Plant Protection (Scientific Journal of Agriculture)

48(2), Summer, 2025

10.22055/ppr.2025.48722.1786

Short Communication

Antibacterial activity of chitosan/thyme essential oil/Copper nano-complexes against *Pseudomonas syringae* pv. *syringae*

M. Azarakhsh [*1], K. Gerivani [2]

1. ***Corresponding Author:** Assistant Professor, Cell and Molecular Biology Department, Kosar University of Bojnord, Bojnord, Iran (mahboobeazarakhsh@gmail.com)
2. M.Sc., North Khorasan Agricultural and Natural Resources Research and Education Center, Bojnourd, Iran

Received: 7 January 2025 Revised: 22 April 2025 Accepted: 3 May 2025

Abstract

Pseudomonas syringae pv. *syringae* (*Pss*) is the causal agent of bacterial canker disease in stone, pome and kiwi fruit trees, which causes much damage to these hosts. Using biodegradable nanoparticle complexes is a new approach against bacterial cankers. This research investigated the antibacterial activity of chitosan nanoparticles (CNPs)/Copper/thyme essential oil (EO) nano-complexes against this bacterium. In this regard, the physicochemical characteristics of chitosan nanoparticles and nano-complexes were analyzed by dynamic light scattering (DLS) and Fourier-transform infrared spectroscopy (FTIR) methods. The DLS results showed that the nanoparticles had unimodal size distribution with an average particle size of 190 to 254 nm. The FTIR analysis confirmed the interaction among the components of nano-complexes. To evaluate the antibacterial activity of the nano-complexes, the minimum inhibitory concentration (MIC) was measured against *Pss*. The results showed that the nano-complexes had relatively high antibacterial activity against *Pss* compared to their components. In particular, the nano-complexes showed the lower MIC value and, therefore, higher antibacterial activity compared to thyme EO, Copper, and CNPs alone. These results suggest a synergistic interaction among the components, indicating that the nano-complexes could be a promising eco-friendly option for controlling bacterial canker disease caused by *Pss*. Further research is needed to study the effectiveness of these compounds in the field and to investigate the long-term environmental effects.

Keywords: Plant protection, Nano-complexes, Bacterial canker, Pseudomonas syringae

Associate editor: R. Rezaei (Ph.D.)

Citation: Azarakhsh, M. & Gerivani, M. (2025). Antibacterial activity of chitosan/thyme essential oil/Copper nano-complexes against *Pseudomonas syringae* pv. *syringae*. *Plant Protection (Scientific Journal of Agriculture), 48*(2), 1-11. https://doi.org/ 10.22055/ppr.2025.48722.1786.

گیاه‌پزشکی (مجله علمی کشاورزی)
جلد ٤٨، شماره ٢، تابستان ١٤٠٤
10.22055/ppr.2025.48722.1786

گزارش کوتاه انگلیسی

خواص آنتی باکتریالی نانوکمپلکس کیتوسان/عصاره آویشن/مس برای مقابله با *Pseudomonas syringae* pv. *syringae*

محبوبه آذرخش *[1]، مسعود گریوانی [2]

۱- * **نویسنده مسوول:** استادیار، گروه زیست شناسی سلولی و مولکولی، دانشکده علوم پایه، دانشگاه کوثر بجنورد، بجنورد، ایران (mahboobeazarakhsh@gmail.com)

۲- کارشناسی ارشد، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی خراسان شمالی، بجنورد، ایران

تاریخ دریافت: ۱۴۰۳/۱۰/۱۸ تاریخ بازنگری: ۱۴۰۴/۰۲/۰۲ تاریخ پذیرش: ۱۴۰۴/۰۲/۱۳

چکیده

باکتری (*Pss*) *Pseudomonas syringae* pv. *syringae* به عنوان عامل ایجاد بیماری شانکر باکتریایی در هسته‌داران، دانه داران و کیوی شناخته می‌شود، که خسارات زیادی را به این میزبان‌ها وارد می‌کند. استفاده از کمپلکس نانوذرات زیست تخریب پذیر به عنوان رویکردی نو برای مقابله با این باکتری است. در این تحقیق، فعالیت آنتی باکتریالی نانوکمپلکس نانوذرات کیتوسان/مس/اسانس آویشن در مقابله با این باکتری مورد بررسی قرار گرفت. در این راستا، خواص فیزیکی شیمیایی نانوذرات کیتوسان و نانو کمپلکس‌ها با استفاده از روش‌های پراکندگی نور دینامیکی (DLS) و سنجش مادون قرمز تبدیل فوریه (FTIR) بررسی شد. نتایج DLS نشان داد که نانوذرات مذکور پراکندگی اندازه ذرات یکنواختی داشته و متوسط اندازه ذرات در محدوده ۱۹۰ تا ۲۵٤ نانومتر می‌باشد. تجزیه و تحلیل FTIR برهمکنش بین اجزای نانوکمپلکس‌ها را تایید کرد. به منظور ارزیابی خواص آنتی باکتریالی نانوکمپلکس‌ها، حداقل غلظت بازدارندگی (MIC) آنها در برابر *Pss* اندازه گیری شد. نتایج نشان داد که نانوکمپلکس‌ها خواص آنتی باکتریالی قابل توجهی در برابر باکتری *Pss* در مقایسه با هر کدام از آنها به تنهایی داشت. به طور خاص، نانو کمپلکس‌ها در مقایسه با اسانس آویشن، مس و نانوذرات کیتوسان به تنهایی، مقدار MIC کمتر و بنابراین فعالیت ضد باکتریایی بالاتری را نشان دادند. این نتایج نشان‌دهنده یک تعامل هم افزایی بین اجزا است، که نشان می‌دهد این نانو کمپلکس‌ها می‌توانند گزینه‌های سازگار با محیط زیست برای کنترل بیماری شانکر باکتریایی ناشی از *Pss* باشند. پژوهش‌های بیشتری برای مطالعه اثربخشی این ترکیبات در شرایط طبیعی و تحقیق در مورد اثرات طولانی مدت زیست محیطی آنها مورد نیاز است.

کلیدواژه‌ها: ***حفاظت از گیاه، نانوکمپلکس ها، شانکر باکتریایی، Pseudomonas syringae***

دبیر تخصصی: دکتر رسول رضائی

Introduction

Pseudomonas syringae is one of the most common pathogens of plants which infect almost all economically important crop species (Lamichhane et al., 2018; Xin et al., 2018). Disease caused by new *P. syringae* isolates, continue to threaten global crop production (Scortichini et al., 2012; Sun et al., 2017; Xin et al., 2018; Córdova et al., 2023). For example, bacterial canker disease caused by *P. syringae* pv. *syringae* (*Pss*) affects all parts of stone fruit trees which causes considerable damage to the trees, and reduces yield (Shahryari et al., 2020). Although this disease occurs worldwide, the current known control methods have limited effects.

Copper-based compounds are usually used to manage the diseases, but limited success has been obtained due to their low efficiency. Moreover, their widespread use has led to copper tolerance and environmental concerns (Shahryari et al., 2020). A new approach for managing these plant diseases is using nanoparticle compounds, which show better control with a lower dose and reduced environmental contaminations (dos Santos et al., 2024).

Copper ions (Cu^{2+}) are used against various fungal and bacterial pathogens in agriculture to control different plant diseases (Varympopi et al., 2020; Cruz-Luna et al., 2021). They appear in some copper-based compounds, which have been commercially used for nearly 14 decades (Lamichhane et al., 2018). Copper nanoparticles are more efficient than conventional copper-based compounds in preventing diseases (Yu et al., 2023). Complexing of copper ions with chitosan nanoparticles (CNPs) is a new approach for preparing stable copper-based nanoparticles with antimicrobial activity. For example, it was shown that copper–CNPs demonstrate higher antifungal activity because of the presence of both copper and chitosan ions. The mechanism of the action was explained so that the chitosan component of the nanoparticles can induce the production of enzymes which play a plant defense role and this will improve plant antifungal and antibacterial activity (Yu et al., 2023). Besides, during plant infection, fungi could produce different levels of acids, which results in the acidic pH and protonate the amino groups of chitosan. As a result, the free copper ions are released from the nanostructures. These copper ions could enter into the fungal cells , which, in turn, induce the synthesis of highly reactive hydroxyl radicals, which could destroy biological molecules (Gomes et al., 2023).

Thymus vulgaris L. (common thyme) is an aromatic plant that belongs to the genus Thymus of the Lamiaceae family (Morales, 2002). Thyme has been employed in medicine, food, agriculture, veterinary, and pest control (Tao et al., 2014). Thyme oil extracted from *Thymus vulgaris* has shown inhibitory activities against different bacteria and yeasts (Deans & Ritchie, 1987; Gaysinsky et al., 2008). The major constituents of thyme essential oil (thyme EO), which is rich in phenolic phytochemicals, have been reported to be thymol, carvacrol, and γ-terpinene (Aydın et al., 2005; Ündeğer et al., 2009).

As a safe and approved food additive, Chitosan is a biopolymer composed of D-glucosamine and N-acetyl-D-glucosamine units linked via β (1–4) glycosidic bond. Chitosan is obtained by deacetylation of chitin, a polysaccharide found in crustacean shells and fungi cell walls. The main characteristics of chitosan include antibacterial and antioxidant effects, biodegradability, nontoxicity, mucoadhesivity, and exceptional biocompatibility, (Ali & Ahmed, 2018). Ionotropic gelation is the most widely used method for CNPs synthesis. In this technique, a polyanion like TPP is used to interact with the positively charged amino groups on chitosan, producing CNPs with spherical shape (Granata et al., 2021).

This study investigated the antibacterial activity of thyme EO/Copper ions/CNPs complexes against *Pss*. As mentioned above, each of these components show antibacterial activity, but their complexes are expected to show superior antibacterial activity against this bacterium. To the best of our knowledge, the antibacterial activity of these complexes against *Pss* has not been reported yet. The final aim of this research is to find a more effective and environmentally friendly approach to fight with stone fruit bacterial canker.

Materials and Methods

Material preparation

Chitosan (≥ 92.0% deacetylation, CAS no.: 9012-76-4, EC no.: 618-480-0) was prepared from Tahoorkaran Bahador Pars Company, Shiraz, Iran. CuSo4 ($5H_2O$), Tween 80, and Sodium tripolyphosphate (TPP) were supplied from Merck Company. Thyme plants were harvested from research field of North Khorasan Agricultural Research, Education and Extension Organization, Iran, Bojnord. After shade drying of thyme leaves, their EO was extracted for 180 min using a Clevenger apparatus. After hydro-distillation, water was removed by decantation and the EO obtained was stored at 4°C for further uses.

Preparation of chitosan nanoparticles (CNPs)

Two hundred milligrams of low molecular weight chitosan was dissolved in 50 ml 1% (v/v) acetic acid solution and mixed overnight to prepare a 4 mg/ml chitosan solution. To 10 ml of this chitosan solution, 1 ml of 20 mg/ml tween 80 solution in deionized water was added. CNPs were prepared using spontaneously ionic gelation method. For this purpose, 1 ml of TPP solution (5 mg/ml) was dropwise added into the above chitosan solution while stirring at 700 RPM on the magnet stirrer. The final pH of the system was adjusted to 4.2-4.5 adding 4N NaOH. Nanoparticle system was dialyzed against deionized water using a dialysis membrane with a 12000 g/mol cut off overnight. Afterwards the system was air dried in 40 °C to dry the particles for further investigation. Copper-loaded chitosan particles were prepared using the same procedure. 5 mg/ml of $CuSO_4.5H_2O$ solution was prepared. Two or 4 ml of this solution was added to the chitosan solution before adding TPP. Thyme EO loaded nanoparticles were prepared using the same procedure. After addition of EO mixing was continued for three hours on magnet stirring with a speed of 700 RPM.

Characterization of particle size

The particle size and polydispersity index (PDI) were measured using dynamic light scattering method (Particle Sizing Systems, K-ONE NANO LTD, DLS9900). The samples were analyzed in three replications at 25°C with 90° scattering angle. The samples were dispersed in deionized water. The average particle size was obtained by analyzing three different batches which each of them was measured three times.

Fourier transforms infrared (FTIR) analysis

FTIR analysis was used to confirm the synthesis of nano-complexes. The results were recorded by Thermo Scientific Nicolet iS10 spectrometer. In FTIR spectroscopy the chemical bonds in a molecule are characterized based on their vibration which depends on the elements and types of bonds. During FTIR measurements, an IR beam is subjected to a spot on the specimen. The specimen's transmittance and reflectance of the infrared radiation are measured at different frequencies and translated into an IR absorption plot.

Bacterial strain and inoculums preparation

A bacterial strain of *Pseudomonas syringae* pv. *syringae* (IBRC-M 10702) was obtained from Iranian biological-resource center, Iran, Karaj.

Growth medium and minimum inhibitory concentration assay (MIC)

For the preparation of inoculum, a single colony of the bacteria was transferred from the solid culture media to 5 ml of Muller Hinton (MH) broth and incubate at 37 °C on shaker incubator (120 rpm). Optical density (OD) was measured at 600 nm after 24 hours incubation and adjusted to 0.5 adding sterile deionized water under the Laminar flow cabinet. Ten sterile test tubes were prepared, and 1 ml of MH Broth was added to each one. One ml of nano-complex suspensions was added to the first test tube and after mixing 1 ml of the mixture was transferred to the second test tube and in the same way serial dilution was obtained up to the tenth test tube. This procedure was repeated for positive control (without treatment: adding deionized water) and Bulk controls (Cu, Thyme EO and CNPs). Then 100µl of the bacterial suspension with adjusted OD at 0.5 was added to each test tube. Negative control tubes (without bacteria) were also used. The prepared test tubes were incubated in shaker incubator at 37 °C for 24 hours and the minimum inhibitory concentrations were determined visually. The MIC assay was performed in triplicate with independently grown cultures. The results showed high reproducibility so calculating a means and statistical analysis was unnecessary.

Results and Discussion

Fourier Transforms Infrared (FTIR) Analysis

FTIR spectroscopy uses infrared waves which induce vibration in the chemical bonds and due to this vibration, the presence and absence of functional groups in samples could be examined. The present study performed FTIR analysis to confirm the interaction of chitosan, TPP and Cu^{2+}-thyme EO. In bulk chitosan, a specific peak at 3357 cm^{-1} corresponds to the combined peaks of the -NH2 and -OH group stretching vibration. The characteristic peak at 2917 cm^{-1} was due to the C-H stretching. A peak at around 1400 cm^{-1} is usually attributed either to the deformation of C-H (Cardenas & Miranda, 2004) or the stretching of C-N, (Qu et al., 2011) and finally, the peaks between 1100 and 1000 cm^{-1}, due to the stretching of the glycosidic bond C-O that connects the glucosamine monomers of chitosan (Kim, 2010). CNPs in comparison to bulk chitosan shows sharper peaks at 3357 cm^{-1}. The characteristic peak at 2917 cm^{-1} became sharper and shifted to 2921 cm^{-1}. The characteristic peak at 1412 shifted to 1456, and new peaks at 1734 cm^{-1} and 1634 cm^{-1} appeared in the spectrum of CNPs. As a result of copper interaction with CNPs the induced characteristic peaks at 3355 cm^{-1}, 1633 cm^{-1}, and 1073 cm^{-1} shifted to 3277 cm^{-1}, 1622 cm^{-1}, 1069 cm^{-1}, respectively, which can be considered as a confirmation of complex formation. Copper concentration did not change the FTIR spectrum significantly, and thyme EO presence did not change the FTIR spectrum (Fig. 1). Therefore, the FTIR study showed a shift in characteristic peaks in CNPs and Cu-CNPs compared to bulk chitosan and these results were in line with earlier findings (Saharan et al., 2013; Saharan et al., 2015; M. K. Choudhary et al., 2017).

Dynamic Light Scattering (DLS) Results

Particle size analysis was conducted using dynamic light scattering method which measures the diffusion of particles in solutions to determine their hydrodynamic size. For this purpose, in 10 ml of deionized water, combinations of chitosan, TPP, $CuSO_4$ ($5H_2O$) and thyme EO according to Table 1, were prepared. The results of particle size analysis have been shown in Table 2.

The average particle size, represented by D50 (median diameter), ranges from 190 nm to 254 nm across the samples. The Polydispersity Index (PDI), an indicator of size distribution, is relatively low for most samples, ranging from 0.97 to 1.12. This

suggests that the samples have a relatively narrow size distribution, with most particles falling within a similar size range. Only Sample 2, where TPP was not added, exhibits a higher PDI of 1.41, indicating a broader size distribution.

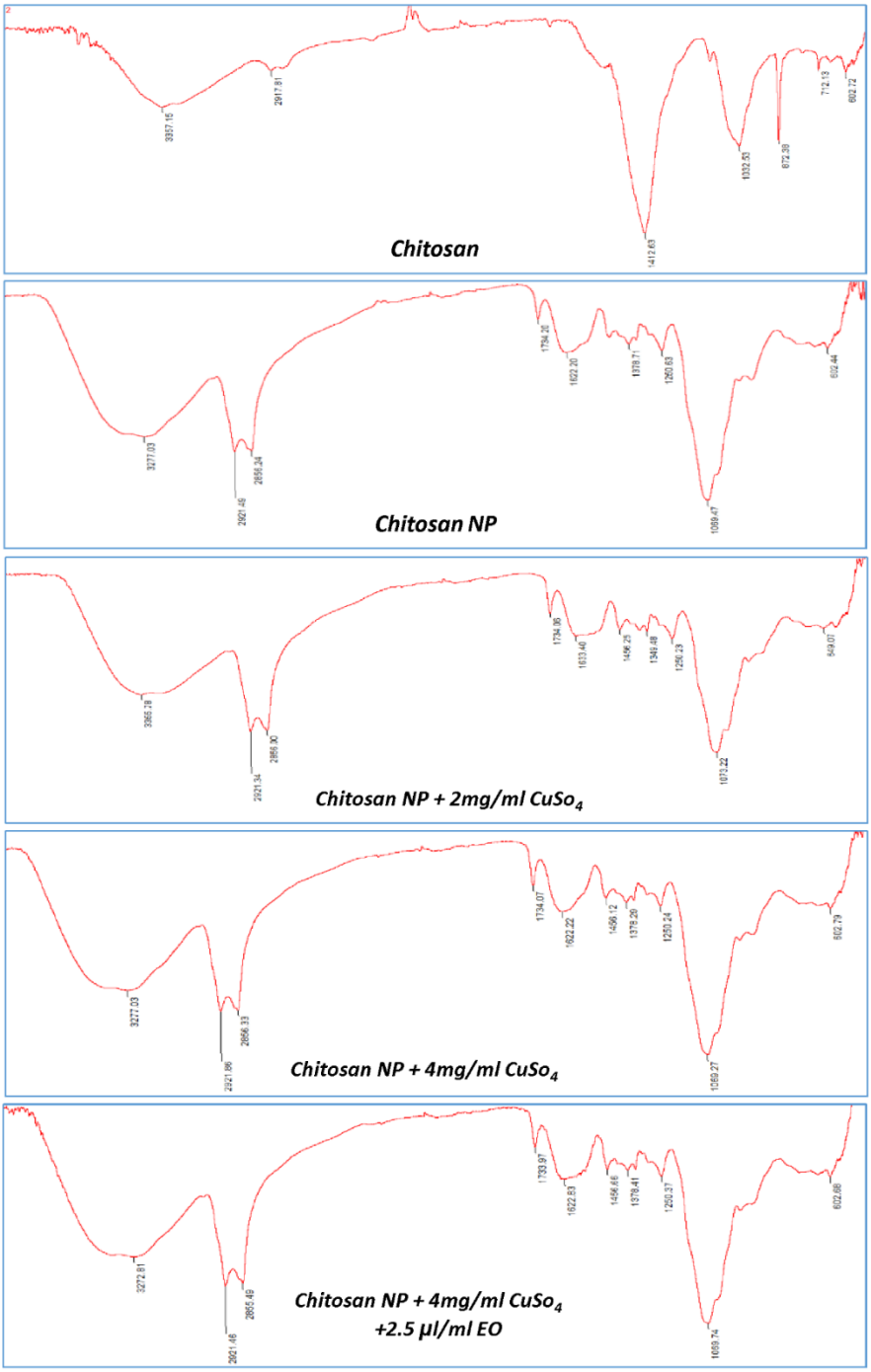

Fig. 1. FTIR spectrum of chitosan, chitosan NP and nano-complexes. In comparison to the bulk chitosan, the characteristic peaks of chitosan were shifted and new characteristic peaks were appeared in chitosan NP spectrum, which can be correlated to the nanoparticle's formation. The copper/chitosan NP interaction was also confirmed by shifting in the characteristic peaks.

Table 1. Concentrations of the main parts of prepared systems for DLS analysis.

Sample No.	Chitosan concentration (mg/ml)	TPP concentration (mg/ml)	CuSO4 (5H2O) concentration (mg/ml)	Thyme EO Concentration (mg/ml)
1	2	0.25	1	0
2	2	0	1	0
3	2	0.25	2	0
4	2	0.25	2	2.5
5	2	0.25	0	0

Table 2. Results of particle size and size distribution analysis for the samples in Table 1.

Sample No.	D10 (nm)	D50 (nm)	D90 (nm)	PDI
1	118	190	330	1.12
2	136	254	495	1.41
3	140	224	370	1.03
4	128	221	351	1.01
5	138	220	352	0.97

Samples 1 and 3-5, which contain chitosan and TPP, exhibit similar particle size distributions compared to Sample 2 (without TPP). This suggests that the presence of TPP, a polyanion, interacts with chitosan, a polycation, to form stable nanoparticles. Adding CuSO4 (Samples 1- 4) did not seem to significantly impact the average particle size or size distribution compared to Sample 5, which lacked CuSO4. Adding $CuSO_4$ ($5H_2O$) concentration from 1 to 2 mg/ml, did not significantly change the particles average size, but interestingly the PDI decreased from 1.12 to 1.03. Sample 4, the only sample containing thyme EO, shows a slightly lower PDI than the other samples. This suggests that the incorporation of thyme EO may contribute to a narrower size distribution.

Overall, the DLS analysis indicated the successful formation of nanoparticles with relatively uniform size distributions in most samples. The presence of TPP appears crucial for nanoparticle formation, while CuSO4 and thyme EO had limited influence on the overall particle size and distribution.

Antibacterial assessment against *Pss*

To assess the antibacterial activity of CNPs/Cu^{2+}/thyme EO complexes against *Pss*, the minimum inhibitory concentration (MIC) for different ratios of these three components was determined. The results of this assessment have been shown in Table 3. In Sample 1, thyme EO had the weakest antibacterial activity. As has been shown in Samples 2-5, $CuSO_4$ ($5H_2O$) solution and CNPs demonstrate lower MIC values and therefor better antibacterial activity in comparison to thyme EO. Adding 1 mg/ml $CuSO_4$ ($5H_2O$) to this CNPs concentration (Sample 6), reduced the MIC value to 0.031 mg/ml. By increasing the $CuSO_4$ ($5H_2O$) concentration to 2 mg/ml (Sample 7), the antibacterial activity has not been improved. At last, as it is shown in Sample 8, addition of thyme EO, did not change the MIC significantly in comparison with CNPs+$CuSO_4$ (Sample 7). However, it showed increased antibacterial activity in comparison with thyme EO, $CuSO_4$ and CNP each alone.

In the absence of CNP (Sample 2), CuSO4 at 2 mg/ml exhibited an MIC of 0.062 mg/ml. When combined with CNP (Samples 6-7), CuSO4 at 1 mg/ml or 2 mg/ml further reduced the MIC to 0.031 mg/ml. This indicates a synergistic effect between CNP and CuSO4 in enhancing antimicrobial activity. In the absence of CNP and CuSO4 (Sample 1), thyme EO had the MIC of 1.25 mg/ml. When combined with CNP and CuSO4 (Sample 8), the MIC was further reduced to 0.031 mg/ml, indicating a synergistic effect among all three components.

Table 3. Minimum Inhibition Concentration for CNP/Cu/Thyme EO complexes with different proportions

Sample No.	Chitosan nanoparticles (CNP) (mg/ml)	$CuSO_4$ ($5H_2O$) (mg/ml)	Thyme essential oil (EO) (mg/ml)	Minimum Inhibition Concentration (mg/ml)
1	0	0	2.5	1.25
2	0	2	0	0.062
3	1	0	0	0.125
4	2	0	0	0.250
5	4	0	0	0.250
6	4	1	0	0.031
7	4	2	0	0.031
8	4	2	2.5	0.031

The effect of Cu^{2+}-CNPs was shown in previous research on pathogenic bacteria to humans or plant pathogenic fungi (Mekahlia & Bouzid, 2009; Manikandan & Sathiyabama, 2015; Tantubay et al., 2015; R. C. Choudhary et al., 2017; Gritsch et al., 2018; Tabesh et al., 2019; Vanti et al., 2020; Ahmed et al., 2021; Omar et al., 2021; Gomes et al., 2023), however it was less studied against the agriculture bacterial pathogens (Hoang et al., 2022; Akdaşçi et al., 2025) and it was not studied before on *P. syringae* pv. *syringae* specifically.

Although the addition of thyme, EO has not increased the antibacterial effect of the nano-complexes, since the thyme EO has pest insect control effect (Barros et al., 2022; Goharrostami et al., 2022), their simultaneous application could also cause pest control effect (in addition to the antibacterial effects). However, further studies are required to confirm the pest control effect.

Conclusion

The antibacterial activity of the CNPs/Cu^{2+}/Thyme EO nano-complexes against *Pseudomonas syringae pv. syringae* as the leading cause of bacterial canker in stone, pome and kiwi fruit trees was investigated. The nano-complexes formation was confirmed by FTIR analysis. DLS analysis showed that nano-complexes had a unimodal size distribution with particle size in the range of 190-254 nm. The minimum inhibitory concentrations of different ratios of these components were determined. Results showed that thyme EO alone does not have significant antibacterial activity against this bacterium compared with CNPs and $CuSO_4$. A synergistic effect of the antibacterial activity of CNPs and $CuSO_4$ was observed so that the MIC value was lower in comparison with each component individually. Combining these three components as an environmentally friendly combination can be suggested as an effective combination to combat bacterial canker in the orchards. However, it should be confirmed further by greenhouse or field tests.

Acknowledgment

The study was supported by Kosar University of Bojnord with the grant number NO.0010271898.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmed, S. B., Mohamed, H. I., Al-Subaie, A. M., Al-Ohali, A. I., & Mahmoud, N. M. (2021). Investigation of the antimicrobial activity and hematological pattern of nano-chitosan and its nano-copper composite. *Scientific Reports, 11*(1), 9540. https://doi.org/10.1038/s41598-021-88907-z

Akdaşçi, E., Duman, H., Eker, F., Bechelany, M., & Karav, S. (2025). Chitosan and Its Nanoparticles: A Multifaceted Approach to Antibacterial Applications. *Nanomaterials*, *15*(2), 126. https://doi.org/10.3390/nano15020126

Ali, A., & Ahmed, S. (2018). A review on chitosan and its nanocomposites in drug delivery. *International Journal of Biological Macromolecules, 109*, 273-286. https://doi.org/10.1016/j.ijbiomac.2017.12.078

Aydın, S., Başaran, A. A., & Başaran, N. (2005). Modulating effects of thyme and its major ingredients on oxidative DNA damage in human lymphocytes. *Journal of Agricultural and Food Chemistry, 53*(4), 1299-1305. https://doi.org/10.1021/jf0402375

Barros, F. A., Radünz, M., Scariot, M. A., Camargo, T. M., Nunes, C. F., de Souza, R. R., et al. (2022). Efficacy of encapsulated and non-encapsulated thyme essential oil (*Thymus vulgaris L.*) in the control of *Sitophilus zeamais* and its effects on the quality of corn grains throughout storage. *Crop Protection, 153*, 105885. https://doi.org/10.1016/j.cropro.2021.105885

Cardenas, G., & Miranda, S. P. (2004). FTIR and TGA studies of chitosan composite films. *Journal of the Chilean Chemical Society, 49*(4), 291-295. https://doi.org/10.4067/S0717-97072004000400005

Choudhary, M. K., Swati, & Saharan, V. (2017). Synthesis, characterization and evaluation of physico-chemical profile of Cu-Chitosan Nanocomposite. *International Journal of Chemical Studies*, *5*(4), 1489-1494.

Choudhary, R. C., Kumaraswamy, R., Kumari, S., Sharma, S., Pal, A., Raliya, R., et al. (2017). Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays L.*). *Scientific Reports, 7*(1), 9754. https://doi.org/10.1038/s41598-017-08571-0

Córdova, P., Rivera-González, J. P., Rojas-Martínez, V., Fiore, N., Bastías, R., Zamorano, A., et al. (2023). Phytopathogenic *Pseudomonas syringae* as a threat to agriculture: perspectives of a promising biological control using bacteriophages and microorganisms. *Horticulturae*, *9*(6), 712. https://doi.org/10.3390/horticulturae9060712

Cruz-Luna, A. R., Cruz-Martínez, H., Vásquez-López, A., & Medina, D. I. (2021). Metal nanoparticles as novel antifungal agents for sustainable agriculture: Current advances and future directions. *Journal of Fungi*, *7*(12), 1033. https://doi.org/10.3390/jof7121033

Deans, S., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. *International Journal of Food Microbiology, 5*(2), 165-180. https://doi.org/10.1016/0168-1605(87)90034-1

dos Santos, O. A. L., dos Santos, M. S., Antunes Filho, S., & Backx, B. P. (2024). Nanotechnology for the control of plant pathogens and pests. *Plant Nano Biology*, 100080. https://doi.org/10.1016/j.plana.2024.100080

Gaysinsky, S., Davidson, P. M., McClements, D. J., & Weiss, J. (2008). Formulation and characterization of phytophenol-carrying antimicrobial microemulsions. *Food Biophysics*, *3*, 54-65. https://doi.org/10.1007/s11483-007-9048-1

Goharrostami, M., Sendi, J. J., Hosseini, R., & Mahmoodi, N. O. A. (2022). Effect of thyme essential oil and its two components on toxicity and some physiological parameters in mulberry pyralid Glyphodes pyloalis Walker. *Pesticide Biochemistry and Physiology, 188*, 105220. https://doi.org/10.1016/j.pestbp.2022.105220

Gomes, D. G., Sanada, K., Pieretti, J. C., Shigueoka, L. H., Sera, G. H., Seabra, A. B., & Oliveira, H. C. (2023). Nanoencapsulation boosts the copper-induced defense responses of a susceptible *Coffea arabica* cultivar against *Hemileia vastatrix*. *Antibiotics, 12*(2), 249. https://doi.org/10.3390/antibiotics12020249

Granata, G., Stracquadanio, S., Leonardi, M., Napoli, E., Malandrino, G., Cafiso, V., et al. (2021). Oregano and thyme essential oils encapsulated in chitosan nanoparticles as effective antimicrobial agents against foodborne pathogens. *Molecules, 26*(13), 4055. https://doi.org/10.3390/molecules26134055

Gritsch, L., Lovell, C., Goldmann, W. H., & Boccaccini, A. R. (2018). Fabrication and characterization of copper (II)-chitosan complexes as antibiotic-free antibacterial biomaterial. *Carbohydrate Polymers, 179*, 370-378. https://doi.org/10.1016/j.carbpol.2017.09.095

Hoang, N. H., Le Thanh, T., Sangpueak, R., Treekoon, J., Saengchan, C., Thepbandit, W., et al. (2022). Chitosan nanoparticles-based ionic gelation method: a promising candidate for plant disease management. *Polymers, 14*(4), 662. https://doi.org/10.3390/polym14040662

Kim, S.-K. (2010). *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*: CRC Press.

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J.-N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. *Agronomy for Sustainable Development, 38*(3), 28. https://doi.org/10.1007/s13593-018-0503-9

Manikandan, A., & Sathiyabama, M. (2015). Green synthesis of copper-chitosan nanoparticles and study of its antibacterial activity. *Journal of Nanomedicine and Nanotechnology, 6*(1), 1. https://doi.org/10.4172/2157-7439.1000251

Mekahlia, S., & Bouzid, B. (2009). Chitosan-Copper (II) complex as antibacterial agent: synthesis, characterization and coordinating bond-activity correlation study. *Physics Procedia, 2*(3), 1045-1053. https://doi.org/10.1016/j.phpro.2009.11.061

Morales, R. (2002). The history, botany and taxonomy of the genus Thymus. In *Thyme* (pp. 15-57): CRC Press.

Omar, H. S., Al Mutery, A., Osman, N. H., Reyad, N. E.-H. A., & Abou-Zeid, M. A. (2021). Genetic diversity, antifungal evaluation and molecular docking studies of Cu-chitosan nanoparticles as prospective stem rust inhibitor candidates among some Egyptian wheat genotypes. *PLoS One, 16*(11), e0257959. https://doi.org/10.1371/journal.pone.0257959

Qu, J., Hu, Q., Shen, K., Zhang, K., Li, Y., Li, H., et al. (2011). The preparation and characterization of chitosan rods modified with Fe3+ by a chelation mechanism. *Carbohydrate Research, 346*(6), 822-827. https://doi.org/10.1016/j.carres.2011.02.006

Saharan, V., Mehrotra, A., Khatik, R., Rawal, P., Sharma, S., & Pal, A. (2013). Synthesis of chitosan based nanoparticles and their in vitro evaluation against phytopathogenic fungi. *International Journal of Biological Macromolecules, 62*, 677-683. https://doi.org/10.1016/j.ijbiomac.2013.10.012

Saharan, V., Sharma, G., Yadav, M., Choudhary, M. K., Sharma, S., Pal, A., et al. (2015). Synthesis and in vitro antifungal efficacy of Cu–chitosan nanoparticles against pathogenic fungi of tomato. *International Journal of Biological Macromolecules, 75*, 346-353. https://doi.org/10.1016/j.ijbiomac.2015.01.027

Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M., & Firrao, G. (2012). *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Molecular Plant Pathology, 13*(7), 631-640. https://doi.org/10.1111/j.1364-3703.2012.00788.x

Shahryari, F., Rabiei, Z., & Sadighian, S. (2020). Antibacterial activity of synthesized silver nanoparticles by sumac aqueous extract and silver-chitosan nanocomposite against *Pseudomonas syringae* pv. *syringae*. *Journal of Plant Pathology, 102*, 469-475. https://doi.org/10.1007/s42161-019-00478-1

Sun, S., Zhi, Y., Zhu, Z., Jin, J., Duan, C., Wu, X., & Xiaoming, W. (2017). An emerging disease caused by *Pseudomonas syringae* pv. *phaseolicola* threatens mung bean production in China. *Plant Disease, 101*(1), 95-102. https://doi.org/10.1094/PDIS-04-16-0448-RE

Tabesh, E., Salimijazi, H., Kharaziha, M., Mahmoudi, M., & Hejazi, M. (2019). Development of an in-situ chitosan-copper nanoparticle coating by electrophoretic deposition. *Surface and Coatings Technology, 364*, 239-247. https://doi.org/10.1016/j.surfcoat.2019.02.040

Tantubay, S., Mukhopadhyay, S. K., Kalita, H., Konar, S., Dey, S., Pathak, A., & Pramanik, P. (2015). Carboxymethylated chitosan-stabilized copper nanoparticles: a promise to contribute a potent antifungal and antibacterial agent. *Journal of Nanoparticle Research, 17*, 1-18. https://doi.org/10.1007/s11051-015-3047-9

Tao, F., Hill, L. E., Peng, Y., & Gomes, C. L. (2014). Synthesis and characterization of β-cyclodextrin inclusion complexes of thymol and thyme oil for antimicrobial delivery applications. *LWT-Food Science and Technology, 59*(1), 247-255. https://doi.org/10.1016/j.lwt.2014.05.037

Ündeğer, Ü., Başaran, A., Degen, G., & Başaran, N. (2009). Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 Chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. *Food and Chemical Toxicology, 47*(8), 2037-2043. https://doi.org/10.1016/j.fct.2009.05.020

Vanti, G. L., Masaphy, S., Kurjogi, M., Chakrasali, S., & Nargund, V. B. (2020). Synthesis and application of chitosan-copper nanoparticles on damping off causing plant pathogenic fungi. *International Journal of Biological Macromolecules, 156*, 1387-1395. https://doi.org/10.1016/j.ijbiomac.2019.11.179

Varympopi, A., Dimopoulou, A., Theologidis, I., Karamanidou, T., Kaldeli Kerou, A., Vlachou, A., et al. (2020). Bactericides based on copper nanoparticles restrain growth of important plant pathogens. *Pathogens, 9*(12), 1024. https://doi.org/10.3390/pathogens9121024

Xin, X.-F., Kvitko, B., & He, S. Y. (2018). *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews Microbiology, 16*(5), 316-328. https://doi.org/10.1038/nrmicro.2018.17

Yu, Y., Liu, H., Xia, H., & Chu, Z. (2023). Double-or triple-tiered protection: prospects for the sustainable application of copper-based antimicrobial compounds for another fourteen decades. *International Journal of Molecular Sciences, 24*(13), 10893. https://doi.org/10.3390/ijms241310893