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Integrated Disease Management Strategy to Enhance Microgreen Production in Regenerative Organic Systems

INTEGRATED DISEASE MANAGEMENT STRATEGY TO ENHANCE MICROGREEN PRODUCTION IN REGENERATIVE ORGANIC SYSTEMS

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Abstract

A greenhouse study was conducted to evaluate the effect of bio-fungicide, RootShield⁺ and ultraviolet-light-C (UV-C) on yield and physiological growth parameters of broccoli (*Brassica oleracea* var. *italica*) microgreens.

Treatments were arranged using a randomized complete block design with four replications. The major soil-borne pathogen was detected by testing infected plants belonging to the phylum of zygomycete. While the tested antipathogenic fungi had a significant effect on the microgreen yield, UV-C application did not affect the pathogen. *Trichoderma* treated with soil received the greatest yield. UV-C can be integral to pest management in organic microgreen production, requiring detailed physiological research.

Keywords: Broccoli; microgreen; regenerative organic system; soil-borne pathogen; UV-C

Introduction

Microgreens are immature vegetable shoots harvested 7 to 21 days after germination once the cotyledonary leaves have developed with one set of true leaves (Galieni et al., 2020; Zhang et al., 2021). These young greens are known for their texture and concentrated flavor, making them popular

additions to salads, sandwiches, and other dishes (Turner et al., 2020). Microgreens can be considered better substitutes for sprouts due to their rich nutritional content and more intense flavor and taste. In addition, microgreens are rich in vitamins (e.g., vitamin C), minerals (e.g., Cu and Zn), and phytochemicals, including carotenoids and phenolic compounds, which act as antioxidants in the human body (Yadav et al., 2019; Zhang et al., 2021). Butkutė et al. (2018) observed up to 3.2 times higher Zn content in microgreens than in raw and sprouted seeds of small legumes. Microgreens showed a total chlorophyll content range of 12.35 to 112.62 mg 100 g⁻¹, richer than sprouts (de la Fuente et al., 2019). Chlorophyll and carotenoids are major photosynthetic pigments of coloration that affect customers' choice of microgreens and economic value (Žnidarčič et al., 2011).

Many microgreen growers struggle to control and optimize climatic factors associated with temperature and humidity. Humidity levels above 75% are linked to bacterial and fungal proliferation on green tissues that lead to yield loss (Hamilton et al., 2023). Other agronomic challenges faced by growers include poor germination rates associated with poor seed quality, unfavorable quality and quantity of light, and lack of sufficient moisture for plant germination and growth (Hernandez-Adasme et al., 2023; Senevirathne et al., 2019). Microgreen growers face additional challenges related to microgreen food safety, regulation, and certification. Around 13% of foodborne outbreaks in the U.S. were linked to fresh produce (Carstens et al., 2019). Fresh produce derived from microgreens is widely known to be exposed to various sources of contamination from soil and irrigation water (Alegbeleye et al., 2018). Microgreens are exposed to foliar and soilborne pathogens that interfere with their growth and development. Pathogenesis is characterized by an overt host-pathogen interaction that leads to the proliferation of infective microbial units throughout the host root or foliar tissues while interfering with their functions. Disease intensity and

proliferation depend on the plant susceptibility level, the pathogens' infectiousness and virulence, and the environment's conduciveness (Agrios, 2005). Therefore, a combination of low resistance in the plant, a virulent pathogen, and a humid environment could trigger a massive pathogenic infestation that would lead to complete crop loss. Vegetables and microgreens are sensitive to root rot which can lead to damping-off on seedlings caused by *Pythium* spp., *Fusarium* spp., or *Rhizoctonia* spp. (Martins et al., 2022; McGehee et al., 2018; Riaz et al., 2021).

Different strategies in the farmers' toolbox can be used to prevent, mitigate, or control diseases. One of the strategies to control plant diseases is using biological approaches that directly target the pathogens. Biological approaches to disease control include an arsenal of plant-derived products, microbial metabolites, and microbial agents. The genus *Trichoderma*, a cosmopolite soilborne filamentous fungus, is widely known and studied for its inherent antagonistic abilities to control plant pathogens and has been widely used as a biocontrol agent (Contina et al., 2017; Harman et al., 2004; Druzhinina et al., 2011). *Trichoderma* spp. used multipronged mechanisms to control soilborne pathogens and include (Harman et al., 2004): (1) direct parasitism of the pathogens; (2) production of hydrolytic enzymes; (3) competition for space and resources; (4) root colonization; (5) promotion of plant growth; and (6) induction of systemic resistance in the host. Varied species of *Trichoderma* have been shown to significantly control nematodes (Contina et al., 2017; Sahebani and Hadavi, 2008), *Fusarium* spp. (Erazo et al., 2021), *Phytophthora* spp., *Pythium* spp. (McGehee et al., 2018), *Rhizoctonia* spp. and insects (Poveda, 2021).

Ultraviolet light is also employed to disinfect microbial contaminants by inhibiting their growth. Among the several types of UV radiation, UV-C (200-280 nm) demonstrates the most potent germicidal impact. Exposure to UV light also leads to delayed spore germination and enhanced resistance to

plant pathogens by facilitating the accumulation of antimicrobial substances, such as terpenoids (Cornell Chronicle, 2019). A groundbreaking discovery highlighting the efficacy of applying UV light during nighttime for fungal eradication has inspired researchers to utilize this method for managing various fungal diseases in horticultural crops (Suthaparan et al., 2012).

In response to the limited information and techniques available for controlling diseases in regenerative organic microgreen production, a study was conducted to develop an integrated approach combining the application of antipathogenic fungi as a biological control and utilizing ultraviolet light as a physical disease management method. Broccoli (*Brassica oleracea*) was chosen as the case study due to its high susceptibility to soil-borne microbial diseases. The observations from this study will serve as the foundation for designing a comprehensive research project to mitigate the impact of various microbial diseases in soil-based microgreen production systems. In this study, we evaluated the indirect effects of anti-pathogenic fungi and UV-C irradiation on microbial infestation through measuring the yield and other physiological growth parameters (such as stem length and root length) of microgreens. Investigation for the impact of treatments on soil-borne pathogenic agents will be studied in the future.

Materials and Methods

Greenhouse conditions

This research was conducted in a greenhouse in Blakeslee, Pennsylvania (USDA plant hardiness zone 5b (-26.1°C to -23.3°C)). The temperature inside the greenhouse was maintained at 24.4°C and the humidity level at 45% throughout the study period. Plants were maintained under 12-hr:12-hr light:dark photoperiod and watered using the bottom tray filling method up to

three times a day based on the requirement. A factorial arrangement (2×2) based on a randomized complete block design was used to assess the influence of Ultraviolet (UV) light and a combination of two isolates of antipathogenic fungi on the yield of microgreens produced from broccoli seeds. The trial was replicated thrice from December 2022 to March 2023. Samples were also collected and dispatched to a laboratory at Plant Disease Clinic, Penn State University, University Park, PA, to identify the source of the plant infestation. The results of pathology tests on damaged plants collected from control units illustrated the presence of a pathogen belonging to the zygomycete (*Rhizopus* or *Mucor*) phylum.

Plant material

Broccoli seeds were acquired from Johnny's Seeds, Fairfield, ME (82% germination capacity). Since broccoli seeds and microgreens are particularly vulnerable to microbial infestations compared to many other crops, this research focuses on using them as a case study. To cultivate broccoli microgreens, seedling trays were filled with 3.4 kg of organic potting mix soil and 18 grams of broccoli seeds were evenly distributed on the soil's surface. After planting, the seeds were immediately irrigated, and the trays were then moved to a dark chamber to facilitate germination. Following a 72-hr germination period, the germinated plants were transferred from the dark chamber to greenhouse benches for a growth duration of 7 to 10 days. Yield assessment was conducted using destructive sampling methods.

Inoculation

RootShield Plus (RS+), an OMRI-listed fungicide approved for organic production, was incorporated into the soil to provide a source of two isolates of *Trichoderma* (*Trichoderma harzianum* strain T-22 and *Trichoderma virens* strain G-41). 5.8 grams of RS+ was mixed with 3.4 kg of potting soil before seeding for each tray treated with *Trichoderma*.

Ultraviolet light exposure

In each replication, three trays of planted broccoli were subjected to UV-C (200-280 nm) exposure at 150 J M^{-2} (at 3 inches from the source of light) for a duration of five seconds using ECO Series Germicidal HVAC Kit (American Ultraviolet, Lebanon, IN). The UV-C exposure duration was selected based on the source of light and exposure distance to achieve microbial reduction without compromising food composition and quality.

Plant growth evaluation

After a period of 10 to 12 days following seeding, the microgreens were manually harvested, and the yield of fresh produce was carefully measured and compared across the various treatments. A day before harvesting, fractional green canopy cover (%FGCC) was measured as an index of green leaf growth. To evaluate %FGCC, photos of the foliage were taken using a smartphone camera and analyzed by the Canopeo application. Green pixels were counted with the program to assess the ratio of green leaves to bare soil, converted to a percentage of canopy cover. The direct effect of treatments was evaluated by comparing the yield and %FGCC in treated trays with non-treated control units.

Statistical analyses

Data analysis was performed with R statistical software. A linear transformed model was used to assess the effect of RootShield and UV light exposure on the growth and development of broccoli. A two-way analysis of variance (ANOVA) was also conducted to investigate the interaction between different groups of treatments at $\alpha < 0.05$. Furthermore, a correlation model was developed to investigate the relationship between canopy cover (%FGCC) and the harvested yield. This correlation model facilitated an understanding of how variations in canopy cover influenced the final yield of the broccoli

microgreens, providing valuable insights for future agricultural practices. One-way ANOVA followed by Tukey's test was also performed to compare the means of measured parameters among the treatments.

Results and Discussion

While the tested antipathogenic fungi significantly affected microgreen yield, UV-C light exposure did not cause a significant change in this measured parameter (Table 1). Experimental units treated solely with *Trichoderma* showed a higher yield, with an average of 25.8 grams of fresh microgreen production (Fig. 1). This amount was followed by 21.5 grams of microgreen production in trays treated with a combination of *Trichoderma* and UV-C light. The observed difference in yield may be attributed to the antagonistic relationship between UV-C light application and antipathogenic fungi. Smaetz-Baron et al. (1997) reported a significant restriction in the germination rate of *Trichoderma* when exposed to UV-C light. However, the effect of UV-C application was found to be non-significant; trays treated with UV-C only produced a slightly higher yield than the non-treated control units.

Source of variation	Yield		%FGCC		Stem length		Root length	
	df	MS ^a	df	MS ^a	df	MS ^a	df	MS ^a
Replications	3	452.4**	3	1052.9*	3	292.6*	3	22.6 ^{NS}
Antipathogenic fungi (<i>Trichoderma</i>)	1	1183.5*	1	2831.8*	1	796.2**	1	3.0 ^{NS}
Ultraviolet light (UV-C)	1	71.0 ^{NS}	1	47.0 ^{NS}	1	81.4 ^{NS}	1	51.0 ^{NS}
<i>Trichoderma</i> *UV-C	1	102.7 ^{NS}	1	280.1 ^{NS}	1	0.32 ^{NS}	1	0.26 ^{NS}

Table 1. Analysis of variance (ANOVA) for the measured parameters among treatments.

Note: ^a NS p > 0.05 *0.01 < p < 0.05 **p < 0.01

The analysis of variance revealed a non-significant interaction between the tested treatments for all the measured parameters (Table 1). A notable impact of the anti-pathogenic fungi on stem length and %FGCC was also observed.

The trays treated with UV-C and *Trichoderma* exhibited the longest stems, with an average length of 59.6 mm. In contrast, the control units had the shortest broccoli microgreens, with an average stem length of 50.86 mm (Table 2).

On the other hand, root length showed no significant differences among the treatments, ranging between 22.41 and 24.96 mm. Despite the non-significant



Fig. 2. Image showing root length of microgreen receiving a) *Trichoderma* and b) no *Trichoderma* treatment.

effect of *Trichoderma* on the length of the microgreens, we observed a well-developed and robust auxiliary root system in trays treated by anti-pathogenic fungi (Fig. 2). At the end of experiment, total root length of microgreen was measured, where root length was increased by four-times in microgreen seeds treated with *Trichoderma* (average = 4 cm) compared to no *Trichoderma* (average = 1cm). Applying anti-pathogenic fungi also led to an average of 26% higher %FGCC than the non-treated units (Table 2). The increased %FGCC indicated a greater number of developed chlorophyll cells, which led to a faster growth rate, expanded canopy coverage, and a higher yield. This relationship was supported by a significant positive correlation between the recorded %FGCC and the harvested yield (Fig. 3).

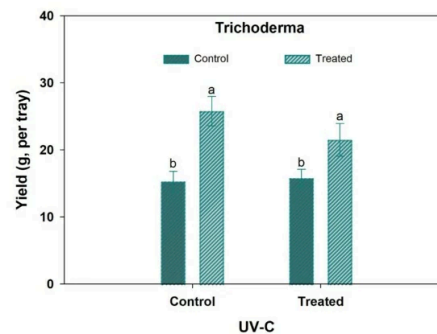


Fig. 1. Mean harvested yield of microgreen among treatments. Mean followed by different lowercase letters are significantly different at $P < 0.05$.

Treatments	Mean Microgreen Stem Length (mm)		Mean Microgreen Root Length (mm)		Mean %FGCC		
	Trichoderma		Trichoderma		Trichoderma		
	Control*	Treated	Control*	Treated	Control*	Treated	
UV-C	Control*	50.8 ^b	57.4 ^{ab}	24.5	25.0	45.7 ^c	61.3 ^a
	Treated	52.8 ^b	59.6 ^a	23.6	22.4	47.8 ^{bc}	55.6 ^{ab}
		F(3, 17) = 3.00, P=0.036		F(3, 17) = 0.43, P= 0.729		F(3, 17) = 6.11, P=0.001	

Table 2. One-way ANOVA results with means for different dependent variables as affected by UV-C and *Trichoderma* treatment.

Note: Mean followed by different lowercase letters are significantly different at $P < 0.05$.

Broccoli inoculated with *Trichoderma* achieved higher growth and development than that with non-amended soil. Our experiment showed that *Trichoderma* inoculants acted as a bio-stimulant to improve plant growth and a biocontrol agent to protect the plant against pathogens. Similar studies have demonstrated that *Trichoderma* spp. can colonize the root epidermis and increase the plant's ability to increase nutrient absorption and significantly increase its growth (Harman, 2006). Plant growth promotion has been related to the ability of *Trichoderma* spp. to secrete and release siderophores that are used to chelate other metals such as Manganese, Nickel, and Zinc and make them available for plant absorption (Saha et al., 2013). Other studies have found that some *Trichoderma* species have the phosphate-solubilizing ability, hormone-like compounds, which may enhance plant nutrient uptake (Lopez-Bucio et al., 2015; Rudresh et al., 2005).

Although this study did not directly evaluate the biocontrol process, the lack of disease development in soil amended with *Trichoderma* indicates that the biocontrol fungi provided a protective layer to the infection court. Previous

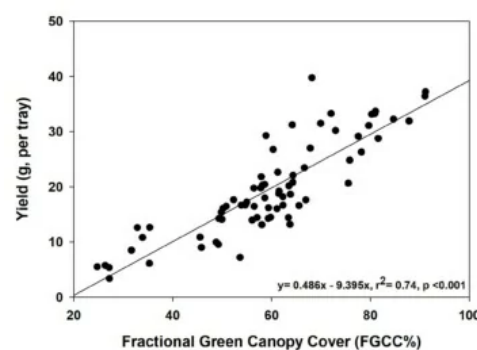


Fig. 3. Microgreen yield following the fractional green canopy cover (%FGCC) from the study.

studies have elucidated the disease control process of *Trichoderma* and involved the production of bioactive compounds, cell wall degrading enzymes, and secondary metabolites (Contreras-Cornejo et al., 2016; Harman, 2006). Other studies have found that *Trichoderma* can directly parasitize the pathogens through hyphae colonization, competition for resources, and induction of systemic resistance in the host plant (Contina et al., 2017; Harman et al., 2004; Van Loon, 2007).

The agroecological services provided by *Trichoderma* spp. are often limited by the soil environmental factors – abiotic elements – and the influence of the soil trophic web – biotic elements. Studies have found that *Trichoderma* proliferation in the medium depended primarily on soil temperature and moisture conditions (Eastburn and Butler, 1987; Widden and Abitbol, 1980). Several biotic factors were found to be closely related to *T. harzianum* distribution in soil, including a positive association with bacterial populations (Hadar et al., 1984). In a recent study, *T. virens* and *T. asperellum* were exposed to ultraviolet light and underwent modifications in their genetic structure, and the obtained mutants could successfully control plant pathogens to a greater extent than the wild types (Alfiky, 2019). However, in our study, the exposure of *T. virens* and *T. harzianum* did not enhance their biocontrol activities or bio-stimulant capacities compared to non-exposed treatment. These ambiguities could be explained by the fact that only specific *Trichoderma* strains, when exposed to UV light, can increase their bio-stimulant and biocontrol activities.

Future research should focus on elucidating mutagenesis events that might occur when microgreens, *T. virens*, and *T. harzianum* are exposed to UV light and to determine the impact on plant growth and development as well as the ability of *Trichoderma* spp. to proliferate in the soil and perform as bio-stimulants and biocontrol agents. Mapping the genetic structure where mutations might occur would help identify specific genes associated with

enhancing the beneficial properties of *Trichoderma* spp. and would potentially add mutagenesis to the toolbox for controlling plant diseases and increasing plant growth. We intend to continue with our line of study and expand it to explore specific interactions between the host plant, the pathogens, the biocontrol agents, and the environment.

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