MYCORRHIZAL AND TRICHODERMA FUNGAL SEED INOCULATION IMPACTING WILDFLOWER GERMINATION AND SEEDLING ESTABLISHMENT

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There have been an enormous number of studies related to the positive impacts of using fungi in organic gardening and turf management. These studies have shown that some fungal strains provide protection against pathogens and stimulates productivity of crops. However, there has been few studies and literature on the adoption of using important fungal strains in the establishment of wildflowers.

This eight week study used an initial *Arbuscular mycorrhiza* and *Trichoderma harzianum* priming seed soak before seed dispersal. The study hypothesized that the inoculation of the seeds would improve germination/emergence, decrease damping off and improve biomass of seedling stand compared to control. Measurements conducted were plant emergence, root to shoot length, and biomass. Uniform rates of seed dispersal and irrigation were maintained for all three tested groups.

Under field conditions the first weeks saw comparatively significant gains in seedling emergence and biomass associated with the Mycorrhizae treated seeds. In the last three weeks under drought conditions the Trichoderma treated group saw comparatively robust growth and finally outperformed both the Mycorrhizal and Control groups.

Introduction

The benefits of wildflower plantings are many. Depending on the wildflower site, economical, educational, recreational, ecological, and aesthetic advantages may exist. There are several ecological benefits of a wildflower meadow. Wildflowers can improve water quality by decreasing amounts of runoff of pesticides, petroleum products, lead, and sediments . Increased water quality and reduction in air pollution can be realized compared to turf areas because of the wildflower plant’s ability to trap and hold pollutants related to its increased vegetative biomass. Once trapped, pollutants are not able to runoff as pollution to the groundwater, thereby improving water quality. Wildflowers anchor soil with deep dense rooting to stabilize eroding slopes, river or stream banks, and beach dunes. Wildflower plantings use little, if any, pesticides thereby the potential for offsite movement of pesticides is decreased. Wildflower meadows can act as greenways, with wildlife using them for food, shelter, and transportation corridors. Wildflower plantings aid in habitat restoration and protection by attracting birds and insects, especially butterflies. From an educational and recreational standpoint, wildflower plantings afford people the opportunity to observe, interact, appreciate, and understand more about natural settings. Wildflower native plantings can be used to educate and offer an opportunity for activities such as walking, running, bird watching, and photography. Native wildflowers store significant amounts more carbon than turf grass which has much shorter roots and thus stores much less carbon.

In urban areas there is an increased interest in using native wildflowers alongside roadways or residences replacing what is most often planted in turf grass. These wildflower plantings or pocket meadows are small, native plantings inspired by the natural landscape. They offer the same ecological benefits as wild meadows, but on a smaller, more manageable scale. One advantage of established pocket meadows is the decrease in maintenance time compared to other flowering gardens or turfgrass. However, many of these small meadow projects are frustrated from the beginning as poor germination of seed or subsequent rooting is not successful. Is it possible that the reduced germination and root development of wildflower seed is linked with ineffective fungal communities at restored sites? Many of these former turfgrass sites are plowed, sprayed with herbicides, or some form of solarization is used to kill grass and weeds. Fungal communities are beneficial to host plants because they collect and deliver soil nutrients, provide drought resistance, and induce resistance to herbivores and pathogens. Therefore, disturbed planting sites may limit the re-establishment of wildflower species that strongly benefit from these fungal communities.

Besides being able to cause burns, headache, nausea, and even lung disease, using pesticides or herbicides are damaging to the environment. The widespread use of chemical fertilizers has led to contamination of the air and soil. The natural microorganisms are harmed and the soil fertility can be destroyed by the use of chemicals in a garden or field. Trichoderma fungi can be a solution to this problem. Trichoderma is a genus, or type, of fungi that acts as an eco-friendly biological control with many benefits. This fungus is found in root and soil ecosystems. Using Trichoderma is a way to repair agrochemical pollution. The benefits of using Trichoderma include enhanced plant growth, accelerating the flowering of plants, and treating seeds with Trichoderma can be used to control disease and promote seedling establishment.

Trichoderma fungus grows on the surface of roots, and its spores can survive in soil. Trichoderma will multiply and continually spread to cover the roots, where chemical fertilizer will only cover where it is applied. Since Trichoderma is able to grow, it is able to protect and enhance the root growth for the entire growing season, while again chemical fungicides are only able to protect the plant for weeks at most.

> *Trichoderma* kills several major root rot fungi: *Pythium*, *Rhizoctonia*, and *Fusarium*. The process is called mycoparasitism. *Trichoderma* secretes an enzyme that dissolves the cell wall of the other fungi. It can then get inside the bad fungi and consume them. The strain used in T-22 secretes much more of the key enzyme, endochitinase, than wild strains, thus T-22 grows better and secretes more enzyme than wild strains. This combination allows it to protect crop roots against root rot fungi in the field. (Concklin, 2024)

Trichoderma accelerates the flowering of the plant and development of flowers. It is considered to be a biostimulant. This fungus allows the plant to absorb and utilize more nutrients as well as support growth regulators which in turn, allows for more rapid growth. Trichoderma is shown to stimulate the uptake of macro- and micronutrients and elements. Aside from the ability to aid in the prolongation of the leaves and shoots, "Trichoderma also stimulates the flowering of plants, formation of leaves, and helps elongate the inflorescence shoots and inflorescences. The number and weight of flowers can increase as well, depending on the concentration and type of Trichoderma the seeds have been treated with. " (Trichoderma spp. 2022) Unlike man-made fungicides, *Trichoderma* does not harm the other useful organisms in the soil.

Stresses such as drought, salinity, flooding, plant pathogens, nutrient deficiency and toxicity limit crop productivity, and are also reasons for food shortages. Fertilizer is one method used to obtain optimum yields, although this often causes environmental issues. Chemical fertilizers, when given for long periods of time, may lead to the deterioration of soil characteristics and fertility, and heavy metals can accumulate in plant tissues. This leads to the nutritional value and edibility of fruits being affected. Researchers have found that instead of using chemical fertilizers, biological fertilizers, such as animal manure, the decaying remains of organic matter, domestic sewage, excess crops and microorganisms (like bacteria and fungi), can be used as a more stable solution. Microorganisms play giant roles in the promotion of plant growth through the regulation of nutrition and hormonal balances, production of plant growth regulators, solubilization of nutrients and induction of resistance against plant pathogens.

Studies have shown that the fungus Mycorrhizae has benefits for plants and improve the plants environmental services as a whole. Mycorrhizae is a complex system of fungi that creates relationships with plant roots. They form filaments (hyphae) that attach to plant roots and draw out nutrients and water from the soil. This in return helps the root system gain nutrients that they would otherwise not access. It has also been researched that these fungi can help increase a plants ability to tolerate different types of environmental stresses.

One specific trait of Mycorrhizae, which is its ability to find and modify nutrients in soil and transform it into a usable nutrient for the species of plant it's attached to, creating resources for that plant to use. There are many other benefits of this Fungi besides this, including: quicker plant establishment, increased access to water and nutrients beyond the root zone, improvement in drought tolerance through increased access to water, improving nutrients availability by breaking down rock, assistance in building and maintaining soil quality and structure, and growing plants in soils with high concentrations of salt and toxic metals.

> A variation of mycorrhizal fungi, Glomeromycota, also known as *Arbuscular mycorrhizal* fungi, are one of the most impactful fungi because they form mutual bonds with the roots of almost 90% of plant species INCLUDING wildflowers. They are also vital to plant health and may determine the compositions of plant communities. These mycorrhizae produce highly branched haustoria, which promotes nutrient exchange with their host root cells. (Huey, 2020)

<u>Literature Review</u>

Biological seed treatment studies has shown effectiveness in protecting seeds from soil-borne pathogens at early stage of plant development and also effectively reducing the dependence on chemical fungicides for diseases management. Seed bio-priming has also shown to promote plant health and stress tolerance. Seed biopriming involves seed coating with beneficial microbes often leading to raid and uniform seed colonization.

A seed biopriming study related significant gains in pea production using Trichoderma fungi.

> The results showed enhanced plant growth in the treatment as compared to the control plants (Figure 1). There was an increase in shoot length, root length, number of leaves, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight by 35.29, 96.49,

> 28.13, 36.10, 146.26, 30.17 and 77.20 %, respectively, as compared to control. (Moussa, et.al. 2023)

A seed biopriming study using Trichoderma fungi in the establishment of tomatoes reported significant gains in disease resistance.

> Microbial biopriming with *T. harzianum* resulted in the enhanced expression of tomato defense-related genes and was accompanied by increased antioxidative enzymic activities. *T. harzianum* primed plants showed 2.71-fold higher systematic acquired resistance (SOD) than control and 1.34-fold (*Fol* + *Th*) higher SOD activity compared to *Fusarium wilt* challenged plants (Andleeb, 2022)

The below study reported significant gains in germination and seedling growth parameters of the perennial *Echinacea purpurea* using mycorrhizal bio primed seed.

> The main cause of raising seed vigor in bio-priming treatments is the boosting germination percentage and seed length. Bio-priming improved germination percentage according to the boosting hormones and organic substances, which improved the total number of germinated seeds and finally promoted the seed vigor. Mycorrhizal fungi, because of having the various hormones and nutrition increased amount of nutrients available for seeds, thus improving seedling growth and germination percentage. The combined use of mycorrhizal fungi and growth-promoting bacteria increases antioxidant production, which increases antioxidant activity, reducing reactive oxygen species against stress and protecting cells against stress. (Monfared, 2023)

The below study reported significant reduction in drought stress in bioprimed seeds of rice with applications of *Trichoderma* fungi.

> The soil application of Trichoderma spp., especially T. harzianum subsided the negative effect of water stress on rice plants and promoted the shoot and root growth and biomass production of rice. The present study has demonstrated that soil application of T. harzianum may efficiently reduce the impact of water stress at least up to 50% water availability levels and may enhance the plant growth and biomass production by 17–21%. (Shah, 2024)

Materials and Methods

The site for this study was selected in May 2023. This site was a former soccer field that had not been used for 4 years. No herbicide or fertilization had been applied doing that period. A soil test of the site revealed no needed amendments. Design Site was covered with two layers of weed barrier for the period of May 2023 to August 2024. This weed barrier was effective in killing all grass and weeds from site. The site was raked gently of dead debris before seed was applied. The site measured 45 feet by 15 feet for a total of 675 sq. ft. The site was equally divided into three equal measurements of 225 sq.ft for each study groups. Site #1 was designated for seed applied that had been soaked in mycorrhizae fungi for 14 hours. Site #2 was a control area where no application of fungi took place before seed application. Site #3 was designated for the seed soaked in trichoderma fungi for 14 hours.

The seed used for this study was a Fall Maximum Seed Mix purchased from American Meadows Seed Co. The mix is a combination of Fall and Summer blooming Perennials and Annuals. Perennials included in seed: Hyssop, Butterfly Weed, Aster Coreopsis, Blanket Flower, Ox Eye Sunflower, Echinacea, Blazing Star, Blue Flax, Lupine, Penstemon, and Bee Balm. Annuals included in seed: Cornflower, Cosmos, Plains Coreopsis, Chinese Forget-Me-Nots, California Poppy, Baby's Breath, Candytuft, Scarlet Flax, Sweet Clover, Red Poppy.

The seed amount applied to each of the 3 study plots was ¼ lb. of seed.

Measurements

Procedures

Two weeks after initial seed dispersal measurements of emerged wildflowers were taken. 16 sq. centimeter samples representing the largest populated plant density from each study plot were extracted. The total dry biomass of each sample was weighed. Fifteen plants with largest root to shoot length from each study group were bundled to measure average length. Eight weeks after initial seed dispersal the same biomass and root to shoot bundle measurements were repeated.

Results

Table One: Seed Plot Measurements

	Plot 1. Mycorrhizae Seed Soak	Plot 2. Control Seed Soak		Plot 3. Trichoderma Seed Soak
Week 2 Plant Coverage %	90%	70%		60%
Week 8 Plant Coverage %	100%	90%		90%
Week 2 Sample Biomass Dry grams	5.7	5.0		2.4
Week 8 Sample Biomass Dry gr.	48.0	20.0		73.0
Week 2 Root to Shoot Length Avg.	8.25	7.62		3.81
Week 4 Root to Shoot Length Avg. cm.	12.0	10.4		11.0

Figure One. Week 2 Root to Shoot and Biomass

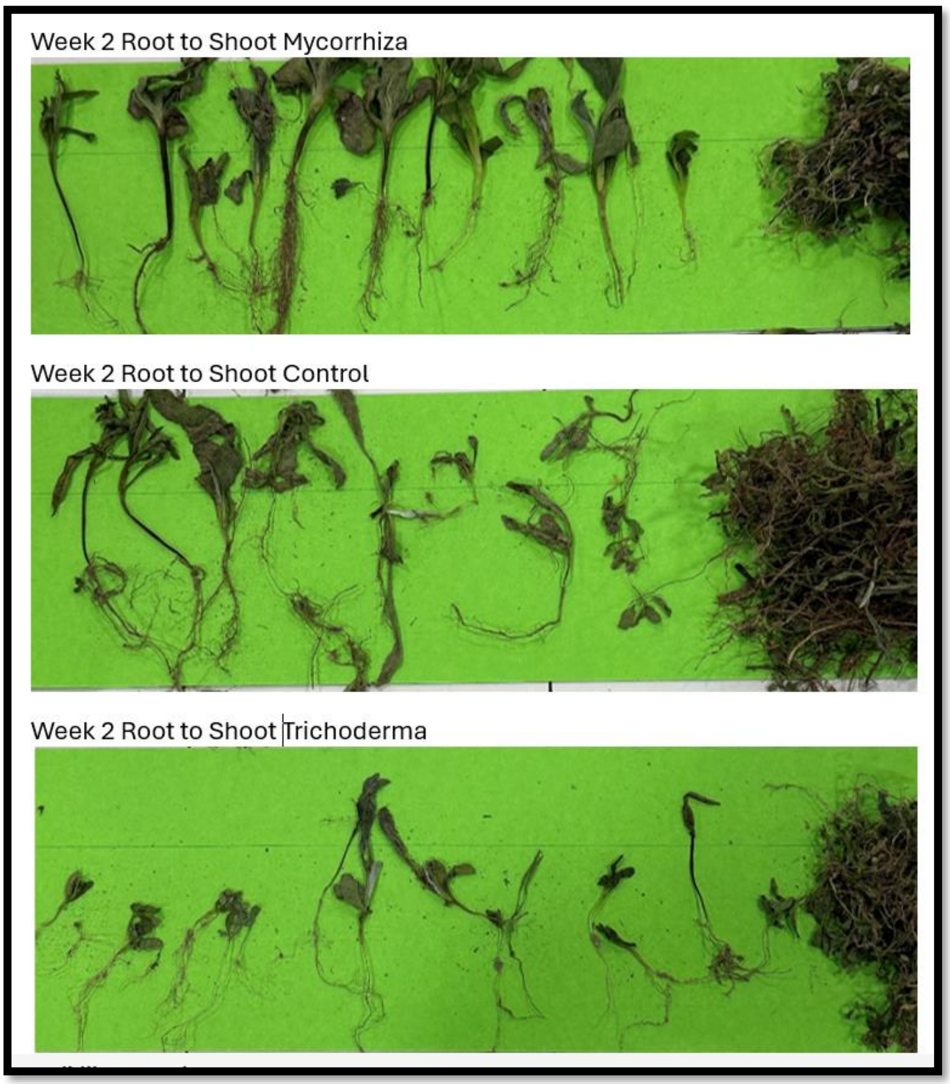

Figure 2 Week 8 Root to Shoot and Biomass

Discussion.

As hypothesized, the seed priming of wildflowers improved seedling emergence and improved biomass of seedling stand. In the first weeks with significant rainfall the Mycorrhiza seed treated site outperformed both the control site and significantly outperformed the Trichoderma site. (See Table One.) Under field conditions of minimal rainfall, the subsequent two weeks and no rainfall the last four weeks the Trichoderma site advanced both in biomass and root to shoot length significantly compared to both the control and Mycorrhiza sites. In eight weeks, the sample biomass of the Mycorrhiza site outperformed the control by 240%, and the sample biomass of the Trichoderma site outperformed the control by 365%.

Conclusion.

The establishment of wildflower/meadow sites can be promoted with the use of the fungus *Arbuscular mycorrhiza* and *Trichoderma harzianum* as a seed priming agent. These beneficial fungi greatly increase the effective rooting area of plants, thereby enhancing plant growth, vigor, and tolerance of environmental extremes. The fungi colonize roots and extend into the surrounding soil forming an essential link between plant and soil resources. This fungal support can be important as many wildflower sites have been plowed or the previous grass/weeds treated with herbicides or solarized. A strong stand of rooted and established wildflowers in the near-term weeks shortly following germination can suppress the encroachment of weeds. Additionally, this study has shown that the fungal support can help plants withstand drought stress. With a fall sowing of wildflower seeds the strong vigor of rooting provided by the fungus would ready plants for spring takeoff. This study used a seed mix of perennials and annuals. Subsequent studies should be undertaken on specific wildflowers.

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