# Plant Growth Enhancement & Fungal Disease Suppression via Copper, Zinc, and Manganese Nanoparticle Foliar Sprays

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## Abstract:

Sensitive crop regions are constantly under environmental stresses that foster plentiful plant disease. Basil plants, for instance, have been victims of *Fusarium oxysporum (F.o.)* wilt for decades, where growth conditions have stimulated progression of this disease, and subsequent crop destruction. A simple and effective treatment that would eradicate *F.o* wilt, while promoting overall plant growth, is needed. Metallic nanoparticles (NPs) have shown to improve plant health and overall crop yield, due to systemic movement through the plant's root system, where the nutritional value of metallic nanoparticles is fully realized. This research investigates whether the "foliar-spray" application of NPs of copper, manganese, and zinc (as oxides) increases the growth rate and crop efficiency of healthy *O. basilicum* plants, and inhibits the adverse effects of *F.o.*, to ultimately devise an easily-applied, simple, and effective treatment to promote increased crop growth. Pre-grown (3") basil plants were first transferred to ~0.8L pots using ProMix-BX soil, which was pre-inoculated with 1-2ml of 1g/L-*F.o* in water. Each plant was then treated with ~2ml foliar spray of the respective nanoparticles. After 6 weeks growth, all three MO-NP treatments produced significant increases (>120%) in biomass, relative to diseased plants; ZnNPs were the most favorable, at 180% increase in biomass relative to untreated, diseased plants. Combined Cu-Zn NP treatment enhanced diseased plants' biomass by 29% and provided a 40% increase in height. Most importantly, diseased-plants outgrew healthy controls by 21%, highlighting the treatment's ability to fully suppress *F.o.*, so that infected plants grow beyond normal, healthy conditions.

## Introduction:

The agricultural industry always welcomes innovation in crop production, efficiency, and resistance to disease. With an ever-evolving earth as well as an increasing global population, sensitive crop regions are constantly under environmental stresses that foster plentiful plant disease. I have always been intrigued by agricultural sustainability. I have a desire to help farmers increase overall crop yield in a business stricken by slim profit margins. Basil plants, for instance, have been victims of *Fusarium oxysporum* wilt for decades, where hot weather, dry soil, and rising soil temperatures all contribute to the growth of this disease, and subsequent crop destruction. In recent years, *Fusarium oxysporum* wilt has become a major problem for many crops, farmers, and gardens. A simple and effective treatment that would eradicate *F. oxysporum (F.o)* wilt, while promoting overall plant growth, is needed. Recent research has indicated that treatment of crop-soil with metallic nanoparticles (NPs) can improve plant health and overall crop yield, by moving through the plant's root system, where the nutritional value of metallic nanoparticles is fully realized. Upon reading these studies, I became fascinated that treatment of crop-soil with metallic nanoparticles can improve plant health and overall crop yield because their small size (10-15 nm) allows for movement through the plant's root system. I later discovered that this works by supplying nutritional value to the xylem and phloem structures of the plant, thereby improving the plant's immune system. For tomato and eggplant, for example, crop yields increased by 60% and 35%, respectively, through the addition of copper (II) oxide nanoparticles (CuNPs). While the addition of metal-NPs to plant soil can increase crop output via systemic entry of the nutrient metal, it is unknown whether the same nanoparticle-metal ions can penetrate the epidermis (due to their small size of 10-15 nm) while initiating a "host defense mechanism" and maintenance of a healthy metabolism. Working against the defenses of those organisms that are invasive to crops, such as *F.o,* is also ideal. Three important proteins involved in nonspecific host defense, plastocyanin, peroxidases, and multi-Cu oxidases, can be fulfilled by Cu NP's. Further, Zn NP's have been attributed to the normal metabolism of two of the most important plant growth hormones, auxin and gibberellin. antimicrobial properties in literature. Manganese oxide "nano-metals" have also shown to be activators of several enzymes in the synthesis of lignin defense compounds. In this research, metal-oxide nanoparticles of zinc, copper, and manganese will each be introduced in a foliar spray, to healthy basil plants *(Ocimum basilicum)*, as well as basil plants that are infected with *F.o* (as a model, common crop disease) to investigate whether each of the metal-nanoparticle treatments can (*i*) increase crop yield for healthy plants, and (*ii*) mitigate, or inhibit the adverse effects of crop disease, to ultimately increase crop production.

## Purpose:

This research will investigate whether the "foliar-spray" application of metal-nanoparticles of copper and zinc (in the form of their oxides) will (i) increase the growth rate and crop efficiency of healthy O. basilicum plants (chosen as a model crop, as they have a relatively short maturity span of 6 weeks), and (ii) inhibit the adverse effects of F.o (a model crop disease), to ultimately devise an easily-applied, simple, and effective treatment to promote increased crop growth.

## Hypothesis:

Foliar sprays of Cu and Zn NPs will promote increased plant growth (i.e. plant height and biomass), while suppressing F.o wilt disease in sweet basil plants.

## Metal-Oxide NP Solution Fabrication

Copper, Zinc, and Manganese nanoparticles (10-15nm) were purchased from Sigma Aldrich as their respective oxides, and used as is to create separate 500ppm (50mg/100ml) solutions in 1% Regulaid surfactant (used to enhance suspension of the nanoparticles for spraying). Also, a third solution of 250ppm CuO/250ppm ZnO was prepared, via equal-volume mixing of the prior solutions. Prior to use as a foliar spray, each NP solution was ultrasonicated for 2 minutes, at an amplitude of 50, using a QSonica ultrasonicator.

## SEM & EDS Analysis of Metal-Oxide NPs:

Each 500ppm metal-oxide nanoparticle solution was analyzed via Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS), to characterize the nanoparticles, for later recognition on basil plants, and to validate purity.

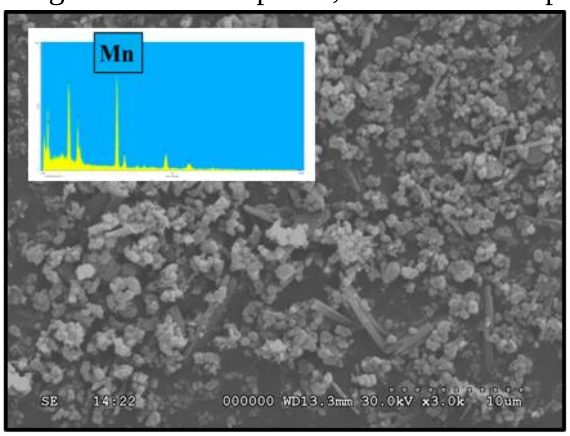



**Fig. 1. 3000x/30kV illumination SEM image of the MnNPs**

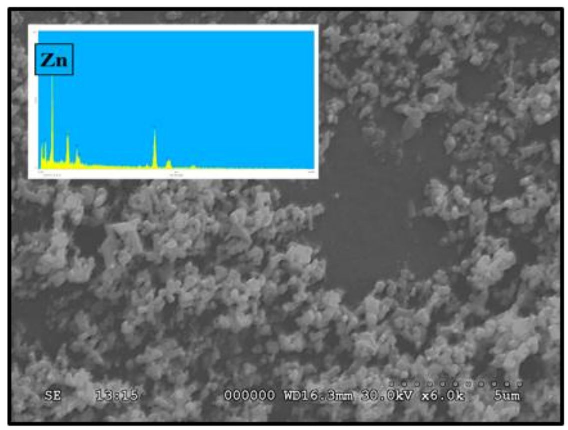
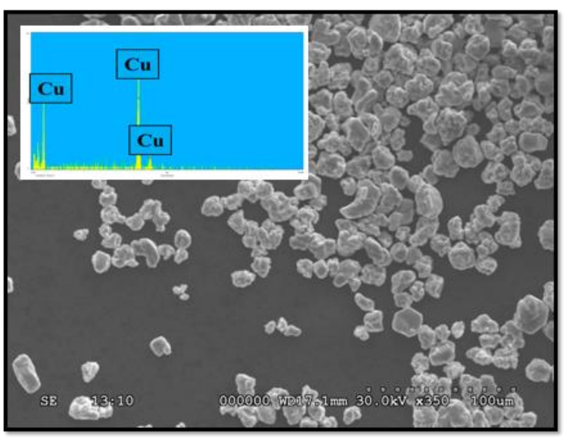



**Fig. 2. 6000x/30kV illumination SEM image of the ZnNPs**




SEM images in Figs. 1-2 highlight the small, separate, nanometer-size particles for MnNPs and ZnNPs, respectively. For the CuNPs (Fig. 3), however, the nanoparticles are aggregated into 10-20 μm bundles, which may affect their absorption by the basil plants, and therefore their function at inhibiting the adverse effects of *F.o.*

## Selection of Sweet Basil as Host Crop:

To evaluate the metal-oxide NP's potential for improved disease management (with Fusarium wilt as the model fungal disease) for common crops, a plant with a relatively short maturity span was needed, so that the influence of the disease, and/or the nanoparticle's inhibition of the same, could be easily measured. Basil was selected, as it has a 6 week maturity span, and also possesses anti-carcinogenic substances.

*Fig 4. Ocimum basilicum are grown to a height of 3 inches, prior to treatment*

*Fig. 5. Basil plant maturity after 6 weeks*

*Fig. 6. An F.o-infected (Control) Basil Plant*

## Phase 1: Design of Basil Plant-NP-Fusarium Experiments:

To create the basil plant configurations for measure of metal-oxide inhibition of *F. o.* on basil plants, each pre-grown (3" tall) plant was transferred to ~0.8L pot using ProMix BX potting soil, which was pre-inoculated with 1-2 ml of 1g/L *F.o* in water. Following, each plant was treated with liberal (~2ml) foliar spray of the respective nanoparticles, while the base of the plant is covered with cardboard, to keep the nanoparticles from entering the underlying soil (Fig. 7).

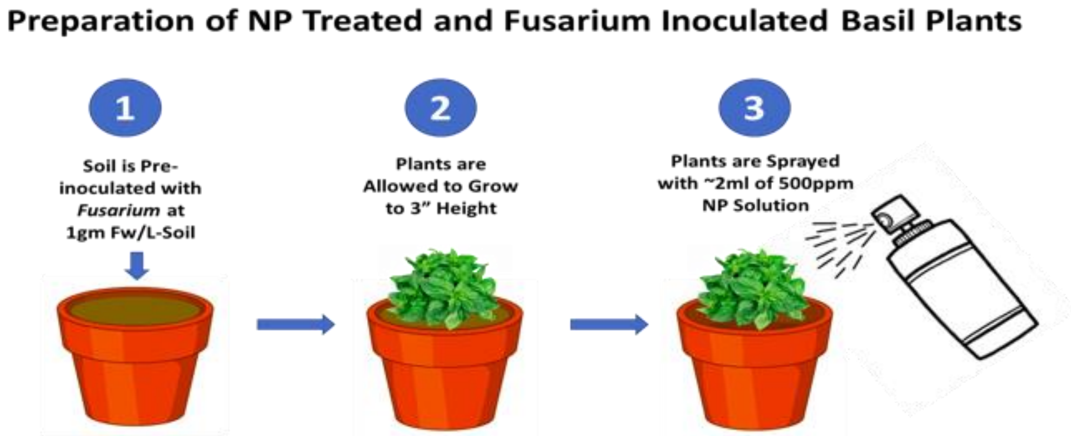



*Fig. 7.*

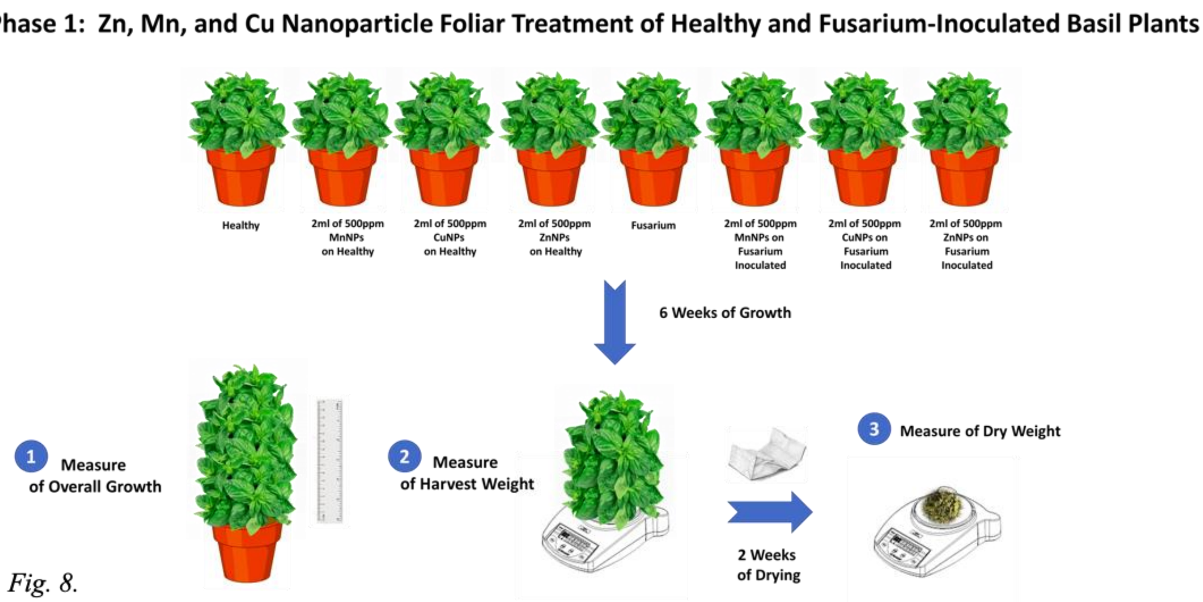



*Fig. 8.*

Basil plant-NP-Fusarium configurations included ZnNP-only, MnNP-only, and CuNP-only, whose weekly growth was measured against a healthy control (Fig. 8), to measure the growth enhancements brought about by the singular NPs for healthy basil plants. Further, weekly growth of ZnNP-*Fusarium*, MnNP-*Fusarium*, and CuNP-*Fusarium* were compared to both healthy controls, as well as plants that were simply inoculated with *Fusarium*. For each plant configuration, 6 plants were included, to increase experimental validity.

## Analysis of Metal-Oxide NP Growth Enhancement & Disease Suppression:

**MO-NP Enhancement of Healthy Basil Growth:** After 6 weeks of growth, healthy plants treated with both CuNPs and MnNPs were 80-92% the height of their non-treated counterparts (Fig. 10), however plant vitality was equal. Those healthy plants treated with ZnNPs, however, were ~5% taller, highlighting ZnNP's tendency to promote basil plant growth for healthy specimens. This is supported by the data for weekly growth measurements (Figs. 11-12), where ZnNP-treated healthy plants consistently outgrow healthy, and other MO-NP-treated, healthy plants.

**MO-NP Enhancement of *F.o.*-Inoculated Basil Growth:** Similar analysis of 6-week growth data for MO-NP treated, *F.o.*-inoculated plants reveals the nanoparticle's ability to promote growth for diseased plants. While not reaching the growth rate of healthy controls, the CuNPs, MnNPs, and ZnNPs promoted 39%, 44%, and 72% growth increases (respectively), versus *F.o.* inoculated (only) controls (Figs. 10 & 13). Once again, ZnNPs appear to be the most favorable to promote growth, regardless of plant health.

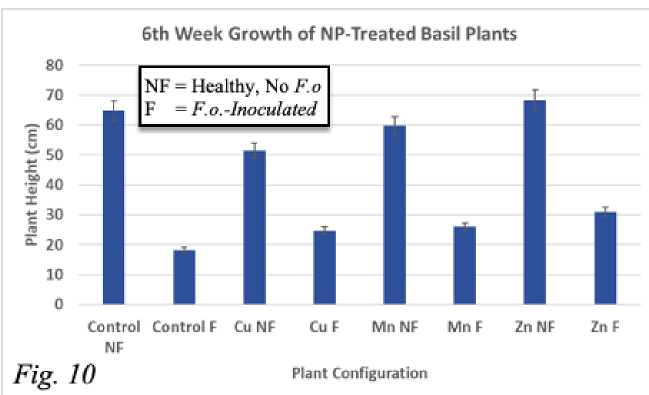


*Fig. 10*

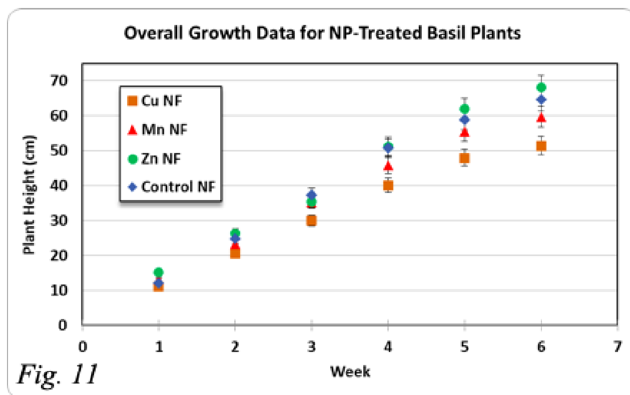


*Fig. 11*

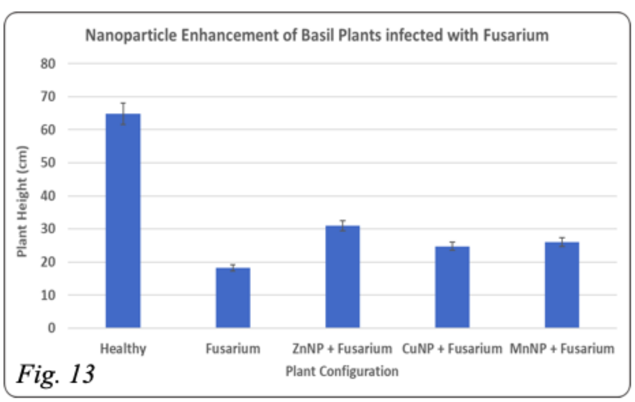


*Fig. 13*

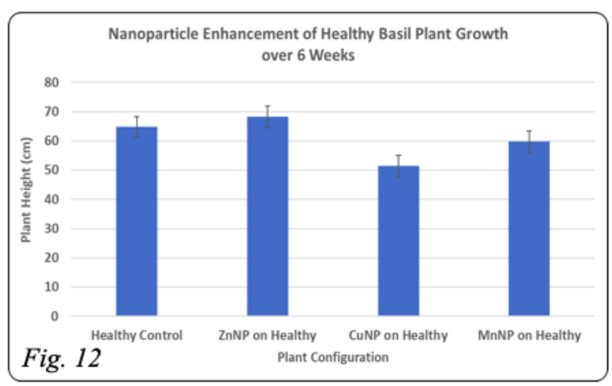


*Fig. 12*

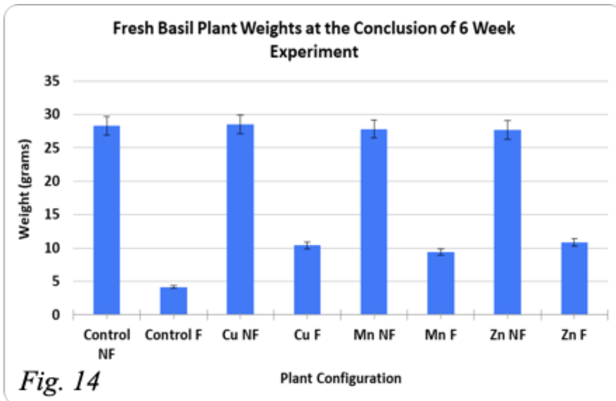



*Fig. 14*

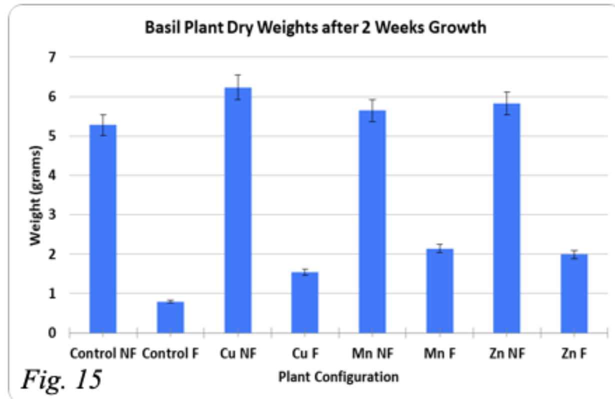



*Fig. 15*

*Figure 9: Growth of intentionally-mixed Basil-NP-Fusarium plants at 6 weeks, immediately prior to final growth measurement, and wet-mass determination.*
*Figs. 10-15: Phase 1 Growth, Wet Mass, and Dry Weight results for Mo-NP treated Basil Plants for both healthy plant, and those inoculated with F. o.*

**MO-NP Enhancement of Healthy Biomass:** After 6 weeks of growth, all plants were cut at the soil level, and their respective wet biomass measured. For healthy plants, growth for those plants treated with CuNPs, MnNPs, and ZnNPs were each comparable to non-treated healthy plants (Fig. 14). As such, the addition of NPs does little to promote overall biomass, while not harming the basil plants. Drying of the fresh biomass (in a paper bag) leads to analysis of dry biomass for healthy plants with those with MO-NP treatment. For dry biomass, CuNPs-treatment provide 17% improvement, relative to untreated, healthy plants, while the MnNP and ZnNPs each provided ~10% improvement (Fig. 15).

**MO-NP Enhancement of *F.o.*-inoculated Biomass:** For freshly-cut, *F.o.*-inoculated basil plants treated with MO-NPs, all three metal treatments increased wet biomass above the *F.o.*-inoculated, non-treated controls, with a 150%, 125%, and 180% improvement, for CuNPs, MnNPs, and ZnNPs, respectively (Fig. 14). This highlights MO-NP-treatment improvement above the disease condition, with Cu and Zn being the most notable. Unfortunately, none of the MO-NP treatments promoted wet-biomass to levels of the healthy controls. Similarly, for dry biomass, all three MO-NP treatments provided some remedy to the *F.o.* inoculation (~100-160%), with MnNPs being the highest (Fig. 15). Collectively, all biomass data highlight the ability of MO-NPs to provide as much as 10-17% increase in healthy basil plant growth. For diseased plants, MO-MP treatments provide 120-180% increase in Basil biomass, however these plants are not restored to a healthy state.

## Phase 2: Design of Basil Cu-Zn NP Fusarium Experiments:

Although all MO-NPs demonstrated increased resistance to Fusarium in basil, both CuNPs and ZnNPs were the most favorable, and as such, formed the basis for phase 2 experiments, where F.o. inoculated basil plants were treated with a 250ppm (of each) foliar spray solution of CuNP-ZnNPs. Like phase 1 experiments, basil plants were grown to 3" height, and transferred to 0.8L pots with Fusarium-inoculated soil, followed by 2ml foliar-spray  treatments of 500ppm CuNPs, 500ppm ZnNPs, and a 250CuNP/250ppm ZnNP mixture. A similar set of experiments was simultaneously carried out for NP-treatment versus healthy control plants (Fig. 16).


*Fig. 16*

## Cu-Zn NP Growth Enhancement & Disease Suppression:

While 500ppm ZnNP treatment of healthy basil plants promoted ~16% increased growth relative to healthy, non-treated controls, growth results from the combined (250ppm each) Cu-Zn NP treatment are highlighted by ~30% increase versus healthy, ~14% more than ZnNP alone (Fig. 17). On a weekly basis (Fig. 18), *Fusarium*-inoculated plants lagged behind the healthy controls. Cu-Zn NP-treated plants, however, equally outgrew both the healthy and disease conditions by ~38% (33 cm for Cu-Zn NP treated, vs. 24 cm for healthy).

For fresh (harvest) weights, post 6-weeks, basil plants treated with the combined Cu-Zn NP treatment outgrew their control counterparts, 10% for the healthy plants, and 29% for the

*Fusarium* disease condition (Fig. 18). Finally, for dry weight measurements, the combined Cu-Zn NP treatment of *Fusarium*-diseased basil plants promoted growth by 42% versus the diseased, untreated control plants (Fig. 19). Most importantly, Cu-Zn NP treated, diseased-plants outgrew healthy controls by 21%, highlighting the treatment's ability to fully suppress, and provide additional growth assistance, to *Fusarium*-infected plants.

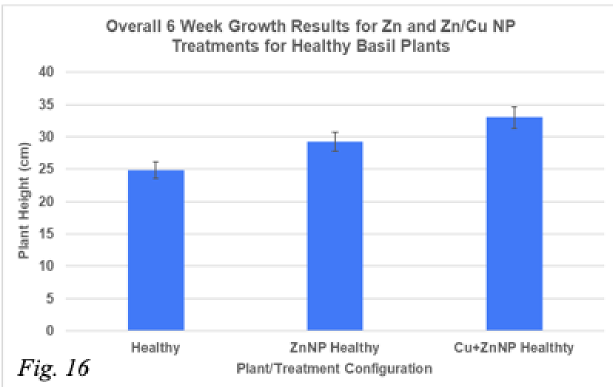



*Fig. 16*

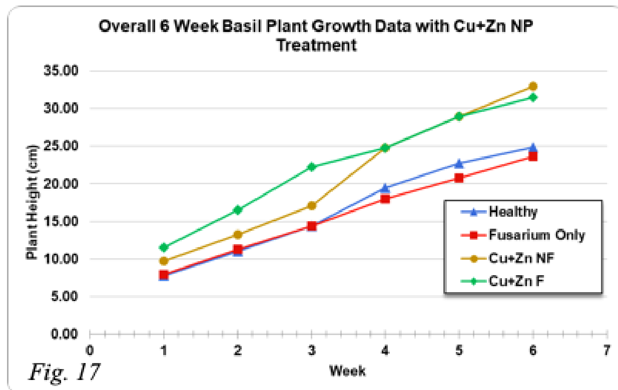



*Fig. 17*

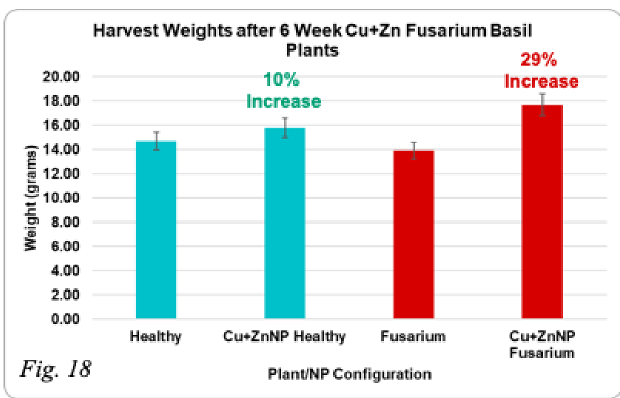



*Fig. 18*

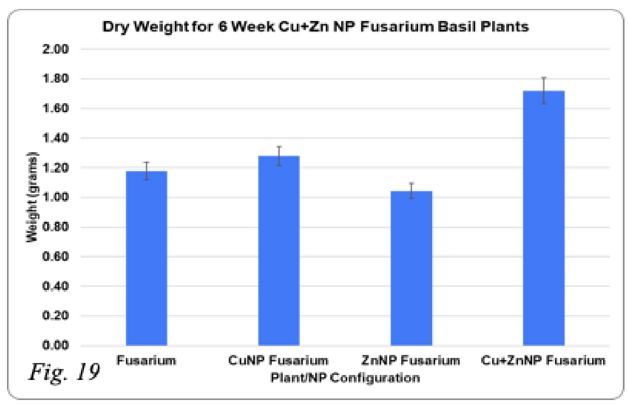



*Fig. 19*

*Figs. 20a-b: (a, left): Cu/Zn NP F.o. plant configuration demonstrates increased growth, relative to plants in (b), which are inoculated with Fusarium.*


## Basil Leaf Absorption of ZnNPs:

Promotion of basil plant growth for both the healthy and Fusarium disease state was evident for all metal oxide NPs, particularly ZnNPs. To investigate whether the ZnNPs are absorbed into the leaf's structure over the days following application of the foliar spray, a basil leaf with newly applied ZnNPs was analyzed for 5 days, post application, using visual microscopy. Visual images of the same plant region over 5 days (Figs 21-23) highlights the disappearance of the ZnNPs, which were absorbed into the basil leaf.

*Fig. 21: (Time = 0) The ZnNP treatment was just applied and allowed to settle on the basil leaf*

*Fig. 22: (Time = 24 hrs) Traces of the ZnNPs are reduced, relative to Time = 0.*

*Fig. 23: (Time = 5 days) The ZnNPs are no longer visible, suggesting their absorption into the epidermis.*

## Measure of Metal-Oxide NP Absorption via SEM and EDS:

For each basil plant configuration, a small leaf, petiole, and stem were removed and examined via SEM and EDS to assess uptake/absorption of the metal nanoparticles through the plant vascular system. In each case, the removed leaf was massed (both wet and dry) and included on the final wet/dry mass data for the plant. In addition, the leaf was examined (both top and bottom) for damage caused by *F.o.*

In the case of the MnNPs, the nanoparticles were located within the petiole of the basil leaf, which, like visual microscopic analysis, confirms that the MnNPs are absorbed by the basil leaf epidermis (Figs 24-25), and enter the plant's vascular system. Similarly, the ZnNPs were found within the phloem and xylem of the basil stem (Figs 26-28), both tube-like vessels that are responsible for food, salts, water, and support. This is direct evidence that the ZnNPs are absorbed via the leaf epidermis, and are transported through the stem with other plant nutrients.

**Detection of Absorbed MnNPs**

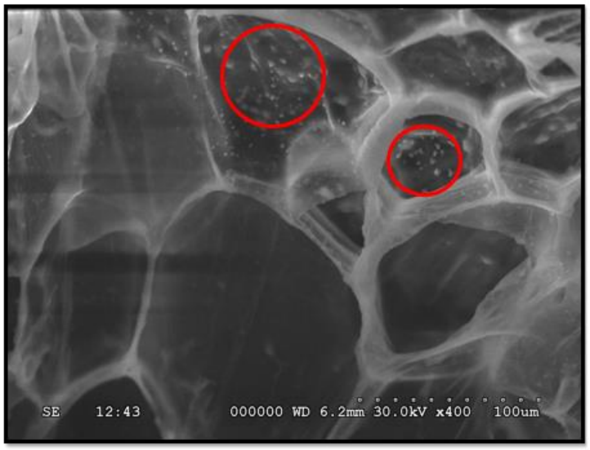



*Fig. 24: 400x/30kV SEM image of the basil plant petiole, where MnNPs were located (highlighted in red)*

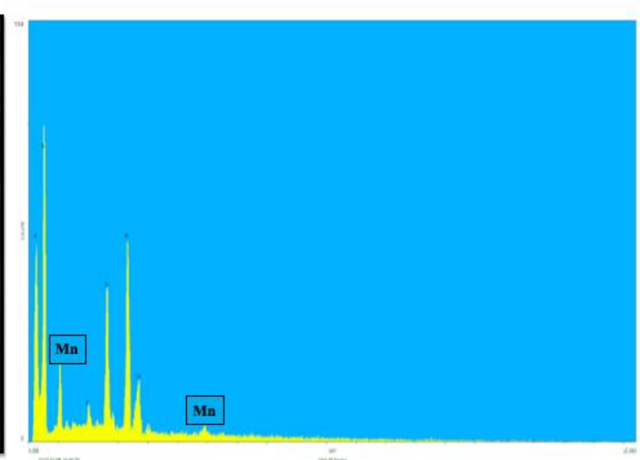



*Fig. 25: EDS analysis of red-highlighted nanoparticles in Fig 24 confirm the presence of MnNPs*

**Detection of Absorbed ZnNPs**

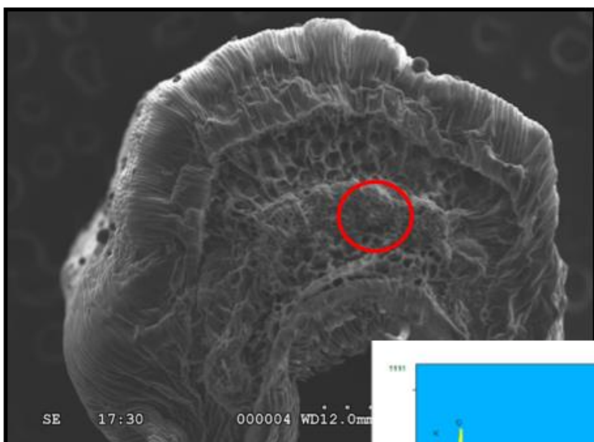



*Fig. 26: 50x/30kV SEM image of the basil plant stem, highlighting the xylem and phloem structures, (highlighted in red)*

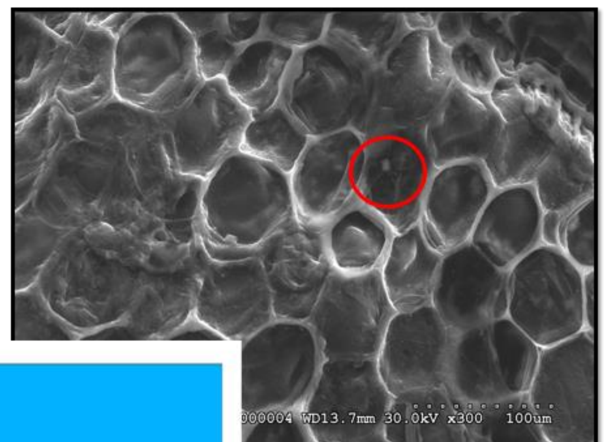



*Fig. 27 (above): 300x/30kV SEM image the xylem and phloem within the basil plant stem, where ZnNPs were located*

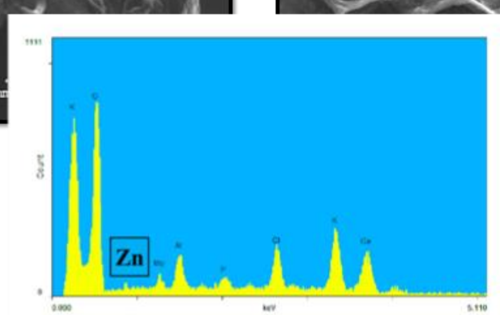



*Fig. 28 (left): EDS analysis of red-highlighted nanoparticle in Fig. 27 confirms the presence of ZnNPs*

## Discussion & Conclusions:

As a new approach to increase crop production, and increase disease resistance, CuO, MnO, and ZnO metal-oxide foliar sprays were investigated for their ability to promote growth in basil plants (our crop model), and provide assistance in the suppression of common plant diseases (using Fusarium wilt as a model fungus). At an applied concentration of 2ml of 500ppm MO-NPs suspension in water, all MO-NPs promoted growth for healthy basil plants. Specifically, all resulted in at least a 10% increase in plant biomass over a 6-week growth cycle, with CuNPs producing the most dramatic increase, at ~17%. Treatment with ZnNPs, on the other hand, produced 5% taller plants. For those plants infected with F.o., treatment with 500ppm MO-NPs produced as much as 40% increase in growth-height relative to an untreated, infected plant, highlighting the treatment's ability in suppressing the dwarfing effects of the fungal disease. Once again, ZnNPs produced the most significant increase in growth-height of diseased plants, at 72%. With regards to biomass, all three MO-NP treatments produced significant increases (>120%) in biomass, relative to diseased plants; ZnNPs were again the most favorable, at 180% increase in biomass relative to untreated, diseased plants. In all cases of Fusarium-infected plants, treatment with MO-NPs could not restore the plants to growth rates of healthy plants.

Since ZnNPs and CuNPs provided the most favorable assistance for both healthy and diseased basil plants, phase 2 of the research focused on treatment with a Cu-Zn NP mix, with additional investigations into the mechanism for NP growth enhancement, and disease suppression. Combined Cu-Zn NP treatment enhanced healthy plant biomass by 10%, and diseased plants by 29%. Regarding growth height, Cu-Zn treatment produced 30% increase in healthy plants, and as much as 40% increase for the diseased crop. Most importantly, for the combined treatment, diseased-plants outgrew healthy controls by 21%, highlighting the treatment’s ability to fully suppress, and provide additional growth assistance, to Fusarium-infected plants beyond healthy conditions.

Finally, visual and SEM microscopy analyses highlight the NP’s absorption into the plant vascular system via the leaf epidermis, with NPs found in the phloem and xylem structures of the plant stem, where they can directly impact plant nutrition.

## Future Research:

Evaluation of various combinations of NPs could yield additional growth benefits, as would change in the relative proportions of NPs within the foliar spray treatment. Lastly, other unexplored metal oxide NPs, such as Ag, have shown promising resistance to disease. These can be similarly applied to *Fusarium*-inoculated basil.

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