Metal oxide nanoparticles for management of Verticillium wilt of eggplant and Fusarium wilt of watermelon

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Nutrition is the first line of defense against disease. Micronutrients protect roots against soilborne diseases by activating enzymes to create defense products.

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>Activates polyphenoloxidases</td>
</tr>
<tr>
<td>Mn</td>
<td>Activates enzymes in the Shikimic acid and Phenylpropanoid pathways</td>
</tr>
<tr>
<td>Zn</td>
<td>Activates superoxide dismutases</td>
</tr>
</tbody>
</table>
• The Obstacles

Increasing micronutrient levels in roots is problematic in neutral soils.

Micronutrients are not basipetally translocated.

When applied to soil they frequently precipitate and become unavailable to the plant.
Nanoparticles of CuO and other metals can move basipetally whereas bulk equivalents do not.

The Hypothesis

Would applying NP of micronutrients to leaves affect growth?

Would these metals be translocated to roots?
First studies were done on Fusarium wilt of tomato

- Two rates (100 ppm or 1,000 ppm) of nanoparticles of Al, Fe, Cu, Mn, Ni, or Zn were sprayed onto tomatoes in the greenhouse.

- Plants were inoculated with *Fusarium*. 
Greenhouse tomato experiments

Area Under the Disease Progress Curve
(disease * days)

- CK
- AlO
- CuO
- FeO
- MnO
- NiO
- ZnO

Stars indicate significant differences.
Verticillium Wilt of Eggplant

Caused by soilborne fungus, *Verticillium dahliae*

Can reduced yields by 30%
Greenhouse experiments

• Would foliarly nanoparticles of Cu, Mn, or Zn suppress Verticillium wilt of eggplant?

• Would they behave the same as their bulked oxide equivalents?
Methods

Nanoparticles of Cu, Mn, and Zn oxides were compared to the bulked oxide equivalent (1.0 mg/ml).

Plants were sprayed, allowed to dry and grown in soil with *V. dahliae*.

Growth and disease were measured.
Comparison of Nanoparticles to their bulked equivalent for effects on fresh weight of eggplants transplants in the greenhouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ck</td>
<td>16</td>
</tr>
<tr>
<td>CuO</td>
<td>14</td>
</tr>
<tr>
<td>NP CuO</td>
<td>18</td>
</tr>
<tr>
<td>MnO</td>
<td>12</td>
</tr>
<tr>
<td>NP MnO</td>
<td>14</td>
</tr>
<tr>
<td>ZnO</td>
<td>12</td>
</tr>
<tr>
<td>NP ZnO</td>
<td>12</td>
</tr>
</tbody>
</table>
Comparison of NP to their bulked equivalent for disease progress on eggplant transplants in the greenhouse.

Area Under Disease Progress curve

- Ck
- CuO
- NP CuO
- MnO
- NP MnO
- ZnO
- NP ZnO
Cu levels in roots of eggplants treated with CuO NP or bulked oxide equivalents

<table>
<thead>
<tr>
<th>Root</th>
<th>Control</th>
<th>Bulked CuO</th>
<th>NP CuO</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/g</td>
<td>30</td>
<td>50</td>
<td>55</td>
</tr>
</tbody>
</table>
Root levels of Cu (µg/g)

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Bulked</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>17% increase</td>
<td>Not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

17% increase
Field Experiment

- Would nanoparticles of Cu, Mn and Zn suppress Verticillium wilt of eggplant?
- Would they affect yield?
Nanoparticle-Verticillium field trial on Eggplant 2013

Treatments
Control
CuO Bulk
CuO NP
MnO Bulk
MnO NP
ZnO Bulk
ZnO NP
Comparison of NP to the bulked equivalent on the canopy progress of eggplants with Verticillium wilt

![Graph showing comparison of treatments](image)

- **Untreated**
- **Bulked Cu**
- **NP Cu**
- **Bulked Mn**
- **NP Mn**
- **Bulked Zn**
- **NP Zn**

**M² * days**

2013
Comparison of Nanoparticles to their bulked equivalent for yield on eggplants with Verticillium wilt.

2013 Yield (kg/plant)
Nanoparticle-Verticillium field trial on Eggplant 2014

Treatments

Control
CuO Bulk
CuO NP
MnO Bulk
MnO NP
ZnO Bulk
ZnO NP
Comparison of NP to the bulked equivalent on the canopy progress of eggplants with Verticillium wilt 2014
Comparison of Nanoparticles to their bulked equivalent for yield on eggplants with Verticillium wilt.

2014 Yield (kg)

- Untreated
- Bulked CuO
- NP CuO
- Bulked MnO
- NP MnO
- Bulked ZnO
- NP ZnO
Conclusions for Field

Experiments (2013 & 2014)

- Nanoparticles of CuO increase yield in both years more than the untreated control and the bulked CuO.
- Fruit skin or flesh did not have elevated levels Cu when compared to controls.
Assume 2,500-3,000 eggplant transplants/A. So, 2,500 seedlings treated with 23 g CuO NP in 23 liters (1000 ppm) applied to run off) = costs $44.00.

We received a 17-31% increase over Bulked CuO (cost $18.40).

Eggplants averages = $17,500 - $20,000/A.

So a $44.00 investment could increase profits $5,526 - $6,315.
Nanoparticle-Verticillium field trial on Eggplant 2015

Treatments

Control
NP CuO 100
NP CuO 250
NP CuO 500
NP CuO 1000
Bulked 1000
Effect of increasing rate of Nanoparticles of CuO on yield of eggplant affected by Verticillium wilt.

2015 Yield (kg)/plant

- Untreated
- NP 100
- NP 250
- NP 500
- NP 1000
- Bulked 1000

NS
Nanoparticle-Verticillium field trial on Eggplant 2016

Treatments

Control
CuO
MnO
ZnO
CuO + MnO
CuO + ZnO
MnO + ZnO
CuO + MnO + ZnO
Nanoparticle-Verticillium field trial on Eggplant 2016

Disease Incidence

- Ck
- CuO + MnO
- MnO + ZnO
- CuO + ZnO
- CuO + MnO + ZnO
Fusarium Wilt of Watermelon

Caused by *Fusarium oxysporum* f. sp. *niveum*

2013 Florida Growables
Watermelon Experiment 1

Would nanoparticles of Cu, Mn, Si, Ti, or Zn increase or decrease Fusarium disease on watermelon.

Compared NP vs Bulked oxides.
Methods

NP or bulked equivalents of Cu, Mn, Si, Ti, or Zn oxides were sprayed onto watermelons in the greenhouse.

Plants were inoculated with conidial drench of *Fon*
Effect of Bulked vs NP of Cu, Mn, Si, Ti and Zn oxides on growth of watermelon infested with Fusarium wilt in the greenhouse.

Fresh Weights (g)
Effect of Bulked vs NP of Cu, Mn, Si, Ti and Zn oxides on the disease progress of watermelon infested with Fusarium wilt in the greenhouse.

Estimates of disease progress (disease * days)
Effect of Cu applied as NP of CuO, Bulked CuO, Kocide 2000, or Cu octoantate on yield (kg/plant) of watermelons

Digests of edible flesh found no differences in Cu levels among Treatment
Methods

NP or bulked equivalent of Cu, Mn, Si, Ti, or Zn oxides were sprayed onto watermelons in the greenhouse.

Plants were inoculated with conidial drench of *Fon*
Comparison of Nanoparticles of Cu, Mn, Si, Ti and and Zinc to their large bulked equivalent for effect on watermelon fresh weights.

<table>
<thead>
<tr>
<th></th>
<th>Fresh Wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticles</td>
<td>21</td>
</tr>
<tr>
<td>Bulked equivalent</td>
<td>18</td>
</tr>
</tbody>
</table>

- **CK**
- **Cu**
- **Mn**
- **Si**
- **Ti**
- **Zn**
Comparison of Nanoparticles of Cu, Mn, Si, Ti and Zn to their bulked equivalents for estimates of disease progress of Fusarium wilt of watermelon.

Estimates of disease progress (disease * days)

- NP
- Bulked
Foliar application of CuO NP (1000 PPM)

Control       NP of CuO
Comparison of Nanoparticles rate of Cu, Mn, Si, Ti and Zinc for on watermelon fresh weights inoculated with FON.

<table>
<thead>
<tr>
<th></th>
<th>AUDPC</th>
<th>Fresh Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>30</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Mn</td>
<td>25</td>
<td>1000 ppm</td>
</tr>
<tr>
<td>Si</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

= 100 ppm

= 1000 ppm
Root copper levels show no significant basipetal translocation of NP of CuO
Field studies 2015

Treatments
1. Control
2. NP of CuO
3. Bulked CuO
4. Kocide 2000
5. Organic Cu soap
   (Cu octanoate)

Applied twice to seedlings in greenhouse
Effect of Cu applied as NP of CuO, Bulked CuO, Kocide 2000, or Cu octoantate on yield (kg/plant) of watermelons

**Kg Fruit/plot**

- Control
- NP CuO: 60
- Bulked CuO
- Kocide 2000
- CU octanoate

Digests of edible flesh found no differences in Cu levels among Treatment
2016 Watermelon NP Studies (Greenhouse)

Fresh weights

![Graph showing fresh weight comparisons between noninoculated and inoculated plants with various compounds.]

July 13 vs. July 21 comparisons:
- **Ck NP CuO**
- **Ck** vs. **NP CuO**

Noninoculated vs. Inoculated with FON.
Field studies 2016

Treatments
1. Control
2. B NP
3. CeO NP
4. CuO NP
5. MnO NP
6. ZnO NP
The effect of nanoparticle (NP) or bulked equivalent rates (Log concentration) of CuO, MnO, or ZnO on the integrated values of the radial colony expansion of *Fusarium oxysporum* on 25% potato dextrose agar over three time points.

Error bars represent the standard error of the mean.
Conclusions

• Treating watermelon with NP of CuO promotes growth, yield and may suppress Fusarium wilt of watermelon.

• Season long effects were observed in 2015 following applications to young transplants.
Conclusions

• Treating seedlings of eggplants and watermelons with NP of CuO promotes growth and yield.

• Season long effects were observed following single or double applications to young transplants.
Acknowledgements

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Questions
Foliar application of CuO NP

*Pythium aphanidermatum* on Chrysanthemum

<table>
<thead>
<tr>
<th>Control</th>
<th>CuO</th>
<th>CuO</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ppm</td>
<td>500 ppm</td>
<td></td>
</tr>
</tbody>
</table>