

BIOCOMPATIBLE MANAGEMENT ON POSTHARVEST DISEASES

Diyana ALEKSANDROVA¹, Gabor ZSIVANOVITS²

¹Agricultural Academy, Fruit Growing Institute - Plovdiv,
12 Ostromila, Plovdiv, Bulgaria

²Agricultural Academy, Institute of Food Preservation and Quality,
Plovdiv, Bulgaria

Corresponding author email: aleksandrova_diyana@abv.bg

Abstract

*In recent years, there has been a trend to research alternative methods for controlling post-harvest fungal pathogens. Using biopolymers and plant extracts is an innovative method that includes many advantages, with human health as a priority. The research includes the efficiency of biopolymer chitosan and extract of grape seed and a combination of them used to control the fungal pathogens *Alternaria* spp. and *Penicillium* spp. in vitro conditions. Grape seed extract has antimicrobial activity, manifested by phenolic compounds that cause oxidation of the cell membranes of pathogens without affecting the plants. As a biological agent, chitosan is a biopolymer natural polysaccharide that has several amino groups, determining its activity against plant pathogens. The best percentage of mycelial inhibition varied according to the two pathogens. The pathogen *Alternaria* spp. was influenced by the combination of chitosan (1%) and grape seed extract (1.5%), the experiment has significant differences between different variants in research. The mycelia of *Penicillium* spp. showed the best result with inhibition by applying chitosan (1%) with a 1 % solution of grape seed extract. The highest percentage of mycelia growth inhibition was achieved on the sixth day of measurement 20.18% in the pathogen *Alternaria* spp. According to the control variant, *Penicillium* spp. has been highest suppressed on the fourth day 15.21% of cultivation of fungal pathogen.*

Key words: biopolymers, chitosan, grape seed extract, antimicrobial activity.

INTRODUCTION

The used of synthetic fungicides for the control of phytopathogenic fungi has traditionally been associated with a number of harmful environmental consequences. These consequences include toxicity to non-target organisms, the development of resistance in pathogen populations, and environmental pollution. In the context of these challenges, the importance of sustainable alternatives such as biocides, which support the transition to more environmentally friendly agricultural practices, is growing (Kumar et al., 2022).

Chitosan, a unique natural biopolymer, is derived from chitin through deacetylation. It is the second most abundant natural polysaccharide, after cellulose, and the most abundant biopolymer of animal origin. It is a non-toxic, biocompatible, biodegradable polymer (Hisham et al., 2024). Chitin's sources are diverse, ranging from terrestrial arthropods (e.g., spiders, scorpions, beetles, cockroaches, and brachiopods) and marine crustaceans (e.g.,

crab, lobster, prawn, and krill) to Mollusca (e.g., squid) and microorganisms (e.g., fungi cell walls - Alishahi & Aider, 2012). Currently, the primary industrial source of chitin is the waste generated by marine food production, particularly the exoskeletons of crabs, lobsters, and shrimps (Leceta et al., 2013). Based on the exciting literature it is also the most environment friendly raw material of the chitosan (Kou et al., 2021).

The management of phytopathogenic fungal *Alternaria alternata* and *Fusarium* spp. diseases through, chitosan nanoparticles (NCTs), showed antifungal properties but lower efficacy, requiring 1000 ppm for complete inhibition (Gharieb et al., 2025).

The potential of incorporating grape seed extract (GSE) into biopolymer coatings to enhance food preservation, particularly focusing on its antioxidant properties and suitability for combating lipid oxidation. Biopolymers offer a compelling alternative to synthetic polymers in food packaging due to their edibility,

biocompatibility, and biodegradability (Ezati & Rhim., 2020; Riahi et al., 2021).

Crucially, biopolymers can serve as carriers for functional compounds, enabling the development of active packaging films with enhanced properties (Roy & Rhim, 2020).

The release rate of bioactive materials from biopolymers often surpasses that of synthetic counterparts, making them ideal for delivering active ingredients. Various active compounds can be incorporated into biopolymers to impart specific functionalities. These include nanoparticles, natural plant extracts and essential oils, and antibiotic drugs (Priyadarshi & Rhim, 2020).

The review of many studies focuses on using natural plant extracts, specifically GSE, for their antioxidant potential. Lipid oxidation is a significant factor contributing to the deterioration of many foods, including peanuts. Therefore, incorporating antioxidant properties into coating formulations is paramount for preserving food quality and extending shelf life. Recent research has explored numerous natural antioxidants derived from agricultural or natural waste streams, highlighting their safety, cost-effectiveness, and potential for value-added utilization (Jiang et al., 2021; Zhang et al., 2020).

GSE has emerged as a promising natural antioxidant for enriching biopolymer coatings (Priyadarshi et al., 2021; Wu et al., 2019).

Grape seeds, a significant byproduct of the wine and grape juice industry, are abundant in polyphenolic compounds, including catechins, epicatechins, procyanidins, and gallic acid (Krishnaswamy et al., 2013; Sogut & Seydim, 2018; Wu et al., 2019).

These compounds contribute to GSE's potent antioxidant activity. Furthermore, GSE established safety and edibility make it an attractive candidate for use in food packaging applications (Krishnaswamy et al., 2013).

The positive effect of biopolymers application on fungal pathogens was confirmed in our previous research (Aleksandrova et al., 2021; Aleksandrova et al., 2019; Zsivanovits, et al., 2022).

This study aims to investigate the potential of biopolymer coatings and plant extracts for controlling post-harvest diseases. Specifically, it will evaluate the inhibitory effects of chitosan

combined with grape seed extract against the fungal pathogens *Penicillium* spp. and *Alternaria* spp.

MATERIALS AND METHODS

Monitoring of infected peach fruit was collected for the experiment in the phytopathology laboratory of Fruit Growing Institute - Plovdiv. The strain of fungal pathogens *Alternaria* spp. and *Penicillium* spp. were isolated from infected peach fruit in the storage period. Pathogens were isolated by standard phytopathological methods of the infected fruit placed on potato dextrose agar. The single spore of the pathogens was cultured on potato dextrose agar (PDA) for two weeks at $25 \pm 1^\circ\text{C}$ for sporulation.

Seven variants of the antifungal action of 1% chitosan and grape seed extract have been studied. The grape seed extract (GSE) was used alone or in combination with chitosan. For the control variant, a Petri dish was used only with PDA and fungal pathogens. The media application includes:

Chitosan–potato dextrose agar (PDA), application 1% of chitosan and pathogen;

GSE (1%) – PDA, GSE 1%, and pathogen;

GSE (1.5%) – PDA, GSE 1.5%, and pathogen;

GSE (2%) – PDA, GSE 2%, and pathogen;

ChGSE (1%) – PDA, 1% of chitosan solution, GSE 1%, and pathogen;

ChGSE (1.5%) – PDA, 1% of chitosan solution, 1.5% GSE, and pathogen;

ChGSE (2%) – PDA, 1% of chitosan solution, GSE 2%, and pathogen.

The development of fungal cultures on the medium was studied by measuring the colony diameter every two days. The percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Inhibition \%} = \frac{(dc-dt)}{dc} \cdot 100, \text{ where:}$$

dc = the average diameter of the fungal colony in the control variant;

dt = the average diameter of the fungal colony of the treatment variant.

Each variant was replaced in five repetitions.

The data obtained was analysed statistically using the SPSS 19.0 program. The data was subjected to Duncan's Multiple Range Test (MRT) at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The fungal pathogens (*Alternaria* spp. and *Penicillium* spp.) were isolated from infected peach fruits during the storage period. The pathogens were cultured on potato dextrose agar (PDA) for two weeks at $25 \pm 1^\circ\text{C}$ for sporulation. A mycelium disc was transferred on PDA media with additional chitosan 1% and extracted from GSE in different concentrations (1%, 1.5% and 2%) and a combination of them.

The dynamic lane in the development of *Alternaria* spp. was measured on each two days (Table 1). During the first report, the control variant which was only media showed the highest value (53.0 mm), indicating the largest colony diameter and the weakest inhibition. Singal application of chitosan 1% was values 52.5 mm. In general, chitosan shows better inhibition than the control variant, but the differences are not statistically significant in all cases (ab).

Table 1. The development of *Alternaria* spp. pathogen on media with additional biopolymers

Variant	After two days (mm)	Inhibition (%)	After four days (mm)	Inhibition (%)	After six days (mm)	Inhibition (%)	After eight days (mm)	Inhibition (%)
Control	53.0±0.6 a	-	65.2±0.3 ab	-	77.3±0.4 ab	-	90.0±0.0 a	-
Chitosan	52.5±0.3 ab	0.94	69.2 ±0.2 a	-6.14	78.2±0.3 ab	-1.17	90.0±0.0 a	0
GSE (1%)	51.2±0.3 ab	3.40	64.7 ±0.5 ab	0.77	74.2±0.4 b	4.01	88.3±0.4 ab	1.89
GSE (1.5%)	45.0±0.4 b	15.09	55.7 ±0.2 b	14.57	61.7 ±0.3 b	20.18	78.7±0.2 b	12.56
GSE (2%)	51.5±0.1 ab	2.83	67.3±0.5 a	-3.22	80.0±0.5 a	-3.49	90.0±0.0 a	0
ChGSE (1%)	51.2±0.5 ab	3.40	65.2±0.4 ab	0	77.3±0.2 ab	0	90.0±0.0 a	0
ChGSE (1.5%)	48.8±0.2 ab	8.68	63.2±0.2 ab	3.07	68.0±0.2b	12.03	77.5±0.5 b	14.00
ChGSE (2%)	51.7 ±0.2ab	2.45	71.2±0.5 a	-9.20	85.5±0.5 a	-10.61	90.0 ± 0.0 a	0

*-Different letters in the same column indicate significant differences ($p < 0.05$).

The variant with GSE showed values ranging from 45.0 mm to 51.5 mm. The concentrations of 1.5% extract from grape seed were 45.0 mm which showed good inhibition of the pathogen. The variants with combinations of chitosan and GSE (512 mm and 517 mm) show inhibition similar to chitosan alone. Significant differences were found between the control variant and the variant with GSE (1.5%).

No inhibition was observed at the control variant with 65.2 mm, compared to variants with chitosan, the highest value was 69.2 mm, after four days. The values of different concentrations of grape seed extract range from 55.7 mm to 67.3 mm. The 1.5% concentration (55.7 mm) continues to show the best inhibition.

After six days, the best inhibition was reported in the extract of grape seed 1.5% (61.7mm) and the combination variant with chitosan (68.0 mm). The both variants have significant differences between GSE (2%) and ChGSE (2%) variants.

After eight days the results indicate that ChGSE (1.5%) were the most effective in suppressing fungal colony, with colony diameters of 77.5mm and 78.7mm.

The highest percentage of inhibition was achieved with GSE (1.5%), reaching 20.18% on the 6th day of the experiment. This combination showed consistently high percentages of inhibition throughout the measurement period, indicating its effectiveness. The media with added only grape seed extract in 1.5% solution also showed good results from the second day, with 15.09% inhibition.

The results of the study on the growth of the fungal pathogen *Alternaria* spp. showed variations in the standard deviation of the measured colony diameters, reflecting the degree of data dispersion. After two days, the standard deviation ranged from ± 0.1 to ± 0.6 , with the highest value observed in the control sample (± 0.6), while the lowest (± 0.1) was recorded in GSE (2%). This suggested relatively stable growth of *Alternaria* spp. in most media, with minimal differences in standard deviation. After four days, the standard deviation varied between ± 0.2 and ± 0.5 , with the lowest (± 0.2) were observed in GSE (1.5%), Chitosan and ChGSE (1.5%).

On six days, the standard deviation again ranged from ± 0.2 to ± 0.5 , with the lowest values (± 0.2) recorded in ChGSE (1%), GSE (1.5%), and

ChGSE (1.5%). After eight days, the fungal colonies in most variants reached the maximum Petri dish diameter (90 mm).

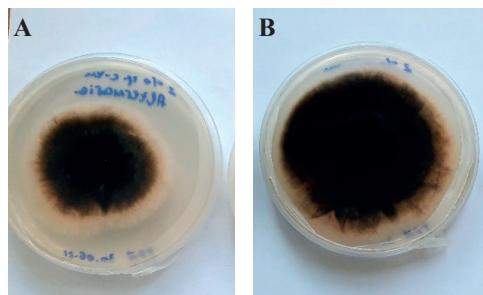


Figure 1. *Alternaria* spp culture on PDA: A - chitosan and 1.5% GSE; B - 1% chitosan

The chitosan inhibits the development of *Alternaria* spp. and also induces phytoalexin production in the host. Such interactions of chitosan with the pathogen and the host suggest that chitosan could be a useful natural material for the postharvest preservation of fruit and vegetables (Reddy et al., 2000). The results of another study Kara et. al., (2018) showed that the lonely application of GSE wasn't induce inhibition of the mycelial growth of *A. alternata*, this position was also confirmed by our results. The management of the fungal pathogen *Penicillium* spp. through the used biopolymers in the control variant was measured with the highest values in all columns. The values of chitosan were close to those of the control, suggesting that chitosan alone haven't been showed significant antimicrobial activity against *Penicillium* spp. under these conditions.

The different concentrations of GSE there was some reduction in values in some of the columns, but the differences were statistically non-significant. With GSE (1.5%), there was a trend towards lower values but also without statistical differences. This suggests that GSE may have been inhibitory effect, but its effect was weak or depends on the concentration and time to application.

Combination of chitosan and GSE, this combination the most pronounced inhibitory effect was observed. Especially with ChGSE (1%) and ChGSE (2%), there was a significant decrease in values, denoted by "b", which indicates a statistically significant difference

from the control. This suggests that the combination of chitosan and GSE has a synergistic effect and leads to better suppression of *Penicillium* spp. growth than each component separately.

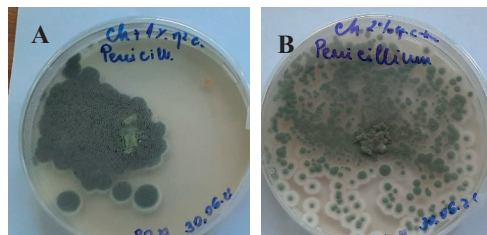


Figure 2. *Penicillium* spp culture on PDA: A - 1% chitosan and GSE 1%; B - 1% chitosan and 2% GSE

Among all the tested variants, chitosan with combination with GSE 1% solution stands out as the most effective inhibitor of *Penicillium* spp. mycelia growth.

Unlike the other variants, ChGSE (1%) demonstrates stable and high levels of inhibition throughout the measurement period, suggesting a sustained and long-lasting effect. This result contrasts with the observed rapid decrease in effectiveness in other variants, particularly GSE (2%), which showed high initial inhibition but quickly lost its activity.

The standard deviation of colony diameters of the fungal pathogen *Penicillium* spp. demonstrated variations. After two days, the standard deviation ranged from ± 0.4 to ± 1.2 , with the highest variability observed in GSE 2% (± 1.2) and the lowest in GSE 1% (± 0.4). This indicated that the growth of *Penicillium* spp. was more uniform in certain media. On the four days, the standard deviation values varied between ± 0.3 and ± 0.9 , with the lowest recorded in GSE 1.5% (± 0.3) and ChGSE (1%) (± 0.3). This suggested that fungal growth in GSE (1.5%) was more consistent compared to ChGSE (2%), where larger variations were observed. The standard deviation on six days ranged from ± 0.3 to ± 0.9 , with the lowest values in media with chitosan (± 0.3) and GSE 1.5% (± 0.4), while the highest (± 0.9) was recorded in the control sample. This showed that *Penicillium* spp. developed more uniformly in media containing chitosan and GSE 1.5%, whereas in the control, growth varied more significantly (Table 2).

Table 2 The development of *Penicillium* spp. pathogen on media with additional biopolymers

Variant	After two days (mm)	Inhibition (%)	After four days (mm)	Inhibition (%)	After six days (mm)	Inhibition (%)	After eight days (mm)	Inhibition (%)
Control	84.5±0.6 a	-	88.1±0.8 a	-	88.7±0.9 a	-	90.0±0.0 a	-
Chitosan	77.7±0.7 a	8.05	88.0±0.4 a	0.11	88.7±0.3 a	0	90.0 ±0.0 a	0
GSE (1%)	76.8±0.4 a	9.11	85.8 ±0.6 a	2.61	86.3±0.5ab	2.7	90.0±0.0 a	0
GSE (1.5%)	79.2±0.6 a	6.27	82.3±0.3 a	6.58	88.3±0.4a	0.45	88.7±0.3 a	1.44
GSE (2%)	73.7±1.2 a	12.78	85.0±0.4 a	3.52	88.3±0.7ab	0.45	88.8±0.5a	1.33
ChGSE (1%)	74.2±0.4a	12.19	74.7±0.3 a	15.21	77.5±0.4b	12.63	79.2±0.5b	11.99
ChGSE (1.5%)	82.3±0.7 a	2.60	85.0±0.4 a	3.52	85.2±0.4ab	3.95	90.0±0.0 a	0
ChGSE (2%)	82.7±0.8 a	2.13	84.7±0.9 a	3.86	87.5±0.6a	1.35	88.8±0.2 a	1.33

^{a-b}Different letters in the same column indicate significant differences ($p < 0.05$).

After eight days of incubation most samples reached the maximum diameter of 90 mm. The biopolymers added to the media in variant ChGSE 1% and GSE 1.5% the fungal growth was inhibited, the colony diameters were smaller, leading to nonzero standard deviations (± 0.2 to ± 0.5). This indicated that these components effectively slowed or restricted fungal growth.

The chitosan demonstrates the highest percentage of inhibition in the first period, but its effectiveness quickly decreases to minimal or zero values thereafter. This suggests that chitosan has a short-term effect on *Penicillium* spp. The extract of grape seeds showed a greater inhibitory effect than chitosan, but its effect was weaker compared to the combination of them. The different concentrations of grape seeds weren't dependent on the inhibitory effect on the concentration of GSE within the studied limits. In the combination variants, the data has a significantly better inhibitory effect than each of the components (chitosan and GSE) separately. They confirmed the synergism effect between the two biopolymers. Grape seed oils and emulsions contain compounds that exhibit an inhibitory effect against fruit rot diseases, including aldehydes, phenols, and ketones. Thymol, carvacrol, and p-anisaldehyde have demonstrated fungicidal activity. Grape seeds are rich in these components, showing the highest inhibitory activity against many post-harvest pathogens (Daferera et al, 2000).

CONCLUSIONS

The present study examines the effect of chitosan and various percentages of grape seed extract on the inhibition of *Penicillium* and *Alternaria* growth. The results reveal significant variations in the effectiveness of the tested compounds, highlighting the importance of selecting appropriate biopolymers and their concentrations for controlling post-harvest pathogens.

The results of Duncan's Multiple Range Test show that the most effective was the combination of chitosan and GSE. The pathogen *Alternaria* spp. was inhibited with 1 per cent of solution chitosan and 1.5% GSE. The best combination of suppression of *Penicillium* spp. was achieved by containing media 1% chitosan and 1% GSE.

ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students – 2".

REFERENCES

- Aleksandrova, D., Zsivanovits, G., Rankova, Z., Malchev, S., & Gande, S. (2021). Influence of biopolymer coatings on post-harvest cherry diseases. *Journal of Mountain Agriculture on the Balkans*, 2021, Vol. 24, No. 6, 401-413.

- Aleksandrova, D., Zsivanovits, G., Rankova, Z., Malchev, S., & Gande, S. (2019). Effect of chitosan edible coating on sweet cherry post-harvest storage pathogens. *3rd International Conference on Biosystems and Food Engineering*.
- Alishahi, A., & Aider, M. (2012). Applications of chitosan in the seafood industry and aquaculture: a review. *Food and Bioprocess Technology*, 5, 817-830.
- Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *Journal of agricultural and food chemistry*, 48(6), 2576-2581.
- Ezati, P., & Rhim, J. W. (2020). pH-responsive chitosan-based film incorporated with alizarin for intelligent packaging applications. *Food Hydrocolloids*, 102, 105629.
- Hisham, F., Akmal, M. M., Ahmad, F., Ahmad, K., & Samat, N. (2024). Biopolymer chitosan: Potential sources, extraction methods, and emerging applications. *Ain Shams Engineering Journal*, 15(2), 102424.
- Jiang, L., Luo, Z., Liu, H., Wang, F., Li, H., Gao, H., & Zhang, H. (2021). Preparation and characterization of chitosan films containing lychee (*Litchi chinensis* Sonn.) pericarp powder and their application as active food packaging. *Foods*, 10(11), 2834.
- Kara, Z., Baykan, M., Doğan, M., & Ege, D. (2018). Effectiveness of grape (*Vitis vinifera* L.) seed extracts on fungi and bacteria management. *Selcuk Journal of Agriculture and Food Sciences*, 32(3), 366-372.
- Krishnaswamy, K., Orsat, V., Gariépy, Y., & Thangavel, K. (2013). Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (*Vitis vinifera*). *Food and Bioprocess Technology*, 6, 441-455.
- Kou, S. G., Peters, L. M., & Mucalo, M. R. (2021). Chitosan: A review of sources and preparation methods. *International Journal of Biological Macromolecules*, 169, 85-94.
- Kumar, A., Choudhary, A., Kaur, H., Guha, S., Mehta, S., & Husen, A. (2022). Potential applications of engineered nanoparticles in plant disease management: a critical update. *Chemosphere*, 295, 133798.
- Gharieb, M. M., Omara, M. S. & Soliman, A. (2025). Antifungal activity of mycogenic selenium nanoparticles conjugated with fungal nano chitosan against three important phytopathogenic fungi. *BioNanoScience*, 15(2), 1-18.
- Leceta, I., Guerrero, P., Ibarburu, I., Dueñas, M. T. & De La Caba, K. (2013). Characterization and antimicrobial analysis of chitosan-based films. *Journal of Food Engineering*, 116(4), 889-899.
- Priyadarshi, R., & Rhim, J. W. (2020). Chitosan-based biodegradable functional films for food packaging applications. *Innovative Food Science & Emerging Technologies*, 62, 102346.
- Priyadarshi, R., Ezati, P., & Rhim, J. W. (2021). Recent advances in intelligent food packaging applications using natural food colorants. *ACS Food Science & Technology*, 1(2), 124-138.
- Reddy, M. B., Angers, P., Castaigne, F. & Arul, J. (2000). Chitosan effects on black mold rot and pathogenic factors produced by *Alternaria alternata* in postharvest tomatoes. *Journal of the American Society for Horticultural Science*, 125(6), 742-747.
- Riahi, Z., Priyadarshi, R., Rhim, J. W. & Bagheri, R. (2021). Gelatin-based functional films integrated with grapefruit seed extract and TiO₂ for active food packaging applications. *Food Hydrocolloids*, 112, 106314.
- Roy, S., & Rhim, J. W. (2020). Preparation of carbohydrate-based functional composite films incorporated with curcumin. *Food Hydrocolloids*, 98, 105302.
- Sogut, E. & Seydim, A. C. (2018). Development of Chitosan and Polycaprolactone based active bilayer films enhanced with nanocellulose and grape seed extract. *Carbohydrate Polymers*, 195(April) 180-188. <https://doi.org/10.1016/j.carbpol.2018.04.071>.
- Wu, Z., Deng, W., Luo, J. & Deng, D. (2019). Multifunctional nano-cellulose composite films with grape seed extracts and immobilized silver nanoparticles. *Carbohydrate polymers*, 205, 447-455.
- Zhang, L., Chen, J., Liao, H., Li, C. & Chen, M. (2020). Anti-inflammatory effect of lipophilic grape seed proanthocyanidin in RAW 264.7 cells and a zebrafish model. *Journal of Functional Foods*, 75, 104217. <https://doi.org/10.1016/j.jff.2020.104217>
- Zsivanovits, G. I., Sabeva, P. G., Petrova, T. V., Momchilova, M. M., Zhelyazkov, S. P., Iserliyska, D. Z. & Aleksandrova, D. V. (2022). Improving the shelf-life of the sweet cherry by multicomponent edible coatings. *Agricultural Sciences/Agrarni Nauki*, 14(33)