The Effect of Wetting Agents and Plant Growth Regulators on Microbial Growth in Culture Medium

Introduction

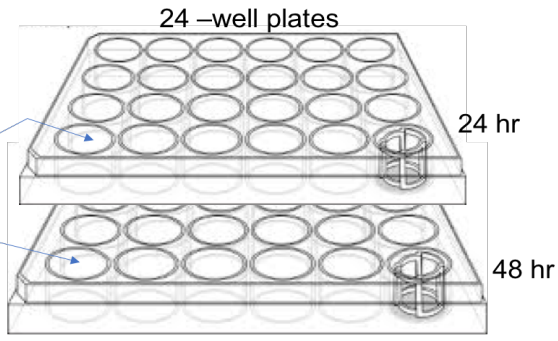
This is a preliminary study that was done to assess the impact of selected wetting agents and plant growth regulators on bacterial growth in culture medium under laboratory conditions. Seven wetting agents and three plant growth regulators were mixed at four concentrations with the growth medium that was inoculated with microorganisms extracted from soil. The bacterial growth was monitored over time by measuring the turbidity of the medium as an indicator of growth. More information on details of the study is given below.

Materials & Methods

We used a generic growth medium for total heterotrophic bacteria called Nutrient broth. The broth contained antifungal additive (nystatin) to prevent fungal interference on bacterial growth. The growth assay was prepared by mixing the various ingredients in a 24-well culture plate as shown in Figure 1. The total assay volume was 1 mL (1000 µL). Each product was added at four levels: 0 (positive control), 10, 20 and 50 µL. The concentrations corresponded to 0, 1, 2 and 5%. The positive control included everything but the product. The negative control, on the other hand, received everything but the microorganisms. After mixing all the ingredients, the plates were shaken in an incubator at 25°C at 150 rpm to facilitate aeration. Two separate plates were set up for the two sampling times, which were 24 and 48 hrs. Measurements for turbidity were taken with a spectrophotometer at 600 nm. All treatments were set-up in duplicate. Sterile phosphate buffer was used to extract microorganisms from soil to be used as inoculants. Some of the products contain surfactants that turned the medium milky upon addition. This interfered with the measurement of turbidity initially. To correct for this, the medium was centrifuged at high speed to separate the bacteria that settled at the bottom. The surfactant was then removed, and the bacteria were subsequently resuspended in sterile phosphate buffer for measurement.

Figure 1. Preparation protocols for the ten turf care products

Treatment	Product type	Preparations (assay ingredients)
Dispatch, Fleet, Magnus, Oars, Prevade, Sixteen 90 and Vivax	Wetting agents	Product (0, 10, 20 or 50 µL) + 1000 µL medium broth + 10 µL microbial source
Anuew, Proxy, Trimmit	Growth regulators	



The absorbance from the negative control was subtracted from the absorbance values of the rest. Analysis of variance was conducted in JMP Pro 13 to compare the mean absorbance values among treatments and concentrations.

Summary of Results & Discussion

- The mean absorbance readings for bacterial growth were significantly ($P < 0.0001$) lower in the broth treated with all the wettings agents except for Sixteen 90 as compared to the positive control (Table 1 and Figures 2).
- The mean absorbance readings for bacterial growth were significantly ($P < 0.0001$) lower in the broth treated with all plant growth regulators except for Anuew as compared to the positive control (Table 2 and Figure 3).
- The effect of the concentration was significant ($P < 0.0001$) but was mainly between the positive control (0 µL) and the rest.
- From the wetting agents, growth was inhibited by Dispatch, Fleet, Oars, Prevade and Vivax (Table 1 and Figure 2).
- From the wetting agents, Sixteen 90 was the only one that did not have any negative impact on bacterial growth. In fact, it seems to have enhanced it at higher concentrations (Table 1 and Figure 2).
- The effect of Magnus appeared to only slow down bacterial growth initially as significantly higher growth was observed at 48 hr than at 24 hr. But the growth was still lower than the positive control at 48 hr.
- From the plant growth regulars, Proxy and Trimmit completely inhibited bacterial growth (Table 2 and Figure 3).
- Anuew slowed down bacterial growth initially (24 hr) but growth was comparable to the positive control at time 48 hr (Table 2 and Figure 3).

Table 1. Mean absorbance for bacterial growth with wetting agents at four concentrations

Time	24 hr				48 hr			
	Concentration (µL)				Concentration (µL)			
	0	10	20	50	0	10	20	50
Product	----------Mean absorbance----------							
Dispatch	0.266	0.036	0.038	0.037	0.623	0.036	0.037	0.036
Fleet	0.370	0.181	0.028	0.036	0.766	0.120	0.079	0.121
Magnus	0.224	0.000	0.000	0.000	0.789	0.273	0.339	0.550
Oars	0.399	0.036	0.043	0.037	0.680	0.078	0.039	0.037
Prevade	0.442	0.038	0.043	0.044	0.768	0.032	0.039	0.060
Sixteen 90	0.188	0.217	0.236	0.217	0.615	0.778	0.985	0.897
Vivax	0.246	0.078	0.111	0.136	0.530	0.112	0.119	0.215

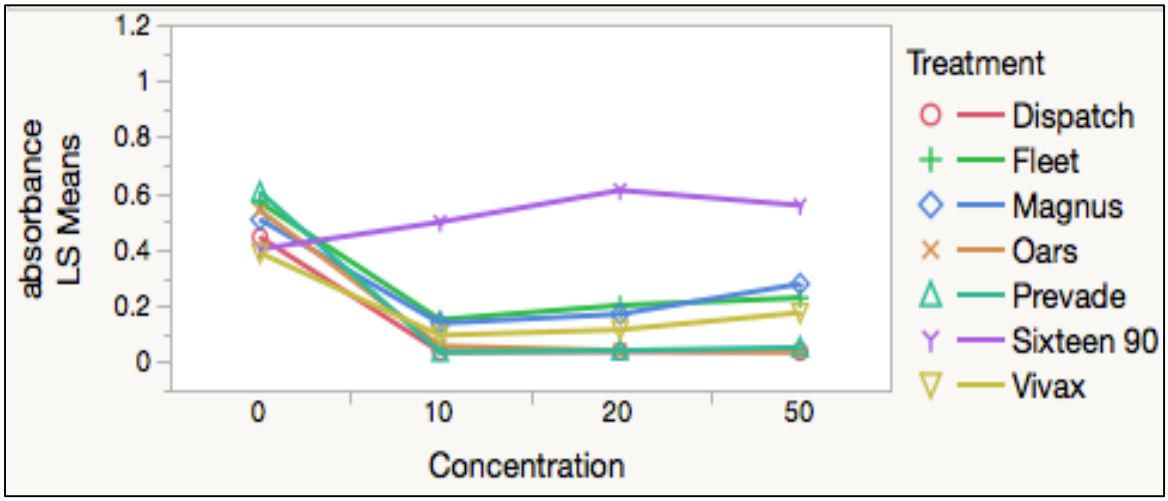


Figure 2. Means plot for absorbance by treatments*concentration of wetting agents after 48 hrs of growth

Table 2. Mean absorbance of bacterial growth with plant growth regulators at four concentrations after 24 and 48 hrs of growth

	24 hrs				**48 hrs**			
	Concentration (ppm)				Concentration (ppm)			
	0	10	20	50	0	10	20	50
Product	--------Mean absorbance----------							
Anuew	0.314	0.100	0.257	0.184	0.614	0.566	0.465	0.502
Proxy	0.337	0.038	0.036	0.035	0.916	0.039	0.057	0.036
Trimmit	0.388	0.017	0.039	0.000	0.598	0.018	0.150	0.006

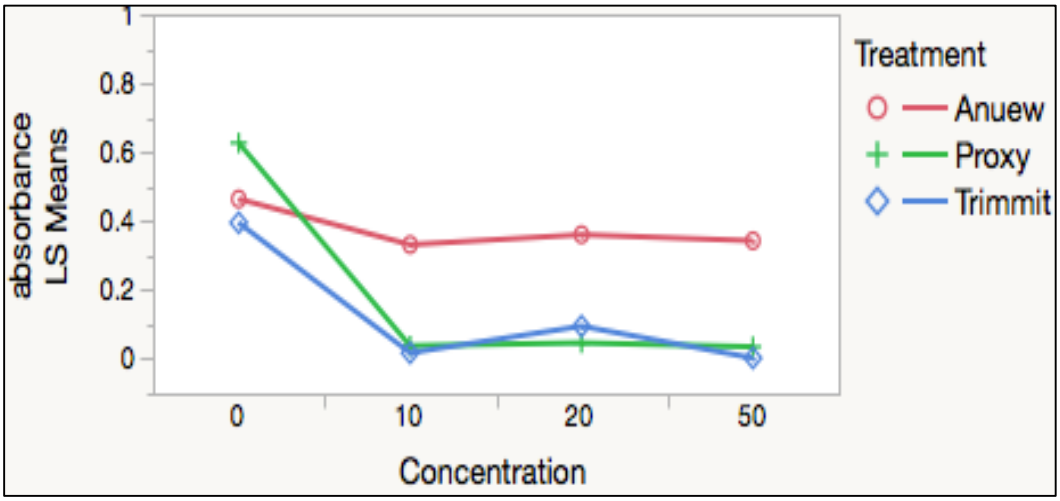


Figure 3. Means plot for absorbance by treatments*concentration of plant growth regulators

Concluding remarks and recommendation

- Dispatch, Fleet, Oars, Prevade, Vivax, Proxy and Trimmit inhibited bacterial growth.
- Sixteen 90 did not show any negative impact on bacterial growth whereas the impacts of Magnus and Anuew appeared to be only temporary.
- The effects of the wetting agents and plant growth regulators in a growth medium might not be reflective of what will happen in soils where there are organic matter and soil particles that can minimize the impact.
- However, we recommend field based studies for the wettings agents and the plant growth regulators that completely inhibited microbial growth to further study their impacts not only on microbial growth but also their impact on microbial functions.