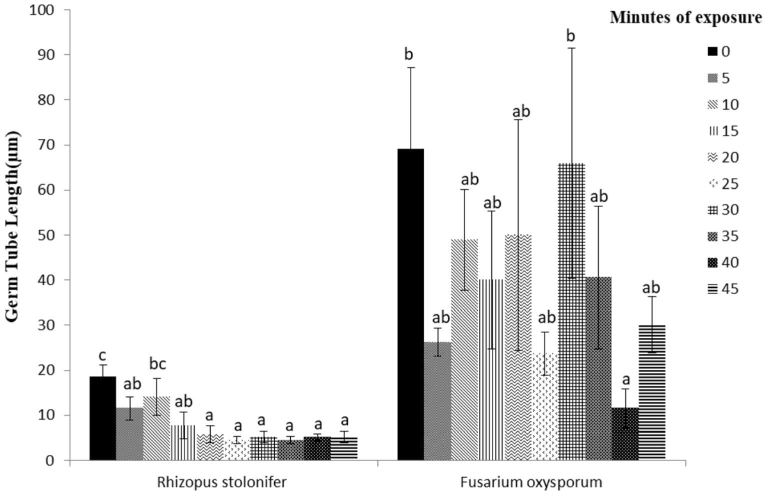

Fig. 2 Germ tube length of *Rhizopus stolonifer* and *Fusarium oxysporum* after exposure to gasoline emission and incubation at 28 °C

the highest exposure periods for the two fungi show the harmful effect of the emissions with prolonged exposure. This might have been due to the high concentration of carbon monoxide and carbon dioxide coupled with the complete absence of oxygen. *R. stolonifer* was reported to grow significantly at 0% oxygen but high concentrations of CO_2 suppressed mycelium growth of the fungus (Wells and Uota 1969). On the contrary in another report, the spores of *R. stolonifer* did not germinate when molecular oxygen was rigorously excluded (Bussel et al. 1969). The initial stimulation observed in this study, may be due to the low levels of inhibitory oxides of carbon which the mold fungus was exposed to at the initial phase. The observed increase in levels of oxides of carbon with time of running the power generator supports this explanation. In addition, *R. stolonifer* being a faster-growing fungus, may have initiated germination in the first few minutes of exposure before the first test at 5 min such that no negative effect was observed at that period of exposure. Germ tube emergence occurred in *F. oxysporum* from 4 h when grown on potato dextrose agar (Kumari et al. 1975), while for *R. stolonifer* , modeling experiments showed that evidences of germination were expected from 30 s at 25 °C in water (Gabler et al. 2004). Longer exposures however had effect as the emitted gas constituents may have altered the chemical composition of the medium after dissolving in water to various extents.

Anaerobic environment, created by the absence of oxygen and elevated CO_2 and CO levels may have led to anoxia within the fungal cells causing the yield of less metabolic energy (Atwell et al. 2015) and energy is needed for growth to take place. This likely inhibited fungal growth measured as spore germination and germ tube extension. Therefore, it was not unexpected that increase in CO and CO_2 concentrations in the atmosphere inhibited growth of the two filamentous fungi tested. The oxides of sulfur and nitrogen which are also reported to be present in gasoline emissions (Tavares et al. 2010) though not detected by the equipment used in this study might have also formed acidic solutions in water causing direct and indirect inhibition of growth. Pure cultures of *Cladosporium cladosporioides* (Fres.) De Vries and *Coniothyrium olivaceum* Bonord were inhibited by sulfur dioxide (SO_2) concentrations of < 0.053 μl/l (Wookey et al. 1991). Concentration dependence of fungal pathogen response to SO_2 revealed that high concentrations were suppressive, while low concentrations caused greater severity of fungal plant diseases (Khan and Khan 2011). There are inconsistent reports on the influence of nitrogen oxides on fungal growth (Depayras et al. 2018; Orr and Nelson 2018; Strohm et al. 2019).

The survival of the two fungi at such long exposure to gasoline emissions is indicative of their tolerance to the unfavorable environment. Despite exposure to emissions, the organisms may therefore still be active in growth for long periods. Their pathogenic abilities in vivo therefore need to be investigated. The oxides of carbon dissolve in water forming weak carbonic acid. The carbonic acid together with other acids formed by emitted oxides of nitrogen and sulfur in solution probably also contributed to inhibition of fungal growth measured as hyphal extension. This is because the acids formed must have lowered the pH of the medium. The varied effects of pH on growth of *F. oxysporum* have been reported (Gordon et al. 2019).

The hydrocarbons in gasoline detected in the emissions most likely also played a role in negatively influencing growth of fungi. The observed decrease in hydrocarbons level with length of running the power generator coinciding with the much higher level of growth in *Fusarium* than *Rhizopus* at the higher exposure periods signify that *Fusarium* thrived better at reduced hydrocarbons concentration even when the concentrations of carbon oxides increased. Aromatic hydrocarbons from marine gas oil (MGO) inhibited growth of soil fungi by stopping hyphal extension (Hughes et al. 2007). In this study, *R. stolonifer* however seemed more affected by CO_2, CO, and hydrocarbon levels of emissions. The consistent reduction in germination of *R. stolonifer* spores compared with fluctuations in that of *F. oxysporum* are evidences of the relative resistances of the fungi, with *R. stolonifer* appearing less susceptible to the negative effects of the emissions than *F. oxysporum*. The fact that the most effective inhibition of germ tube elongation of *R. stolonifer* occurred from 25 min exposure when hydrocarbon content had decreased confirms that all gasses in the emissions had more serious effect on the fungus, though the hydrocarbons might have exerted more effect. Fungi from gasoline polluted soils in liquid cultures showed favorable growth on volatile aromatic hydrocarbons in a combination of low pH and low water activity using the hydrocarbons as their sole carbon and energy sources (Prenafeta-Boldu et al. 2001). The source of carbon in the present study was solely from the emissions which also lowered the pH of the liquid in which the fungi were suspended. It is probable that *R. stolonifer* and *F. oxysporum* employed the emissions for their carbon supply. More fungi isolated from air filters exposed to hydrocarbon-polluted gas streams assimilated volatile aromatic hydrocarbons for their carbon and energy requirements (Prenafeta-Boldu et al. 2006).

For *F. oxysporum* spores, the fluctuating germination percentage with length of exposure after an initial stimulation whereby two peaks of decreased height were observed however, demonstrate the ability of the fungus to adjust to unfavorable environment. Progressive decrease in microbial growth of *Fusarium* sp. occurred on PDA with increase in concentration of crude oil (Nwadinigwe and Obinwa 2006). Gasoline, itself from crude oil, probably played a role in negatively influencing growth of fungi in the present study. The initial reduction in germination and germ tube length of *F. oxysporum* suggest that the organism being slower growing than *R. stolonifer* was from the onset more affected by the unfavorable environment created by gasoline emissions. Thereafter, *F. oxysporum* physiologically adjusted and adapted, even though growth was inhibited in both fungi. *F. oxysporum* adjusted better to the unfavorable environment in its vegetative growth, so the fungus may survive longer than *R. stolonifer* especially as hyphal fragments, when gasoline emissions pollute the environment. This was evidenced by the longer germ tubes of *F. oxysporum* even at high exposure periods.

Conclusions

Generator emissions affected the growth of both fungi tested causing reductions. The aspect of growth more affected in *R. stolonifer* was the germ tube extension while for *F. oxysporum* spore germination was more affected. The multiple peaks observed with increasing length of exposure for spore germination and germ tube growth for both fungi are evidences of their attempt to adjust to the unfavorable environment created by gasoline emissions. Both organisms may likely survive such adverse conditions for long periods even though their growth may decrease significantly. Exposure of infected plants to gasoline generator fumes may therefore play a role in alleviating fungal disease severity.

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Availability of supporting data and materials
All data generated or analyzed during the study are included in this published article.

Authors' contributions
The data was collected and statistically analyzed by KPB. The manuscript was prepared by AAT who also supervised the laboratory work, while all authors including OOO read and approved the manuscript. BKP's contribution was 40%, ATA contributed 40% while OOO contributed 20% to the work.

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KPB is a graduate student of Environmental Biology while ATA and OOO hold positions as lecturers in biology with interest in postharvest pathology/mycology. Both AAT and OOO are members of the Mycological Society of Nigeria.

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