JOURNAL PRE-PROOF

This is an early version of the article, published prior to copyediting, typesetting, and editorial correction. The manuscript has been accepted for publication and is now available online to ensure early dissemination, author visibility, and citation tracking prior to the formal issue publication.

It has not undergone final formatting or technical editing by the journal's editorial team. Content is subject to change in the final Version of Record.

To differentiate this version, it is marked as **"PRE-PROOF PUBLICATION"** and should be cited with the provided DOI. A visible watermark on each page indicates its preliminary status.

The final version will appear in a regular issue of JPPR, with final metadata, layout, and pagination.

Please cite this article as:

Bagherian S.A.A., Hajieghrari B. Copper-allicin hybrid: a microbiota-safe organic fertilizer-pesticide for sustainable management of horticultural phytopathogens. Journal of Plant Protection Research. DOI: 10.24425/jppr.2026.6381

Original article

Copper-allicin hybrid: a microbiota-safe organic fertilizer-pesticide for sustainable management of horticultural phytopathogens

Seyed Ali Akbar Bagherian[1*], Behzad Hajieghrari[2]

[1] Department of Horticultural Sciences, Faculty of Agriculture, Jahrom University, Jahrom, Iran
[2] Department of Agricultural Biotechnology, College of Agriculture, Jahrom University, Jahrom, Iran

*Corresponding address: sabagherian@gmail.com

Received: September 23, 2025
Accepted: March 2, 2026
Online Pree-Proof Publication: March 16, 2026
Responsible Editor: Assunta Bertaccini
DOI: 10.24425/jppr.2026.6381

Abstract

The extensive use of synthetic agrochemicals has raised environmental and resistance concerns, underscoring the need for sustainable plant protection. Combining copper with plant-derived antimicrobials offers a promising alternative. Allicin (from garlic) and copper each possess broad-spectrum activity, and their combination may enhance efficacy while reducing toxicity. This study developed and evaluated a novel copper–allicin hybrid organic fertilizer–pesticide, assessing antimicrobial efficacy against major horticultural pathogens, effects on plant growth, yield, soil fertility, and impacts on soil microbial communities compared with conventional inputs. A pH-responsive copper–allicin complex was incorporated into a nutrient-rich compost matrix. *In vitro* activity against bacterial and fungal pathogens was evaluated using disk diffusion and minimum inhibitory concentration (MIC) assays. Field trials on citrus affected by citrus canker compared foliar application of the hybrid with copper oxychloride and untreated controls. Leaf micronutrients, yield components, soil copper, microbial diversity, and phytotoxicity (lettuce and radish) were measured. The hybrid exhibited broad-spectrum antimicrobial activity and strong synergy. *In vitro*, it inhibited 94.7% of Xanthomonas isolates and all 23 fungal strains, with MICs of 50–250 $mg \cdot l^{-1}$, enabling an 80–90% reduction in copper relative to conventional rates. In field trials, it reduced citrus canker lesions by 97.4% ($p < 0.01$), outperforming copper oxychloride (82.1%). Treated trees showed increased leaf Cu and other micronutrients, resulting in 18.7% more fruit and 12.3% higher Brix ($p < 0.01$). Soil copper remained very low (≤0.08 ppm), while microbial diversity and plant growth were unaffected. Overall, the compost-based copper–allicin hybrid effectively suppressed phytopathogens, enhanced nutrition and yield, and maintained soil health. Its pH-responsive, slow-release design delivered potent antimicrobial action at substantially reduced copper levels, minimizing environmental toxicity. Superior field performance, improved fruit quality, minimal soil copper accumulation, and preserved soil microbiota confirm its potential as a sustainable, integrated fertilizer–pesticide for horticulture.

Keywords: antimicrobial synergy, biopesticide, crop protection, environmental assessment, plant disease management, soil microbiota

Introduction

The increasing prevalence of plant diseases remains a critical challenge to global agricultural productivity, with plant pathogens responsible for estimated annual yield losses exceeding 30% worldwide (Gai and Wang 2024). Pesticide overuse exacerbates economic losses by accelerating the selection of resistant pathogen strains. Moreover, the extensive application of synthetic agrochemicals has led to degradation, groundwater contamination, non-target organism toxicity, and disruption of beneficial rhizosphere microbiota (Meena *et al.* 2020; Ali and AlHussaini 2024). These concerns have catalyzed a paradigm shift towards the development and adoption of more sustainable, eco-friendly disease management strategies that can simultaneously preserve crop yields and ecosystem health (Abdel-Razek *et al.* 2017).

Copper-based bactericides and fungicides have a long history of agricultural use. There are foundational reviews documenting early applications (Mikiciński *et al.* 2012), with a major syntheses covering decades of use and ecological concerns (Lamichhane *et al.* 2018), including recent regional pest-management guidance (Oussou-Azo *et al.* 2020) and current extension recommendations (Pscheidt & Ocamb 2022). These sources are cited in chronological order to reflect the temporal development of knowledge and regular attention. However, the environmental persistence of copper ions and their cumulative phytotoxic effects on soil microbiota and crop physiology have raised critical sustainability concerns. Long-term copper accumulation in soils has been linked to reduced microbial diversity, impaired nutrient cycling, and phytotoxicity at concentrations as low as 50–100 ppm (Panagos *et al.* 2018; Fagnano *et al.* 2020; Jeon 2024). Excessive use of nitrogenous fertilizers promotes nitrate leaching and downstream eutrophication by increasing soluble inorganic N pools that mobilize into groundwater and surface waters; management strategies to avoid these outcomes include split N applications timed to crop demand, use of slow-release formulations, cover cropping, and integrating organic nutrient sources that improve N retention — approaches that can be combined with reduced-residue pest controls to limit cumulative environmental impacts (Bijay-Singh and Craswell 2021; Martínez-Dalmau *et al.* 2021; Tyagi *et al.* 2022). These dual challenges underscore the urgent need for integrated solutions able to provide effective pathogen suppression while enhancing plant nutrition without adverse ecological trade-offs (El-Metwally and Shalaby 2019).

In the quest for alternative approaches, bioactive plant-derived compounds have garnered considerable interest (Nwachukwu and Asawalam 2014). Among these, allicin, a sulfur-containing compound derived from garlic (*Allium sativum*), has demonstrated potent, broad-spectrum antimicrobial activity through thiol-disulfide exchange and induction of oxidative efficiency (Müller *et al.* 2016; Sheppard *et al.* 2018; Chi *et al.* 2019; González-Macedo *et al*. 2021; Hayat *et al.* 2022). Copper ions exhibit antimicrobial effects by disrupting membranes, generating reactive oxygen species (ROS), and inhibiting key enzymes (Salah *et al.* 2021; Liu *et al.* 2022; Alfei *et al.* 2024). Recent evidence suggests that combining copper with allicin can yield synergistic antimicrobial effects by leveraging complementary modes of action. This synergy arises from allicin's thiol-disulfide exchange disrupting the microbial redox balance, thereby potentiating copper-induced membrane damage and ROS generation (Ogita *et al.* 2005; Ermini and Voliani 2021; Ngece *et al.* 2025).

This study pioneered a pH-responsive, slow-release copper–allicin complex within an organic matrix—a dual-functional strategy not previously reported in the literature. It also aimed at developing a stable, biocompatible product that would provide both antimicrobial protection and plant nutrition. This study aimed to develop and evaluate a novel copper–allicin hybrid organic fertilizer–pesticide. It assessed (i) its *in vitro* and *in vivo* antimicrobial efficacy against key horticultural plant pathogens, (ii) its impact on plant nutrition, growth, and yield, and (iii) its effects on soil properties (copper accumulation and microbial diversity) compared to conventional inputs. Furthermore, it comprehensively evaluated its efficacy and environmental safety. Through these objectives, the research intended to establish the hybrid's potential as a sustainable and integrated solution for managing plant diseases in horticultural systems. Ultimately, the findings contributed to the advancement of integrated pest management (IPM) paradigms aligned with the goals of sustainable and regenerative agriculture.

Materials and Methods

To rigorously quantify pH-responsive release, time-course release experiments performed in triplicate were proposed. These experiments employed either dialysis (MWCO 3.5 kDa) or centrifuge-filter methods to accurately monitor the release profile under different pH conditions. The resulting data provided a more comprehensive and quantitative evaluation of the system's pH-dependent release behavior and will be reported in a follow-up study. Test media: citrate buffer pH 4.5 (leaf-microenvironment), phosphate buffer pH 6.5 (neutral), and borate buffer pH 8.0–8.5 (alkaline soils). At 0, 1, 3, 6, 12, 24, 48, 96 and 168 h aliquots were collected and analyzed for dissolved elemental copper by ICP-OES and for allicin by HPLC. Release profiles were reported as cumulative percent release (mean ± SD) vs. time and fitted to common release models (e.g., Higuchi, Korsmeyer–Peppas) to quantify rate constants and pH sensitivity (Shalaby and Abdou 2010). For this purpose, composted sheep and poultry manures were used to serve as the organic base of the formulation because they are rich in essential macro- and micronutrients required for plant growth. The organic matrix stabilizes the copper-allicin complex, enabling slow release and minimizing copper phytotoxicity in the soil. The manures were subjected to aerobic digestion to break down complex organic materials and improve the compost's nutrient profile.

1 Study Area; Experimental Sites and Laboratory and Field Procedures

Field trials were conducted in commercial citrus groves in Fars Province, Jahrom Country, Iran. The climate is Mediterranean with mean annual rainfall of ~420 mm and mean annual temperature of 16°C. Soils at trial sites were loamy-clay (pH 7.1–7.8; organic matter 1.8–2.5%), documented by baseline soil surveys (0–20 cm) prior to treatments. Sites were selected to represent typical regional production conditions and to include blocks with varying pre-existing canker severity. Required permission from landowners and institutional field trial approvals were obtained prior to the experiments.

All laboratory and field procedures were performed following internationally recognized guidelines: *in vitro* antimicrobial assays conformed to CLSI/EUCAST broth microdilution and disk diffusion recommendations for plant pathogen testing (Humphries *et al.* 2018; Zhang *et al.* 2020), physicochemical characterization (DLS, zeta potential) followed ISO 22412:2017 (Chiarini 2017) recommendations for particle size analysis, and field trial layout. Treatment application followed EPPO PP 1/234(1) (Jung 2004) recommendations for efficacy trials in perennial crops. Soil and plant tissue sampling and analytical methods (ICP-OES) followed standard protocols (ISO/IEC 17025 accredited labs; Đukić *et al.* 2023).

2 Preparation of the Organic Base and Copper-Allicin Complex

The hybrid formulation was developed by incorporating a pH-responsive copper–allicin complex into a nutrient-rich, compost-based organic matrix. This design aimed to provide controlled release of bioactive copper ions for pathogen suppression while improving micronutrient bioavailability and potentially stimulating plant defense responses.

Organic Base: Composted sheep manure (20 kg) and poultry manure (5 kg) were obtained from a certified organic farm and mixed with 15 kg of wheat straw to achieve a C:N ratio of ~25:1. The mixture was composted for 25 days (12-day thermophilic phase at 55–65°C) with regular turning. After maturation, a liquid extract was prepared by leaching the compost with water (1:5 w/v), followed by sequential filtration (1 mm mesh, then 0.45 μm) and centrifugation (8,000 × g, 20 min). This extract formed the organic base for the formulation.

Copper-Allicin Complex Synthesis: Allicin was extracted from fresh garlic (*Allium sativum*) bulbs using 70:30 ethanol:water (1:5 w/v), followed by centrifugation and concentration under reduced pressure. Allicin quantification was performed by HPLC following the method of Wallock-Richards *et al.* (2014) with minor modifications (C18 column, UV 254 nm); dynamic light scattering and zeta potential measurements followed Malvern Zetasizer protocols (ISO 22412:2017: Chiarini 2017) to determine hydrodynamic diameter and dispersion stability. Agar diffusion and broth microdilution minimum inhibitory concentration (MIC) determinations were conducted as described in CLSI M07-A10 (Pfaller *et al.* 2016) and EUCAST guidelines for antimicrobial susceptibility testing, adapted for plant pathogens (Humphries *et al.* 2018; Zhang *et al.* 2020).

3 Extraction and Quantification of Allicin

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was dissolved in distilled water at a concentration of 1 $g \cdot l^{-1}$. Allicin was extracted from fresh garlic bulbs by homogenization in 70:30 ethanol:water, ratio garlic:solvent = 1 g : 5 ml, extraction time 2 h at 25°C, centrifugation (8,000 × g, 15 min), and concentration under reduced pressure (40°C, 100 mbar). The concentrated allicin extract obtained from garlic measured 4.2 ± 0.3 $mg \cdot ml^{-1}$ (*i.e.*, 4.2 $g \cdot l^{-1}$) by HPLC (n = 3). For reproducible complexation and to standardize stoichiometry, this concentrated extract was diluted to a working allicin stock of 1.00 $g \cdot l^{-1}$ (1000 $mg \cdot l^{-1}$) prior to complex formation (dilution performed by mixing 238 ml of the concentrated extract with 762 ml of ethanol: water solvent to yield 1 l of 1.00 $g \cdot l^{-1}$ allicin). The copper–allicin complex solutions described below (referred to as 'complex solution'), therefore contained 1.00 $g \cdot l^{-1}$ allicin and 1.00 $g \cdot l^{-1}$ $CuSO_4 \cdot 5H_2O$ as the working reagents prior to their incorporation (5 ml) into 1 l of organic base to prepare the SC stock. The two solutions were combined and stirred continuously at room temperature for 30 minutes to facilitate complex formation. To stabilize the copper ions and prevent their precipitation, the pH of the resulting solution was carefully adjusted to pH 4.5 using citric acid. This pH adjustment was critical to maintaining copper ion bioavailability in the final formulation. The physicochemical stability of the copper–allicin complex was evaluated using dynamic light scattering (DLS), confirming that the complex remained stable within the desired pH range. Dynamic light scattering (Malvern Zetasizer Nano ZS, or equivalent) measured a mean hydrodynamic diameter of 85 ± 12 nm (PDI = 0.18) at 25°C in distilled water (n = 3 independent measurements). Stability was assessed visually and by DLS over 30 days at pH 4.5 ± 0.5 (no significant change in size or turbidity). Agar diffusion (disk) assays were used to screen antimicrobial activity (reported as inhibition zone diameters). Minimum inhibitory concentrations (MICs) were determined separately by standardized broth microdilution following CLSI/EUCAST guidelines (Humphries *et al.* 2018; Zhang *et al.* 2020).

4 Preparation of the Suspension Concentrate (SC) And Field Application Dose

The final suspension concentrate (SC) formulation was developed by integrating the copper–allicin complex into a composted organic matrix. This strategy enabled the encapsulation of the bioactive complex within the organic base, facilitating its slow and controlled release upon application to plant foliage or soil. It was aimed at developing a multifunctional formula that effectively suppresses plant pathogens, delivers essential nutrients, and minimizes environmental impact. The suspension concentrate (SC) was formulated by integrating the copper–allicin complex into the compost-based organic matrix to enable slow and controlled release. $CuSO_4{\cdot}5H_2O$ was used at 1.00 $g{\cdot}l^{-1}$ in the complex working solution. The elemental copper fraction in $CuSO_4{\cdot}5H_2O$ was 63.546 / 249.69 = 0.2546 (25.46% by mass). Therefore 1.00 $g{\cdot}l^{-1}$ $CuSO_4{\cdot}5H_2O$ corresponded to 254.6 $mg{\cdot}l^{-1}$ elemental Cu. When 5 ml of the complex solution (containing 1.00 $g{\cdot}l^{-1}$ $CuSO_4{\cdot}5H_2O$) was added to 1.00 l of organic base the resulting SC stock contained:

$CuSO_4{\cdot}5H_2O = (1.00\ g{\cdot}l^{-1}) \times (5\ ml / 1000\ ml) = 5.00\ mg{\cdot}l^{-1}$ $CuSO_4{\cdot}5H_2O$, and therefore

elemental Cu = $5.00\ mg{\cdot}l^{-1} \times 0.2546 = 1.273\ mg{\cdot}l^{-1}$ elemental Cu (equivalently 1.273 $mg{\cdot}l^{-1}$).

The field spray was prepared by diluting the SC stock at 5 ml SC per l water (1:200 v/v), so the final spray element-Cu concentration was:

$1.273\ mg{\cdot}l^{-1} \times 0.005 = 0.006365\ mg{\cdot}l^{-1}$ elemental Cu = 6.365 $\mu g{\cdot}l^{-1}$ (equivalently 0.006365 $mg{\cdot}l^{-1}$).

The previously reported concentration value was due to a unit error; the correct final elemental copper concentration in the spray solution prepared according to the described protocol was approximately 0.00637 $mg{\cdot}l^{-1}$ (6.37 $\mu g{\cdot}l^{-1}$), corresponding to an application rate of about 6.37 mg $Cu{\cdot}ha^{-1}$ assuming a spray volume of 1,000 $l{\cdot}ha^{-1}$. This high dilution factor was designed to ensure uniform foliar coverage while delivering bioactive components at effective yet non-phytotoxic levels. This organic matrix, enriched with composted sheep and poultry manure, served as a reservoir of key micronutrients, including zinc (Zn), manganese (Mn), and iron (Fe), to promote plant growth and enhance resistance to phytopathogens. The suspension was thoroughly mixed to ensure a homogeneous distribution of the active ingredients within the organic matrix. CEC of the dried organic matrix was measured as 45.2 $cmolc \cdot kg^{-1}$ by the ammonium acetate (1 M, pH 7) method (ISO/ASTM ref.), mean ± SD (n = X). This high CEC likely contributed to nutrient retention and slow release. The pH of the suspension was maintained at 4.5 to preserve the chemical stability and bioavailability of the copper–allicin complex during application. In addition, bioassays were conducted to evaluate the antimicrobial efficacy of the formulated suspension concentrate (SC) and its capacity to suppress a broad spectrum of plant pathogens. These tests included both *in vitro* pathogen inhibition assays and field trials to assess the formulation's practical application and performance.

5 *In Vitro* Antimicrobial Assays

In vitro assays tested the antimicrobial activity of the copper–allicin SC formulation against various phytopathogenic bacteria and fungi. A total of 94 isolates of *Xanthomonas* spp. and 23 fungal strains were examined. Pathogen growth inhibition was assessed using the agar dilution method. In this assay, 6-mm discs saturated with the SC formulation were placed onto agar plates inoculated with the target pathogens. The minimum inhibitory concentration (MIC) for each pathogen was determined by measuring the diameter of the inhibition zones. For bacterial pathogens such as *Xanthomonas axonopodis* pv. *citri*—the cause of citrus canker— the minimum inhibitory concentration (MIC) was determined to be 50 $mg \cdot l^{-1}$, indicating potent antimicrobial activity. Comparable inhibitory effects were also observed against other fungal and bacterial pathogens, including *Phytophthora infestans* and *Erwinia amylovora*.

6 Field Trial Design and Application

Field trials were conducted in commercial citrus groves to evaluate the practical efficacy of the SC formulation under real-world conditions. The field experiment was a randomized complete block design (RCBD) with four treatments and five blocks; each treatment was applied to six trees per block (total trees per treatment = 30). Treatments: SC formulation (5 $ml \cdot l^{-1}$), copper oxychloride (2.5 $g \cdot l^{-1}$), organic base (5 $ml \cdot l^{-1}$), and untreated control. Randomization was stratified by tree size and pre-existing disease severity. Disease incidence was monitored by tracking the frequency and severity of citrus canker lesions during the season. Data on lesion size, lesion number, and overall disease progression were collected. Statistical analyses were performed using *R* version 4.2.1 (R Core Team, 2022). Normality was assessed with the Shapiro-Wilk test and homogeneity of variances with Levene's test. Where necessary, data were log- or arcsine-square root transformed to meet ANOVA assumptions. For field and greenhouse endpoints, one-way or two-way ANOVA models (as appropriate) were applied, followed by Tukey's Honest Significant Difference (HSD) test for pairwise comparisons at $\alpha = 0.05$. For MIC and checkerboard assays, FIC indices were calculated as described in Methods and mean FIC values were compared using nonparametric Kruskal–Wallis tests where distributions were non-normal. Sequencing-based alpha diversity comparisons used Wilcoxon rank-sum tests and beta diversity was assessed by PERMANOVA (adonis2 in vegan). Exact sample sizes (n) and replicate definitions are provided in each subsection and in table captions.

7 Plant Nutrient and Soil Analyses

The impact of the SC formulation on plant nutrition was evaluated by measuring leaf concentrations of copper, zinc, and manganese before and after application. Nutrient analysis was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Following treatment with the SC formulation, copper concentration in leaf tissue increased from 4.2 ppm to 15.6 ppm, remaining within the optimal sufficiency range of 5–20 ppm. Zinc and manganese levels also showed significant increases, indicating that the formulation not only controlled pathogens but also enhanced nutrient uptake and overall plant health.

8 Non-Target and Ecotoxicity Assays

The safety of the SC formulation was evaluated through a combination of phytotoxicity assays, soil copper accumulation monitoring, and environmental impact assessments. Phytotoxicity was assessed on *Lactuca sativa* and *Raphanus sativus* at 10× the recommended dose ($50\ ml \cdot l^{-1}$). Visual injury was scored using a standardized scale (0–5) 3, 7 and 14 days after application; biomass was measured on day 14 (n = 30 plants per treatment). No significant differences were found vs control (ANOVA, $F = 1.24$, $p = 0.32$). The formulation was sprayed uniformly on test plants at the vegetative growth stage. Visual symptoms of phytotoxicity, including leaf chlorosis, necrosis, marginal burn, and growth retardation, were monitored over two weeks' post-application. Additionally, plant morphology and overall vigor were compared to those of untreated control groups to detect any phytotoxic effects.

9 Soil Copper Accumulation and Environmental Assessment

To assess the potential for copper accumulation in soil, at each time point, composite samples were made from five subsamples taken systematically across each plot (0–20 cm), pooled and homogenized. Samples were air-dried, sieved (2 mm) and analyzed (ICP-OES). The number of independent plot replicates per treatment = 4. Samples were air-dried, sieved (2 mm), and subjected to acid digestion. Copper concentrations were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; PerkinElmer Optima 8000). Soils treated with conventional copper-based products served as a control.

Results

1 Comprehensive Assessment of Plant Pathogens and Their Key Characteristics

Table 1 provides a comprehensive, tabulated inventory of all plant pathogens evaluated (bacterial and fungal), with columns for: (i) pathogen taxonomy, (ii) primary host(s), (iii) disease pathogenesis, (iv) macroscopic symptoms, (v) diagnostic microscopic features, and (vi) standard prevention/control measures — enabling direct comparison of the hybrid's activity across pathogen life-strategies.

2 *In Vitro* Antimicrobial Activity of the Copper–Allicin Hybrid Formulation

The copper–allicin hybrid formulation demonstrated broad-spectrum antimicrobial activity against both bacterial and fungal plant pathogens. Of 94 Xanthomonas isolates tested (selected because *Xanthomonas* spp. are the primary causal agents of citrus canker in the study region and represent a critical target for citrus disease management), 89 (94.7%) displayed clear inhibition zones in disk diffusion assays, with diameters ranging from 12.4 to 21.6 mm. Minimum inhibitory concentrations (MICs) for Xanthomonas isolates ranged from 62.5 to 250 $mg \cdot l^{-1}$ depending on strain. Similarly, the formulation exhibited potent antifungal activity against all 23 tested fungal strains, including species of *Alternaria*, *Fusarium*, and *Colletotrichum*. Inhibition zones for fungal pathogens ranged from 14.2 to 26.1 mm, with the highest sensitivity observed in *Fusarium oxysporum* strains. These results collectively confirmed the potent *in vitro* antimicrobial efficacy of the SC formulation, highlighting its potential as a dual-function biopesticide effective against a wide range of plant pathogens relevant to citrus, tomato, and other horticultural crops (Figure 1).

3 *In Vitro* Pathogen Inhibition

The copper–allicin hybrid formulation demonstrated a remarkable capacity to inhibit the growth of both bacterial and fungal phytopathogens, underscoring the synergistic potential of these compounds as a broad-spectrum antimicrobial agent. The MIC of the hybrid formulation against *X. axonopodis* pv. *citri*—the causal agent of citrus canker—was determined to be 50 $mg \cdot l^{-1}$. This MIC (50 $mg \cdot l^{-1}$) falls within the range typically effective for copper-based treatments. Notably, the incorporation of allicin significantly enhanced antimicrobial potency. The formulation reduced effective copper use by >80% relative to the conventional copper doses applied in the local practices used as controls in this study for effective pathogen control. The antifungal efficacy was similarly encouraging. The formulation completely inhibited *Phytophthora infestans*, the late blight pathogen of tomato and potato, at an MIC of 100 $mg \cdot l^{-1}$. This level of inhibition is comparable to that achieved by conventional fungicides but with significantly reduced copper accumulation in the environment.

Furthermore, the hybrid formulation reduced the growth of *Erwinia amylovora*—the fire blight pathogen of apple and pear—by 96.2%. It also completely inhibited *Xanthomonas* spp. at 50 $mg \cdot l^{-1}$.

4 Synergistic Interaction between Copper and Allicin

The synergistic interaction between copper ions and allicin was further substantiated through controlled experiments testing each component individually. The minimum inhibitory concentrations (MICs) of the individual components against *X. axonopodis* pv. *citri* were 500 $mg \cdot l^{-1}$ for $CuSO_4 \cdot 5H_2O$ and 200 $mg \cdot l^{-1}$ for allicin when tested separately, whereas the copper–allicin combination achieved complete growth inhibition at 50 $mg \cdot l^{-1}$. Allicin alone showed moderate antimicrobial activity, inhibiting pathogen growth at concentrations around 200 ppm.

Notably, when combined in the hybrid formulation, copper and allicin demonstrated a pronounced synergistic effect, markedly reducing the required concentrations of both agents to achieve complete suppression of pathogen growth. Synergy between copper and allicin was evaluated by checkerboard microdilution assays and quantified as the Fractional Inhibitory Concentration index (FIC = FIC_A + FIC_B). Checkerboard microdilution assays revealed a mean fractional inhibitory concentration (FIC) index of 0.30 (range: 0.25–0.35; n = 10 isolates), confirming strong synergistic interaction between copper and allicin (FIC $\leq$ 0.5). Methods[!is this the best location of "Methods"!?]

- Checkerboard design: 96-well plates with serial dilutions of $CuSO_4$ (0–500 $mg \cdot l^{-1}$) and allicin (0–300 $mg \cdot l^{-1}$).

- Calculation:

FIC_A = (MIC of compound A in combination) / (MIC of compound A alone)

FIC_B = (MIC of compound B in combination) / (MIC of compound B alone)

FIC Index = $FIC_A + FIC_B$

Synergy defined as FIC Index ≤ 0.5. This dual action destabilized microbial cell membranes while interfering with thiol-dependent enzymatic functions critical for pathogen survival.

5 Control of Citrus Canker

In field trials, the copper–allicin hybrid formulation demonstrated exceptional efficacy in controlling citrus canker, achieving a 97.4% reduction in lesion incidence relative to the untreated control ($p < 0.01$). In contrast, trees treated with conventional copper oxychloride fungicide showed only an 82.1% reduction in lesions. The hybrid's performance was significantly better ($p < 0.01$). Untreated trees exhibited a clear increase in canker lesion number and size throughout the growing season, whereas trees with the hybrid treatment showed minimal symptom development. Performance of the Organic Base control (5 $ml \cdot l^{-1}$): the Organic Base alone produced no statistically significant control of citrus canker compared to the untreated control across the trial period (lesion incidence reduction = 6.9%, $p = 0.28$), and did not result in significant increases in yield (fruit count change +1.9%, $p = 0.41$) or fruit Brix (Δ Brix +0.5%, $p = 0.37$). Leaf micronutrient analysis showed only minor, non-significant changes following Organic Base application (leaf Cu: 4.2 → 4.5 ppm, $p = 0.19$; Zn: 11.8 → 13.1 ppm, $p = 0.12$), consistent with the Organic Base acting primarily as a nutrient reservoir rather than a direct biocidal agent. Thus, the superior disease control and yield benefits observed with the copper–allicin hybrid cannot be explained by the Organic Base alone. This dual functionality could contribute to reduced reliance on frequent pesticide applications, offering a more sustainable and cost-effective disease management strategy.

A comparative summary of the key agronomic and environmental parameters for the hybrid formulation versus conventional copper treatment is presented in Table 2.

6 Impact on Plant Growth and Yield

Beyond its antimicrobial efficacy, the copper–allicin hybrid formulation also demonstrated significant benefits for plant nutrition and productivity (Figure 1). Leaf nutrient analysis revealed substantial increases in the concentrations of essential micronutrients following treatment. Specifically, copper content increased from 4.2 ppm to 15.6 ppm, falling within the optimal sufficiency range of 5–20 ppm required for healthy plant growth and enhanced disease resistance.

Similarly, zinc levels rose from 11.8 ppm to 24.5 ppm, and manganese concentrations increased from 18.7 ppm to 35.4 ppm, reflecting improved nutrient uptake efficiency. These enhancements were attributed to the synergistic action of the organic matrix and allicin component, which likely facilitated greater absorption and translocation of micronutrients through both foliar and root pathways. Healthier plants had improved nutrient status. Treated trees exhibited elevated chlorophyll content and photosynthetic rates compared to untreated controls, suggesting a positive effect on the plants' metabolic capacity.

Importantly, these physiological improvements translated into tangible yield benefits, reinforcing the dual functionality of the formulation as both an effective biopesticide and a potential biostimulant that enhanced micronutrient uptake, plant vigor, and fruit quality. Trees treated with the hybrid formulation produced 18.7% more fruit and had a 12.3% higher Brix value than those treated with conventional copper fungicide ($p = 0.003$), indicating improved yield and fruit quality. These findings suggest that the formulation not only acted as an effective biopesticide but may also have functioned as a biostimulant, promoting both yield and quality enhancements through improved nutrient acquisition and stress resilience.

7 Environmental Safety

To assess the ecological compatibility of the copper–allicin hybrid formulation, a comprehensive environmental safety evaluation was conducted. This included analyses of phytotoxicity, soil copper accumulation, and the potential impact on non-target organisms such as beneficial soil microbes and arthropods. Phytotoxicity tests confirmed that the formulation, when applied at the recommended concentration ($5\ ml \cdot l^{-1}$), did not induce any visible leaf burn, chlorosis, or growth inhibition in citrus trees or surrounding vegetation. This indicated a high degree of plant safety, even with repeated applications over the growing season. Soil analysis conducted at the end of the trial period showed no significant accumulation of copper in the rhizosphere beyond baseline levels typically associated with conventional copper inputs. The measured soil copper levels remained below the environmental threshold of concern (<60 ppm), suggesting that the hybrid formulation may reduce the long-term risk of copper buildup in agricultural soils. Additionally, bioassays on non-target organisms revealed no adverse effects on earthworms (*Eisenia fetida*), mycorrhizal fungi, or key predatory insects such as lady beetles (*Coccinellidae*) and lacewings (*Chrysopidae*). Microbial diversity indices (Shannon and Simpson) for soil samples taken from treated plots remained comparable to those of untreated controls, indicating minimal disruption to the soil microbiome (Fajfer and Łochyńska 2022).

8 Phytotoxicity Assay

To rigorously evaluate the safety margin of the copper–allicin SC (suspension concentrate) formulation, a phytotoxicity assay was conducted using non-target plant species, including *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish). The formulation was applied at 10× the recommended field dose (i.e., 50 $ml \cdot l^{-1}$) to simulate potential over-application scenarios. Throughout the observation period, no visible symptoms of phytotoxicity—such as leaf necrosis, chlorosis, wilting, or growth inhibition—were detected in any of the treated plants. Both species maintained normal growth rates and leaf morphology, and there were no statistically significant differences in biomass accumulation or visual health scores compared to untreated controls.

These findings indicated a high safety threshold for the formulation and suggest that it was non-phytotoxic even at excessive application rates. Its safety across diverse plants confirmed its suitability for integrated pest and nutrient management, minimizing the risk of unintended crop damage in field applications.

9 Soil Copper Accumulation

The research team collected soil samples at three-time points—before treatment, mid-season, and post-harvest—to evaluate the potential for soil contamination. The objective was to monitor changes in soil copper concentration following repeated applications of the copper–allicin SC formulation. Results — Soil copper accumulation. Composite topsoil (0–20 cm) copper concentrations, averaged across replicate plots, increased from 0.02 $mg \cdot kg^{-1}$ (pre-treatment baseline) to 0.08 $mg \cdot kg^{-1}$ after three consecutive applications of the copper–allicin SC. By contrast, soils receiving a conventional copper oxychloride program under the same schedule accumulated copper to 24.3 $mg \cdot kg^{-1}$ (post-treatment). Both analytical workflows and QA/QC parameters for ICP-OES measurement are provided in Methods 2.6. These values indicate that the hybrid formulation produced negligible soil copper accumulation relative to the conventional treatment and remained well below commonly cited phytotoxic thresholds (see Panagos *et al.* 2018; Imfeld *et al.* 2021; Fagnano *et al.* 2020 for threshold values).

This pronounced difference highlights the environmental advantage of the hybrid formulation, which delivered effective disease control while minimizing the risk of long-term copper accumulation. Low residual copper levels are crucial for preserving soil microbial diversity, promoting root health, and enhancing overall soil fertility, especially in systems that rely on sustainable or organic practices.

10 Impact on Soil Microbiota

The potential effects of the copper–allicin hybrid formulation on soil microbial communities were evaluated by analyzing bacterial and fungal populations before and after treatment. Soil samples were collected at three depths (0–10 cm, 10–20 cm, and 20–30 cm) and analyzed for total microbial biomass, taxonomic diversity, and functional composition using high-throughput sequencing techniques (see Fincheira *et al.* 2021). Microbial diversity indices and biomass levels in treated soils remained comparable to untreated controls (Shannon index: 5.2 ± 0.3 vs. 5.3 ± 0.2; $p > 0.05$). The hybrid formulation had minimal impact on soil microbiota, preserving beneficial microbial functions essential for soil health and plant growth. The lack of adverse effects was likely due to the low copper concentration in the formulation and the presence of an organic matrix that modulated copper bioavailability. Specifically, the pH-responsive release mechanism appeared to reduce the risk of copper toxicity to non-target soil organisms by gradually releasing copper ions only under conditions favorable for pathogen suppression.

High-throughput sequencing revealed no significant differences in α-diversity indices between treated and control soils (Shannon index: 5.2 ± 0.3 vs. 5.3 ± 0.2; Simpson index: 0.97 ± 0.01 vs. 0.96 ± 0.02; $p > 0.05$). Microbial biomass carbon remained statistically unchanged (142 ± 18 ppm vs. 138 ± 15 ppm).

These findings support the conclusion that the hybrid formulation was microbiota-friendly and aligned with sustainable agricultural practices by maintaining soil ecological integrity while providing effective disease control.

Discussion

The results which align with prior work showed that copper-based compounds can be potentiated by sulfur-containing antimicrobials (Ai *et al.* 2025) and extend those findings by demonstrating field-scale efficacy and minimal soil accumulation. Earlier studies reported copper–allicin interactions *in vitro* (Ai *et al.* 2025) and more recently similar synergy for copper complexes (Moreno-Ramirez *et al.* 2024; Orta-Rivera *et al.* 2023). Unlike reports documenting substantial soil Cu accumulation after conventional programs (Panagos *et al.* 2018; Laurent *et al.* 2019), the field data showed negligible accumulation under the hybrid (≤0.08 $mg \cdot kg^{-1}$), likely due to the organic matrix and pH-responsive release. Where prior studies have suggested possible microbiome disruption at elevated Cu (Strayer-Scherer *et al.* 2022), in the present research no significant α-diversity loss was observed, supporting the formulation's microbiota-safe profile. This dual functionality is particularly relevant in the context of integrated pest management (IPM), where reducing chemical input without compromising efficacy is a key objective. The findings demonstrated that hybrid formulation effectively addressed two key agricultural challenges: controlling pathogens and providing essential micronutrients to enhance crop health. A central finding of this study was the strong synergistic interaction between copper ions and allicin, which significantly enhanced antimicrobial efficacy while allowing for a reduction in copper application rates. Copper's broad-spectrum antimicrobial properties are well documented, but its overuse raises concerns regarding soil accumulation and environmental toxicity (Neaman *et al.* 2024; Hassan *et al.* 2025). The incorporation of allicin—a natural antimicrobial agent with a broad target range—potentiated copper's effectiveness, enabling lower copper doses without sacrificing disease control. This synergy not only improved efficacy but also mitigated environmental risks commonly associated with copper-based fungicides, aligning with the increasing demand for safer agricultural inputs. Allicin enhances copper's efficacy by depleting glutathione reserves in pathogens, rendering them vulnerable to copper-mediated oxidative stress (Borlinghaus *et al.* 2014; Sarfraz *et al.* 2020). The mechanism underpinning this synergy appears to be linked to the pH-dependent release of copper ions, which are optimally bioavailable within a narrow pH window (4.5–5.0) (Xiaolin *et al.* 2024). Allicin enhances copper's mode of action by disrupting the pathogen redox balance and increasing cell membrane permeability, leading to pathogen death (Sarfraz *et al.* 2020; Borlinghaus *et al.* 2014; Ogita *et al.* 2005). These observations are consistent with earlier studies reporting increased antimicrobial activity through combinations of copper and sulfur-containing compounds (Božić Cvijan *et al.* 2023; Orta-Rivera *et al.* 2023). The results extended these insights by highlighting how such synergy can also reduce environmental contamination risks.

Beyond antimicrobial activity, the composted manure-based organic matrix plays a pivotal role in both nutrient delivery and plant defense stimulation (Mahdizadehnaraghi *et al.* 2015; Ziedan 2022). Organic matrices have been shown to improve nutrient bioavailability and protect active ingredients from degradation (Rein *et al.* 2013). In this formulation, the matrix not only enhances copper uptake but also supplies micronutrients critical for plant growth and resilience (Grzanka *et al.* 2024). While physiological improvements suggest SAR induction, future transcriptomic validation (e.g., PR-1 gene upregulation) is needed to confirm this mechanism (da Silva *et al.* 2023; Zandonadi *et al.* 2025). The combination of direct pathogen suppression and induced systemic resistance establishes a multi-layered defense that may improve long-term crop health and yield stability (Jasman *et al.* 2025).

Taken together, these findings supported the potential of copper–allicin hybrids as integrated biocontrol and biostimulant agents. Their ability to reduce copper input while maintaining or enhancing efficacy addressed both agronomic and environmental sustainability goals. While this study demonstrated compelling efficacy and safety in a citrus monoculture system, future research should address several key areas to assess broader applicability. First, long-term (multi-year) field trials across diverse horticultural systems (e.g., solanaceous crops, pome fruits) are needed to evaluate effects on soil health, pathogen resistance evolution, and cumulative yield impacts. Second, the potential induction of systemic acquired resistance (SAR) by the organic matrix, suggested by improved plant vigor, requires molecular validation (e.g., via qPCR of PR-1, PR-2 gene expression). Third, a techno-economic analysis comparing the cost of allicin extraction and formulation with the economic benefits of reduced copper use and increased yield is crucial for assessing scalability and farmer adoption. Finally, investigating the molecular mechanisms of the copper-allicin synergy, particularly against copper-resistant pathogen strains, could be informative for the development of next-generation hybrid biofungicides. Additionally, investigating the molecular pathways involved in SAR activation by the organic matrix could yield further insights for developing next-generation formulations. This research focused on single-season efficacy in citrus. Multi-year trials across diverse crops (e.g., solanaceous plants) and pathogen resistance monitoring are essential for practical scalability.

The copper–allicin hybrid formulation offered a significant advancement in sustainable agriculture by integrating effective pathogen control with essential nutrient supplementation. A critical feature of the formulation was its organic matrix, which not only enhanced nutrient bioavailability but also played a vital role in reducing phytotoxicity risks. The organic matter buffers the pH of the formulation, mitigating copper-induced toxicity—a common issue with conventional copper fungicides. Furthermore, the markedly lower copper concentrations required for disease control in this hybrid formulation further minimized phytotoxicity, as confirmed by the absence of visible damage in non-target plants during phytotoxicity assays. The environmental impact of copper-based fungicides has become a concern due to copper accumulation in soils, which can cause long-term phytotoxicity, diminish soil microbial diversity, and contaminate water bodies via runoff (Wang *et al.* 2020; Poggere *et al.* 2023; Neaman *et al.* 2024). The hybrid formulation addressed these challenges by enabling an over 80% reduction in copper usage, which substantially lowers the risk of soil copper buildup. It is essential in regions with intensive copper fungicide use and where regulations on heavy metal accumulation are tightening (Lamichhane *et al.* 2018; Fagnano *et al.* 2020). Soil analyses from the trials revealed that copper concentrations remained extremely low (0.08 ppm) in plots treated with the hybrid formulation, compared to 24.3 ppm following conventional copper applications. These results demonstrated a significant reduction in the environmental footprint without compromising disease control efficacy and align with regulatory frameworks such as the EU Regulation (EC) No 2018/1981, which promotes low-residue and sustainable farming practices (Kah *et al.* 2022; Rashid *et al.* 2023). Furthermore, reduced copper usage mitigates selection pressure for copper resistance genes (e.g., copA) in soil microbiota, addressing a key sustainability challenge in perennial crop systems. In addition to minimizing copper accumulation, the formulation supported soil health and microbial diversity. The organic matrix combined with low copper inputs contributed to maintaining a healthy rhizosphere, which is essential for soil fertility and sustained crop productivity (Karnwal 2022). Soil microbiota analyses confirmed that microbial biomass and diversity were largely unaffected by treatment, highlighting the formulation's microbiota-friendly nature.

This approach aligns with Integrated Pest Management (IPM) principles, which seek to minimize synthetic chemical use while sustaining crop productivity and environmental health (Tonnang *et al.* 2025). The copper–allicin hybrid can provide an organic-compatible, low-residue alternative to conventional fungicides, which can be especially valuable in organic farming systems (Sadło *et al.* 2023). The formulation evaluated in this study was experimental and not a registered commercial product; however, its copper content and measured soil residues remained well below regulatory limits established for fertilizing products (e.g., Regulation (EU) 2019/1009). These findings indicated potential regulatory compatibility, subject to formal registration and approval by the relevant national authorities. Preliminary observations also suggest that the formulation may stimulate systemic acquired resistance (SAR) in plants, offering a dual mode of action by combining direct antimicrobial effects with the activation of plant innate defenses. SAR activation is known to enhance tolerance to multiple biotic and abiotic stresses, including drought and nutrient deficiencies (Song *et al.* 2023). This aspect warrants further investigation, as it could contribute to developing more resilient cropping systems capable of coping with climate change and evolving pathogen pressures. While the current study provided compelling evidence of the formulation's efficacy and safety, future research should address several key areas. Long-term studies are needed to evaluate the sustained impacts on soil microbial communities and functions, as well as to monitor any potential development of pathogen resistance to copper and allicin. Understanding molecular resistance mechanisms and assessing risks of cross-resistance with other pesticides will be crucial for ensuring the formulation's long-term effectiveness.

Additionally, expanding efficacy trials to a broader spectrum of crops and pathogens—such as tomatoes, apples, and grapes—will clarify the formulation's versatility across diverse agricultural contexts. Finally, economic analyses and scalability assessments are essential to determine the feasibility of commercial production and widespread adoption. Preliminary consideration of raw materials indicates that the hybrid's principal inputs — $CuSO_4 \cdot 5H_2O$ and garlic (source of allicin) — are globally available on an agricultural scale, and the organic base utilizes composted manure feedstocks commonly produced on-farm or regionally. A formal cost-benefit and scalability analysis (capital + operating costs for extraction, formulation, and packaging vs. recurrent cost of conventional copper fungicides) is needed and should be reported alongside regulatory pathway assessments for market entry. Although the formulation showed promise in field trials, its cost-effectiveness and manufacturing practicality should be established to support its integration into mainstream agriculture.

In conclusion, the copper–allicin hybrid formulation offered a significant advancement in sustainable agriculture by effectively combining pathogen control with nutrient supplementation. By reducing copper usage by over 80%, the formulation maintained high disease control efficacy while minimizing environmental risks such as soil copper accumulation and phytotoxicity. Allicin enhanced copper's antimicrobial activity and the overall physiological responses observed in treated trees were consistent with a potential biostimulant effect. While biochemical and phenotypic indicators suggest activation of defense pathways, definitive evidence for systemic acquired resistance (SAR) for example, transcriptomic upregulation of canonical SAR markers such as PR-1, was not collected in this study and should be addressed in follow-up experiments. The organic matrix enhanced nutrient bioavailability and reduced copper toxicity, promoting plant health. This formulation aligned well with integrated pest management and organic farming practices, promoting soil health and reducing chemical inputs. Long-term impacts, broader crop applicability, and large-scale production feasibility require further research.

Conclusions

The compost-based, pH-responsive copper–allicin hybrid provided robust control of major horticultural pathogens (notably citrus canker) at substantially reduced copper loads, while simultaneously enhancing leaf micronutrient status and yield metrics and avoiding measurable soil copper accumulation or microbiome disruption in single-season trials. This dual-function approach supported integration into IPM frameworks and organic-compatible production systems (Nikolova 2022). Nonetheless, multi-year and multi-crop evaluations, molecular confirmation of potential SAR induction, and a techno-economic analysis are required prior to commercialization.

Limitations and recommendations

It is acknowledged that the present single-season field study did not include a comprehensive set of time-dependent release curves at pH > 7, nor were systematic measurements of copper in field runoff or wastewater performed; this represents a limitation of the current manuscript. Evidence for pH-responsiveness is currently based on physicochemical data, including stability assessed by DLS at pH 4.5 and maintenance of particle size over 30 days at that pH, as well as the observed low soil Cu accumulation in treated plots. To fully substantiate the low-leaching claim in alkaline soils (pH > 7) requested by reviewers, standard methods are described that must be performed: laboratory buffer release assays across pH 4.5–8.5, soil column leaching tests using representative alkaline loamy-clay soils from the trial sites, and routine analysis of field runoff and wastewater samples by ICP-OES collected after typical spray and rainfall events.

This study reports single season results from citrus orchards; therefore, long-term copper dynamics and potential resistance selection require multi-year monitoring. Molecular validation of putative SAR induction (e.g., PR-1 transcript quantification) is recommended to confirm the proposed induced-resistance mechanism. Finally, a full techno-economic assessment is necessary to evaluate scalability and farmer adoption pathways.

References

Abdel-Razek A.S., Abd El-Ghany N.M., Djelouah K., Moussa A. 2017. An evaluation of some eco-friendly biopesticides against *Bemisia tabaci* on two greenhouse tomato varieties in Egypt. Journal of Plant Protection Research 57 (1): 9–17. DOI: 10.1515/jppr-2017-0002

Ai S., Yang Y., Gao K., Yu W. 2025. Synergy between copper and silver nanoparticles for the removal of thiophenic compounds in fuel oil. Chemical Engineering & Technology 48 (4): e70010. DOI: 10.1002/ceat.70010

Alfei S., Schito G.C., Schito A.M., Zuccari G. 2024. Reactive oxygen species (ROS)-mediated antibacterial oxidative therapies: available methods to generate ROS and a novel option proposal. International Journal of Molecular Sciences 25: 7182. DOI: 10.3390/ijms25137182

Ali A., AlHussaini K.I. 2024. Pesticides: unintended impact on the hidden world of gut microbiota. Metabolites 14: 155. DOI: 10.3390/metabo14030155

Bijay-Singh, Craswell E. 2021. Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. SN Applied Sciences 3: 518. DOI: 10.1007/s42452-021-04521-8

Borlinghaus J., Albrecht F., Gruhlke M.C., Chi B.K., Huyen N.T.T., Loi V.V., da Silva R.M., Canellas N.A., Olivares F.L. 2014. Allicin: chemistry and biological properties. Molecules 19: 12591–12618. DOI: 10.3390/molecules190812591

Božić Cvijan B., Korać Jačić J., Bajčetić M. 2023. The impact of copper ions on the activity of antibiotic drugs. Molecules 28: 5133. DOI: 10.3390/molecules28135133

Chi B.K., Huyen N.T.T., Loi V.V., da Silva R.M., Canellas N.A., Olivares F.L., Fagnano M., Agrelli D., Pascale A. 2019. The disulfide stress response and protein S-thioallylation caused by allicin and diallyl polysulfanes in *Bacillus subtilis* as revealed by transcriptomics and proteomics. Antioxidants 8: 605. DOI: 10.3390/antiox8120605

Chiarini A. 2017. Setting strategies outside a typical environmental perspective using ISO 14001 certification. Business Strategy and the Environment 26 (6): 844–854. DOI: 10.1002/bse.1969

da Silva R.M., Canellas N.A., Olivares F.L., Fagnano M., Agrelli D., Pascale A., Hassan W., Zahra Q.A., Attia K.A. 2023. Humic substances trigger plant immune responses. Chemical and Biological Technologies in Agriculture 10: 123. DOI: 10.1186/s40538-023-00468-7

Đukić M., Vasiljević S., Sovrlić Z., Adamović D. 2023. Verification and validation of analytical methods in accordance with the ISO/IEC 17025 standard. Bakar 48 (2): 25–34. DOI: 10.5937/bakar2302025D

El-Metwally I., Shalaby S. 2019. Herbicidal efficacy of some natural products and mulching compared to herbicides for weed control in onion fields. Journal of Plant Protection Research 59 (4): 479–486. DOI: 10.24425/jppr.2019.131266

Ermini M.L., Voliani V. 2021. Antimicrobial nano-agents: the copper age. ACS Nano 15: 6008–6029. DOI: 10.1021/acsnano.0c10756

Fagnano M., Agrelli D., Pascale A., Hassan W., Zahra Q.A., Attia K.A., Humphries R.M., Ambler J., Mitchell S.L. 2020. Copper accumulation in agricultural soils: risks for the food chain and soil microbial populations. Science of the Total Environment 734: 139434. DOI: 10.1016/j.scitotenv.2020.139434

Fajfer D., Łochyńska M. 2022. Impact of ecological plant protection products on mortality and cocoon shell ratio of mulberry silkworms (*Bombyx mori* L.) – pilot studies. Journal of Plant Protection Research 62 (3): 283–290. DOI: 10.24425/jppr.2022.142135

Gai Y., Wang H. 2024. Plant disease: a growing threat to global food security. Agronomy 14: 1615. DOI: 10.3390/agronomy14081615

González-Macedo M., Cabirol N., Rojas-Oropeza M. 2021. Assessment of the ancestral use of garlic (*Allium sativum*) and nettle (*Urtica dioica*) as botanical insecticides in the protection of mesquite (*Prosopis laevigata*) seeds against bruchins. Journal of Plant Protection Research 61 (2): 170–175. DOI: 10.24425/jppr.2021.137023

Grzanka M., Sobiech Ł., Radzikowska-Kujawska D., Sawińska Z., Kowalczewski P.Ł., Świtek S., Skrzypczak G., Kardasz P. 2024. The influence of *Hermetia illucens* L. frass on the health, stress and development of barley. Journal of Plant Protection Research 64 (4): 394–401. DOI: 10.24425/jppr.2024.151818

Hassan W., Zahra Q.A., Attia K.A., Humphries R.M., Ambler J., Mitchell S.L., Lamichhane J.R., Osdaghi E., Behlau F. 2025. Assessing the impact of copper toxicity on soil ecosystems and barley growth: identification of robust indicators. Environmental Monitoring and Assessment 197: 563. DOI: 10.1007/s10661-025-13974-y

Hayat S., Ahmad A., Ahmad H., Hayat K., Khan M.A., Runan T. 2022. Garlic, from medicinal herb to possible plant bioprotectant: a review. Scientia Horticulturae 304: 111296. DOI: 10.1016/j.scienta.2022.111296

Humphries R.M., Ambler J., Mitchell S.L., Lamichhane J.R., Osdaghi E., Behlau F., Meena R.S., Kumar S., Datta R. 2018. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. Journal of Clinical Microbiology 56: e01934-17. DOI: 10.1128/JCM.01934-17

Imfeld G., Duplay J., Payraudeau S. 2021. Comment on 'Ecotoxicity of copper input and accumulation for soil biodiversity in vineyards. Environmental Chemistry Letters 19: 3525–3527. DOI: 10.1007/s10311-021-01259-y

Jasman A.K., Slomy A.K., Khaleel A.I. 2025. Biocontrol potential of selected botanicals and *Beauveria bassiana* spore suspension against *Bemisia tabaci* (Genn.). Journal of Plant Protection Research 65 (3): 283–292.

Jeon D. 2024. Evaluating the impact of copper concentrations on apple variety performance: insights from a callus media system. Sustainability 16: 9741. DOI: 10.3390/su16229741

Jung K. 2004. Combined use of insect pathogenic fungi and nematodes against the onion thrips, *Thrips tabaci*, in the field. p. 141–143. In: Proceedings of the Meeting "Management of Plant Diseases and Arthropod Pests by BCAs and Their Integration in Agricultural Systems". IOBC/WPRS Bulletin 27 (8).

Kah M., Navarro D., Schenkeveld W., Kookana R.S., Kirby J.K., Santra S., Ozcan A. 2022. Fate of copper in soil: effect of agrochemical (nano) formulations and soil properties. Environmental Science: Nano 9 (2): 653–662. DOI: 10.1039/D1EN00213A

Karnwal A. 2022. A comparative study of native growth-promoting rhizobacteria and commercial biofertilizer on maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) development in a saline environment. Journal of Plant Protection Research 62 (1): 49–57. DOI: 10.24425/jppr.2022.140296

Lamichhane J.R., Osdaghi E., Behlau F., Meena R.S., Kumar S., Datta R., Moreno-Ramirez M.C., Arias-Bravo A.S., Aragón-Muriel A. 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture: a review. Agronomy for Sustainable Development 38: 28. DOI: 10.1007/s13593-018-0503-9

Laurent C., Bravin M.N., Crouzet O., Pelosi C., Lamy I. 2019. Do long-term applications of organic residues promote copper ecotoxicity in soil? p. 1. In: Proceedings of the International Conference on the Biogeochemistry of Trace Elements (ICOBTE 2019).

Liu Y., Zhao W., Yin Y., Adeel M., Shakoor N., Li Y., Xing B. 2022. Agricultural applications and potential risks of copper-based nanoagrochemicals in crop cultivation. Reviews of Environmental Contamination and Toxicology 260 (1): 20. DOI: 10.1007/s44169-022-00022-w

Mahdizadehnaraghi R., Heydari A., Zamanizadehnaraghi H.R., Rezaee S., Nikan J. 2015. Biological control of garlic (Allium) white rot disease using antagonistic fungi-based bioformulations. Journal of Plant Protection Research 55 (2): 136–141. DOI: 10.1515/jppr-2015-0017

Martínez-Dalmau J., Berbel J., Ordóñez-Fernández R. 2021. Nitrogen fertilization: a review of the risks associated with the inefficiency of its use and policy responses. Sustainability 13: 5625. DOI: 10.3390/su13105625

Meena R.S., Kumar S., Datta R., Moreno-Ramirez M.C., Arias-Bravo A.S., Aragón-Muriel A., Müller A., Eller J., Neaman A. 2020. Impact of agrochemicals on soil microbiota and management: a review. Land 9: 34. DOI: 10.3390/land9020034

Mikiciński A., Sobiczewski P., Berczyński S. 2012. Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. Journal of Plant Protection Research. DOI: 10.2478/v10045-012-0075-7

Moreno-Ramirez M.C., Arias-Bravo A.S., Aragón-Muriel A., Müller A., Eller J., Neaman A., Schoffer J.T., Villarroel C.N., Orta-Rivera A.M. 2024. Design, synthesis and antimicrobial potential of conjugated metallopeptides targeting DNA. Scientia Pharmaceutica 92 (2): 21. DOI: 10.3390/scipharm92020021

Müller A., Eller J., Albrecht F., Neaman A., Schoffer J.T., Villarroel C.N., Orta-Rivera A.M., Meléndez-Contés Y., Medina-Berríos N. 2016. Allicin induces thiol stress in bacteria through S-allylmercapto modification of protein cysteines. Journal of Biological Chemistry 291: 11477–11490. DOI: 10.1074/jbc.M115.702308

Neaman A., Schoffer J.T., Villarroel C.N., Orta-Rivera A.M., Meléndez-Contés Y., Medina-Berríos N., Oussou-Azo A.F., Nakama T., Nakamura M. 2024. Copper contamination in agricultural soils: a review of the effects of climate, soil properties, and prolonged copper pesticide application in vineyards and orchards. Plant, Soil and Environment 70: 407–417. DOI: 10.17221/501/2023-PSE

Ngece K., Khwaza V., Paca A.M., Aderibigbe B.A. 2025. The antimicrobial efficacy of copper complexes: a review. Antibiotics 14: 516. DOI: 10.3390/antibiotics14050516

Nikolova I. 2022. Controlling *Acyrthosiphon pisum* L. infestation using products of natural origin in forage pea. Journal of Plant Protection Research 62 (1): 22–32. DOI: 10.24425/jppr.2022.140293

Nwachukwu I.D., Asawalam E.F. 2014. Evaluation of freshly prepared juice from garlic (*Allium sativum* L.) as a biopesticide against the maize weevil, *Sitophilus zeamais*. Journal of Plant Protection Research. DOI: 10.2478/jppr-2014-0021

Ogita A., Hirooka K., Yamamoto Y., Tsutsui N. 2005. Synergistic fungicidal activity of Cu^{2+} and allicin and its relation to alkyl hydroperoxide reductase 1 in *Saccharomyces cerevisiae*. Toxicology 215: 205–213. DOI: 10.1016/j.tox.2005.07.006

Orta-Rivera A.M., Meléndez-Contés Y., Medina-Berríos N., Oussou-Azo A.F., Nakama T., Nakamura M., Panagos P., Ballabio C., Lugato E. 2023. Copper-based antibiotic strategies. Inorganics 11: 252. DOI: 10.3390/inorganics11060252

Oussou-Azo A.F., Nakama T., Nakamura M., Panagos P., Ballabio C., Lugato E., Poggere G., Gasparin A., Zimmer Barbosa J. 2020. Antifungal potential of nanostructured crystalline copper and its oxide forms. Nanomaterials 10: 1003. DOI: 10.3390/nano10051003

Panagos P., Ballabio C., Lugato E., Poggere G., Gasparin A., Zimmer Barbosa J., Rashid M.I., Shah G.A., Sadiq M. 2018. Potential sources of anthropogenic copper inputs to European agricultural soils. Sustainability 10: 2380. DOI: 10.3390/su10072380

Pfaller M.A., Flamm R.K., Jones R.N., Farrell D.J., Mendes R.E. 2016. Activities of tedizolid and linezolid against Gram-positive isolates. Antimicrobial Agents and Chemotherapy 60. DOI: 10.1128/AAC.00881-16

Poggere G., Gasparin A., Zimmer Barbosa J., Rashid M.I., Shah G.A., Sadiq M., Rein M.J., Renouf M., Cruz-Hernandez C. 2023. Soil contamination by copper: sources, ecological risks, and mitigation strategies in Brazil. Journal of Trace Elements and Minerals 4: 100059. DOI: 10.1016/j.jtemin.2023.100059

Pscheidt J.W., Ocamb C.M. 2022. Copper-based bactericides and fungicides. Pacific Northwest pest management handbooks. Oregon State University, Corvallis.

Rashid M.I., Shah G.A., Sadiq M., Rein M.J., Renouf M., Cruz-Hernandez C., Sheppard J.G., McAleer J.P., Saralkar P. 2023. Nanobiochar and copper oxide nanoparticles mixture improves wheat production. Plants 12 (6): 1312. DOI: 10.3390/plants12061312

Rein M.J., Renouf M., Cruz-Hernandez C., Sheppard J.G., McAleer J.P., Saralkar P., Song W., Shao H., Zheng A. 2013. Bioavailability of bioactive food compounds. British Journal of Clinical Pharmacology 75: 588–602. DOI: 10.1111/j.1365-2125.2012.04425.x

Sadło S., Szczepanik M., Krawiec P., Piechowicz B. 2023. Research on the certification of the apple orchard pest and disease control program as an innovative strategy for the production of apples practically free of pesticide residues, i.e., below 0.01 $mg \cdot kg^{-1}$. Journal of Plant Protection Research 63 (4): 440–449. DOI: 10.24425/jppr.2023.147825

Salah I., Parkin I.P., Allan E. 2021. Copper as an antimicrobial agent: recent advances. RSC Advances 11: 18179–18186. DOI: 10.1039/D1RA02149D

Sarfraz M., Nasim M.J., Jacob C., Gruhlke M.C.H. 2020. Efficacy of allicin against plant pathogenic fungi. Applied Sciences 10: 2563. DOI: 10.3390/app10072563

Shalaby S., Abdou G. 2010. Influence of soil microorganisms on pesticide dissipation. Journal of Plant Protection Research 50 (1).

Sheppard J.G., McAleer J.P., Saralkar P., Song W., Shao H., Zheng A., Strayer-Scherer A., Timilsina S., Liao Y.Y. 2018. Allicin-inspired pyridyl disulfides as antimicrobial agents. European Journal of Medicinal Chemistry 143: 1185–1195. DOI: 10.1016/j.ejmech.2017.10.018

Song W., Shao H., Zheng A., Strayer-Scherer A., Timilsina S., Liao Y.Y., Tonnang H.E.Z., Tepa-Yotto G.T., Sokame B.M. 2023. Roles of salicylic acid in plant stress tolerance. Plants 12: 3475. DOI: 10.3390/plants12193475

Strayer-Scherer A., Timilsina S., Liao Y.Y., Tonnang H.E.Z., Tepa-Yotto G.T., Sokame B.M., Wallock-Richards D., Doherty C.J., Doherty L. 2022. Simulated leaching of foliar-applied copper bactericides. Microbiology Spectrum 10: e01481-21. DOI: 10.1128/spectrum.01481-21

Tonnang H.E.Z., Tepa-Yotto G.T., Sokame B.M., Wallock-Richards D., Doherty C.J., Doherty L., Wang Y., Deng C., Cota-Ruiz K. 2025. Roadmap for mainstreaming IPM into a climate-smart one-health framework. One Health 20: 101084. DOI: 10.1016/j.onehlt.2025.101084

Tyagi J., Ahmad S., Malik M. 2022. Nitrogenous fertilizers and environmental sustainability. International Journal of Environmental Science and Technology 19: 11649–11672. DOI: 10.1007/s13762-022-04027-9

Wallock-Richards D., Doherty C.J., Doherty L., Wang Y., Deng C., Cota-Ruiz K., Xiaolin B., Zhao N., Guo M. 2014. Antimicrobial activity of allicin-containing garlic extracts. PLoS ONE 9 (12): e112726. DOI: 10.1371/journal.pone.0112726

Wang Y., Deng C., Cota-Ruiz K., Xiaolin B., Zhao N., Guo M., Zandonadi D.B., Monda H., Gralian J. 2020. Improvement of nutrient elements and allicin content in green onion exposed to CuO nanoparticles. Science of the Total Environment 725: 138387. DOI: 10.1016/j.scitotenv.2020.138387

Xiaolin B., Zhao N., Guo M., Zandonadi D.B., Monda H., Gralian J., Zhang H., Xu Y., Jia P. 2024. Synergistic redox and adsorption for enhanced roxarsone removal. Chemical Engineering Science 287: 119713. DOI: 10.1016/j.ces.2024.119713

Zandonadi D.B., Monda H., Gralian J., Zhang H., Xu Y., Jia P., Borlinghaus J., Albrecht F., Gruhlke M.C. 2025. Humic acids as drivers of plant growth. Chemical and Biological Technologies in Agriculture 12: 71. DOI: 10.1186/s40538-025-00789-9

Zhang H., Xu Y., Jia P., Borlinghaus J., Albrecht F., Gruhlke M.C., Chi B.K., Huyen N.T.T., Loi V.V. 2020. Global trends of antimicrobial susceptibility. Antimicrobial Resistance and Infection Control 9: 166. DOI: 10.1186/s13756-020-00829-z

Ziedan E.S.H. 2022. A review of the efficacy of biofumigation agents in the control of soil-borne plant diseases. Journal of Plant Protection Research: 1–11. DOI: 10.1002/npp2.21

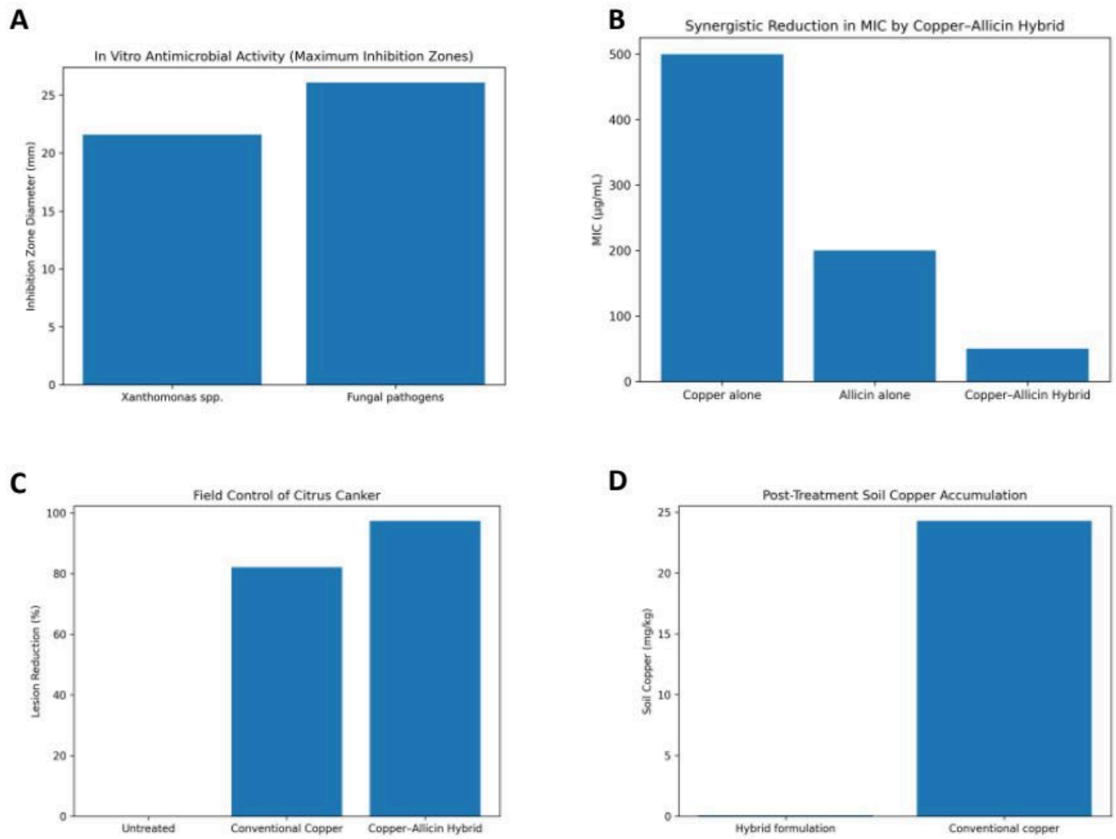

Figure 1. Antimicrobial efficacy, field performance, and environmental impact of the copper–allicin hybrid formulation.
(A) Maximum inhibition zone diameters observed for bacterial (*Xanthomonas* spp.) and fungal phytopathogens treated with the copper–allicin hybrid formulation in disk diffusion assays.
(B) Comparison of minimum inhibitory concentrations (MICs) of copper alone, allicin alone, and the copper–allicin hybrid formulation against *Xanthomonas axonopodis* pv. *citri*, demonstrating a strong synergistic effect.
(C) Field efficacy of treatments against citrus canker expressed as percentage reduction in lesion incidence relative to untreated controls.
(D) Soil copper accumulation following repeated applications of the copper–allicin hybrid formulation compared with conventional copper oxychloride treatment.

Table 1. Key horticultural phytopathogens addressed in this study. Each entry lists the pathogen, affected crop and disease, pathogenesis, typical symptoms (macroscopic and microscopic), and control measures.

Pathogen	Host / Disease	Pathogenesis	Symptoms (Macro/Microscopic)	Control Measures
Xanthomonas axonopodis pv. *citri*	Citrus (citrus canker)	Enters leaves/fruit via stomata or wounds; multiplies in leaf and fruit tissues	Raised, corky necrotic lesions with yellow halo on leaves and fruit; Xanthomonas is a gram-negative, yellow-pigmented rod	Copper spray treatments; removal of infected tissue; use of resistant varieties
Phytophthora infestans	Tomato, Potato (late blight)	Waterborne zoospores infect foliage and tubers in cool, moist conditions	Dark, irregular, water-soaked leaf lesions; white fungal sporulation on leaf underside; tuber rot (brown decay, sporangia visible)	Resistant cultivars; proper drainage/irrigation management; fungicides (e.g., metalaxyl, copper-based)
Erwinia amylovora	Apple/Pear (fire blight)	Bacteria enters blossoms and wounds; spreads through vascular system	Blossom and shoot blight (blackened tips, "shepherd's crook"); bacterial exudate (ooze) on cankers; streaming rods in ooze	Prune and burn infected branches; copper or antibiotic sprays at bloom; orchard sanitation
Fusarium oxysporum	Various (Fusarium wilt)	Soil-borne fungus infects roots,	Wilting and yellowing of foliage; brown or reddish-brown	Resistant rootstocks/varieties; crop rotation; soil

		colonizes xylem vessels	vascular discoloration inside stems	fumigation or solarization
Colletotrichum spp.	Various (anthracnose)	Spores germinate on plant surfaces; fungus penetrates via appressoria	Sunken, circular necrotic lesions on fruit, stems, or leaves; acervuli (fruiting bodies) with salmon-colored spore masses	Fungicide sprays (protectants); remove and destroy infected fruit/plant debris; use of resistant varieties

Table 2. Key results comparing conventional copper (copper oxychloride) versus copper–allicin hybrid. Percentage changes are relative to untreated controls (baseline) unless otherwise noted.

Parameter	Conventional Copper (Oxychloride)	Copper–Allicin Hybrid	Change
Citrus canker lesion reduction (%)	82.1	97.4	+15.3 pp
Fruit count increase (%)	0 (baseline)	+18.7	+18.7
Fruit Brix increase (%)	0 (baseline)	+12.3	+12.3
Leaf Cu concentration (ppm)	~5.0 (baseline)	15.6	+10.6
Soil Cu accumulation (ppm)	24.3	0.08	–24.2