International Journal of Agricultural Technology 2025 Vol. 21(2):685-696
DOI: https://doi.org/10.63369/ijat.2025.21.2.685-696
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

In vitro biocontrol potential of natural substance combination against plant pathogens

Somsri, A.[1], Saelao, P.[2], Thongsen, N.[2], Kenkhunthot, T.[2], Pilasombut, K.[3], Urairong, H.[4] and Rumjuankiat, K.[4*]

[1]Department of Plant Resources, College of Industrial Sciences, Kongju National University, Yesan 32439, Republic of Korea; [2]Faculty of Agricultural Innovation, Rangsit University, Pathum Thani 12000, Thailand; [3]Office of Administrative Interdisciplinary Program on Agricultural Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand; [4]College of Agricultural Innovation and Food Technology, Rangsit University, Pathum Thani 12000, Thailand.

Somsri, A., Saelao, P., Thongsen, N., Kenkhunthot, T., Pilasombut, K., Urairong, H. and Rumjuankiat, K. (2025). *In vitro* biocontrol potential of natural substance combination against plant pathogens. International Journal of Agricultural Technology 21(2):685-696.

Abstract The antimicrobial potential of chitosan (CHT), banana peel vinegar (BPV) and plant-derived extracts from basil leaves (BE), fingerroot (FRE), and mangosteen peel (MSE) was evaluated against *Diaporthe phaseolorum*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Curvularia* sp., and *Xanthomonas campestris* pv. *campestris* (Xcc)**.** Among these, MSE and FRE exhibited the highest efficacy, with minimum inhibitory concentrations (MIC) of 0.006 mg/ml and 0.048 mg/ml, respectively. Both extracts also inhibited Xcc, showing partial synergistic effects with a fractional inhibitory concentration index (FICI) of 0.625**.** These results highlight the antimicrobial potential of natural compounds, offering valuable insights for developing sustainable strategies in plant disease management and biofungicide formulation.

Keywords: Biocontrol, Natural substance, Plant diseases

Introduction

Plant diseases pose a significant threat to the agriculture sectors as a considerable threat to farming production capacity. Pathogens cause various important plant diseases resulting in crop yield and quality losses (Laura *et al*., 2017). Pathogenic fungi can also release metabolites and harmful toxins during the infection process (Riseh *et al*., 2022). Therefore, effective management of crop plant diseases is paramount to mitigate huge economic losses. Chemical pesticides have been widely applied to control plant diseases due to their easy application, broad spectrum of action, and relatively low investment (Kumar *et al*., 2014). However, the overuse and misuse of synthetic pesticides to increase plant productivity and resistance against agricultural pathogens have negatively

[*] **Corresponding Author**: Rumjuankiat, K.; **Email:** kittaporn.r@rsu.ac.th

impacted human health, the environment, and toxicity to non-target organisms (Kumar and Gupta 2012, Lengai *et al*., 2020, Riseh *et al*., 2022).

Under the changing agriculture scenario, biological control agents have attracted widespread interest as alternative pest management options (Lengai *et al*., 2020). Natural substances as biocontrol agents are now extensively applied in the plant diseases management and inhibit a wide range of plant pathogens. Plant extracts, especially Thai herbal extracts, offer defense mechanisms against pathogens and pests, with crude extracts applied successfully to inhibit fungal and bacterial infections (Rueangrit *et al*., 2019). Alsultan *et al.* (2016) revealed that antibacterial activity of mangosteen leaf extracts inhibited *Pseudomonas syringe* pv. *tomato* and *Xanthomonas oryzae* pv. *oryzae* effectively, whereas mangosteen (*Garcinia mangostana* L.) pericarp extracts also showed antimicrobial potency to control Anthracnose, a postharvest disease in "Kluai Hom Tong" banana (Musa (AAA group)) by the fungus *Colletotrichum musae* (Montri *et al*., 2020). Fingerroot or Kachi was also found to be a powerful fungicidal agent**,** showing inhibition of fungal plant pathogens namely *Colletotrichum gloeosporioides*, *Dothiorella* sp., *Lasiodiplodia theobromae*, *Pythium* sp. and *Pestalotiopsis* sp. (Rattanakreetakul *et al*., 2005). Previous studies screened the antifungal activity of basil extract against important phytogenic fungi, *F. oxysporum* f. sp. *vasinfectum* and *Curvularia* sp. (Silva *et al.,* 2023).

Besides plant extracts, other natural substances are also widely used for plant disease management. Chitosan is gaining attention as an alternative biocontrol agent that has a broad spectrum of antimicrobial properties with various pathogens including *Fusarium thiochromans* (Hua-Li *et al.,* 2017), *Alternaria solani* (Sathiyabama *et al.,* 2014), *Alternaria tenuissima* (Sathiyabama *et al*., 2014), *Penicillium expansum* (Wang *et al*., 2014) and *Aspergillus ochraceus* (Huang *et al*., 2021). Chitosan also has excellent properties of biodegradability and non-toxicity (Badawy and Rabea, 2011), can regulate anti-oxidation and immune functions and has been widely applied in the fields of medicine, food, pesticides, and plant protection (Wang *et al*., 2014, Huang *et al*., 2021). Vinegar is also a powerful natural antimicrobial agent, with applications mostly to inhibit food spoilage pathogens. However, information concerning vinegar is unavailable for plant disease management.

The study assessed the antimicrobial properties of natural substances against plant pathogenic fungi and bacteria that cause plant diseases.

Materials and methods

Preparation of natural antimicrobial substances

Plant crude extracts

Two Thai local plants, *Ocimum basilicum* L. (sweet basil) and *Boesenbergia rotunda* (L.) Mansf. (fingerroot) and one Thai fruit, *Garcinia mangostana* L. (mangosteen) were collected as leaves and the whole peel (outer and inner peels), respectively. The method was modified from Niño *et al.* (2012). The three samples were first cleaned and washed with water to remove all impurities. After rinsing with distilled water, the mangosteen peel was chopped into small pieces (1.0 × 1.0 cm^2). Each plant sample was oven-dried at 50ºC with forced air for 48 h. The dried samples were ground to a fine powder and aliquots were extracted by 95% ethanolic maceration at a ratio of 1:3 (sample: ethanol) for 48 h at RT. Subsequently, all samples were filtrated with Whatman No.1 filter paper to give ethanolic solutions and then evaporated using a vacuum rotary evaporator (Heidolph Laborota 4000, Germany) to collect crude ethanolic extracts of each plant. All the crude extracts were weighed, dissolved in dimethylsulfoxide (DMSO), adjusted to 100 mg/ml, and kept at 4°C until further study.

Chitosan

The shells of *Penaeus monodon* shrimp were thoroughly cleaned using running tap water, followed by rinsing with distilled water to eliminate any remaining impurities. After the washing step, the shells were chopped into small pieces (0.5 × 0.5 cm^2), then boiled in water for 30 minutes and subsequently dried at 60°C for 4 hours. The extraction of chitosan was carried out using a modified procedure based on the methods described by Rakkhumkaew and Pengsuk (2018) and Yodsenee et al. (2020). During the demineralization step, the shrimp shells were immersed in 2M hydrochloric acid (HCl; QRëC, New Zealand) at a 1:20 (w/v) ratio, with continuous stirring for 30 minutes at room temperature. The decalcified shells were then filtered, washed with ionized water until neutral pH was reached, and dried at 60°C overnight. To remove proteins, the dried shells were treated with 2M sodium hydroxide (NaOH; Sigma-Aldrich, Germany) at a 1:20 (w/v) ratio under constant stirring for 2 hours at room temperature. Following this, the shells were filtered, rinsed to neutrality with ionized water, and dried overnight at 60°C. For decolorization, the shrimp shells were treated with 95% (v/v) ethanol for 10 minutes, followed by washing and drying at 60°C overnight. The transformation of chitin into chitosan was achieved through deacetylation, where chitin was exposed to 50% (w/w) NaOH at a 1:100 (w/v) ratio and stirred at 100°C for 2 hours. The resulting product was filtered, washed with distilled water until neutralized, and subsequently dried at 60°C for 24 hours. The chitosan solution was then prepared following the protocol of

Rakkhumkaew and Pengsuk (2018), wherein 1 mg of chitosan was dissolved in 1 ml of 0.1% (v/v) acetic acid and stored at 4°C for further analysis.

Banana peel vinegar

Banana peel vinegar was produced according to Byarugaba-Bazirake *et al.* (2014) with slight modifications. Banana peels were cut into small pieces and boiled in water at a ratio of 1:1 (w/v). After that, the mixture was cooled, homogenized in an electric blender, filtrated into a sterilized glass bottle, and added with 10% (v/v) of *saccharomyces cerevisiae*. The alcohol fermentation process took 2 weeks at room temperature. Then, the banana peel wine from the previous process was fermented again by *Acetobacter aceti* at a ratio of 1:1 (v/v) for 1 month at RT.

Antimicrobial activity

The antimicrobial activities of three ethanolic plant crude extracts, chitosan and 4% acetic acid were evaluated by the spot culture growth inhibition assay according to España *et al.* (2017) and Rakkhumkaew and Pengsuk (2018) with a slight modification.

All tested plant pathogens, *Curvularia* sp., *Colletotrichum gloeosporioides*, *Fusarium oxysporum, Diaporthe phaseolorum*, and *Xanthomonas campestris* pv. *campestris* (Xcc), were obtained from the Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, Thailand. The fungal inhibition activity was investigated using 10 µl of natural antimicrobial substances consisting of mangosteen peel crude extract (MSE; 100 mg/ml), basil crude extract (BE; 100 mg/ml), finger root crude extract (FRE; 100 mg/ml), chitosan (CHT; 1 mg/ml) and 4% (v/v) banana peel vinegar (BPV). They were separately spotted onto the surface of a freshly prepared potato dextrose agar plate (PDA; TM Media, India), which contained a mycelial piece of 6 mm in diameter, using a sterile cork borer from a 7-day-old culture of *C. gloeosporioides, Curvularia* sp*., D. phaseolorum* and *F. oxysporum*, whereas Xcc was used as the test microorganism for the antibacterial assay. This strain was transferred into 10 ml of nutrient broth (Merck, Germany) and incubated at 30ºC overnight. In brief, 10 µl of each natural antimicrobial substance was spotted onto the surface of nutrient agar plates which were swabbed with freshly grown Xcc (10^7 CFU/ml) and incubated at 30ºC overnight. Then, the plates were investigated for fungal and bacterial growth by the clear zone diameter of growth inhibition.

Determination of the minimal inhibitory concentration (MIC)

The MIC values were carried out according to the modified method explained by Sharma *et al.* (2020) and Roudbary *et al.* (2023). The MICs of MSE (100 mg/ml) and FRE (100 mg/ml) were determined by the microdillution method. Briefly, 100 µl of different concentrations of MSE and FRE were serially diluted two-fold, and containing medium was put to separate wells of sterile 96-well microtiter plates (Falcon, USA). The mixing wells of MSE and FRE were spotted onto the surface of a PDA medium plate which already contained a mycelial piece of *C. gloeosporioides, Curvularia* sp*., D. phaseolorum* and *F. oxysporum*, and swabbed NA medium with Xcc. DMSO solution without MSE and FRE was used as a negative control. The MIC was read visually and recorded as the lowest concentration of MSE and FRE to inhibit the tested microorganisms.

Evaluation of synergism in MSE and FRE

Different kinds of interactions (synergism, antagonism or additive effect) between the plant crude extracts (MSE and FRE) and tested microorganisms were determined using a checkerboard microdilution test according to the modified method of Nikkhah *et al.* (2017). The assessed concentrations of MSE (100 mg/ml) and FRE (100 mg/ml) were performed using a two-fold serial dilution. The first well in each row of the sterile 96-well microtiter plates contained MSE and FRE. Each test was performed in duplicate, and the fractional inhibitory concentration index (FICI) was as follows:

$$FICI = FIC_{(MSE)} + FIC_{(FRE)}$$

Where $FIC_{(MSE)}$ = MIC of MSE in combination/MIC of MSE alone, $FIC_{(FRE)}$= MIC of FRE in combination/MIC of FRE alone, FICI was calculated as the sum of $FIC_{(MSE)} + FIC_{(FRE)}$ for double combinations, with combinations considered as follows: Synergy ($FICI < 0.5$), addition ($0.5 \leq FICI \leq 1$), indifference ($1 < FICI \leq 4$), and antagonism ($FICI > 4$).

Results

Antimicrobial effect of natural substances on plant pathogens

The antimicrobial capacity of natural extracts, comprising chitosan (CHT), banana peel vinegar (BPV) and three herbal crude extracts of mangosteen peel (MSE), basil leaves (BE) and fingerroot (FRE) were initially investigated against different fungal strains and bacteria by the agar well

diffusion method, as shown in Table 1. Our findings revealed that almost every natural agent presented antimicrobial potency against both pathogenic fungi and bacteria causing plant diseases. The MSE and FRE treatments showed the broadest spectrum of action and exhibited growth of all tested organisms including *C. gloeosporioides, Curvularia* sp*., D. phaseolorum, F. oxysporum* and Xcc, whereas CHT showed antifungal capability to inhibit the growth of *Curvularia* sp. and *F. oxysporum.* The plate of BPV treatment displayed an inhibition zone for *X. campestris*, with BE appearing as a weak antimicrobial substance, presenting no inhibition zones for all test organisms.

Table 1. Preliminary screening of antimicrobial activity using crude extracts of Thai herbal plant, chitosan, and banana peel vinegar

Test organisms	Antimicrobial substance				
	MSE	**BE**	**FRE**	**CHT**	**BPV**
Fungi					
C. gloeosporioides	+	-	+	-	-
Curvularia sp.	++	-	+	+++	-
D. phaseolorum	+	-	+	-	-
F. oxysporum	+	-	+	+	-
Bacteria					
Xcc	+++	-	++	-	+

Results were interpreted as - no inhibition, + inhibition diameter between 1 and 3 mm, ++ inhibition diameter between 4 and 6 mm, and +++ inhibition diameter of more than 7 mm. Each extract (10 µl /disk) was tested in triplicate. Mangosteen peel extract (MSE), basil extract (BE), Fingerroot extract (FRE), Chitosan (CHT), and Banana peel vinegar (BPV).

Determination of minimum inhibitory concentration (MIC)

MSE and FRE, which showed the most powerful antimicrobial properties against all tested organisms, were selected to observe the minimum inhibitory concentration (MIC) of antimicrobial agents. MIC values of MSE against the plant pathogens ranged from 0.006-6.250 mg/ml (Table 2). Xcc showed the highest susceptibility with MIC value 0.006 mg/ml, followed by *Curvularia* sp. and *D. phaseolorum* with MIC values of 0.780 and 3.120 mg/ml, respectively whereas *C. gloeosporioides* and *F. oxysporum* were the most resistant strains to MSE with the same MIC value of 6.250 mg/ml. For FRE, MIC values ranged from 0.048 to 50.00 mg/ml, indicating lower antimicrobial potency compared to MSE. X. campestris was still the most sensitive strain to herbal plant extracts among the tested microorganisms with MIC values of 0.048 mg/ml. By contrast, *Curvularia* sp., *D. phaseolorum* and *F. oxysporum* were the most resistant photogenic fungi in the MSE treatment, with MIC values of 50.00 mg/ml, followed by *C. gloeosporioides* at 25.00

mg/ml. Overall, MSE was demonstrated as a stronger antimicrobial agent than FRE.

Table 2. The minimum inhibitory concentration (MIC) of plant extracts against the test microorganism

Test organisms	MIC (mg /ml)	
	MSE	FRE
Fungi		
C. gloeosporioides	6.250	25.00
Curvularia sp.	0.780	50.00
D. phaseolorum	3.120	50.00
F. oxysporum	6.250	50.00
Bacteria		
Xcc	0.006	0.048

Evaluation on the synergistic effect of MSE/FRE combination

The synergistic effect of MSE and FRE was determined in combination with both crude extracts to evaluate the antimicrobial potency using FICI indices. FICI scales were indicated as follows: synergy (FICI < 0.5), addition (0.5 ≤ FICI ≤ 1), indifference (1 < FICI ≤ 4), and antagonism (FICI > 4). The combination of MSE/FRE showed an antagonism effect for *C. gloeosporioides*, *D. phaseolorum* and *F. oxysporum*, indicating low effectiveness for antifungal substances with FICI values over 4 (Table 3). The plate of *Curvularia* sp. had an indifferent result for FICI 2.125. Xcc was the only susceptible bacterial strain to the MSE/FRE mixture, indicating partial synergy (FICI=0.625).

Table 3. The synergistic capacity of MSE and FRE against plant pathogens

Test organisms	Treatment	MIC alone	MIC in combination	FIC	FICI	Inter action
C. gloeosporioides	MSE	6.25	25.0	4.0	4.125	antagonism
	FRE	25.0	3.125	0.125		
Curvularia sp.	MSE	0.78	0.0976	0.125	2.125	indifference
	FRE	50.00	100	2.0		
D. phaseolorum	MSE	3.13	12.5	4.0	4.50	antagonism
	FRE	50.00	25.0	0.5		
F. oxysporum	MSE	6.25	25.0	4.0	4.50	antagonism
	FRE	50.00	25.0	0.5		
Xcc	MSE	0.006	0.0031	0.5	0.625	partial synergy
	FRE	0.049	0.006	0.125		

Interpretative criteria for the FIC index were as follows: synergy, FICI < or = 0.5, partial synergy, FICI 0.51 to 0.75, indifference, FICI 0.76 to 4.0, and antagonism, FICI > 4.0 (Ko *et al*., 2017).

Discussion

Crop plants are regularly targeted by several of pathogens and pests during both pre- and post-harvest stages, resulting in significant food and economic losses globally (Wang *et al*., 2017). Sustainable and organic agriculture requires eco-friendly disease management approaches to improve the quality of farm produce. Currently, various natural substances have been used, with some contributing to the development of novel plant-based biopesticides for agriculture production (Jamiolkowska, 2020). In this study, we selected five natural materials comprising mangosteen peel (MSE), basil leave (BE), fingerroot (FRE), chitosan (CHT), and banana peel vinegar (BPV) based on ethnobotanical data and traditional uses for antimicrobial substances. The antimicrobial activity of these natural substances was first screened using simple agar-bioassays, with further analyses including MIC and the synergistic effect. Among the selected biocontrol agents, MSE and FRE proved to be superior inhibitors of all plant pathogens, whereas CHT effectively repressed the growth of *Curvularia* sp. and *D. phaseolorum* and BPV showed antibacterial potency only for Xcc. BE had no effect on the test microorganism because aqueous extraction is less effective compared to other solvents such as ethanol and methanol (Lima *et al*., 2019).

The mode of action for the antimicrobial potency of each natural material has been previously explained. Lopez-Moya *et al*. (2019) pointed out that CHT was a versatile compound with antimicrobial activity, and this polymer reduced the germination of various plant pathogenic and mycoparasitic fungi (such as *Fusarium* sp. and *Colletotrichium* sp.). CHT inhibited DNA/RNA synthesis and interrupted protein synthesis of chitosan-sensitive fungi (Verlee *et al.,* 2017), whereas BPV inhibited only gram-negative bacteria. This finding consistent with previous studies which reported that acetic acid possessed high antibacterial potency for gram-negative bacteria (Ryssel *et al.,* 2009). The antimicrobial activity of organic acids primarily arises from their undissociated forms (Malicki *et al*., 2004) that diffuse through the bacterial cell wall, causing a reduction in cytoplasmic pH (Plumridge *et al*., 2004) and inhibit nutrient uptake of pathogen cells (Novodvorska *et al.,* 2016). Various studies have proved that weak acids are broad-spectrum antimicrobials, with effects on different types of pathogenic microbes (Plumridge *et al.,* 2004). BPV tends to be fungistatic rather than fungicidal due to the low percentage of acetic acid. For plant extracts, the highest antimicrobial activity of FRE and MSE contributed to the activity of phenolic compounds with excellent biological activities, especially antimicrobial properties. FRE contains at least 5 compounds as geraniol, camphor, ocimene, eucalyptol and camphene which act against fungal plant pathogens including *F. oxysporum* and *C. gloeosporioides*

(Eng-Chong *et al*., 2012). MSE is rich in xanthones, a class of polyphenolic compounds, along with other bioactive substrances comprising tannins, flavonoids, saponins and anthocyanins (Widyarman *et al*., 2019). Several studies elucidated that xanthones from mangosteen pericarp crude extract showed potential to inhibit phytogenic fungi such as *F. oxysporum*, *Alternaria tenuis* and *Drechslera oryzae* (Moopayak and Tangboriboon, 2020). Flavonoid compounds from mangosteen peel also had a strong inhibitory effect against pathogens such as Xcc, *Fusarium* sp., phytophagous insects and nematodes (Tran *et al*., 2021; Abate *et al*., 2022). Therefore, MSE and FRE were considered the most effective antimicrobial agents to determine MIC, as shown in Table 2. Our findings revealed that both extracts uniformly inhibited the growth of most pathogens with MIC values 0.006-6.250 mg/ml and 0.048-50.00 mg/ml, respectively. The MSE treatment showed the highest inhibition of mycelial growth for all plant pathogens compared to FRE. Therefore, MSE and FRE were combined to measure their synergistic effect. Synergism is a process whereby some compounds of natural agents cooperate to achieve a combined effect greater than the sum of their separate effects (Pezzani *et al*., 2019).

Many studies have recorded those combinations of plant extracts or plant extracts combined with antibiosis revealed synergistic activity against pathogens (Rueangrit *et al.,* 2019). Hakalová *et al.* (2022) reported the combined effect of thyme and clove against the seed-borne bacterium Xcc that causes back rot disease in cabbage. Medina-López *et al*. (2016) reported synergistic activities of the combination of two antifungal fractions obtained from *Jacquinia macrocarpa* extracts and *Baccharisglutinosa* against *Fusarium verticillioide Aspergillus flavus.* In our study, the combination of FRE+MSE showed antagonism in the inhibition of *C. gloeosporioides*, *D. phaseolorum* and *F. oxysporum*, resulting in reduced additive effect (Caesar and Cech 2019). Indifference was also observed for *Curvularia* sp. and partial synergy was recorded in culture plates of Xcc, implying that the combination of both crude extracts effectively repressed the growth of Xcc *in vitro* (Table 3). Previous studies have revealed that Xcc was responsible for black rot in crucifers (Fontana *et al*., 2021). This disease is one of the most widespread and difficult to control because the bacterium has a single polar flagellum that can penetrate the plant and reach the vascular system (Singh *et al*., 2018, Qi *et al.,* 2020). The effects of combinations of these two herbal extracts have not been previously addressed. Our study reported the synergistic activity of FRE and MSE combinations as beneficial information to further evaluate the use of these plant extracts for plant disease management in greenhouse or field studies.

Acknowledgments

The author would like to offer particular thanks to the College of Agricultural Innovation and Food Technology, Rangsit University, Pathum Thani 12000, Thailand.

References

Abate, M., Pagano, C., Masullo, M., Citro, M., Pisanti, S., Piacente, S. and Bifulco M. (2022). Mangostanin, a xanthone derived from garcinia mangostana fruit, exerts protective and reparative effects on oxidative damage in human keratinocytes. Pharmaceuticals, 15(1):84.

Alsultan, Q. M. N., Sijam, K., Rashid, T. S. and Ahmad, K. B. (2016). GC-MS analysis and antibacterial activity of mangosteen leaf extracts against plant pathogenic bacteria. American Journal of Plant Sciences, 7:1013-1020.

Badawy, M. E. and Rabea, E. I. (2011). A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. International Journal of Carbohydrate Chemistry, 2011:1-29.

Byarugaba-Bazirake, G., Byarugaba, W., Tumusiime, M. and Kimono, D. (2014). The technology of producing banana wine vinegar from starch of banana peels. African Journal of Food Science and Technology, 5:1-5.

Caesar, L. K. and Cech, N. B. (2019). Synergy and antagonism in natural product extracts: when 1+1 does not equal 2. Natural Product Reports, 36:869-888.

Eng-Chong, T., Yean-Kee, L., Chin-Fei, C., Choon-Han, H., Sher-Ming, W., Li-Ping, C. T., Gen-Teck, F., Khalid, N., Abd Rahman, N., Karsani, S.A., Othman, S., Othman, R. and Yusof, R. (2012). Indonesian mangosteen fruit (*garcinia mangostana* l.) peel extract inhibits *streptococcus mutans* and *porphyromonas gingivalis* in biofilms *In vitro*. Evidence-Based Complementary and Alternative Medicine, 2012:473637.

España, M. D., Arboleda, J. W., Ribeiro, J. A., Abdelnur, P. V. and Guzman, J. D. (2017). Eucalyptus leaf byproduct inhibits the anthracnose-causing fungus *Colletotrichum gloeosporioides*. Industrial Crops and Products, 108:793-797.

Fontana, R., Caproni, A., Buzzi, R., Sicurella, M., Buratto, M., Salvatori, F., Pappadà, M., Manfredini, S., Baldisserotto, A. and Marconi, P. (2021). Effects of *Moringa oleifera* Leaf Extracts on *Xanthomonas campestris* pv. *campestris.* Microorganisms, 9:2244.

Hakalová, E., Čechová, J., Tekielska, D. A., Eichmeier, A. and Pothier, J. F. (2022). Combined effect of thyme and clove phenolic compounds on *Xanthomonas campestris* pv. *campestris* and biocontrol of black rot disease on cabbage seeds. Frontiers in Microbiology, 13.

Hua-Li, X., Yang, B., Yuan-Yuan, Z., Calderón-Urrea, A., Hu-Jun, W., Lu-Mei, P., Yi, W. and Yong-Cai, L. (2017). Effects of elicitors on trichothecene accumulation and Tri genes expression in potato tubers inoculated with *Fusarium sulphureum*. European Journal of Plant Pathology, 148:673-685.

Huang, X., You, Z., Luo, Y., Yang, C., Ren, J., Liu, Y., Wei, G., Dong, P. and Ren, M. (2021). Antifungal activity of chitosan against *Phytophthora infestans*, the pathogen of potato late blight. International Journal of Biological Macromolecules, 166:1365-1376.

Jamiołkowska, A. (2020). Natural compounds as elicitors of plant resistance against diseases and new biocontrol strategies. Agronomy, 10:173.

Ko, S. J., Kim, M. K., Bang, J. K., Seo, C. H., Luchian, T. and Park, Y. (2017). *Macropis fulvipes* venom component Macropin exerts its antibacterial and anti-biofilm properties by damaging the plasma membranes of drug resistant bacteria. Scientific Reports, **7**:16580.

Kumar, S. and Gupta, O. (2012). Expanding dimensions of plant pathology. JNKVV Res. J, 46:286-293.

Kumar, S., Thakur, M. and Rani, A. (2014). Trichoderma: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. African Journal of Agricultural Research , 9:3838-3852.

Laura, O., Beatrice, C., Cinzia, F. and Luca, R. (2017). Chitosan in agriculture: A new challenge for managing plant disease. Biological Activities and Application of Marine Polysaccharides, A. S. Emad. Rijeka, IntechOpen**:** Ch. 2.

Lengai, G. M. W., Muthomi J. W. and Mbega, E. R. (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific African, **7**: e00239.

Lima, M. D. C., De Sousa, C. P. , Fernandez-Prada, C., Harel, C., Dubreuil, J. and De Souza, E. (2019). A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. Microbial Pathogenesis, 130:259-270.

Lopez-Moya, F., Suarez-Fernandez, M., and Lopez-Llorca, L. V. (2019). Molecular mechanisms of chitosan interactions with fungi and plants. International Journal of Molecular Sciences, 20:332.

Malicki, A., Zawadzki, W., Bruzewicz, S., Graczyk, S. and Czerski, A. (2004). Effect of formic and propionic acid mixture on *Escherichia coli* in fish meal stored at 12 C. Pakistan Journal of Nutrition, 3:353-356.

Medina-López, C. F., Plascencia-Jatomea, M., Cinco-Moroyoqui, F. J., Yépiz-Gómez, M. S., Cortez-Rocha, M. O. and Rosas-Burgos, E. C. (2016). Potentiation of antifungal effect of a mixture of two antifungal fractions obtained from *Baccharis glutinosa* and *Jacquinia macrocarpa* plants. Journal of Environmental Science and Health*,* Part B, 51:760-768.

Niño, J., Mosquera, O. M. and Correa, Y. M. (2012). Antibacterial and antifungal activities of crude plant extracts from Colombian biodiversity. Revista de Biologia Tropical, 60:1535-1542.

Nikkhah, M., Hashemi, M., Najafi, M. B. H. and Farhoosh, R. (2017). Synergistic effects of some essential oils against fungal spoilage on pear fruit. International Journal of Food Microbiology, 257:285-294.

Montri, N., Leujantuak, P., Bunya–atichart, K. and Deewatthanawong, R. (2020). Control of anthracnose in "Kluai Hom Tong" banana (*Musa* (AAA group)) using mangosteen pericarp crude extract. Thai Journal of Agricultural Science, 53:228-235.

Moopayak, W. and Tangboriboon, N. (2020). Mangosteen peel and seed as antimicrobial and drug delivery in rubber products. Journal of Applied Polymer Science, 137:49119.

Novodvorska, M., Stratford, M., Blythe, M. J., Wilson, R., Beniston, R. G. and Archer, D. B. (2016). Metabolic activity in dormant conidia of *Aspergillus niger* and developmental changes during conidial outgrowth. Fungal Genetics and Biology, 94:23-31.

Pezzani, R., Salehi, B., Vitalini, S., Iriti, M., Zuñiga, F. A., Sharifi-Rad, J., Martorell, M. and Martins, N. (2019). Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective. Medicina (Kaunas),55(4).

Plumridge, A., Hesse, S. J., Watson, A. J., Lowe, K. C., Stratford, M. and Archer, D. B. (2004). The weak acid preservative sorbic acid inhibits conidial germination and mycelial growth of *Aspergillus niger* through intracellular acidification. Applied and Environmental microbiology, 70:3506-3511.

Qi, Y. H., Huang, L., Liu, G. F., Leng, M. and Lu, G. T. (2020). PilG and PilH antagonisticallycontrol flagellum-dependent and pili-dependent motility in the phytopathogen *Xanthomonas campestris* pv. *campestris*. BMC Microbiology, 20:1-14.

Rakkhumkaew, N. and Pengsuk, C. (2018). Chitosan and chitooligosaccharides from shrimp shell waste: characterization, antimicrobial and shelf life extension in bread. Food Science and Biotechnology, 27:1201-1208.

Rattanakreetakul, C., Jamkratoak, S., Leksomboon, C., Farungsang, N. and Farungsang, U. (2005). Chemical properties of essential oil from Krachai and their antifungal activity. Proceedings of 43rd Kasetsart University Annual Conference, Thailand, 697 p.

Riseh, R. S., Hassanisaadi, M., Vatankhah, M., Babaki, S. A. and Barka, E. A. (2022). Chitosan as a potential natural compound to manage plant diseases. Int J Biol Macromol, 220:998-1009.
Rueangrit, S., Eakjamnong, W. and Dethoup, T. (2019). Hidden synergistic effects of the combinations of plant extracts against plant pathogenic fungi. Journal of Biopesticides, 12:51-60.
Roudbary, M., Alimohammadi, A., Tavallaei, M. R., Zarimeidani, R and Nikoomanesh, F. (2023). Antifungal activity of *Thymus kotschyanus* extract: An *in vitro* study on the expression of *CDR1* and *CDR2* genes in clinical isolates of *Candida albicans*. Journal of Herbal Medicine, 38(100644).
Ryssel, H., Kloeters, O., Germann, G., Schäfer, T., Wiedemann, G. and Oehlbauer, M. (2009). The antimicrobial effect of acetic acid: an alternative to common local antiseptics. Burns, 35:695-700.
Sathiyabama, M., Akila, G and Charles, R. E. (2014). Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer. Archives of Phytopathology and Plant Protection, 47:1963-1973.
Sharma, K., Guleria, S., Razdan, V. K. and Babu, V. (2020). Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. Industrial Crops and Products, 154(112569.
Silva, M. S. B. d. S., Rodrigues, A. A. C., Oliveira, A. C. S. d., Silva, E. K. C., Dias, L. R. C. and Costa, N. d. J. F. (2023). Plant extracts in the control of plant pathogens seeds and fusariosis in okra. Revista Ceres, 70:124-131.
Singh, S., Dey, S., Bhatia, R., Batley, J. and Kumar, R. (2018). Molecular breeding for resistance to black rot *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson in Brassicas: recent advances. Euphytica, 214:1-17.
Tran, T. H., Le, H. T., Nguyen, H. M. , Tran, T. H., Do Thi, T., Nguyen, X. C. and Ha, M. T. (2021). Garcinoxanthones SV, new xanthone derivatives from the pericarps of Garcinia mangostana together with their cytotoxic and antioxidant activities. Fitoterapia, 151: 104880.
Verlee, A., Mincke, S. and Stevens, C. V. (2017). Recent developments in antibacterial and antifungal chitosan and its derivatives. Carbohydrate Polymers, 164:268-283.
Wang, L., Wu, H., Qin, G. and Meng, X. (2014). Chitosan disrupts *Penicillium expansum* and controls postharvest blue mold of jujube fruit. Food Control, 41:56-62.
Wang, M., Thomas, N. and Jin, H. (2017). Cross-kingdom RNA trafficking and environmental RNAi for powerful innovative pre-and post-harvest plant protection. Current Opinion in Plant Biology, 38:133-141.
Widyarman, A. S., Lay, S. H., Wendhita, I. P., Tjakra, E. E., Murdono, F. I. and Binartha, C. T. O. (2019). Indonesian mangosteen fruit (*Garcinia mangostana* L.) peel extract inhibits *streptococcus* mutans and *porphyromonas gingivalis* in Biofilms *In vitro*. Contemp Clin Dent, 10:123-128.
Yodsenee, K., Showpanish, K., Sonhom, N., Pilasombut, K., Prachom, N., Sathitkowitchai, W., Buathong, R. and Rumjuankiat, K. (2020). Encapsulation of *Pediococcus pentosaceus* RSU-Nh1 into pectin-sodium alginate and chitosan coating. Technology, 16:207-222.

(Received: 25 September 2024, Revised: 3 March 2025, Accepted: 12 March 2025)