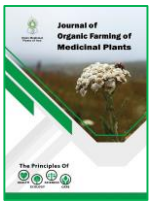
Journal of Organic Farming of Medicinal Plants: September 2024; 3 (1): 44-49
Available online at http://www.jofmp.areeo.ac.ir



Original Paper

In vitro evaluation of bio control agents and botanicals against mulberry root rot pathogen *Fusarium oxysporum*

Hadi Rahanandeh [a], Nadieh Nazari[b], Ezzatullah Sadaqat Far [c], Mahsa Moshayedi[d], Saeid Chavoshi[e]

[a]*Department of Plant protection, Ra.C., Islamic Azad University, Rasht, Iran*
[b] *Graduated in Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch*
[c] *Department of Plant protection, Ar.C., Islamic Azad University, Arak, Iran*
[d] *Member of the Agricultural Engineering System of Guilan Province*
[e] *Department of Agronomy and Plant Breeding, Ar.C., Islamic Azad University, Arak, Iran*

ARTICLE INFO

Corressponding Author:
Hadi Rahanandeh
Rahanandeh20@yahoo.com

Received: 28 February 2025
Accepted: 29 April 2025

Keywords:
Plant extract
Mulberry root rot
Antagonist bacteria
Fusarium oxysporum

ABSTRACT

Mulberry is only source of food for the silkworm (Bombyx mori) L. Mulberry is sensitive to soil borne diseases among them root rot is fast spreading disease caused by F. oxysporum. Pathogenes can be managed by synthetic chemical fungicides but the use of synthetic fungicides results in Toxic resedues on the growth and development of silkworms. In this study, the effect of antagonistic biological agents for the management of mulberry root rot pathogen was investigated. The extract of five herbs is used: garlic, peppermint, tea, thyme, and neem. The extracts were tested at three concentrations of 10, 10-1, 10-3 and three solvents of ethanol, methanol and water. The effect of the extracts in a certain concentration was investigated based on the method of mixing with the culture medium. Bacteria were purified from soil using serial dilution method and were examined based on dual culture, volatile compounds, extracellular extract, and antibiotics. The highest inhibition rate was observed in extracts obtained from ethanol solvent. Ethanolic extract of all plants except peppermint showed 100% inhibition. The least effect was observed for extracts dissolved in water. The highest inhibition rate water solvent was observed in neem extract with 58.34. In different solvents, the lowest inhibition rate was observed in extracts obtained from peppermint. 100 bacterial isolates were isolated from soil samples, of which five isolates showed antagonistic ability in various tests. The top bacteria were identified based on biochemical and molecular factors and belonged to the two genera Pseudomonas and Bacillus.

1. Introduction

Mulberry leaves are very beneficial for silkworm nutrition and mulberry fruit is very beneficial for human health (Sultana and Kim, 2016). Mulberry blight is one of the important and destructive diseases in mulberry cultivation that can cause severe yield and quality reduction. This disease is usually caused by fungal agents such as *Fusarium* spp., *Botryosphaeria* spp., *Phomopsis* spp. and *S. sclerotiorum* (Yu *et al.*, 2016). In recent years, biological control based on microorganisms has been considered as a potential and sustainable alternative for combating plant pathogens. Controlling fungal diseases such as *Fusarium* in mulberry trees using a combination of beneficial bacteria and plant extracts is an effective and environmentally friendly method. *Fusarium* can cause *Fusarium* wilt, which damages the roots and vessels of the plant and reduces the yield of mulberry trees (Agrios, 2005). The use of antagonist bacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* as biological control agents can inhibit the growth of *Fusarium* fungi due to the production of antifungal compounds such as siderophores, lytic enzymes and secondary metabolites (Compant *et al*., 2005). On the other hand, plant extracts containing bioactive compounds such as allicin (in garlic), thymol (in thyme) and azadirachtin (in neem) also have strong antifungal properties and can directly inhibit the growth of the fungus (Pandey *et al*., 2016). The combination of these two methods (beneficial bacteria and plant extracts) can have a synergistic effect and provide better disease control (Singh et al., 2020). For example, the simultaneous use of *Pseudomonas fluorescens* and garlic extract in field trials has shown that this combination can effectively prevent the development of *Fusarium* disease and improve plant health (Kumar *et al*., 2018). These

methods are not only environmentally friendly, but also prevent the development of resistance in fungi and are safe for humans and other beneficial organisms (Bhalerao *et al*., 2015). However, there are also challenges such as the need for more repetition and slower effect compared to chemical pesticides, which requires more careful management (Rana *et al*., 2017). Overall, the use of beneficial bacteria and plant extracts as a biological control method can help maintain the health of mulberry trees and reduce dependence on chemical pesticides. The aim of this study was to investigate the effect of antagonistic bacteria and plant extracts on inhibiting the growth of *Fusarium oxysporum.*

2. Materials and Methods

2.1 Preparation, propagation and storage of pathogenic fungus

The pathogenic fungus was obtained from the Islamic Azad University, Rasht Branch, and its cultivation was carried out on PDA medium and propagated. Then, the original sample was stored in test tubes containing triglyceride.

2.2 Extraction of neem, tea, peppermint, thyme and garlic with aqueous solvent:

The fresh leaves were placed in an oven at 70 degrees for 3 days, then 40 grams of the dried leaves were powdered, mixed with 150 ml of distilled water, and placed on a distillation apparatus for 15 minutes. Then the resulting solution was filtered through Whatman filter paper. The resulting extract was passed through a 0.45 Millipore filter and poured into a sterile glass jar. Flowers were used instead of leaves for the thyme extract and Garlic powder was used for garlic extract (Rahanandeh, & Sedaghat far 2022).

2.3 Ethanol and methanolic extraction of neem, tea, peppermint, thyme and garlic

First, 10 grams of dried leaves were soaked in 100 ml of solvent, then soaked on a shaker for 24 hours at room temperature. The solution was passed through filter paper and after filtering the extract, it was placed in a distillation apparatus and the temperature was raised to 50 degrees to concentrate the solution. Then the extracted extract was sterilized through a 0.45 Millipore filter. Flowers were used instead of leaves for the thyme extract and Garlic powder was used for garlic extract (Rahanandeh, & Sedaghat far 2022).

2.4 Preparation of extract concentrations

A series of dilutions of 10, 10^{-1}, and 10^{-2} were prepared using water.

2.5 Isolation, purification and identification of antagonist bacteria

20 g of rhizosphere soil was mixed with 500 mL of sterile distilled water and the mixture was diluted by adding 1 mL of the mixture to 9 mL of sterile distilled water to create four serial dilutions (101 , 10−1, 10−2 and 10−3). Then, 100 µL of each dilution was transferred and spread on the surface of nutrient agar (NA) plates. After incubation at 28°C for 48 h, representative colonies that were different in shape and colour on the culture medium were selected and purified in a test tube containing NA, numbered and were incu bated for 2 days at 28°C (Rahanandeh *et al*., 2017).

2.6 Antagonistic property of separate microorganisms

The antagonistic capacity of microorganism in the laboratory was examined using the dual culture method of Hagedron et al. (1989). The production of volatile antifungal compounds was examined using Montealegra Method (Montealegra et al. 2003), and the production of antibiotics was based on Kraus and Loper (1990) method. The experiment was arranged in RCBD with 3 replications (Rahanandeh *et al*., 2017). Differences in treatment means were compared using Duncan's Multiple Range Test.

2.7 Identification of bacterial isolates Biochemical identification.

Selected bacterial isolates with Fungicidal activity were characterized and identified (Williams et al., 1989). The morphological and cultural characteristics of selected bacterial isolates were examined on nutrient agar medium. The morphological characters analysed included shape, size, Gram reaction, endospore characteristics and pigmentation. In addition, motility in nutrient broth was also tested and growth on nutrient agar at 4°C and 40°C were investigated. Biochemical characteristics (nitrate reaction, casein hydrolysis, Voges–Proskauer test, methyl red test, indole production, fermentation and assimilation of carbohydrates, gelatin liquefaction, arginine dihydrolase and starch hydrolysis) were also studied (schaad et al., 2001).

2.8 Molecular identification.

DNA was extracted from selected bacterial isolates using the SinaClon extraction kit (Iran). The bacterial 16S rDNA was amplified using the universal primers (P1/F 5' –AGA GTT TGA TCC TGG TCA GAA CGC T–3' and P6/R 5' –TAC GGC TAC CTT GTT ACG ACT TCA CCC C–3').. The sequencing results were compared using the NCBI Basic Local Alignment Search Tool (BLAST) program and 16S rDNA gene similarity was evaluated using GenBank data. A phylogenetic tree was drawn using the MEGA 11 phylogenetic program according to the UPGMA method.

2.9 Testing the effect of bacteria and plant extracts on silkworms

Table 1- Comparison of the percentage of interaction effect of plant extracts on *F. oxysporum* in laboratory experiments

Concentration / Treatment	10	10^{-1}	10^{-2}	10	10^{-1}	10^{-2}	10	10^{-1}	10^{-2}
		Water solvent			Ethanol solvent			Methanol solvent	
Tea	35.43b	27.13a	4.25a	100a	22.5b	2.5b	100a	48.31a	18.75b
Neem	58.34a	35a	4.25a	100a	43.75a	15a	100a	37.5ba	27.5a
Garlic	47.93b	31.63a	2.5a	100a	25b	10a	91.25ab	27.5b	13.78c
Thyme	23.09c	8.38b	2.13a	100a	22.5b	8.2b	100a	27.5b	13.75c
peppermint	27.84c	12.5b	4.25a	62.5b	16.25b	2.5b	85.21c	20b	5cd
control	0d	0c	0a	0c	0c	0c	0d	0c	0d

According to Tukey's test, means with similar letters in column do not have a significant difference at the 1% probability level

Table 2- Laboratory test results of antagonist bacteria

Treatment	Percentage of inhibition in dual culture	Percentage of inhibition in volatile compounds	Average Percentage inhibition of extracellular metabolites	Percentage of inhibition in Antibiotic	Percentage of live larvae
B. subtilis (H1)	46.41b	41.33c	41.53a	90.32a	88.3c
P. flourescens (H4)	49.81b	36.11d	37.06b	78.18b	99.55a
P. megaterium (H16)	66.58a	47.35b	43.38a	89.09a	72.05d
B. coagulans (H24)	58.23a	100a	36.36b	81.81b	99.6a
B. subtilis (H25)	59.68a	100a	30.56c	89.09a	98.10b
Control	0c	0e	0d	0d	100a
Mancozeb	-	-	-	-	

Text numbers are mean percent inhibition of triplicates. According to the method of least significant difference (LSD), the averages followed by the same letters in the same column do not differ significantly from each other.

All bacteria and extracts were fed to the worms as part of their diet (sprayed on the leaves 24 hours before feeding), and the control treatment was only water sprayed on the leaves. This experiment was conducted in 3 repetitions, with 7 silkworms in each repetition, and at the end, the number of worms that had cocoons and live cocoons was evaluated (Moshayedi *et al*., 2017).

3. Results

3.1 Dual culture test

The antagonistic action of selected bacterial bio control agents against *F. oxysporum* was tested through dual culture technique. Based on the observations of radial growth of the bio agents and fungus, the per cent inhibition was calculated. The results are presented in Table 2. Among the bacterial bio agents tested against *F. oxysporum* the *Bacillus subtilis* (H25), *Bacillus coagulans* (H24) and *Priestia megaterium* (H16) were significantly superior over control with 59.88, 58.23 and 66.58 per cent mycelial inhibition respectively. This is followed by *B. subtilis* (H1) with 46.4 per cent mycelial inhibition followed by *Pseudomonas fluorescence* (H4) with 49.81 per cent mycelial inhibition, percent (Table 2).

3.2 Volatile metabolites

To show the effect of anti-fungal volatile compound on the decrease in mycelia growth of the fungus, the thickness of fungus colonies was studied. The strains of *B. coagulans* (H24) and *B. subtilis* (H25) with 100% decrease in growth was significantly the highest while the strain of *P. fluorescence* (H4) with 36.11% was the least to decrease in mycelia growth of the fungus (Table 2).

3.3 Antibiotic

Antagonistic bacterial strains showed significant inhibition of mycelium growth of the fungus. *B. subtilis* (H1), *P. megaterium* (H16) and *B. subtilis* (H1) strains caused a 90.32%, 89.09 and 89.09 decrease in mycelial growth respectively while *B. coagulans*(H24) and *P. fluorescens*(H4) caused 81.81% and 78.18 decrease and the least effect on the decrease in mycelial growth (Table 2).

3.4 Filtered extracellular liquid metabolites with millipore

The best strain against pathogenic *F. oxysporum* were *B. subtilis*(H1) and *P. megaterium*(H16), which were statistically categorized in group A with inhibition percentage of 41.53% and 43.38%, respectively. *B. subtilis*(H25) showed the least inhibition (Table 2).

3.5 Effect of antagonistic bacteria on the method of the treatment of Mulberry seedlings

Disease severity of *F. oxysporum* showed that *P. fluorescens*(H4) had the least severe infection and *B.*

subtilis(H25) and Mancozeb fungicide were recognized as the strain with the least controlling effect on disease severity(Table 2).

3.6 The results of the inhibitory effect of extract of plants on F. oxysporum growth

In all plant extracts, the percentage of inhibition of *F. oxysporum* growth decreased with decreasing concentration. In all three solvents, neem showed the highest inhibition. Ethanol solvent in four plants, neem, tea, garlic and Thyme, at the first concentration, was able to completely inhibit the growth of *F. oxysporum*. Extracts obtained from water solvent had the least inhibition of the growth of fungal hyphae on the surface of the Petri dish. Extracts obtained from garlic had the least effect on the vegetative growth of *Fusarium*. In examining the effect of plant extracts on larvae, it was observed that none of the extracts added to the diet had a negative effect on the larvae(Table 1).

3.7 Identifying top bacteria

Based on molecular, biochemical, and blast investigations at the site NCBI, they were:
B. subtilis (H1), *B. coagulans* (H24), *B. subtilis* (H25), *P. megaterium* (H16) and *P. flourescens* (H4).

4. Discussion

The first antagonistic effect of selected bacterial biocontrol agents against *F. oxysporum* was tested using the dual culture method. In the present study, the identified isolates belonged to Pseudomonas and Bacillus. In the dual-culture experiment, these bacteria inhibited the vegetative growth of the fungus from 48.41% to 66.58%. These findings were similar to the results of Sundaramoorthy et al. (2012) who evaluated the protective effects of compatible endophytic bacterial strains *Bacillus subtilis* and *Pseudomonas fluorescens* against wilt disease caused by *Fusarium solani*. The results showed that *B. subtilis* and *P. fluorescens* were compatible and effectively inhibited the growth of the *F. solani*. Similarly, Seetha et al. (2010) screened different bio control agents under in vitro conditions and found that *P. fluorescens* showed maximum inhibition of the mycelial growth (95%) against *F. solani*. Studies have shown that volatile compounds produced by *Pseudomonas* and *Bacillus*, such as phenazine, isoburnol, and DAPG, have the ability to inhibit the growth of plant pathogenic fungi such as Fusarium and Rhizoctonia (Haas & Défago, 2005; Ongena & Jacques, 2008). In this study, different isolates showed different abilities in inhibiting the growth of the pathogenic fungus. Isolates *B. coagulans* (H24) and *B. subtilis* (H25) had the greatest effect, and isolate *P. flourescens* (H4) had the lowest inhibition rate. Beneficial bacteria such as *Pseudomonas* and *Bacillus*, through extracellular secretions, cause the breakdown of the fungal cell wall, inhibit the growth of mycelium, and induce systemic resistance in the plant, helping to control diseases caused by *Fusarium* (Kwak et al., 2018). In the present study, this control mechanism showed the lowest inhibition rate, and bacterium *P. megaterium* showed the highest control rate. Antibiotic production by antagonistic bacteria is one of the most important mechanisms for the biological control of plant pathogenic fungi, especially *Fusarium* spp. Bacteria such as *Pseudomonas* spp. and *Bacillus* spp. produce a variety of antifungal compounds that directly inhibit fungal growth. For example, Pseudomonas effectively inhibits the growth of Fusarium by producing antibiotics such as 2,4-diacetylphloroglucinol, and *Bacillus* effectively inhibits the growth of *Fusarium* by producing antifungal lipopeptides such as surfactin and iturin. These compounds play a key role in the control of plant diseases by disrupting the structure of the fungal cell wall, inhibiting metabolic activities, and inducing systemic resistance in the plant (Köhl et al., 2019). In this study, the minimum effectiveness was more than 78.5%, which is similar to the results of other studies. Some Bacillus strains can cause mortality of silkworm larvae by producing toxic compounds such as lipopeptides (such as surfactin and itroin) (Chowdhury et al., 2015). On the other hand, Pseudomonas strains may also negatively affect larval health and development by altering the balance of the gut microbiome or inducing an immune response (Qin et al., 2022; Wang et al., 2017). Therefore, the use of these bacteria in silkworm rearing environments should be done with caution and after careful consideration of the strains and concentrations used. In the present study, bacteria did not have a negative effect on the larvae. In a study, it was shown that thyme extract has a control ability of 75%-80% and 50%-60% on *Fusarium* fungus in the laboratory and greenhouse, respectively (Šegvić Klarić et al., 2007). In the present study, the extract of this plant in two solvents, ethanol and methanol, completely prevented the growth of the fungus, which indicates the high presence of thymol and carvacrol in this plant tissue, and due to the greater solubility of these compounds in alcohol than in water, the inhibition rate of alcoholic solvents was higher (Alizadeh, et al. 2020). Tea extract, due to its phenolic compounds, especially catechins, has a good inhibitory effect on fungi, especially *Fusarium*, and alcoholic solvents have a greater ability to extract these substances (Rahanandeh et al., 2017). In this study, the water solvent also had the lowest inhibition rate. The use of neem extract to control *Fusarium* spp. has been successful in numerous studies. For example, in a study on tomatoes infected with *Fusarium oxysporum* f. sp. *lycopersici*, the use of neem

extract at a concentration of 300 ppm reduced root rot and leaf yellowing symptoms by 50%-60% (Almeida et al., 2017). In another study on banana, neem extract at a concentration of 400 ppm inhibited the growth of *Fusarium oxysporum* f. sp. *cubense* by 65% and significantly reduced disease symptoms (Kumar et al., 2020). Also, in chickpea, neem extract at a concentration of 300 ppm inhibited the growth of *Fusarium oxysporum* f. sp. *ciceris* by 75% (Goel et al., 2016). In the present study, it had a control of 58% to 100% in three different solvents, which is consistent with previous results, indicating the inhibitory power of this extract. Garlic extract has strong antifungal properties against *Fusarium* spp. fungi due to its sulfur compounds such as allicin, ajuene, and diallyl disulfide. Studies have shown that garlic extract at concentrations of 100 to 500 ppm can inhibit the mycelial growth of *Fusarium oxysporum* by 70-80% (Wang et al., 2014). These compounds reduce the pathogenic activity of the fungus by destroying the fungal cell wall, inhibiting key enzymes, and inducing oxidative stress (Yang et al., 2023). In a study on tomatoes, the use of garlic extract reduced the symptoms of root rot and leaf yellowing by 50-60% (Li et al., 2018). In this study, garlic extract was also effective in inhibiting the growth of the pathogen, but its effectiveness was lower. The greatest effectiveness was observed with the extract in ethanol solvent. In the study of Rahman (2007), it was shown that alcoholic solvents are more suitable for extracting garlic compounds, which is consistent with the results of this study. Peppermint extract has strong antifungal properties against *Fusarium* spp. fungi due to its bioactive compounds such as menthol, carvacrol and limonene. Studies have shown that peppermint extract at concentrations of 100 to 400 ppm can inhibit the mycelial growth of *Fusarium oxysporum* by 60-70% (Raut et al., 2021). In the present study, it had a relatively small effect, which may be due to the drying method and the type of peppermint, and different types of peppermint have different amounts of active ingredients.

5. Conclusion

Based on the results of this study, bacteria can be used as biological control agents for this disease, which has a good control on the pathogen and does not have a negative effect on the silkworm larvae. Plant extracts had a good effect on the pathogenic fungus at their highest concentration. In the extracts from the five plants studied, alcoholic solvents, especially ethanol, had a better effect on extracting substances and controlling.

Acknowledgements

The authors of the article would like to thank the National Tea Research Institute for providing the laboratory.

References

Alizadeh, M., Vasebi, Y. & Safaie, N. (2020). Microbial antagonists against plant pathogens in Iran: A review. *Open Agriculture*, *5*(1), 404-440. DOI: 10.1515/opag-2020-0031

Almeida, F. A. C., et al. (2017). "Neem extract as a potential fungicide for controlling Fusarium wilt in tomato plants. *Crop Protection*, 92, 109-115. https://doi.org/10.1016/j.cropro.2016.10.012.

Bhalerao, T. S., Kelkar, T. S, & Dey, U. (2015). Plant extracts: A novel approach for controlling plant pathogens. *International Journal of Current Microbiology and Applied Sciences*, 4(1), 105-112.

Compant, S., Duffy, B., Nowak, J., Clément, C. & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*, 71(9), 4951-4959.

Goel, N., Anukrati, K. & Paul, P.K. (2016). Anti-phytopathogenic and SAR inducing properties of Neem: A review. *Journal of Chemical and Pharmaceutical Sciences*, *9*(4), pp.2547-2555.

Haas, D. & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3(4), 307-319.

Ongena, M. & Jacques, P. (2008). Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. *Trends in Microbiology*, 16(3), 115-125.

Kwak, M. J., et al. (2018). Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology*, 36(11), 1100-1109.

Köhl, J., Kolnaar, R. & Ravensberg, W. J. (2019). Biocontrol of plant diseases by microbial consortia: Mechanisms, applications, and perspectives. *Frontiers in Microbiology*, 10, 2846. DOI: 10.3389/fmicb.2019.02846

Kumar, S., Kumar, R. & Khan, A. (2018). Medicinal plants and their uses in agriculture: A review. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 1815-1821.

Pandey, A. K., Singh, P. & Tripathi, N. N. (2016). Chemistry and bioactivities of essential oils of some Ocimum species: An overview. *Asian Pacific Journal of Tropical Biomedicine*, 4(9), 682-694.

Moshayedi, M., Rahanandeh, H. & Hamzeh, A. (2017). In vitro evaluation of some fungicides and tea extract against Pestalotia sp. and Colletotrichum sp., the causal agents of leaf spot and anthracnose of azalea. *journal of ornamental and horticultural plants*. 1: 45-51.

Qin, L., Qi, J., Shen, G., Qin, D., Wu, J., Song, Y., ... & Xia, Q. (2022). Effects of microbial transfer during food-gut-feces circulation on the health of Bombyx mori. *Microbiology Spectrum*, *10*(6), e02357-22.

Rahanandeh, H., Moshayedi, M. & Hamzeh, A. (2017). Biological control of onion pink root using antagonistic strains of bacteria. *Philippine Journal of Crop Science*. 42 (3), 71-78.

Rahanandeh, H. & Sedaghat far, E. (2022). The inhibitory effect of five plant extracts on the pathogen of mulberry dieback disease (*Sclorotinia sclerotiorum*) in laboratory conditions. *Applied Plant Protection*.11(1), 29-34.

Rana, M., Sharma, A. & Sharma, S. (2017). Challenges in biological control of plant pathogens. *Journal of Plant Diseases and Protection*, 124(6), 529-538.

Raut, L.S., Dalvi, S.M. & Rakh, R.R. (2024). Antifungal Potential of Bacillus subtilis subsp. inaquosorum RLS76 for Management of Fusarium Wilt Disease of Bt-Cotton. *Current Agriculture Research Journal*, *12*(1).

Schaad, N.W., Jones, J.B. & Chun, W. (2001). Laboratory guide for the identification of plant pathogenic bacteria (No. Ed. 3, pp. xii+-373).

Seetha, R.J., Raja, G.R.C. & Ramanjaneyulu, R. (2010). Evaluation of Certain Plant Extracts and Antagonists Against Fusarium solani and Alternaria tenuissima, the Incitants of Root Rot and Die-Back Diseases of Mulberry. *International Journal of Industrial Entomology and Biomaterials*, 20(1), pp.1-5.

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Pieckova, E. & Pepeljnak, S. (2007). Antifungal activity of thyme (Thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings. *Letters in applied microbiology*, 44(1), 36-42.

Singh, D., Sharma, R. R. & Khanna, K. (2020). Biological control of Fusarium wilt: *A review*. *Journal of Plant Pathology*, 102(2), 315-328.

Sultana, R. & Kim, K. (2015). *Bacillus thuringiensis* C25 suppresses popcorn disease caused by *Ciboria shiraiana* in mulberry (*Morus australis* L.). *Biocontrol Science and Technology*, *26*(2), 145–162. https://doi.org/10.1080/09583157.2015.1084999

Rahman, K. (2007). Garlic and aging: New insights into an old remedy. *Ageing Research Reviews*, 6(1), 46-62.

Sundaramoorthy, S., Raghuchandr, T., Raghupathi, N. & Samiyappan, R. (2012). Combinatorial effect of endophytic and plant growth-promoting rhizobacteria against wilt disease of Capsicum annuum L. caused by Fusarium solani. *Biological Control*. 60(1), 59-67.

Wang, Q., Sun, Z., Ma, S., Liu, X., Xia, H. & Chen, K. (2022). Molecular mechanism and potential application of bacterial infection in the silkworm, Bombyx mori. *Developmental & Comparative Immunology*, 131, p.104381.

Yang, X., Bai, S., Wu, J., Fan, Y., Zou, Y., Xia, Z., Ao, J., Chen, T., Zhang, M. & Yang, R. (2023). Antifungal activity and potential action mechanism of Allicin against Trichosporon asahii. *Microbiology Spectrum*, 11(3), pp.e00907-23.

Yu, C., Hu, X., Deng, W., Li, Y., Han, G. & Ye, C. (2016). Soil fungal community comparison of different mulberry genotypes and the relationship with mulberry fruit sclerotiniosis. *Scientific reports*, *6*(1), p.28365.