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RESPONSE OF SOIL FUNGAL POPULATION TO A FUNGICIDE FORMULATION AT THE SPERMOSPHERE OF MAIZE

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Abstract
A laboratory and a screenhouse experiments were respectively conducted to assess the effects of the fungicide Apron Star ® containing thiamenthoxam (20%), metalaxyl (20%), and carbendazim (10%) on maize spermosphere microbial community, as well as on maize seedling performance. Soil was sampled from the spermosphere of fungicide-dressed maize seeds planted in plastic jars in the laboratory and analyzed to determine fungal population. Germination and seedling growth of maize seeds treated with fungicide, and planted in plastic pots in the screehouse were also monitored. Results indicated that fungicide reduced fungal population in the bulk soil whereas fungal population was stimulated by the interaction of fungicide with the maize seed spermosphere. Results further showed that treatment of seeds with fungicide before planting significantly enhanced seedling establishment (30.9% increase in plant height at 4 weeks after planting). It was therefore concluded that Apron Star ® is potent against pathogenic fungi that may adversely affect maize seed germination and seedling establishment. Furthermore, application of this fungicide as seed dressing agent is not likely to pose an overall detrimental effect on the soil community of bacteria and fungi.

Keywords: *Fungicide, spermosphere, microbial community, seed dressing*

Introduction
An array of chemical fungicides are used to control soil-born pathogens and their use is very crucial to the future of crop production. Seed dressing fungicides are used in the form of dust, slurry and soaking treatments (Agrios, 1997), and have long been applied to cereal seeds to prevent seed decay, damping-off and seedling blight, and soil-borne fungi (Kadege and Lyimo, 2015). Even though they engender effective and efficient control of soil-borne fungi, pesticides have been discovered to cause serious environmental problems and may be toxic to non-target organisms (Annonymous, 2005). These chemicals alter soil microbial population and activity as well as the biochemical processes mediated by the organisms (Rabie, 1997). The use of chemical fungicides had however been on the increase because a proper substitute has not been found. The population, activity and diversity of soil microorganisms are more pronounced in regions of the soil where they can access nutrients and material able to support their growth and metabolic activities. One of such zones of nutrient abundance is the spermosphere. The spermosphere is that volume of soil directly affected by the presence of the sown seed. In this region, the overall chemical, biological, and physical properties of the soil are affected to varying degrees. Like the rhizosphere, the spermosphere is colonized by an astounding number of microorganisms, and many of these plant-associated microorganisms can haveprofound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases, and productivity (Mendes *et al*., 2013). The spermosphere soil being a micro soil ecosystem, the spermo-fungal community may respond differently to the presence of a fungicide. The present study therefore seeks to examine the effects of a fungicide formulation applied as seed dressing slurry on the spermosphere fungal population as well as on the growth of maize plant.

Materials and Methods
All the solvents and other chemicals used were analytical grades and obtained from Pascal Chemical Company Ltd. (Akure, Nigeria) unless otherwise specified. The variety of maize planted (TZEE W POP STR C4) was obtained from the International Institute of Tropical Agriculture, (IITA) Ibadan. The soil used was collected from the Crop Type Museum of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure Nigeria ($7^0$16´N,$5^0$12´E), and soil was collected from an area in the field having no herbicide treatment history during the last two decades. The fungicide (Apron Star ®) used was obtained from local agro dealers in Akure. The formulation contained thiamenthoxam (20%), metalaxyl (20%), and carbendazim (10%).

Soil Sample Preparation and Incubation

Soil samples were collected from a depth of 0-15 cm in the field and brought to the laboratory in sealed polyethylene bags. In the laboratory, the samples were sieved using (2mm) to remove plant materials, soil microfauna, debris and stones. After sieving, the soil samples were homogenized and brought to a water holding capacity (WHC) of 60% by first wetting the soil to field capacity after which the moisture content was adjusted to 60% by oven-drying. A portion of the soil (200g) was weighed and transferred into plastic jars. The remaining soil (4kg) was potted and transferred to the screen house.

To dress the maize seeds with Apron Star ®, 1 kg maize seed was weight into a bowl and moistened with water. 3.3g of the fungicide powdered formulation was added and the mixture was stirred thoroughly in order to properly rub the fungicide slurry on the seeds. In the laboratory experiment, 400 treated seeds were counted and added to the 200 g soil in the plastic jar. This amounted to 2 seeds per gram of soil. This was done to achieve several overlaps of spermosphere soils inside the jar (SPERMOSPHERE + FUNGICIDE). In a separate jar, 400 untreated seeds were added to 200 g soil (SPERMOSPHERE + NO FUNGICIDE). The third jar contained 200 g soil with no seed planted, but the soil was treated with same quantity of fungicide estimated to be received by 400 maize seeds (BULK SOIL + FUNGICIDE). The last treatment involved a jar that received only 200 g soil with neither seeds planted nor fungicide treatment (BULK SOIL + NO FUNGICIDE). Each of the treatments was replicated three times and the soil together with treatments was incubated for two days. Soil samples were collected from each of the jars for the determination of yeast and mold population at the end of the incubation period.

The screen house trial involved sowing the treated maize seeds in 4l pot containing 4 kg soil each. Two seeds were sown, which was later thinned to one seedling per pot at 2 WAP. The pots were watered once in two days, no fertilizer was applied, and emerged weeds were removed by hand-picking. Maize growth parameters (plant height and number of leaves per plant) were measured at two weeks interval beginning from 3WAP up to 8 WAP when the experiment was terminated.

Enumeration of Soil Fungal Population

Numbers of fungal spores were estimated by soil dilution technique on yeast extract and Potato Dextrose Agars as isolation media for yeast and mold respectively.

To achieve serial dilution, 5 grams of soil was suspended in 150 ml Erlenmeyer flask containing 95 ml of sterilized distilled water to obtain a 10^{-1} dilution and was kept under shaking conditions at 120 rpm for 15 minutes. From the flask 1 ml of suspension was transferred to 9 ml water blank to make 10^{-2} dilution. The water blank was vortexed and then again 1 ml of the suspension was transferred to a new water blank (9 ml) tube to obtain 10^{-3} dilution. In the similar manner dilutions were made up to 10^{-5}.

The constituents of the Potato Dextrose Agar (gL^{-1}) were peptone 5.0, potato extract 5.0, dextrose 10.0, Agar 20.0, and Distilled water 1000.0 ml at pH 6.5. Potato extract was replaced with yeast extract to formulate the yeast agar. A mixture of 1g soil and 10mL of saline solution was shaken on a mechanical shaker for 10 minutes to dislodge fungal propagules into the solution. This was followed by serial dilutions to the concentrations of 10^{-5}. 0.5 mL of the aliquot was spread on Potato dextrose or yeast extract agars to isolate mold and yeast spores respectively, and this was incubated at 28^{0C} for 4 days. Dilution factors of 5 was used to determine the yeast and mold spore forming units (SFU).

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) and means were separated using the Tukey Test at 5% level of probability (p= 0.05) Figures were plotted using Microsoft excel.

Results

The main effects of individual factor regardless of the other are indicated in figure 1. Dressing maize seeds with fungicide before sowing was found to reduce soil yeast population (7.14%) with reference to the untreated soil. There was also significant alteration in yeast count in the soil sampled at the spermosphere of maize. Yeast population increased by 31.4% in the maize seed spermosphere compared to the bulk soil.

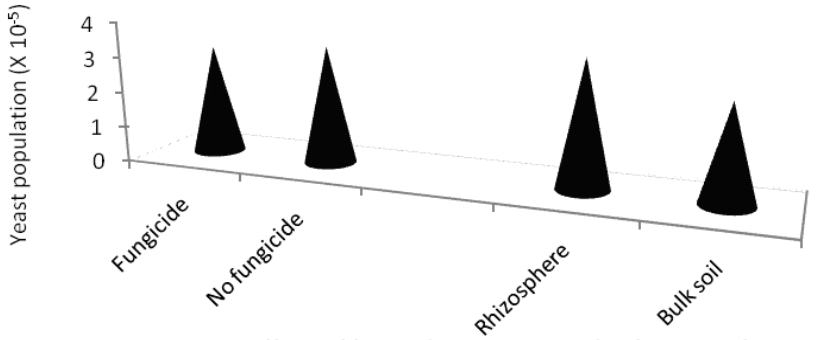

Figure 1. Main effects of fungicide treatment and soil zone on the soil yeast population

The highest yeast population was obtained in the spermosphere soil of maize seed dressed with the fungicide (Figure 2), whereas treating soil with fungicide away from the spermosphere significantly lowered yeast count by 37.9 %.

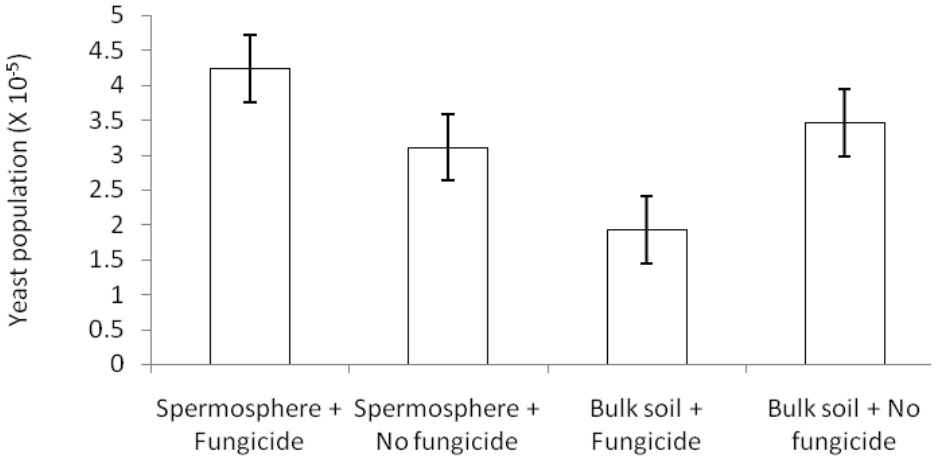

Figure 2. Interactions effects of soil zone and fungicide application on soil yeat population

Considering the main effects of the fungicide treatment regardless of the soil factor on soil mold population, seed dressing with fungicide was found to increase mold population compared to the untreated control (Figure 3). Soil sampled in the spermosphere of maize also contained higher population of mold than obtained in the bulk soil.

Similar to yeast population, presence of fungicide at the spermosphere of maize seed significantly raised mold population compared to when the sperrmosphere soil was not treated (Figure 4). Adding fungicide to the spermosphere soil also significantly increased mold population compared with treating the bulk soil with fungicide. However, when no fungicide was added, mold population was higher in the bulk soil compared to the spermosphere.

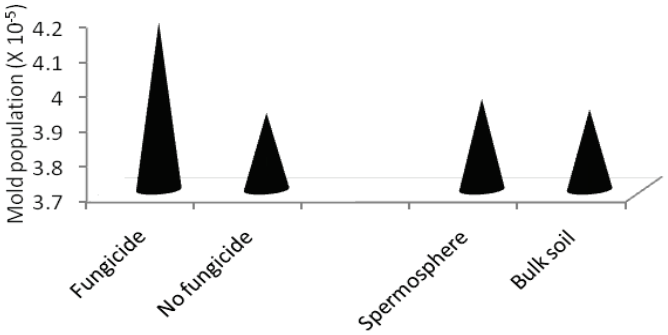

Figure 3. Main effects of fungicide treatment and soil zone on the soil mold population

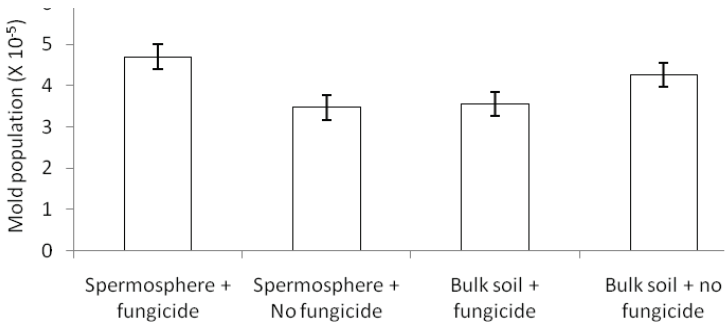

Figure 4. Interactions effects of soil zone and fungicide application on soil mold population

The effects of fungicide treatment on the growth parameters of maize are presented in figures 5 and 6. The fungicide treatment increased maize plant height from the beginning of sampling to the termination of the experiment at 8 weeks after planting (WAP) compared to the control treatment (Figure 5). The increased in maize plant height caused by the fungicide treatment was however only significant at 3 and 4 WAP (17.7 and 30.9% respectively). Average number of leaves counted from the third week up to the termination of the experiment was not influenced in any consistent manner by the fungicide treatment (Figure 6). Treatment of seeds with fungicide was however found to cause increase in the number of leaves at the termination of the experiment.

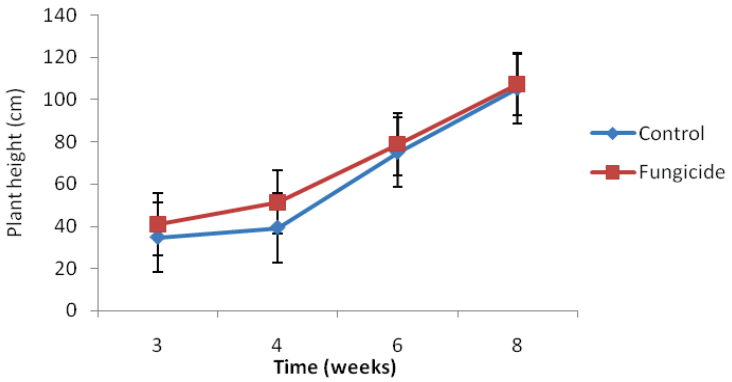

Figure 5. Effects of fungicide treatment on maize plant height (cm)

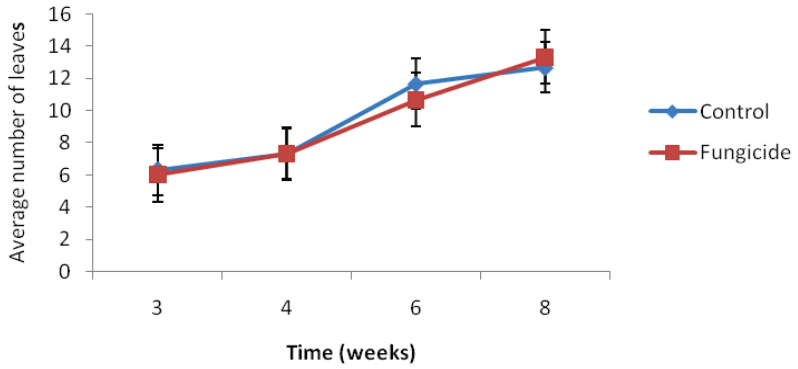

Figure 6. Effects of fungicide treatment on number of leaves of maize

Discussions

This study has clearly demonstrated that fungal species react differently to the fungicide formulation used in this experiment. The fungicide (Apron Star ®) contained thiamenthoxam (20%), metalaxyl (20%), and carbendazim (10%), yet it reduced yeast population, whereas the number of mold cells increased due to the presence of the fungicide. This was an indication that yeast was susceptible while the mold population exhibited resistance to the fungicide formulation. The increase in mold population might have resulted from selection pressure. The rise in soil mold population above the control treatment observed with fungicide application might also be due to the fact that the fungicide or its metabolites and the dead yeast cells killed by the fungicide served as a source of carbon or energy source for the surviving mold for cell proliferation (Tu and Miles, 1976). Application of pesticides to soils has been reported to inhibit some sensitive species while others rapidly appear to replace the sensitive species subsequently maintaining the metabolic integrity of the soil (Goswami *et al*., 2013). When pesticides are applied to the soil at sufficient and optimum concentrations, susceptible organisms are killed or reduced in number. However, the microbial population increases quickly to reach a level far in excess of the untreated soil due to reduction in microbial competition (Adams, 2001) and use of cell debris of killed cells by the survivor population (Goswami *et al*., 2013; Adejoro, 2016).

The study further indicated that community interactions among organisms also play a role in the responses of fungi to fungicide application. This was evident in the differential responses of fungal population at the bulk soil and the maize seed spermosphere soil. The spermosphere is known to be a hot spot of microbial activities. This is caused by an increased nutrient supply for microorganisms, since the sown seed releases organic compounds, which are capable of escaping from the endosperm through the seed coat into the immediate surroundings of the seed (Brimecombe et al. 2007).The spermosphere is therefore a zone with a high microbial diversity. An important consequence of the high diversity is an intense microbial activity (Hrynkiewicz, 2011) and interactions. Interaction among microorganisms is bound to affect species population because of the interdependence of organisms at the different trophic levels.

The fate of the fungicide appeared to be affected by the compounds released by maize seed because a stimulation of fungal population was observed in the spermosphere instead of the repression of population noticed by treating the bulk soil with the fungicide. This stimulation suggested a rapid breakdown (a shortened half-life) of the fungicide at the spermosphere to support an earlier observation that planting soils with maize increased the survival of an atrazine degrading consortium and enhanced the transformation of atrazine to hydroxyatrazine (Alvey and Crowley, 1996). Observation of higher mold population in the bulk soil compared to the spermosphere when fungicide was not added further stressed the strong interaction that occurred between the fungicide and spermosphere of maize seed.

The Significant difference in plant height between the treated and the control maize plants at the early stages of growth was an indication that the fungicide caused maize seedling to establish properly compared to the untreated maize stands. This confirmed the findings of Dumitrass (1982), who indicated that fungicidal treatment of maize seed with carboxin, metalaxyl and thiram results in vigorous growth and therefore greater height over the untreated control. This

scenario also suggested that the seed dressing agent stimulated maize growth at this early stage.

Conclusion and Recommendation

Our study revealed that the commonly used seed dressing fungicide - Apron Star ® containing thiamenthoxam (20%), metalaxyl (20%), and carbendazim (10%) is able to suppress yeast population, but its antifungal activity reduced at the spermosphere due to interaction with the spermosphere microbial environment. The study however confirmed that application of this fungicide produced more vigorous maize seedlings. It is therefore concluded that Apron Star ® is potent against pathogenic fungi that may adversely affects maize seed germination and seedling performance. Further studies are however recommended to confirm response of fungi to this fungicide at the species level. This will help to ascertain if beneficial fungi are adversely affected or not.

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