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# Control of algal growth on greenhouse surfaces using commercial algaecides

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**ABSTRACT**: Greenhouses and nurseries provide ideal environments for facilitating the formation of nuisance algal mats. Algal growth poses safety concerns to horticulturists and stimulates the propagation of unwanted plant pests and pathogens. To date, few strategies and data are available to effectively manage algal problems. The effectiveness of five algaecides was tested on two varying surfaces of greenhouses *in situ* to elucidate the efficacy of chemical methods of removing algae. Moreover, *Nostoc commune* (Vaucher ex Bornet & Flahault) was treated on ceramic tiles *in vitro*, as it is a common alga in greenhouses and nurseries. We found that each algaecide had different effects, depending on the chemical applied, the surface to which the chemical was applied, and finally the types of algae that were targeted. Algaecides across the surfaces tested demonstrated that algal cell characteristics and communal makeup played an important role in algaecide efficacy, where mucilaginous algae were replaced by sheath-forming filamentous cyanobacteria. We found sodium carbonate peroxyhydrate to be the most effective chemical in terms of controlling *Nostoc* on tarp, gravel, and ceramic surfaces.

**Keywords**: *Nostoc commune*, DNA, cyanobacteria, herbicides, management

## Introduction

Nuisance algae are a common problem in greenhouses and are an occupational hazard due to their ability to excrete mucilage-rich substances on surfaces (Santamaria, 2016). These algae can enter a greenhouse via horticulturists' shoes or utensils, aerial transport, or through irrigation systems that use water contaminated with algae. Once in a greenhouse, algae grow quickly and produce large biomass due to nutrients (fertilizers), light, high humidity, warm temperatures, and other factors. The algae also benefit from nutrients introduced by irrigation, especially when the water contains excess nutrients from runoff (Latimer et al., 1996). Algae may also be heat tolerant where soil purification is of no assistance to their removal (Bollen, 1969). Furthermore, irrigation from ponds often contains other microorganisms (Stewart-Wade, 2011) and these can increase the growth of algae as a result of symbiotic relationships. These factors lead to the prominent and robust growth of algae in greenhouses (Granke and Hausbeck, 2010).

Algal growth occurs throughout the year on many surfaces including floors, gravel, tarp, or bound to cooling and water systems. The algal mats formed complicate the working environment for greenhouse and nursery workers. The mats growing on the ground create slippery and hazardous conditions (Mergel and Dickey, 2007). Growers are in danger of slipping and falling because of the moist and mucus-like conditions induced by algal mats, a risk that could cost a company sizable sums in workman compensation fees (Goldberg, 2018). In addition to the hazards of algae mats, algae also harbor plant pathogens and lure pest insects, such as fungus gnats and shore flies. The pests attracted feed on algae while destroying commercial plants inside the nurseries and greenhouses (Keates et al., 1989). Algal mats produce many compounds that can be either beneficial or harmful (Singh et al., 2016). These bioactive compounds, mostly produced by cyanobacteria (blue-green algae), can be toxic and are found in the mats (Kleinteich et al., 2018). The toxins produced by the cyanobacteria can affect crops and consequently bioaccumulate in the plant and are of concern to human consumption (Galey et al., 1987; Vänninen and Koskula, 1998). In order to reduce the risks, there is a need for improved management strategies to reduce unwanted algae growth.

To date, there are a number of products aimed at removing the algal problem faced by greenhouse facilities. Products for the removal of algae include commercially available oxidizing agents, quaternary ammonia products, and inorganic acids (Chase and Osborn, 1984). Although products are available, their efficacy in treating the complex algal communities found on various greenhouse surfaces requires evaluation. The aim of this study was to conduct a preliminary assessment of the efficacy of several commercial algaecides used in treating algae found on multiple surfaces in greenhouse facilities. Since algae are often found growing on ceramic surfaces, tarps, and gravel in commercial nurseries and greenhouses, we investigated the control of algal growth coverage on these surfaces using five retailed algaecides.

## Materials and Methods

### *In situ* field study

Laboratory tape and fluorescent spray paint were used to section 0.5 m$^2$ of greenhouse tarp and gravel substrate. A total of six plots were sectioned and labeled with waterproof markers. Chemical application involved mixing each formulation to the recommended concentration using deionized water in dark spray bottles. Chemicals included copper sulfate [Crystal Blue, Sanco

Chemicals (1 ppm)], hydrogen dioxide [Zerotol® 2.0, Biosafe Systems (1.74 µL mL$^{-1}$)], pelargonic acid [AXXE®, Biosafe Systems (6 %)], sodium hypochlorite (germicidal bleach) [HDX™ (0.825 % v:v)], and sodium carbonate peroxyhydrate [TerraCyte®Pro, Biosafe systems (0.059 mL mL$^{-1}$)]. Commercial bleach for home use usually includes 5.25 % sodium hypochlorite and is applied at the rate of 0.5 % in greenhouses; thus, germicidal bleach (8.25 %) was applied at the rate of 0.825 % to match the dilution of bleach used in greenhouses.

Each greenhouse plot received a single application consisting of 40 sprays, while the control plot received no chemical application. Images of algal growth were taken every seven days for 28 days and modified in Adobe Photoshop (2017.1.1). Once the cropped images had been obtained, they were uploaded into the iLastik (1.3.0) software tool for image analysis. Live and dead algae material (known from microscopic analyses) were manually labeled with two colors as follows: green (live) and red (dead). Material not considered algae was also labeled red. Converted images were then uploaded into a pixel analysis software program (cool PHP tools.com) using the color extract feature to calculate color pixel percentages. Percent coverage of live and dead algae were then calculated based on the initial coverage on day 0 prior to chemical applications. A total of five formulations and one control treatment were used in this study. No replicates were made as this was a preliminary study.

### *In vitro* laboratory study

For the laboratory studies, the rough and coarse unglazed underside of autoclaved (sterilized) ceramic tiles were placed in petri dishes, submerged in 10 mL of $BG11_0$ (nitrogen-free media) and inoculated with 1ml of seed culture of *Nostoc commune* Vaucher ex Bornet & Flahault (strain UTEX B1621), which is a common nuisance alga found in greenhouses. Ceramic tiles were cultured in petri dishes at 25 °C under constant lighting (50 µmol) for two weeks until algae covered the surfaces. Media were replenished during the study in order to keep the tile submerged. Plates were incubated at 25 °C under constant lighting for 21 days and analyzed every 7 days after chemical application. Each tile received a single treatment (two consecutive sprays of each chemical); tiles were then imaged and analyzed as described above.

### Molecular and morphological analysis

In order to assess degradation of algal DNA, 10 mg of algal biomass was removed from each ceramic tile and placed in 1.5 mL Eppendorf tubes which were subjected to freeze-thaw cycles three times for cell lysis. Samples were then processed for DNA using the UltraClean Microbial DNA isolation kit (Qiagen, USA). DNA extracts were amplified with PCR primers for cyanobacteria using the 16S rRNA gene: forward primer CYA359F - GGG GAA TTT TCC GCA ATG GG and reverse primer CYA 781Ra – GAC TAC TGG GGT ATC TAA TCC CAT T (Nübel et al., 1997). PCR reactions were carried out the following parameters: 3 min at 95 °C for initial denaturation and 35 cycles with 95 °C for 30 s, 57 °C for 1 min, and extension at 72 °C for 80 s. PCR reactions were run on a thermocycler (ProFlex PCR System; Applied Biosystems; Life Technologies). PCR products were processed on a 1.5 % agarose gel and visualized using AlphaImager HP (Cell Biosciences). Presence and absence of bands were used as an indicator of *N. commune* status. In order to ascribe species to genera, algae mats were dissected, then visualized and imaged under a microscope (AmScope; 18 mp microscope digital camera, Optitec-YG-100). Morphology based inferences were assessed using several algae guides (Bellinger and Sigee, 2015; Komárek, 2013; Komárek and Anagnostidis, 1999; 2005), and the dominance of certain taxa was determined by their relative abundance through morphological observation.

## Results

### *In situ* algaecide application

Results from the *in-situ* field application of algaecides on either tarp or gravel are found in Tables 1 and 2 and Figures 1A-F and 2A-F. Whether on gravel or tarp, the control received no chemical application. During the first week after application, the chemicals most effective at reducing algae growth on tarp were sodium hypochlorite and copper sulfate with 31.0 and 19.4 % decreases in coverage, respectively (Table 1). Conversely, sodium carbonate peroxyhydrate, pelargonic acid, and hydrogen dioxide resulted in an increase in algae coverage by

**Table 1** – Results of formulations on plots (0.5 m$^2$) containing algae expressed as percentage (%) coverage of live algae on tarp for every week over 28 days. Negative values indicate growth of algae, and positive values percentage decrease in algae growth/ coverage.

| | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|
| Copper sulfate | 19.40 | 15.55 | 21.52 | 51.00 |
| Hydrogen dioxide | –4.25 | 25.23 | 0.92 | –11.83 |
| Pelargonic acid | –40.48 | 29.97 | 36.35 | 53.18 |
| Sodium hypochlorite | 31.00 | 9.02 | 60.20 | 60.08 |
| Sodium carbonate peroxyhydrate | –41.87 | 28.63 | 80.72 | 67.41 |

**Table 2** – Results of formulations on plots (0.5 m$^2$) containing algae expressed as percentage (%) coverage of live algae on gravel for every week over 28 days. Negative values indicate growth of algae, and positive values percentage decrease in algae growth/ coverage.

| | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|
| Copper sulfate | 7.99 | –29.68 | –2.73 | –0.61 |
| Hydrogen dioxide | 44.57 | 43.96 | 25.10 | 70.90 |
| Pelargonic acid | –67.24 | 0.078 | -66.38 | –11.32 |
| Sodium hypochlorite | –37.09 | –20.58 | –18.54 | 9.79 |
| Sodium carbonate peroxyhydrate | 8.66 | –5.35 | 9.95 | 45.57 |

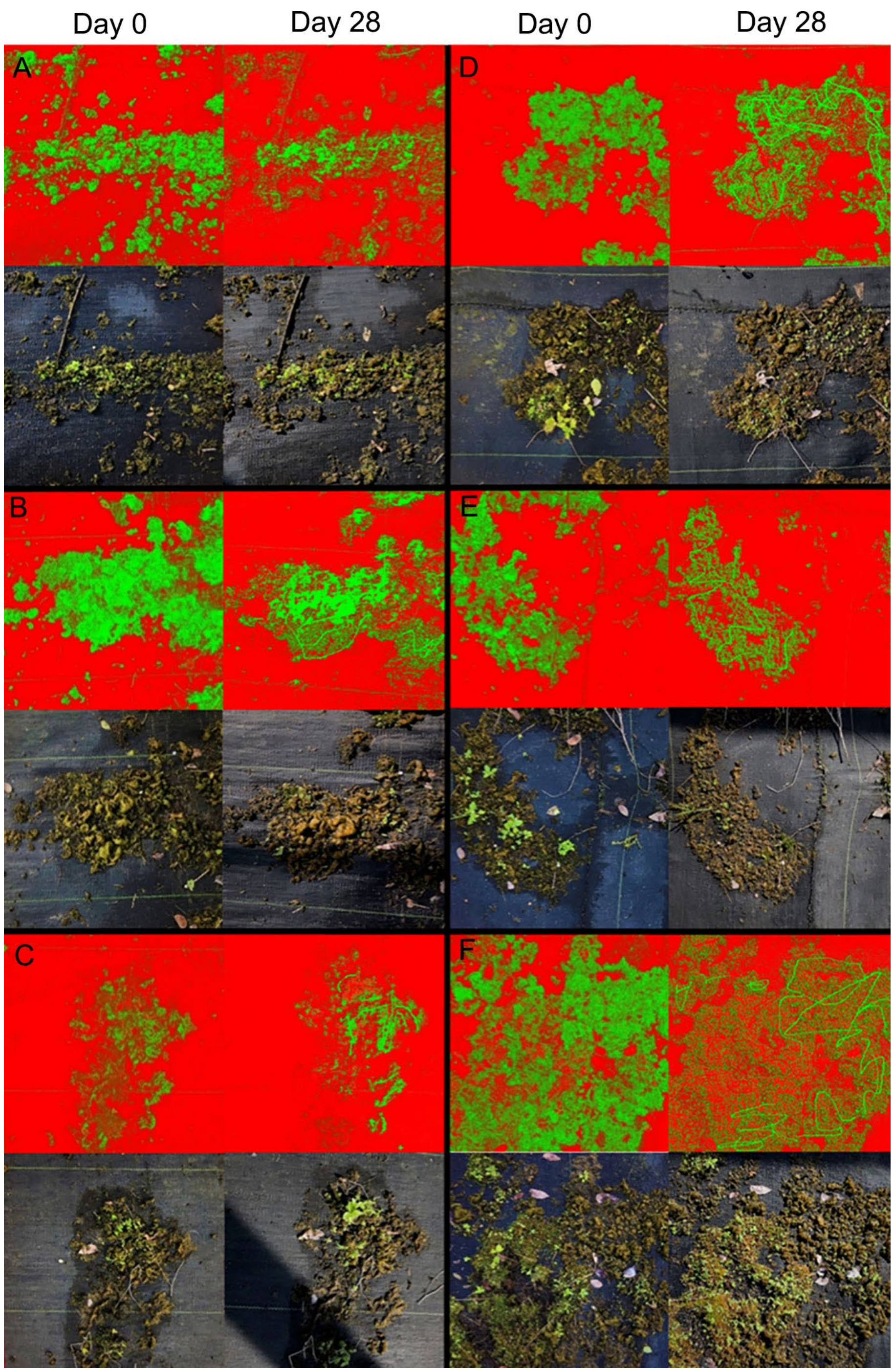


**Figure 1** – Results from field studies involving algae growing on 0.5 m² of tarp showing color-pixelated images (top) and live images (bottom). Red pixilation indicates dead or non-algae material and green pixilation live algae growth from before and after 28 days of application. Algaecides used in this experiment include A) control, B) copper sulfate, C) hydrogen dioxide (Zerotol® 2.0), D) pelargonic acid (AXXE®), E) sodium hypochlorite (germicidal bleach), and F) sodium carbonate peroxyhydrate (TerraCyte®Pro).

41.9, 40.5, and 4.3 %, respectively. By the second and third week after application, all of the chemicals tested had reduced the algae coverage on the tarp. On day 14, pelargonic acid, sodium carbonate peroxyhydrate, and hydrogen dioxide showed the greatest decrease with 29.9, 28.6, and 25.2 % less coverage, respectively. On day 21, the highest to lowest percentage decrease found after chemical application was sodium carbonate

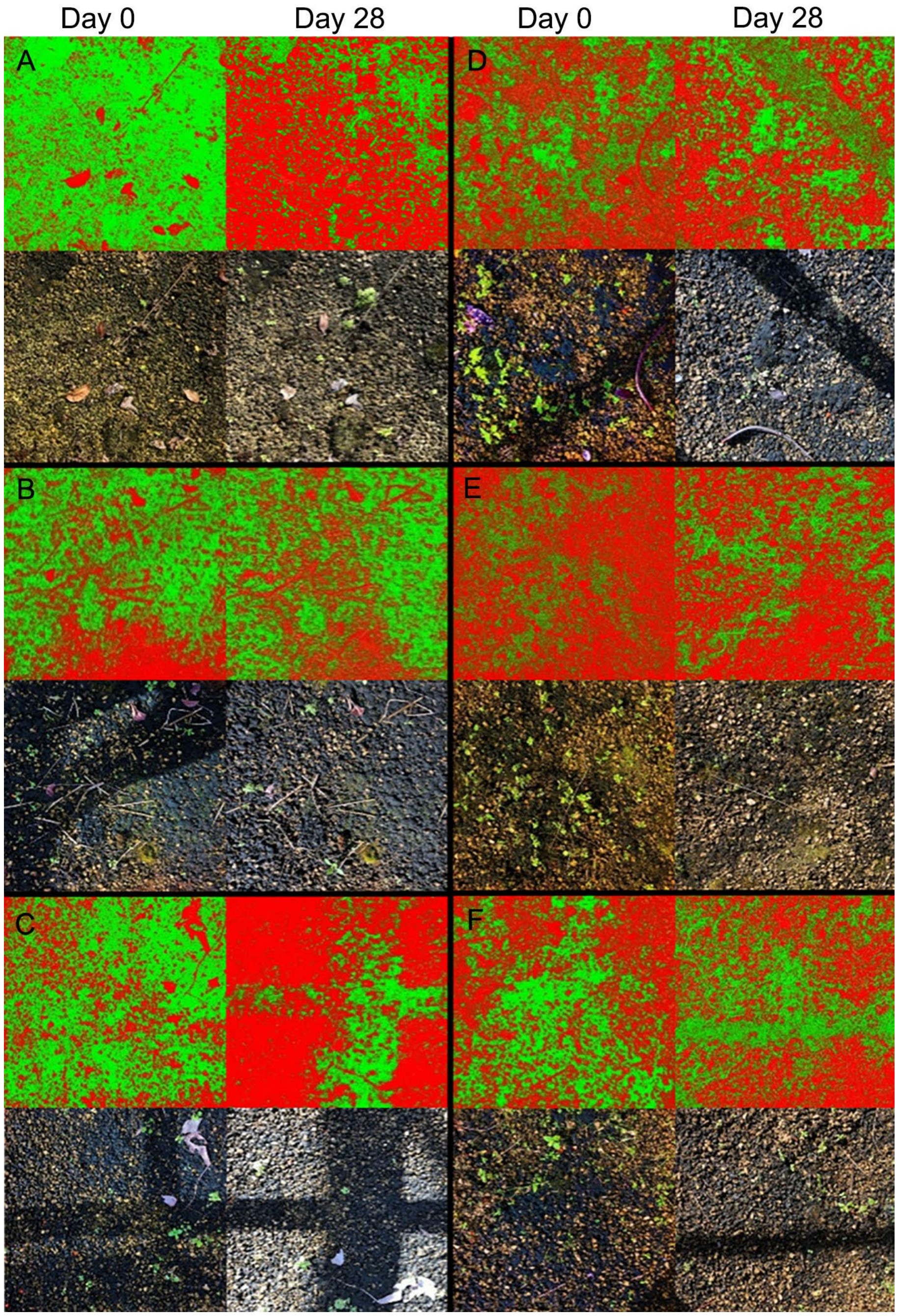


**Figure 2** – Results from field studies involving algae growing on 0.5 $m^2$ of gravel showing color-pixelated images (top) and live images (bottom). Red pixilation indicates dead or non-algae material while green pixilation represents live algae growth and live images from before and after 28 days of application. Algaecides used in this experiment include A) control, B) copper sulfate, C) hydrogen dioxide (Zerotol® 2.0), D) pelargonic acid (AXXE®), E) sodium hypochlorite (germicidal bleach), and F) sodium carbonate peroxyhydrate (TerraCyte®Pro).

peroxyhydrate, sodium hypochlorite, pelargonic acid, copper sulfate, and hydrogen dioxide ranging from 80.7 to 0.9 % decrease in coverage. The greatest suppression on tarps on day 28 was observed using sodium carbonate peroxyhydrate (67.4 % decrease) (Figure 1F). The second and third most effective formulations were sodium hypochlorite and pelargonic acid with a 60.1 % and 53.1 % decrease, respectively (Figure 1E and D). Comparatively, copper sulfate resulted in a moderate 51.0 % decrease in algae coverage on tarps (Figure 1B). In contrast, hydrogen dioxide was the least effective at removing algae and had an increase of 11.8 % coverage (Figure 1C).

By the end of the first week of application the chemicals most effective at reducing algae growth on gravel were hydrogen dioxide, sodium carbonate peroxyhydrate, and copper sulfate registering 44.6, 8.7, and 7.9 %, respectively (Table 2). Pelargonic acid and sodium hypochlorite had an increase in growth by 67.2 and 37.1 %, respectively, in the first week. By day 14, the most effective chemicals were hydrogen dioxide with a 43.9 % decrease in coverage and pelargonic acid with 0.1 %. Copper sulfate, sodium hypochlorite, and sodium carbonate peroxyhydrate alternatively demonstrated an increase in algae growth by 29.7, 20.6, and 5.4 %, respectively. By day 21, hydrogen dioxide and sodium carbonate peroxyhydrate showed decreases in coverage of 25.1 and 9.9 %, respectively. On the other hand, pelargonic acid, sodium hypochlorite, and copper sulfate had increases in growth of 66.4, 18.5, and 2.7 %, respectively. Twenty-eight days after application, the greatest percentage decrease was observed with hydrogen dioxide with a 70.9 % decrease in algae coverage (Figure 2C), while the second and third most effective formulations were sodium carbonate peroxyhydrate and sodium hypochlorite with decreases of 45.5 % and 9.7 %, respectively (Figure 2F and E). Pelargonic acid and copper sulfate, however, showed an increase in algal growth with 11.3 and 0.61 %, respectively (Figure 2D and B).

### *In vitro* algaecide application

The results of the laboratory studies involving *N. commune* grown on ceramic tiles (0.5 cm²) are shown in Table 3 and Figure 3A-F. During the first week of treatment, pelargonic acid, sodium carbonate peroxyhydrate, and sodium hypochlorite had the highest percentage decreases at 98.0, 79.5, and 29.9 %, respectively. On the other hand, copper sulfate and hydrogen dioxide showed increases in growth with 41.2 and 3.8 %, respectively. By day 14 of the treatment, pelargonic acid, sodium carbonate peroxyhydrate, and sodium hypochlorite continued to have the highest decreases in growth at 95.8, 95.6, and 31.4 %, respectively. Hydrogen dioxide also showed a decrease in growth registering 0.7 %. The highest percentage coverage decrease on day 21 was observed with sodium carbonate peroxyhydrate (98.0 %). The plates treated with sodium carbonate peroxyhydrate killed and removed all of the algae material from the ceramic surface (Figure 3F). The second and third most effective formulations were pelargonic acid and sodium hypochlorite with 95.9 % and 26.1 % decreases, respectively (Figure 3D and E). Hydrogen dioxide resulted in a percentage decrease of only 0.6 % (Figure 3C), and copper sulfate had an increase in growth of approximately 62.9 % (Figure 3B).

### Molecular results from *in vitro* studies

All laboratory ceramic tiles inoculated with *N. commune* contained environmental genomic DNA (includes fungi, bacteria, and eukaryotes) (Table 4). After amplifying the extracted environmental genomic DNA with primers specific to filamentous cyanobacteria (16S rRNA), especially *Nostoc*, we found that the plates treated with sodium carbonate peroxyhydrate did not contain any cyanobacterial DNA that could stem from *N. commune*. The remaining plates, copper sulfate, sodium hypochlorite, hydrogen dioxide, and pelargonic acid were not effective in removing DNA from *N. commune* (Figure 3A-E).

**Table 3** – Laboratory study results of formulations on plots of ceramic tiles (5 cm²) containing *Nostoc commune* expressed as percentage (%) coverage of live algae every week over 21 days. Negative values indicate growth of algae, and positive values percentage decrease in algae growth/coverage.

| | Day 7 | Day 14 | Day 21 |
|---|---|---|---|
| Copper sulfate | –41.17 | –55.68 | –62.91 |
| Hydrogen dioxide | –3.77 | 0.66 | 0.55 |
| Pelargonic acid | 98.03 | 95.84 | 95.89 |
| Sodium hypochlorite | 29.91 | 31.43 | 26.12 |
| Sodium carbonate peroxyhydrate | 79.49 | 95.57 | 98.03 |

**Table 4** – Molecular results of greenhouse plots indication of presence (+) or absence (–) of either total DNA extracted, or amplified cyanobacterial 16S rRNA gene.

| | Total DNA | 16S rRNA |
|---|---|---|
| Copper sulfate | + | + |
| Hydrogen dioxide | + | + |
| Pelargonic acid | + | + |
| Sodium hypochlorite | + | + |
| Sodium carbonate peroxyhydrate | + | – |

### Cyanobacterial community structure

There were differences in the algal community between tarp and gravel. The genera dominant on gravel were *Aulosira*, *Chroococcus*, *Scytonema*, and *Stigonema*. On tarp, *Aphanocapsa*, *Aphanothece*, *Aulosira*, *Gloeothece*, *Nostoc*, *Phormidium*, and *Scytonema* dominated. On gravel, cyanobacteria with pigmented hard sheaths were observed, whereas tarp was favorable to cyanobacteria with mucilage.

## Discussion

The perpetual greenhouse nuisance algal issue can lead to serious worker injuries and requires effective management strategies. Chemical methods for removing algae from surfaces often include chlorine, copper, and hydrogen dioxide. The chemicals can either be helpful or not depending on the application and the different algae present. Chemical products are often added in a blanket fashion in attempts to remove all components of algal mat communities. There is a lack of information regarding efficacy of chemicals labeled to kill algal mats and the species they target, leaving horticulturists with limited management strategies (Fausey, 2003). In this study, the effects of five algaecides traditionally used in

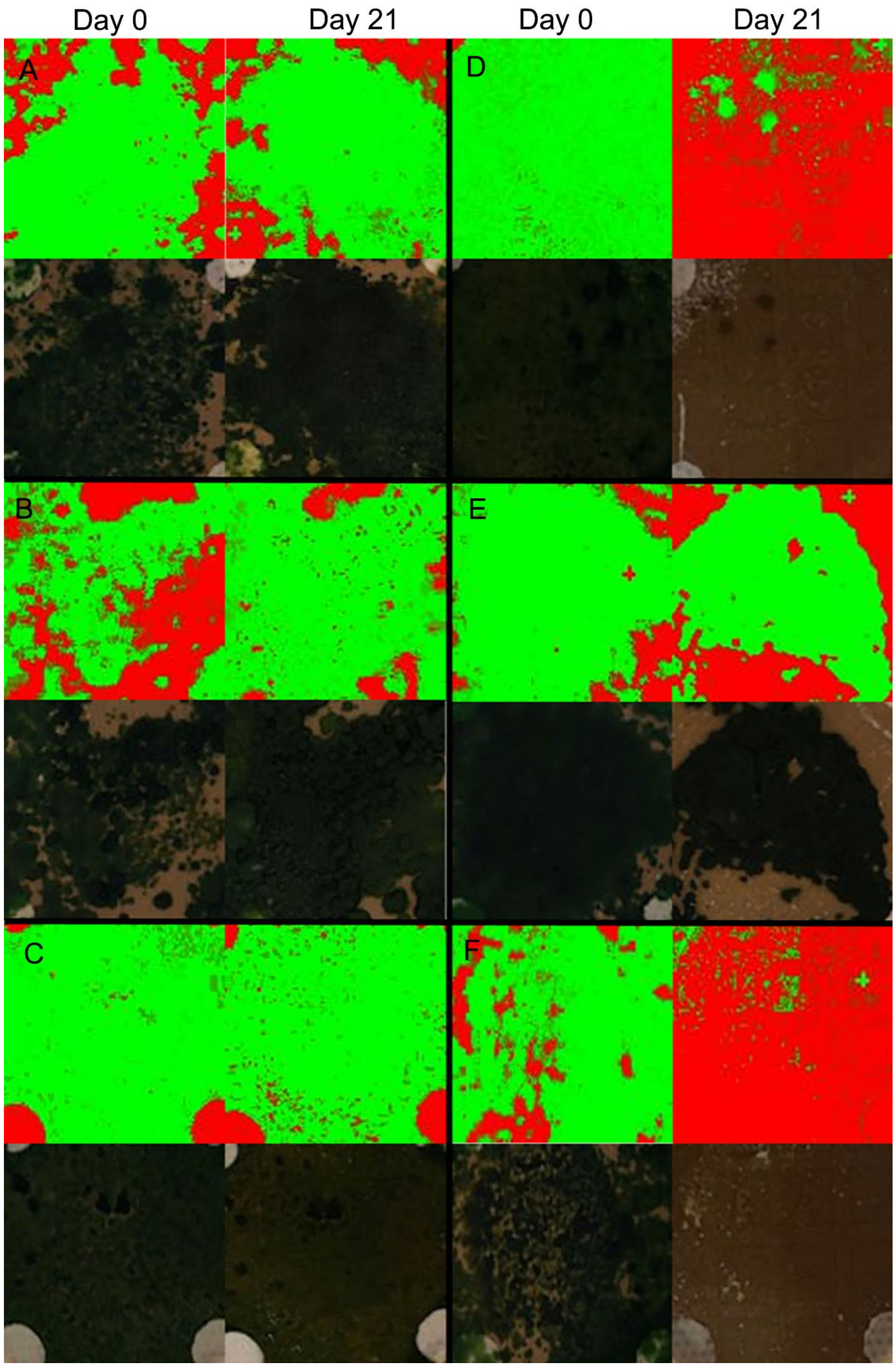


**Figure 3** – Results from laboratory studies involving *Nostoc commune* grown on 5 $cm^2$ ceramic tiles using live (bottom) and color-pixelated (top) images. Red pixilation indicates dead or non-algae material and green live algae growth from before and after 21 days of application. Algaecides used in this experiment include A) control, B) copper sulfate, C) hydrogen dioxide (Zerotol® 2.0), D) pelargonic acid (AXXE®), E) sodium hypochlorite (germicidal bleach), and F) sodium carbonate peroxyhydrate (TerraCyte®Pro).

the removal and eradication of algae were compared on three different surfaces including *in situ* field gravel, tarp and ceramic tiles *in vitro*.

The main factors that influence the actions of chemicals on combating greenhouse algae include 1) the type of chemicals used, 2) the material in which the algae are growing, and 3) the species of algae that are targeted. From our studies, sodium carbonate peroxyhydrate was the most effective at reducing algae growth. From the tarp material, sodium hypochlorite, pelargonic acid, and copper sulfate were also moderately successful in removing algae growth (Table 1). Hydrogen dioxide was not an effective agent when applied to algal mats found on tarp, possibly because it is rapidly oxidized. In contrast, on gravel, hydrogen dioxide was the most effective in reducing algal cover. Hydrogen dioxide penetrates into the gravel, suppressing algae bound onto gravel. In addition to hydrogen dioxide, sodium carbonate peroxyhydrate was also moderately efficient in removing algae from the gravel surface. On the other hand, copper sulfate, pelargonic acid, and sodium hypochlorite were not successful in removing algae (Table 2). Copper sulfate and pelargonic acid stimulated growth in our study. Although not scrutinized here, the mode of action of any individual algaecide on specific algal species is a significant contributor to its effectiveness (Ma et al., 2002; Bohme et al., 1981; Sabba and Vaughn, 1999), which is important since dominant algal communities can vary from surface to surface.

From the *in situ* studies, results show that the chemicals targeted *Nostoc*, which was dominant or present on tarp and gravel, respectively (Figure 4B and D). By isolating each laboratory plate from uncontrolled environmental variables, we found that hydrogen dioxide, sodium hypochlorite, pelargonic acid, and sodium carbonate peroxyhydrate were increasingly effective against *N. commune* grown on ceramic tiles. However, copper sulfate increased growth coverage on the ceramic surface over 21 days. Copper sulfate indicated signs of hormesis, and growth was stimulated rather than reduced (Figure 3B; Figure 4E). Copper sulfate appeared to fertilize the algae, resulting in more pigmented cells than the control (Figure 4E). Other herbicidal compounds, when applied in low concentrations, have also resulted in hormesis when applied to cyanobacteria (Shen et al., 2009).

When observing the raw extracted DNA, all of the plates contained DNA whether fungal, bacterial, or algal in nature. However, once the DNA from the ceramic plates was amplified using the specific filamentous cyanobacteria primer, only sodium carbonate peroxyhydrate resulted in the complete destruction of *Nostoc commune* genetic material. The complete degradation and eradication of *N. commune* genetic material appears to be essential in preventing cyanobacteria from reestablishing growth after chemical application.

In addition to the effects of the chemicals applied, the source material where the algae are growing affects the results. Copper sulfate and pelargonic acid were effective against algae found on tarp but not effective against those found on gravel. Hydrogen dioxide was effective on gravel but not on tarp material. Interestingly, sodium carbonate peroxyhydrate was effective at reducing the algae surface coverage on both tarp and gravel surfaces. Possible differences between these two surfaces are that chemicals applied to tarps may have been more exposed to elemental variation, whereas gravel presented an uneven porous surface allowing chemical persistence. Gravel and tarp also provide dissimilar surfaces amenable to differing species of algae which potentially react differently to each chemical. In the greenhouses tested, the prevailing algal taxon found on tarp was *Nostoc*, whereas *Scytonema* dominated the gravel surfaces.

The inherent disparity between species of algae found on either tarp or gravel could play a major role in algaecide efficiency. The dominant genera of the two substrates differed and these differences in community makeup could have affected the way in which the chemicals performed. Each chemical had a different result based on the characteristics of an individual alga or the overall algal community. From the microscopy images of the treated gravel and tarp (Figure 4A-G), a number of important observations were made. When observing the control, we saw a healthy *Nostoc* with thick mucilage (Figure 4A). Conversely, chemicals including hydrogen dioxide and sodium carbonate peroxyhydrate physically stunted the growth of *Nostoc*, as observed in the yellowing and shrinking from mucilage dehydration

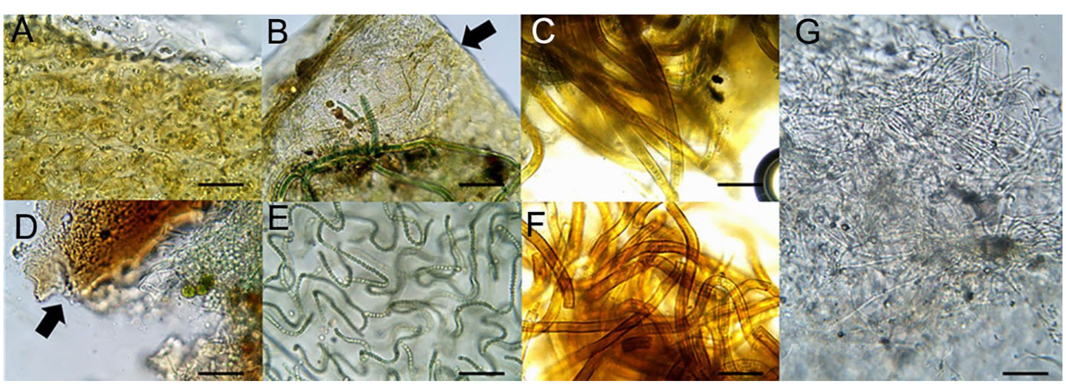


**Figure 4** – Microscopy brightfield images of algae found on either tarp or gravel surfaces 28 days after application of algaecides. Images are: Control on tarp (A), Hydrogen dioxide on tarp (B), (D) and gravel (C), copper sulfate (E), and sodium carbonate peroxyhydrate (Terracyte®Pro) on gravel (F) and tarp (G). Arrows indicate reduced *Nostoc* mucilage. Scale bar represents 10 µm.

(Figure 4B and D). In contrast, these same chemicals could not penetrate the hard, pigmented sheath around *Scytonema* and failed to kill off algal cells that bore into gravel (Figure 4C and F). As a result of reducing the growth of specific algae like *Nostoc* but equally, not affecting algae such as *Scytonema*, different algal communities emerged after application. From these experiments, it was evident that certain algae were killed off, reducing the competition between algae. The reduced competition between algae allowed other algal taxa to dominate, such as *Leptolyngbya* and *Scytonema* (Figure 4B, D and G). As a result, certain chemicals may not effectively algal mats, but allow for persistent contamination through a changing community structure and reestablishment of eradicated cyanobacteria. The cellular developmental stage of an alga has also been considered to be a major factor in species' endurance or susceptibility to algaecides (Pearlmutter and Lembi, 1986).

In this study, five common algaecides used in greenhouses and nurseries for the control of algae were assessed for their efficacy in treating algal contamination to provide an understanding for horticulturists to target the various forms of growth on gravel, tarp, and ceramic surfaces. It seems that chemicals have different results depending on the surface applied, such as in the case of hydrogen dioxide and sodium hypochlorite, with different results on gravel and tarp. We found sodium carbonate peroxyhydrate to be the most effective formulation on all surfaces examined here, specifically for the removal of *Nostoc* species, while sodium hypochlorite is moderately effective on both tarp and gravel surfaces. Sodium carbonate peroxyhydrate is effective at penetrating the surfaces assessed here and renders the surfaces free of contamination by degrading the genetic material. Our results indicate that a better understanding of algal community structure found on varying surfaces is a prerequisite for efficacious management practices in curbing algae contamination in greenhouses and nurseries.

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## Authors' Contributions

**Conceptualization**: Berthold, D.E.; Marble, C.; Laughinghouse IV, H.D. **Data acquisition**: Berthold, D.E.; Elazar, A.; Lefler, F.W.; Laughinghouse IV, H.D. **Data analysis**: Berthold, D.E.; Elazar, A.; Lefler, F.W.; Marble, C.; Laughinghouse IV, H.D. **Design of methodology**: Berthold, D.E.; Marble, C.; Laughinghouse IV, H.D. **Writing and editing**: Berthold, D.E.; Elazar, A.; Marble, C.; Laughinghouse IV, H.D.

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