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Research article

Comparative analysis of copper and zinc based agrichemical biocide products: materials characteristics, phytotoxicity and in vitro antimicrobial efficacy

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Abstract: In the past few decades, copper based biocides have been extensively used in food crop protection including citrus, small fruits and in all garden vegetable production facilities. Continuous and rampant use of copper based biocides over decades has led to accumulation of this metal in the soil and the surrounding ecosystem. Toxic levels of copper and its derivatives in both the soil and in the run off pose serious environmental and public health concerns. Alternatives to copper are in great need for the agriculture industry to produce food crops with minimal environmental risks. A combination of copper and zinc metal containing biocide such as Nordox 30/30 or an improved version of zinc-only containing biocide would be a good alternative to copper-only products if the efficacy can be maintained. As of yet there is no published literature on the comparative study of the materials characteristics and phyto-compatibility properties of copper and zinc-based commercial products that would allow us to evaluate the advantages and disadvantages of both versions of pesticides. In this report, we compared copper hydroxide and zinc oxide based commercially available biocides along with suitable control materials to assess their efficacy as biocides. We present a detailed material characterization of the biocides including morphological studies involving electron microscopy, molecular structure studies involving X-ray diffraction, phytotoxicity studies in model plant (tomato) and antimicrobial studies involving surrogate plant pathogens (*Xanthomonas alfalfae* subsp. *citrumelonis, Pseudomonas syringae* pv. *syringae and Clavibacter michiganensis* subsp. *michiganensis*). Zinc based compounds were found to possess comparable to superior antimicrobial properties while exhibiting significantly lower phytotoxicity when compared to copper based products thus suggesting their potential as an alternative.

Keywords: Copper; Zinc; biocide; pesticide; agriculture; antimicrobial; copper toxicity

1. Introduction

Modern day agriculture requires extensive application of pesticides and agricultural biocides for attaining multiple objectives including but not limited to preventing and treating microbial origin diseases, vector-borne diseases and other seasonal diseases. Specifically, yield loss in food crop production would have a significant effect on both food availability and food prices thereby directly affecting the global hunger levels. Achieving higher food productivity per acre of land is also a necessity to feed the ever-increasing human population as per the United Nations. Pesticides and biocides have been helpful in checking most of the devastating microbial diseases and thereby are used extensively across the world to prevent crop loss. Particularly, copper (Cu) based biocidal compounds such as Cu hydroxide, Cu oxychloride, Cu chelates have been used in citrus groves, field crops, small fruits and vegetable farms for prevention of microbial diseases [1,2]. Wide use of these biocides in the past few decades has resulted in accumulation of copper residues at alarming levels in the soil and in surrounding ecosystem [3-5]. Specifically, leaching of copper into the surrounding ecosystem particularly water reservoirs has raised serious concerns [4,6]. For Cu, the maximum contaminant level in drinking water is 1.3 parts per million (ppm) and soil clean up level is 600 ppm as regulated by the United States Environment Protection Agency (USEPA). Many citrus and tomato growers, due to aggressive (up to 1000 ppm) and multiple use of Cu per season will soon reach the Cu soil clean up level and therefore no longer be able to protect their crops using Cu based bactericides/fungicides [USEPA 1999]. Another concern is the rise in the levels of copper resistance among plant pathogens that are exposed to copper based pesticides over a long period of time [7,8]. Though complete elimination of copper based pesticides may take decades until a suitable alternative is found but efforts are underway where zinc supplementation is employed to reduce the copper content in pesticides. Some commercial products such as Nordox 30/30 has both copper and zinc in equal percentage concentration and has shown to be effective as pesticides [2].

Though various copper and zinc based pesticides and related compounds are commercially available as agricultural biocides, there has never been a comparative study done on their material characteristics and their corresponding antimicrobial properties. In this manuscript, we show a head-to-head comparison of the properties of various pesticides that are either copper and/or zinc based. This, we believe would help fill the void in the scientific literature about the material characteristics of copper and zinc based pesticides and their potential as an environmentally viable pesticide. Moreover, the potential of zinc-based compounds to act as a replacement for copper based pesticides can only be unveiled by these kind of comparative studies. To this end, based on *in vitro* studies, we show in this manuscript that the zinc-based compounds have comparable if not superior antimicrobial properties over copper based pesticides but still exhibit significantly lesser phytotoxicity. Morphological characteristic studies suggest that the differences in their micro/nano structures could play a role in determining the antimicrobial properties of these materials. Recent evidence suggests that nanoparticles of various sizes at the nanoscale level might affect their *in planta* trafficking and henceforth their site of action on target pathogens [9]. The antimicrobial properties of these various biocides were screened against three different bacteria to cover a wide range of crops where these biocides are applied to control the infections. Overall, it appears that zinc based compounds could be a viable alternative to traditional copper based biocides especially suitable in conditions where copper resistance have been documented.

2. Materials and Methods

2.1. Materials

All compounds were purchased from commercial vendors and utilized without any further purification: Kocide® 3000 (DuPont, USA), Copper Sulfate (CQ Concepts, USA), Nordox 30/30 (Brandt, USA), Zinc Oxide 400 and Zinc Oxide 800 (Zinc oxide LLC, USA), Surround WP (NovaSource, USA) and Resazurin sodium salt (Acros organics, USA). *Xanthomonas alfalfae* subsp. *citrumelonis* (ATCC 49120), *Pseudomonas syringae* pv. *syringae* (ATCC 19310) and *Clavibacter michiganensis* subsp. *michiganensis* (ATCC 10202) were purchased from ATCC (U.S. Department of Agriculture (USDA) with permits P526P-12-04060 and P526P-15-01601).

2.2. Material characterization

2.2.1. Scanning electron microscopy

The morphology of the biocides was observed under a scanning electron microscope (SEM) at a constant operating voltage of 15 kV (SEM, Ziess ULTRA-55 FEG SEM). Sample preparation involved dispersing all the samples on to a glass slide substrate. The glass slide was gold coated using a sputter coater (Emitech K550) for 90 seconds at 20 mA to prevent sample-charging related issues. All SEM samples were prepared at total metallic concentration of 800 ppm (copper/zinc) wherever applicable; equivalent to the concentration in the range used in citrus crop protection [10]. The samples were gold coated twice using a sputter coater (Emitech K550) for 90 seconds at 20 mA for the low resolution SEM analysis, while the samples were gold coated for 45 seconds at 40 mA for the high resolution SEM to prevent sample-charging related issues.

2.2.2. X-Ray diffraction

The crystallographic arrangement and the compositional properties of the sample were evaluated using an X-ray diffractometer (XRD, Empyrean Series 2 X-Ray Diffraction System). The XRD analysis was performed using CuKα for an angular interval of 5° to 90°. All characterization studies were performed at Material Characterization Facility (MCF) at the University of Central Florida. All samples were prepared at a metallic concentration of 800 ppm of either copper or zinc.

2.3. Antimicrobial studies

The antimicrobial properties of the compounds were studied using an array of standard microbiological techniques including determining the Minimum Inhibitory Concentration (MIC) and a bacterial viability assay. Samples were tested against Gram-negative *Xanthomonas alfalfae* subsp. *citrumelonis* strain F1 (ATCC 49120, a citrus canker surrogate), Gram-negative *Pseudomonas syringae* pv. *syringae* (ATCC 19310, causative agent of bacterial speck in Lilac, almond, apricots, peaches and wild beans among others) and Gram positive *Clavibacter michiganensis* subsp. *michiganensis* (ATCC 10202, causative agent of bacterial wilt and canker in *Tomato sp*). *X. alfalfae* and *P. syringae* were maintained with nutrient agar and broth while *C. michiganensis* was grown with brain heart infusion (BHI) media. All bacteria were grown at 26 ºC in a shaking incubator (150 rpm).

Minimum inhibitory concentration (MIC) was carried out using broth microdilution assay in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11]. The accuracy of the MIC determination was improved by adding 10 μL of resazurin dye (0.0125% w/v) per 100 μL well volume and observing color changes (blue to pink for live organisms). This allowed reducing any error caused by the cloudiness of the material and by the controls with broth. The absolute quantitative bactericidal activities of each compound were determined by colony forming unit (CFU) assay. Treatment of bacteria with materials was carried out following the same procedure as described in the MIC assay. A range of concentrations (31–500 µg/mL) was chosen from MIC results and used for CFU quantification. Each sample was serially diluted in 1x PBS and plated on nutrient or BHI agar at 26 ºC. After 48 hrs of incubation, individual colonies were counted and CFU/mL were determined and expressed in logarithmic scale.

2.4. Toxicity studies

Phytotoxicity studies of compounds (ZnO 400, ZnO 800, $CuSO_4$, Kocide® 3000, Nordox 30/30, Surround WP) were carried out to ascertain potential plant tissue damage from material usage. Studies were conducted using 25 days-old Florida heat-tolerant (91) Heirloom Tomato (Solanum *lycopersicum*), a model fruit plant purchased from a local Home Depot. Phytotoxicity studies were conducted inside a Panasonic Environmental Test Chamber (Model MLR-352H) to control light intensity, humidity and temperature cycling to simulate summer conditions (85% RH, 34 ºC). Formulations (in triplicates for each sample/concentration) were sprayed at metallic concentrations of 500 and 1000 µg/mL of active agent at 8:00 am before temperatures raises, and observations were taken at 72 hr post spray application. In case of Surround WP (a non-biocide control), the concentration was applied at field spray rate i.e., 0.5 lbs/gallon of water. Formulations were sprayed on plants until run-off and approximately 300 mL of spray volume/plant was required to completely cover the entire plant.

2.5. Statistical analysis

The difference in the bacterial numbers was statistically analyzed by using ANOVA and the means were expressed after Tukey's correction. For the numbers to be statistically significant, $P < 0.05$ was considered the limit.

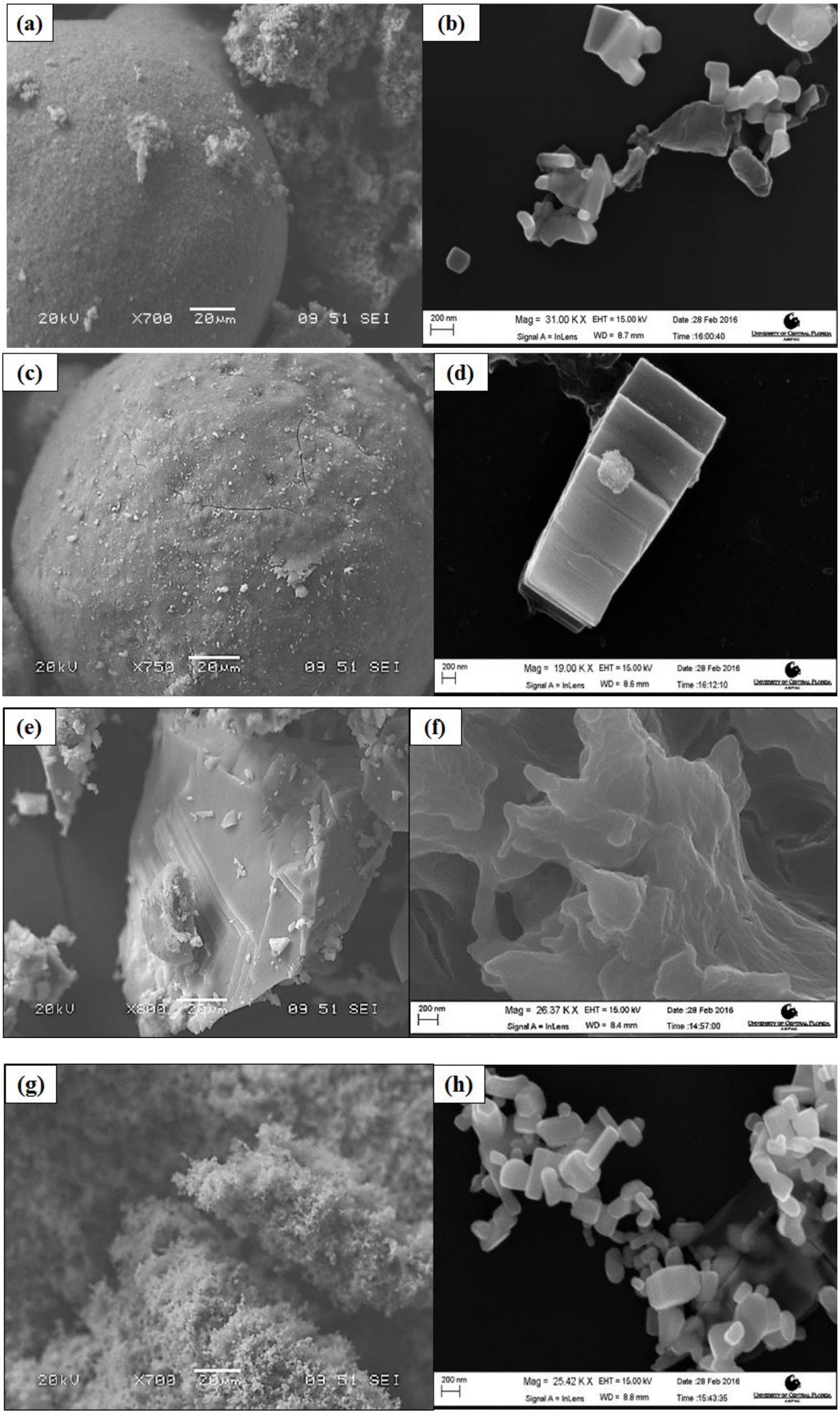
3. Results and Discussion

3.1. Material morphology and structure characterization

The morphological studies of the samples under high and low resolution SEM provided an insight into the structure of the biocides at the sub-micron level. High resolution SEM indicated that the materials—Nordox 30/30, Kocide® 3000, ZnO 800 and ZnO 400 dispersed well on to the glass substrate and comprised of individual nanoparticles that in turn were bound together to form the macroscopic structures as seen from the low resolution SEM images (Figure 1). However, on comparing the corresponding high and low resolution SEM images of Cu Sulfate and Surround WP, it is observed that the material did not disperse well on to the glass substrate. It is to be noted that the non-conductive surface of Surround WP supported very high charging as observed in both the high and low resolution SEM analysis despite the gold coating. Furthermore, the XRD analysis carried out on the biocides indicate that Copper Sulfate, Nordox 30/30, Kocide® 3000, ZnO 800 and ZnO 400 are all crystalline materials with well-defined diffraction peaks. Surround WP was however found to be amorphous. Surround WP was used as a non-metal crop protectant that acted as a control. The mode of action of Surround WP differs from the metal based Nordox 30/30 and Kocide® 3000 as the predominant component of Surround WP is EPK (Edgar Plastic Kaolin) clay.

3.1.1. Nordox 30/30

Diffraction peaks (Figure 2a) from the sample matched with a distinct combination of Cuprite (JCPDS: #05-0667) and Zincite (JCPDS: #36-1451) having cubic and hexagonal lattice structures respectively. Since the maximum intensity peak of the sample superimposed the highest intensity peak of the individual constituents—ZnO and Cu_2O, Nordox 30/30 sample is inferred to be a composite material. Morphological investigation of low-resolution SEM images suggests (Figure 1a) spherical-shell like structure of copper oxide particles and zinc oxide particles with a broad range of particle diameters of 30–150 μm. However, from high resolution SEM (Figure 1b), it is seen that Nordox 30/30 comprises of individual particles attached together with an average particle size range of 150–500 nm.



(a)
20kV X700 20µm 09 51 SEI
(b)
200 nm
Mag = 31.00 K X EHT = 15.00 kV
Signal A = InLens WD = 8.7 mm
Date :28 Feb 2016
Time :16:00:40
(c)
20kV X750 20µm 09 51 SEI
(d)
200 nm
Mag = 19.00 K X EHT = 15.00 kV
Signal A = InLens WD = 8.6 mm
Date :28 Feb 2016
Time :16:12:10
(e)
20kV 09 51 SEI
(f)
200 nm
Mag = 26.37 K X EHT = 15.00 kV
Signal A = InLens WD = 8.4 mm
Date :28 Feb 2016
Time :14:57:00
(g)
20kV X700 20µm 09 51 SEI
(h)
200 nm
Mag = 25.42 K X EHT = 15.00 kV
Signal A = InLens WD = 8.8 mm
Date :28 Feb 2016
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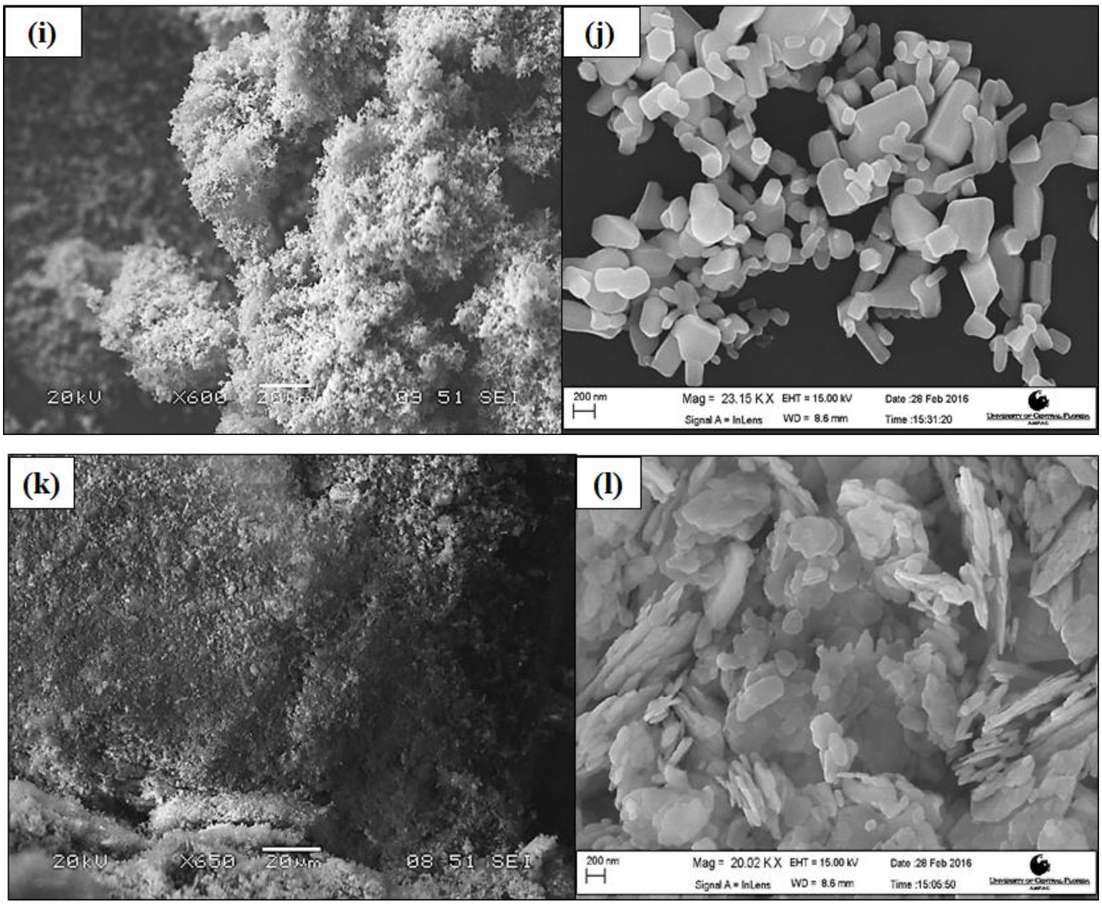

Figure 1. Morphological studies: SEM images of (a) Nordox 30/30 dry sample as purchased revealing micron (30–150 μm) size spheres (b) Nordox 30/30 when dissolved in aqueous solution at a total metallic concentration of 800 ppm for crop applications (such as citrus protection) displaying sub-micron (150–500 nm) size irregular shaped cylindrical rods (c) Kocide® 3000 dry sample as purchased revealing micron (~100 μm) size spheres (d) Kocide® 3000 dissolved in aqueous solution at a total metallic concentration of 800 displaying micron (~2–3 μm) size irregular shaped rectangular rods (e) copper sulfate dry sample as purchased compared revealing micron size irregular shards (f) copper sulfate when dissolved in aqueous solution at a total metallic concentration of 800 ppm displaying micron (20–100 μm) size irregular shaped amorphous gel (g) ZnO 800 dry sample as purchased revealing sub-micron sized irregular shapes, (h) ZnO 800 when dissolved in aqueous solution at a total metallic concentration of 800 ppm displaying sub-micron (300–900 nm) size irregular shaped cylindrical rods (i) ZnO 400 dry sample as purchased revealing sub-micron sized irregular shapes (j) ZnO 400 when dissolved in aqueous solution at a total metallic concentration of 800 ppm displaying sub-micron (200–600 nm) (k) Sorround WP dry sample as purchased revealing micron sized amorphous irregular shapes (l) Surround WP when dissolved in aqueous solution at a concentration of 600 ppm revealing sub-micron size irregular shaped layered shards.

3.1.2. Kocide® 3000

The Orthorhombic lattice structure of Copper hydroxide (JCPDS:#13–0420)) matched with the XRD peaks of the commercial pesticide—Kocide® 3000. Despite indexing the distinct diffraction peaks of the sample, the relatively higher noise to signal ratio (compared to Nordox 30/30) suggests the combination of an amorphous mixture to the crystalline copper hydroxide sample (Figure 2b) which prevents the sample from undergoing well defined diffraction patterns plausibly due to the partial impregnability of the X-Rays into the sample. The higher intensity diffracted at very low angles validates this claim. Although the low resolution SEM image (Figure 1c) revealed spherical shell based particles of copper hydroxide with an average particle size of 100 μm, the high resolution SEM data (Figure 1d) on Kocide® 3000 consists of well dispersed particles of the biocide in the nanoscale, that are aggregated together to form distinct individual particles of the size range of 2–3 μm.

3.1.3. Copper Sulfate

XRD analysis of copper sulfate provided very high number of diffracting peaks matching with the highly crystalline triclinic structure (Figure 2c) of Chalcanthite ($CuSO_4·5H_2O$, JCPDS: #70-1823). The sharp edges of the sample, as observed from the low resolution SEM images (Figure 1e) represent an average particle of the entire composition with well-defined edges. Higher resolution image of the sample (Figure 1f) shows the lack of dispersion of the sample on the glass substrate. The absence of individual particles of the samples, as seen amongst other biocides suggests that the copper sulfate samples were highly concentrated. It is noted that an average particle size range of this biocide is from 20 μm to 100 μm.

3.1.4. Zinc Oxide 800 and Zinc Oxide 400

A perfect match with the Hexagonal lattice structure of Zinc Oxide (JCPDS: #79-0206) suggests that the samples ZnO 800 and ZnO 400 (Figure 2d and Figure 2e) are pure, identical and non-composite by nature, unlike other biocide samples investigated in this study. However, the distinction between these samples is highly conspicuous through morphological studies. Comparison of low-resolution SEM images of both samples suggested that these Zinc based biocides are aggregation of individual Zinc Oxide particles (Figure 1g and Figure 1i). However, comparing high-resolution SEM images of these samples (Figure 1h and Figure 1j) suggests a distinctive size difference amongst both samples, though both of them are a highly networked aggregation of ZnO particles. An average particle size range of 300–900 nm was observed for Zinc Oxide 800 and 200–600 nm particle size range for Zinc Oxide 400.

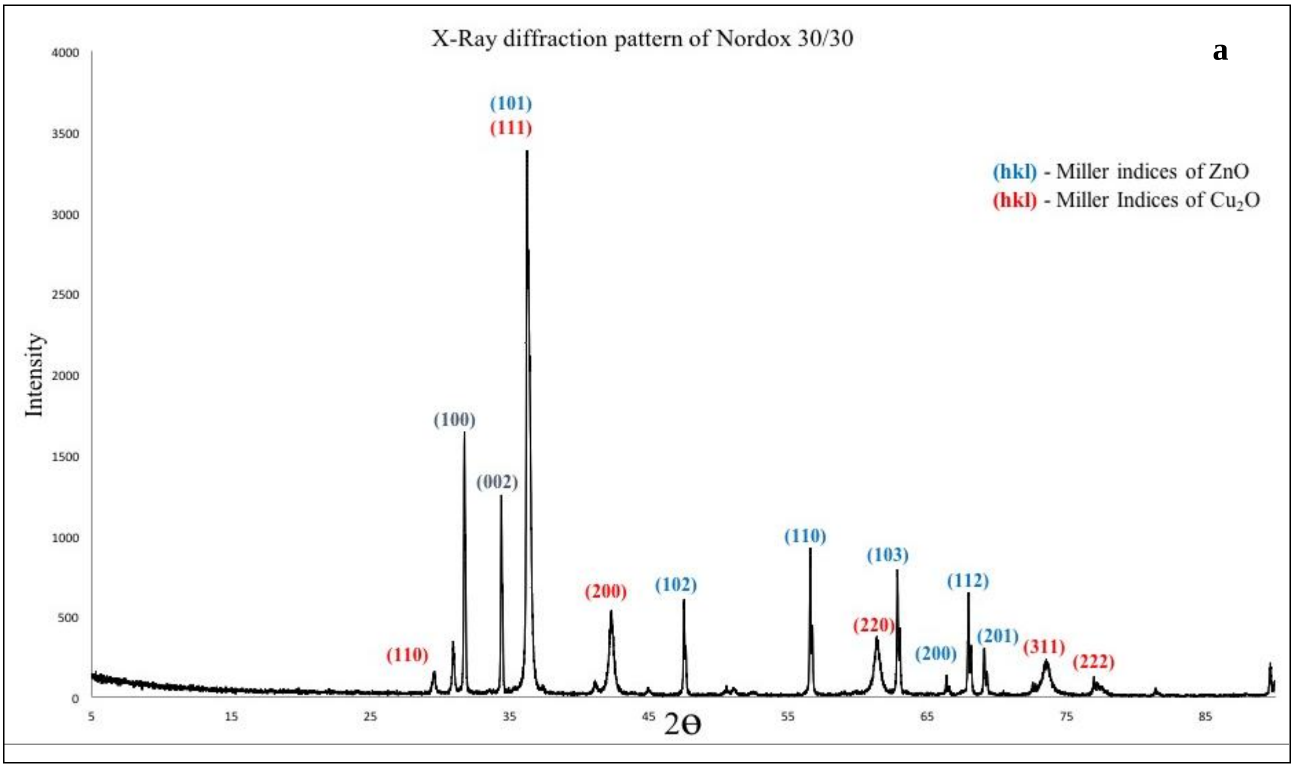

Figure 2a. XRD spectra of Nordox 30/30 exhibited diffraction peaks indicating Cuprite (JCPDS: #05-0667) and Zincite (JCPDS: #36-1451) which display Cubic and Hexagonal lattice structures.

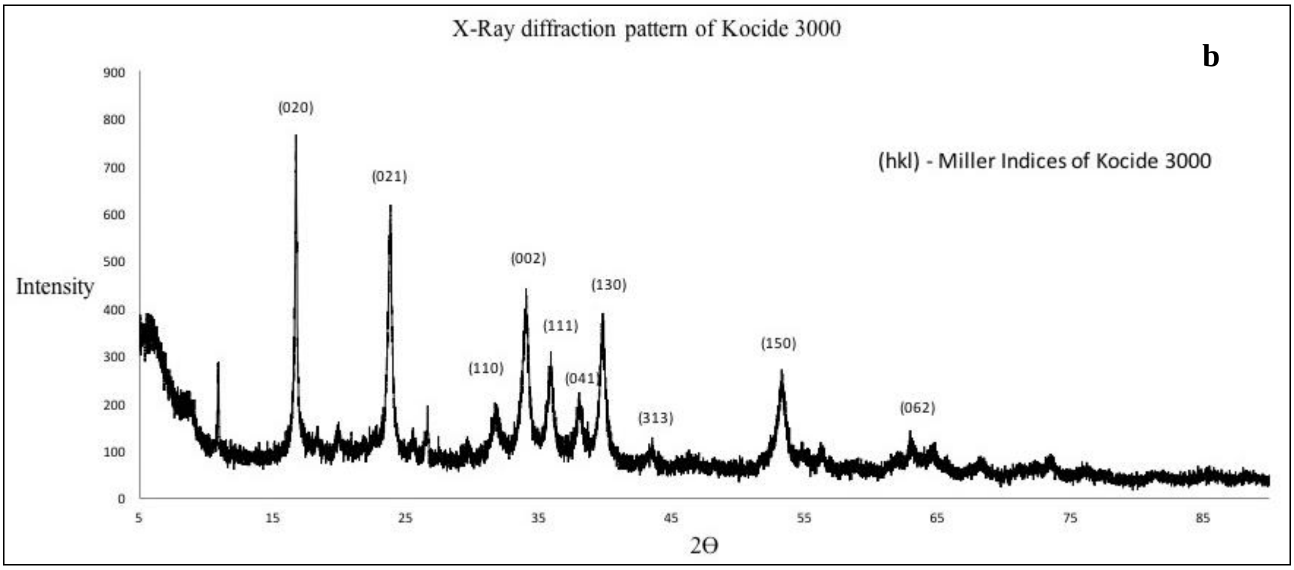

Figure 2b. XRD spectra of Kocide® 3000 exhibited diffraction peaks indicating Copper hydroxide (JCPDS:#13–0420) which displays a Orthorhombic lattice structure.

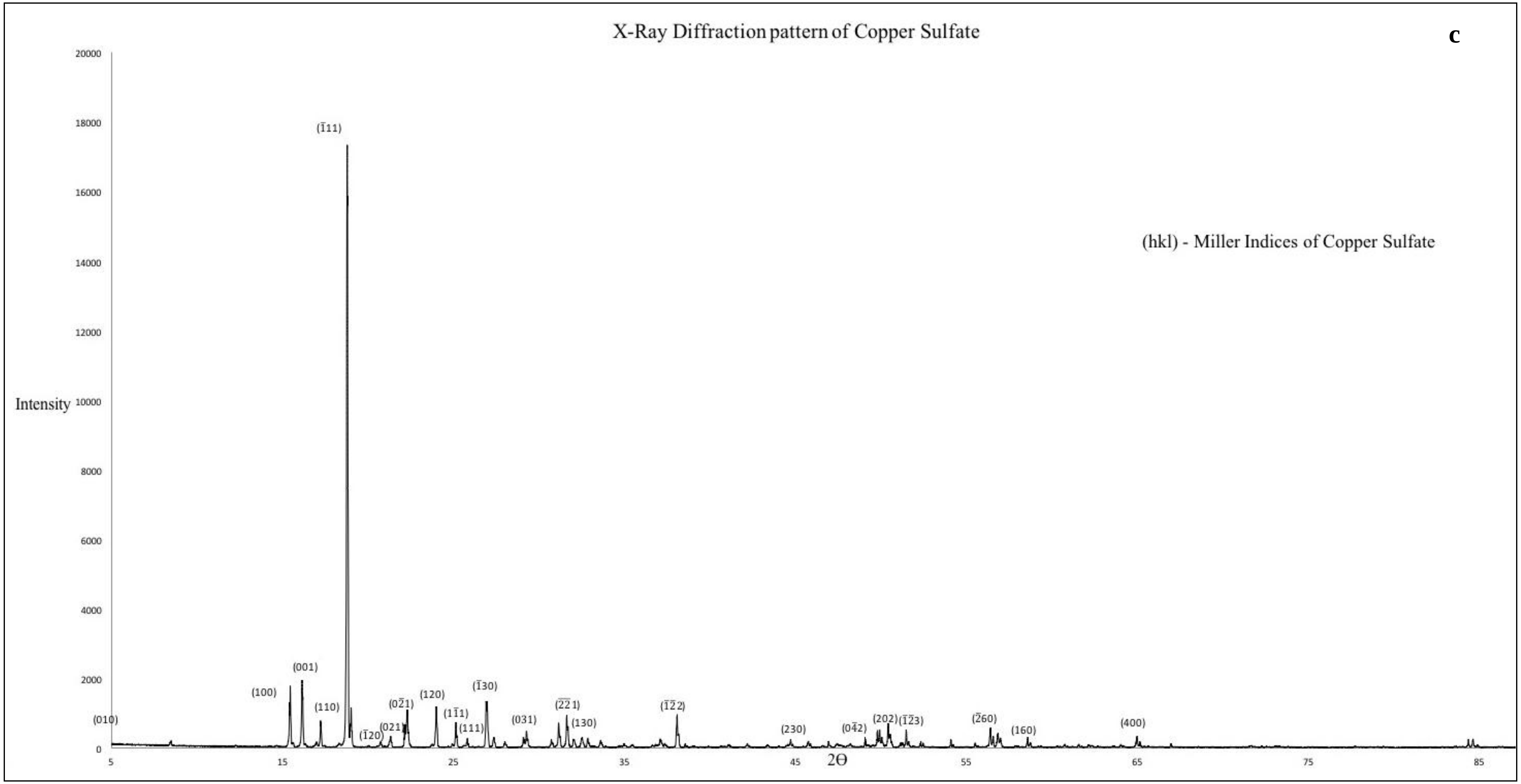

Figure 2c. XRD spectra of (c) copper sulfate exhibited diffraction peaks indicating Chalcanthite ($CuSO_4 \cdot 5H_2O$, JCPDS: #70-1823) which displays a crystalline Triclinic structure.

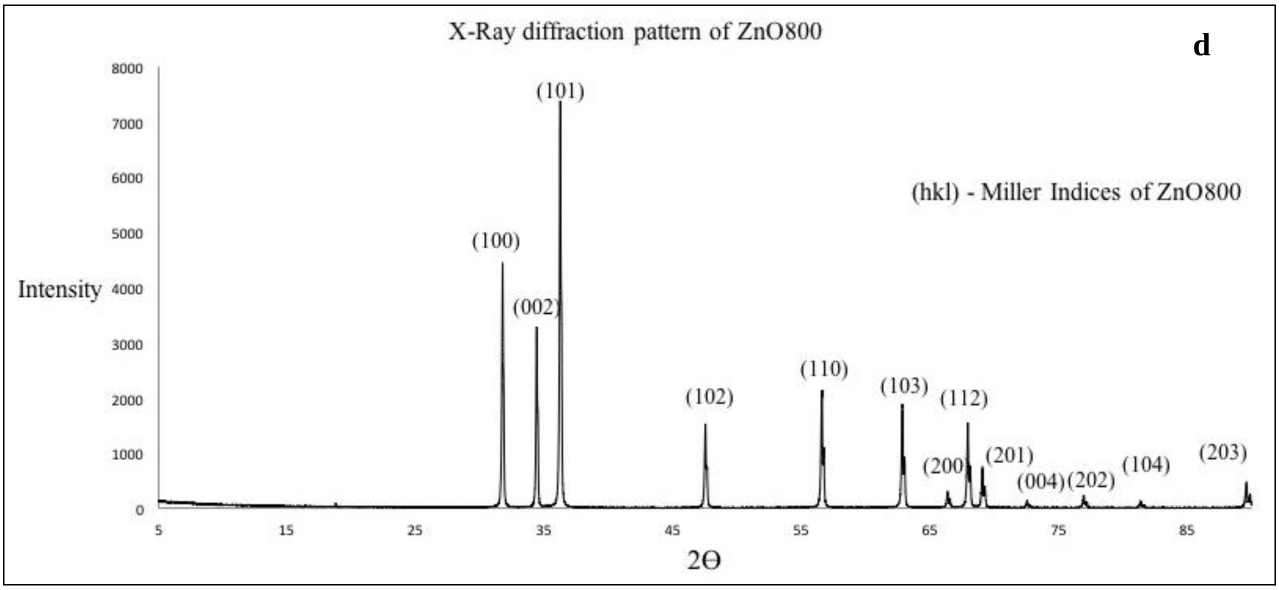

Figure 2d. XRD spectra of ZnO 800 exhibited diffraction peaks indicating Zinc Oxide (JCPDS: #79-0206) which displays a Hexagonal lattice structure.

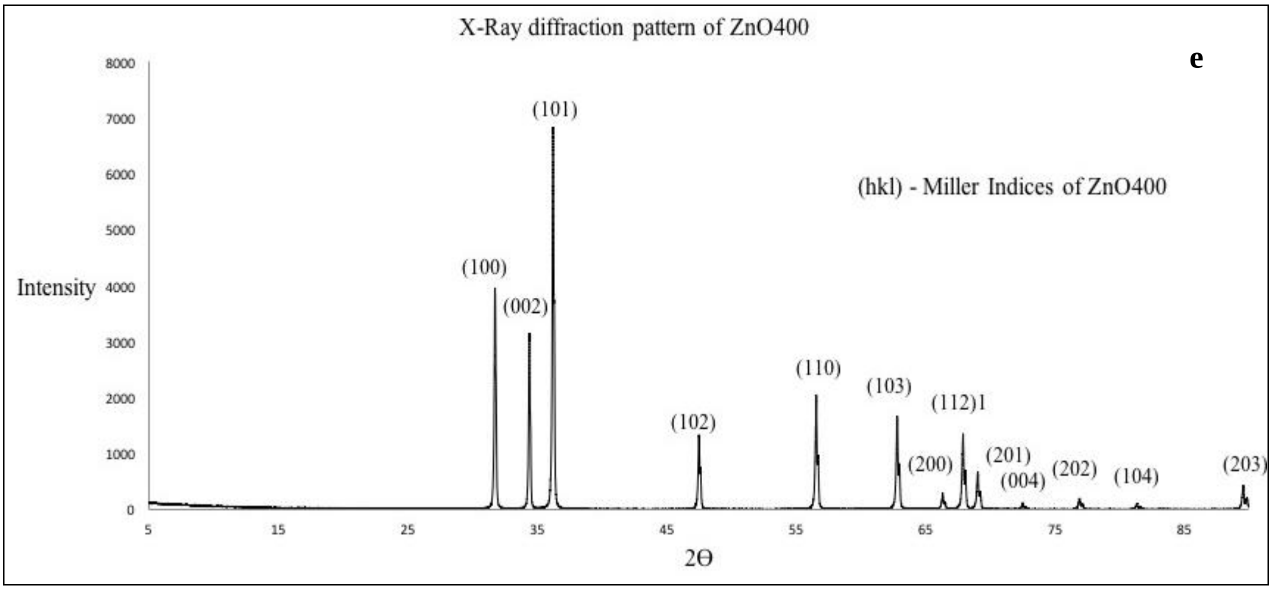

Figure 2e. XRD spectra of ZnO 400 exhibited diffraction peaks indicating Zinc Oxide (JCPDS: #79-0206) which displays a Hexagonal lattice structure.

3.1.5. Surround WP

Due to the very high noise to signal ratio, the diffracted peaks (Figure 2f) were found to be indistinctive amongst all signal and noise peaks which suggested the presence of a very abundant mixture of an amorphous sample in the Surround WP crop protectant. Further, the high and low resolution SEM data shows an extensive network of closely aggregated particles of an uneven size range throughout the sample (Figure 1k & 1l). The lack of an order or periodicity in the size complimented the XRD data that accounted for the amorphous behavior of this biocide.

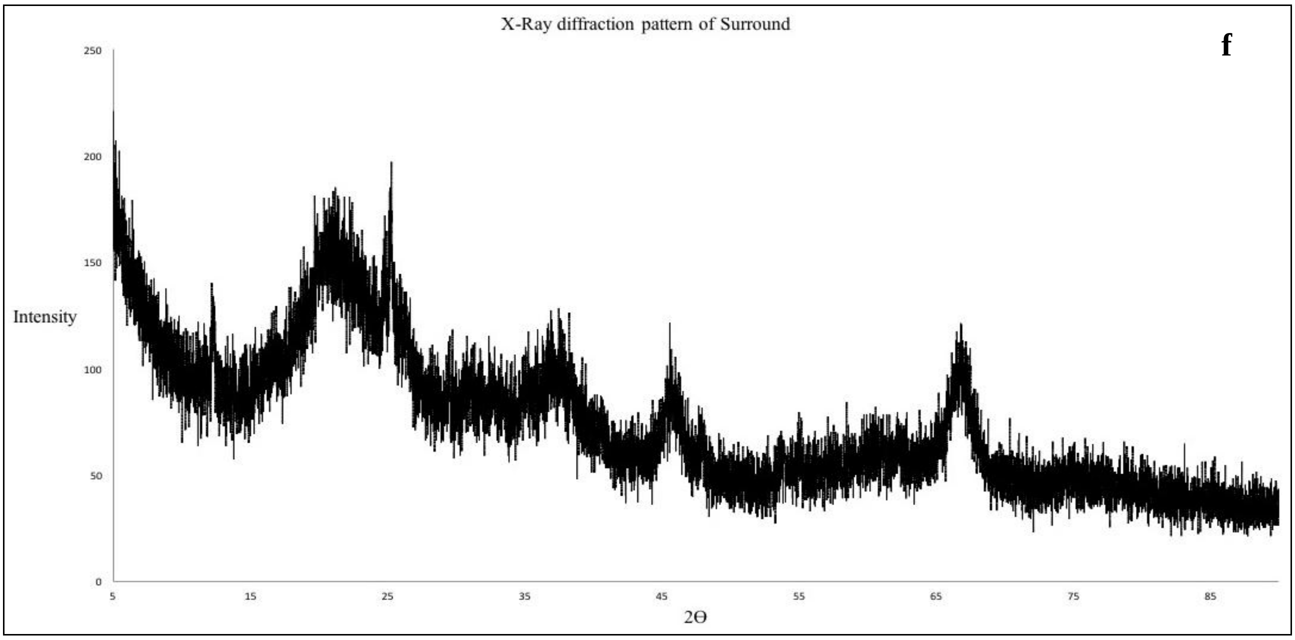

Figure 2f. XRD spectra of Surround WP exhibited diffraction peaks with very high noise to signal ratio indicating a very amorphous material.

Although it was found that the samples differed by individual particle size ranges as mentioned previously, the biocides—Nordox 30/30 and Kocide® 3000 were found to be a composite material. The contrast between high and low resolution SEM data of Kocide® 3000 validated the addition of an amorphous binder in low quantities that were enough to mask the diffraction peaks as observed from the XRD data (Figure 2). In contrast, it is observed that the zinc oxide samples had well defined particle edges and boundaries that account for the perfect crystallinity as reflected by the XRD plot of biocides ZnO 800 and ZnO 400.

3.2. Antimicrobial studies

The antimicrobial properties of commercially available biocides were evaluated by determining the Minimum Inhibitory concentration (MIC) and a bacterial viability assay. Testing against Gram-negative *Xanthomonas alfalfae* subsp *citrumelonis* (ATCC 49120), *Pseudomonas syringae pv. syringae* and Gram-positive *Clavibacter* michiganensis *subsp. michiganensis* revealed MIC values of 125–500 µg/mL for all compounds, with copper (Cu) compounds having higher values such as the case with Kocide® 3000 (250–500 µg/mL) and copper sulfate (125–250 µg/mL) (Table 1). Zinc materials generally exhibited lower MICs with Nordox 30/30 having a value of 125–250 µg/mL while ZnO 400 and 800 both exhibited a MIC of 62.5–125 µg/mL. The higher MIC values of Cu can be attributed to development of resistance of *Xanthomonas* sp and *Pseudomonas* sp to Copper [8]. From Figure 3 and Table 1, it appears that copper sulfate demonstrates a stronger antimicrobial efficacy than Kocide® 3000. Copper hydroxide is the Cu source for Kocide® 3000 and is less water-soluble leading to lower Cu bioavailability than copper sulfate. It is known that Cu (I) is a stronger antimicrobial agent compared to Cu (II) [12,13] and this can be observed in Nordox 30/30 that has better MIC values than Kocide® 3000 [12]. Nordox 30/30 is comprised of cuprous oxide and zinc oxide, thereby providing multiple mechanisms of killing that may explain their increased efficacy particularly bacteria that are resistant to copper and its derivatives. Both MICs and Figure 3 reveal that Nordox 30/30 displayed improved killing over both Cu only materials. Zinc oxide 400 and 800 exhibited strong antimicrobial properties (Table 1 and Figure 3), indicating a lack of resistance of these species to refined zinc particles. Improved efficacy of ZnO 400 to ZnO 800 demonstrated in these materials can be attributed to reduced particle size resulting in higher surface area and increased bioavailability. Further, between the two ZnO particles, the smaller sized ZnO 400 (400 nm sized particles) appears to exhibit better antimicrobial efficacy than the ZnO 800 (800 nm sized nanoparticle) at least against two bacteria viz. *X. alfalfae* and *P. syringae* (Figure 3).

Table 1. Minimal Inhibitory Concentration of agricultural pesticides against various model plant pathogens.

Sample name	*Xanthomonas alfalfae subsp. citrumelonis* (ATCC 49120)	*Pseudomonas syringae pv. syringae* (ATCC 19310)	*Clavibacter michiganensis subsp. michiganensis* (ATCC 10202)
	MIC (μg/mL)	MIC (μg/mL)	MIC (μg/mL)
Kocide® 3000	250–500	250	250
Copper sulfate	250	250	125–250
Nordox 30/30	125–250	125–250	125–250
ZnO 400	125	62–125	62–125
ZnO 800	125	125	62–125

3.3. Phytotoxicity studies

Tests conducted on tomato plants showed severe phytotoxicity for $CuSO_4$, Nordox 30/30 and Kocide® 3000 when treated at 1000 ppm while ZnO 400, ZnO 800 and Surround WP exhibited no toxicity at the same concentration (Figure 3). Since Surround WP is composed of kaolin clay (with no active ingredient), as expected there was no phytotoxicity observed (Figure 4g). To determine the difference in toxicity of these materials, the concentration of 1000 ppm (well above the therapeutic concentration of 200–500 ppm of metallic copper or zinc) was used in these studies. The rationale behind choosing tomato plants was that they are highly sensitive to phytotoxicity and also for the fact that very high levels of Kocide® 3000 are used against bacterial spot disease in tomato these days [14]. The toxicity by copper may be attributed from the disruption of photosynthetic electron transport [15] resulting in leaf burn and discoloration (Figure 4). Copper is an important cofactor for plants, however excess use can disrupt the plant homeostasis and impair the growth [16]. This might explain why there is toxicity observed in copper containing pesticides when applied at higher concentrations. Contrary, zinc is considered to be an essential micronutrient for the plants [17] and can be translocated to younger leaves/fresh shoots if present more than adequately [18]. The zinc based pesticides thus appear to be safer to plant tissues even at high concentrations though we do not know the exact mechanism yet in the samples discussed in this manuscript. Particularly, the ZnO based Zinc nanoparticles; ZnO 400 and ZnO 800 were not toxic at the highest concentrations (1000 ppm) used. It is noteworthy to mention that these two materials exhibited exceptional antimicrobial efficacy with MIC values as low as 125 ppm. This broad therapeutic window would be very helpful in usage of these zinc-based materials against resistant pathogens. Size, chemical composition, surface energy and concentration of different nanomaterials also appear to play a vital role in exerting chemical or physical toxicity in plants. Nanomaterial interaction, surface penetration and translocation within different plant species can help in plant protection, growth and in turn their safety in use as pesticides [9,19,20].

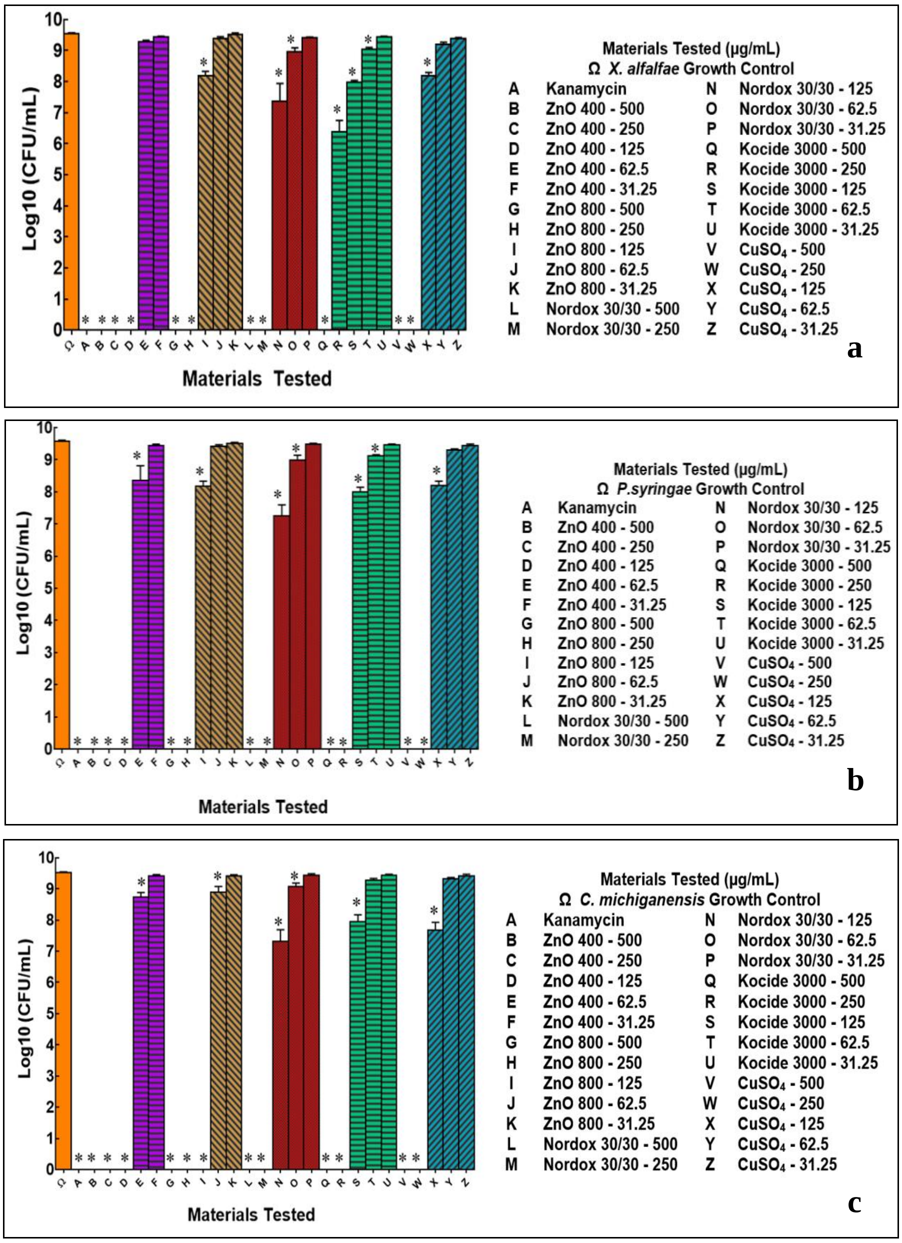

Figure 3. **Colony Forming Unit Assay: Antimicrobial effect of agricultural pesticides against (a)** ***X. alfalfae*** **(b)** ***P. syringae*** **and (c)** ***C. michiganensis*** **viability.** All the compounds were screened for a wide range of metallic concentrations (from 500–31 µg/mL of metallic copper or zinc) Cu compounds exhibited biocidal effects at 250 µg/mL and above while Zn based compounds displayed effects at 125 µg/mL and above. A classical antibiotic kanamycin (50 µg/mL) was used as a control for bacterial killing. The asterisk mark (*) denotes data significantly different from the growth control with $P < 0.05$ using Prism 6.1 two-way Anova.

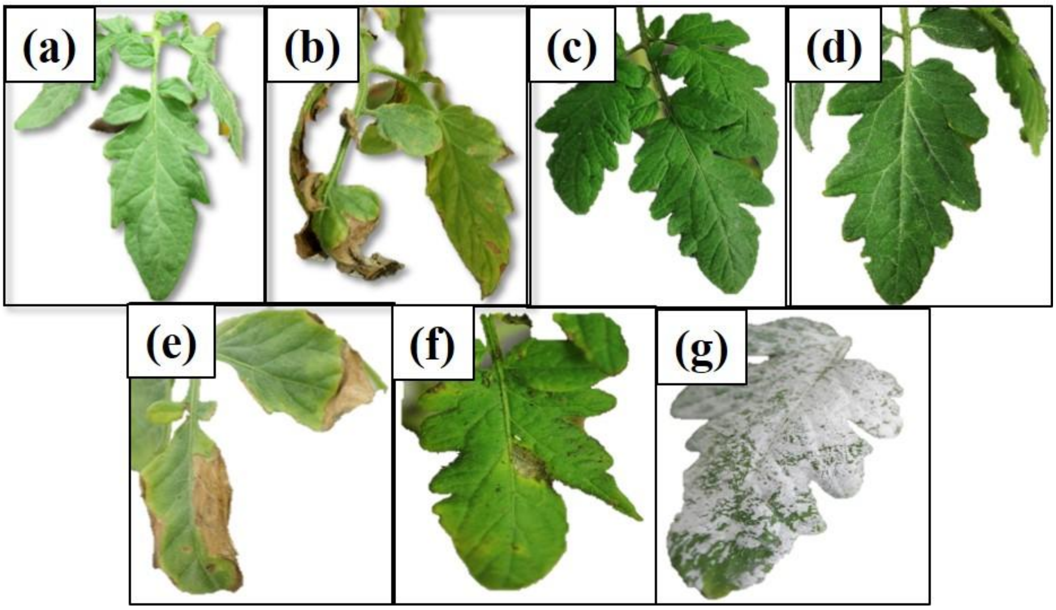

Figure 4. Phytotoxicity in tomato plants: Tomato plants (Solanum *lycopersicum*) exposed to various copper and zinc based pesticides and observations made after 72 hours of exposure. Any change in color from green to brown suggests signs of phytotoxicity. As evident in this figure, both ZnO 400 (c) and ZnO 800 (d) did not produce any toxicity even at 1000 ppm of metallic concentration. Whereas, all the copper containing products (Copper sulfate (b), Kocide® 3000 (e) and Nordox 30/30 (f)) exhibited phytotoxicity (leaf burns) at 1000 ppm metallic concentration. Surround WP (non copper and non zinc) material (g) did not exhibit any phytotoxicity when sprayed at field rate (600 ppm of clay). It is to be noted that the spray rate that is used in this experiment for Kocide® 3000 and Nordox 30/30 is the same concentration that is used in citrus groves where the leaves are more resistant to phytotoxicity and so, these concentrations (1000 ppm) are safe for citrus plants. Tomato plants were chosen for their higher sensitivity to phytotoxicity and also to simulate conditions where copper containing products are used at very high concentrations to contain bacterial spot disease in tomato plants.

4. Conclusion

Overall, it appears that zinc based compounds could be better candidates as biocides. Zinc materials have better safety profile compared to copper based pesticides for highly sensitive tomato plants in the samples that were discussed in this manuscript. Though copper based pesticides have a broad range of antimicrobial efficacy, zinc based biocides appear to be a potential supplement to reduce the complete dependence on copper based pesticides. Thus, zinc based plant protectants appear to be a potential substitute for copper based biocides where there are restrictions to use copper products beyond recommended levels. Additionally, this report would motivate further applied research to develop potent Cu alternatives and as well as improving efficacy of current Cu compounds through nanoscale engineering.

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Conflict of interest

The authors declare there is no conflict of interest.

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