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Research Article

Bacterial Allies in Agricultural Defense: Evaluation of *Xenorhabdus* and *Photorhabdus* Supernatants Against *Phytophthora infestans* and *Monilinia laxa*

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ABSTRACT

Fungal phytopathogens represent a significant threat to global agriculture, affecting crop productivity and food security. *Phytophthora infestans* (Mont.) de Bary and *Monilinia laxa* (Aderh. & Ruhland) Honey (1945) are two pathogens that cause blights and brown rot, respectively, economically vital crops like potato and stone fruits. Sustainable management strategies are crucial for mitigating these threats. *Xenorhabdus* and *Photorhabdus* bacteria produce various secondary metabolites with different biological activities. This study investigated the antifungal activity of cell-free supernatants of *Xenorhabdus* and *Photorhabdus* bacteria against *P. infestans* and *M. laxa*. *Xenorhabdus cabanillasii* and *Xenorhabdus szentirmaii* exhibited antifungal capability at 5% and have been found to have the potential for use as biocontrol agents, whereas *Photorhabdus kayaii* showed relatively low antifungal activity against two tested phytopathogens. These findings underscore the importance of exploring biocontrol agents in integrated pest management practices.

Keywords: *Natural products, biological control, antifungal, plant pathogenic fungi*

Tarımsal Mücadelede Bakteriyel Müttefikler: Xenorhabdus ve *Photorhabdus* bakterilerine ait süpernatantların *Phytophthora infestans* ve *Monilinia laxa* türlerine karşı etkinliklerinin belirlenmesi

ÖZ

Fungal fitopatojenler, tarım verimliliğini ve gıda güvenliğini etkileyerek tarımsal üretimde ciddi bir tehdit oluşturur. *Phytophthora infestans* (Mont.) de Bary ve *Monilinia laxa* (Aderh. & Ruhland) Honey (1945) gibi patojenler, ekonomik olarak önemli tarım ürünlerinde sırasıyla solgunluk ve kahverengi çürüklere neden olan önemli fitopatojenlerdir. Bu fitopatojenlerin etkilerini hafifletmek için sürdürülebilir yönetim stratejileri geliştirmek hayati önem taşımaktadır. *Xenorhabdus* ve *Photorhabdus* cinslerine ait bakterilerin pek çok farklı biyolojik aktiviteye sahip sekonder metabolitler ürettiği bilinmektedir. Bu çalışma, *Xenorhabdus* ve *Photorhabdus* bakterilerinden elde edilen süpernatantların *P. infestans* ve *M. laxa*'ya karşı antifungal aktivitesini belirlemek amacıyla yapılmıştır. Xenorhabdus cabanillasii ve Xenorhabdus szentirmaii türleri %5 konsantrasyonda antifungal aktivite göstermiş ve bu iki türün biyolojik mücadele ajanı olarak kullanılabilme potansiyelleri olduğu belirlenmiş; *Photorhabdus kayaii* türünün ise nispeten daha düşük bir antifungal aktiviteye sahip olduğu görülmüştür. Bu bulgular entegre zararlı yönetimi uygulamalarında biyolojik mücadele ajanlarının keşfinin önemini ortaya koymaktadır.

Anahtar Kelimeler: *Doğal ürünler, biyolojik mücadele, antifungal, bitki patojeni fungus*

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I. INTRODUCTION

Fungal phytopathogens pose a significant threat to agriculture, affecting crop yields and food security on a widespread scale. Phytopathogenicfungi can infect various parts of plants, including leaves, stems, roots, and fruits, leading to a range of diseases, such as rust, blights, and wilts, resulting in reduced crop quality and yield losses. *Phytophthora infestans* (Mont.) de Bary, one of the most aggressive pathogens, causes a disease called blight, mostly affecting two economically important Solanaceae species: potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). The disease causes wilting, damping-off, chlorosis, root rot, and the rotting of other organs [1]. This pathogen is best known for causing the notorious Irish potato famine in the 1840s [2]. *Monilinia laxa* (Aderh. & Ruhland) Honey (1945) causes brown rot in stone fruits in the European Mediterranean areas. The disease leads to notable reductions in yield stemming from both flower and twig blight during infection of flowers and fruit rot at various stages; preharvest, harvest, and postharvest. Postharvest losses tend to be more severe than preharvest losses [3].

Effective strategies to manage and control plant fungal diseases are crucial for sustaining global food production and ensuring a stable and secure food supply. Although chemical pesticides are often highly effective in quickly controlling and managing pest populations, they pose health risks to nontarget organisms and cause soil, water, and air pollution. Moreover, the frequent use of chemicals to control pests can trigger insecticide resistance [3]. Integrated approaches, including the use of resistant crop varieties, cultural practices, and biological control agents, are essential for mitigating the effects of these diseases and safeguarding the world's food systems.

One of the most common biocontrol agents used in integrated pest management (IPM) programs is entomopathogenic bacteria. *Xenorhabdus* spp. and *Photorhabdus* spp. (Fam: Morganellaceae) are Gram-negative, rod-shaped bacteria that are symbiotically associated with the entomopathogenic nematode (EPN) genera *Steinernema* and *Heterorhabditis*, respectively [4]. These two entomopathogenic bacteria are present in the infective juvenile (IJ) stages of EPN. After IJs infect the soil-dwelling insect host, these bacteria are released into the insect hemolymph. Both bacteria genera produce various secondary metabolites not only to help them kill the host and degrade the host, but also to help them protect the host cadaver from other competitive organisms. These natural products are considered reservoirs of innovative insecticidal, antibacterial, and antifungal compounds [5] and can replace existing hazardous chemical pesticides. Previously, various studies have investigated the effects of different *Xenorhabdus* and *Photorhabdus* cell cultures or cell-free supernatants (CFS) against different fungal pathogens [6-10]. The objective of this study was to determine the antifungal activity of *Xenorhabdus* and *Photorhabdus* supernatants against two economically important fungal pyhtopathogens, *M. laxa* and *P. infestans*, under laboratory conditions.

II. MATERIAL AND METHODS

A. FUNGAL PATHOGENS

The fungal pyhtopathogens P. *infestans* and *M. laxa* used in this study were obtained from Prof. Özlem Abacı Günyar (Ege University, Faculty of Science, Department of Biology). Pathogen cultures were maintained on Potato Dextrose Agar (PDA) (Merck) at 25° C. Using a sterile transfer tube, a 5-mm-diameter mycelia plug was removed from the fungal stock cultures and placed centrally on top of a Petri dish containing PDA. All Petri dishes were incubated at 25°C in the dark and subcultured every two weeks until they were used in experiments.

B. OBTAINING SYMBIOTIC BACTERIA AND CELL-FREE SUPERNATANTS

Xenorhabdus cabanillasii Tailliez et al. 2006 (JM26-1), *Xenorhabdus szentirmaii* Lengyel et al. 2005 (DSMZ16338), and *Photorhabdus kayaii* Machado et al. 2018 (DSMZ15194) were used in the experiments. These bacteria have been known to produce various secondary metabolites which can have antifungal effects on different pathogenic fungi species. Bacteria species were obtained from Dr. Helge Bode (Max Planck Institute, Marburg, Germany) and stored in 20% glycerol at −80°C until use in the experiments. Bacteria were subcultured from stock cultures on Luria-Bertani (LB) (Neogen®) agar at 30°C for 48 h. Each bacterial species was inoculated into separate flasks containing 10 ml of LB broth and then placed into an incubator set at 30°C and 150 rpm for overnight cultivation. To obtain cell-free supernatants, 0.3 ml of overnight cultures were inoculated into 100 ml of LB broth and incubated at 30°C and 150 rpm for 5 days. After 5 days, cultures were centrifuged at 4°C and 10000 rpm for 15 minutes and filtered using a 0.22 µm syringe filter (Sartorius Minisart® Syringe Filter). Cell-free supernatants were kept at 4°C for up to 5 days until they were used in experiments.

C. IN VITRO LABORATORY EXPERIMENTS

Potato Dextrose Agar (PDA) was prepared according to the manual of the manufacturer and autoclaved at 121°C for 15 min. After allowing the medium to cool down to 45-50°C, the bacterial supernatants were added at 5%(v/v) and mixed thoroughly. For the negative control group, only sterile bacterial growth medium (LB) was added, and for the positive control group, trans-cinnamic acid (TCA), a known antifungal agent, was used. A TCA stock culture was prepared by dissolving 12.7 g of TCA in 100 ml ethyl alcohol. A 5% TCA of this stock solution was added to the PDA [11]. Using a sterile transfer tube, a 5-mm-diameter mycelia plug was removed from the fungal cultures and placed centrally on top of each Petri dish. All Petri dishes were incubated at 25°C in the dark, and the colony diameter was measured after 7 and 14 days of incubation. The area of the agar plug in the middle of the plates was excluded in the measurements. Five Petri dishes were used for each treatment, and the experiments were repeated three times on different days.

D. STATISTICAL ANALYSIS

To determine differences between treatment means, data were analyzed by one-way ANOVA. All data were subjected to post hoc Tukey HSD means separation ($\alpha = 0.05$) (SPSS 23.0 IBM Corp., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

Monilinia laxa was more susceptible to the tested bacterial CFS than *P. infestans*. The cell-free supernatant of *X. cabanillasii* and TCA (positive control) completely suppressed the growth of *M. laxa* after 7 and 14 days (Figure 1).

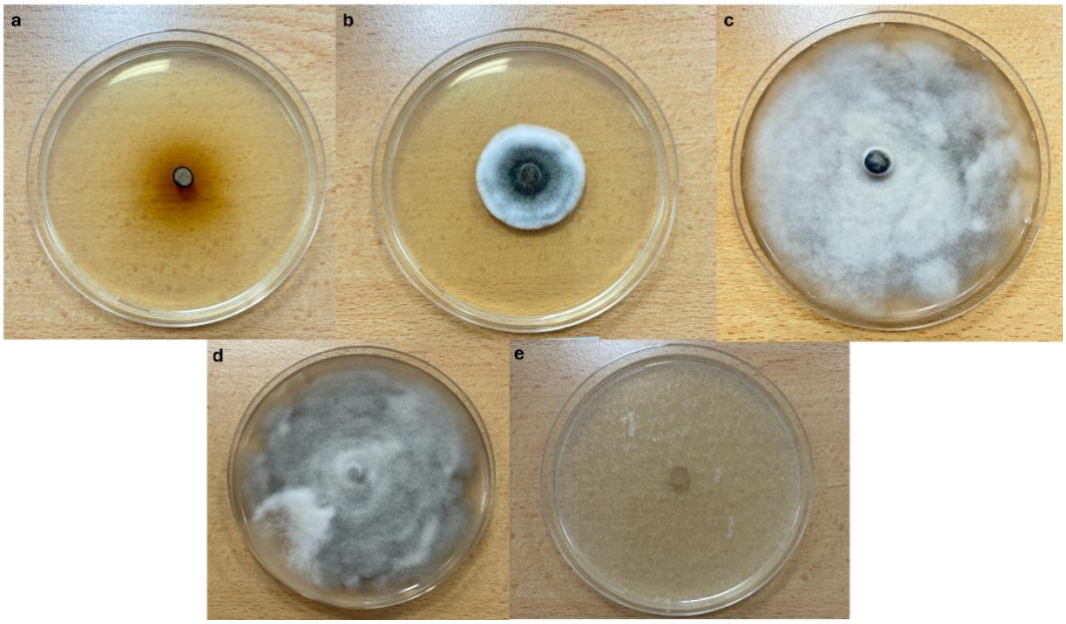

Figure 1. *Growth of Monilinia laxa on agar plates including the cell-free supernatant of Xenorhabdus cabanillasii* ***(a),*** *Xenorhabdus szentirmaii* ***(b),*** *photorhabdus kayaii* ***(c)****, control* ***(d), and*** *TCA* ***(e)*** *after 14 days of incubation.*

The antifungal effect of *X. szentirmaii* was significantly different from the negative control after both 7 (F=6023.776; df=4; P<0.005) and 14 days (F=7854.895; df=4; P<0.005). *Photorhabdus kayaii* CFS showed relatively low antifungal activity, and it was not statistically different from the negative control group (Figure 2).

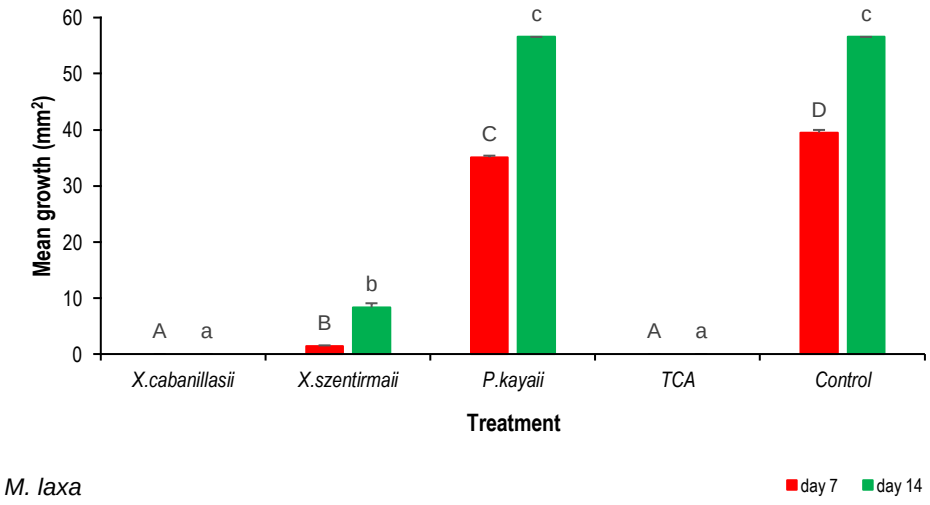

Figure 2. *Antifungal effects of Xenorhabdus cabanillasii, Xenorhabdus szentirmaii, and Photorhabdus kayaii cell-free supernatants against Monilinia laxa. Different letters above the bars indicate statistical significance (Tukey's HSD test, α =0.05).*

For *P. infestans*, there was a statistically significant difference between treatments (for 7 days F=954.729; df=4; P<0.005, for 14 days F= 2136.429; df= 4; P<0.005). *Xenorhabdus cabanillasii* CFS showed the highest antifungal activity during the 14-day period. *X. szentirmaii* CFS and TCA significantly reduced the fungal growth of *P. infestans* on PDA, whereas the suppression by *P. kayaii* CFS was not significantly different from that of the negative control group (Figure 3).

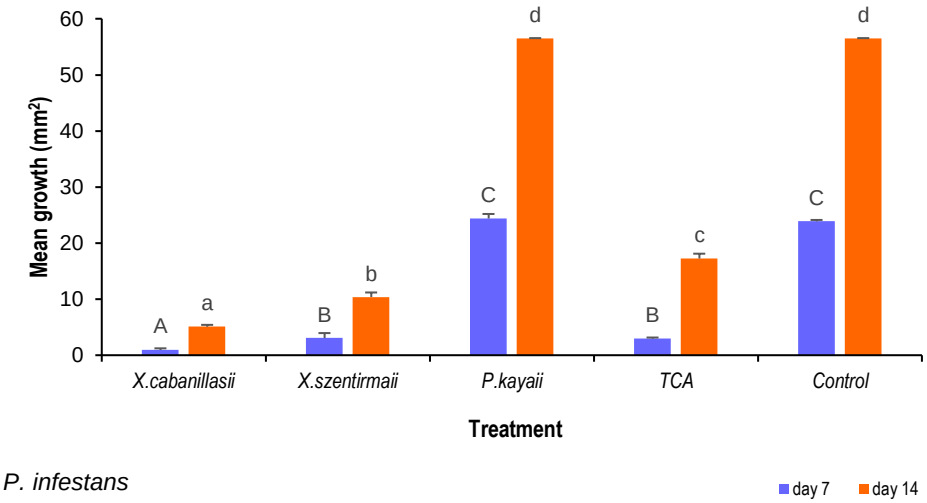

Figure 3. *Antifungal effects of Xenorhabdus cabanillasii, Xenorhabdus szentirmaii, and Photorhabdus kayaii cell-free supernatants against Phytopaththora infestans. Different letters above the bars indicate statistical significance (Tukey's HSD test, α =0.05).*

Effects of different *Xenorhabdus* and *Photorhabdus* species on CFS were assessed against two important fungal pathogens; *M. laxa* and *P. infestans*. The data indicated that *X. cabanillasii* and *X. szentirmaii* were more effective in suppressing fungal growth than *P. kayaii*, *with X. cabanillasii* being the most effective species against these two phytopathogens.

Several secondary metabolites with insecticidal, antifungal, antibacterial, nematicidal, antiprotozoal, and cytotoxic activities have been identified in *Xenorhabdus* and *Photorhabdus* species [12-14]. Each of these metabolites helps the bacteria to compete for space, nutrients, and other resources, resulting in the preservation and bioconversion of the host cadaver as well as fostering the reproduction and development of EPNs. The production of secondary metabolites varies among species and strains [15]. *Xenorhabdus* species are known to produce several secondary metabolites, including xenematides, xenocoumacins, fabclavines, cabanillasin, nemaucin, pristinamycin, xenortides, rhabdopeptides, bicornitun, PAX peptides, rhabduscin and bacteriocins [16], whereas anthraquinone, benzaldehyde, carbapenem, GameXpeptides, indole, kollisin A, pyrone, rhabduscin and stilbenes were isolated from *Photorhabdus* species [13].

Previously, several studies have demonstrated the antifungal activities of different *Xenorhabdus* and *Photorhabdus* species. Ng and Webster found that the metabolites of *Xenorhabdus bovienii* (A2) had an antifungal effect against *P. infestans* at a concentration of 0.1 mg/mL [17]. Hazır et al. evaluated the efficacy of the *Xenorhabdus* and *Photorhabdus* species CFS and TCA against *Fusicladium carpophilum*, *Fusicladium effusum*, *Monilinia fructicola*, *Glomerella cingulata*, and *Armillaria tabescens* [9]. They found TCA to be the most effective treatment, and similarly to this study, the CFS of *Xenorhabdus* spp. exhibited stronger suppressive effects on plant pathogenic fungi compared to *Photorhabdus* spp. In 2014, Bock et al. isolated TCA from *Photorhabdus luminescens* bacteria, which are mainly isolated from *Cinnamon* spp. plants. TCA is known to be an effective antifungal compound [7]. As an interesting result, pure TCA, which was used as a positive control for this study, was more effective than CFS against *P. kayaii* in controlling both fungal pathogens. This may be due to the lower TCA concentrations in the *Photorhabdus* CFS. In a recent study, the CFS of 16 *Xenorhabdus* and *Photorhabdus* strains were evaluated against the fungal phytopathogens *Sclerotinia sclerotiorum* and *X. szentirmaii*, which showed the highest fungicidal activity with an inhibition rate of >98% [18]. The in vitro activities of *Xenorhabdus* and *Photorhabdus* CFS against *Cryphonectria parasitica*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* were determined in 2021 [11]. Similar to the findings of this study, the authors also found that *Xenorhabdus* species were more

effective than *Photorhabdus* in suppressing fungal plant pathogen growth. However, contrary to this study, they found *that X. szentirmaii was* more effective than *X. cabanillasii* against all tested phytopathogens. They also showed that the antifungal compound isolated from *X. szentirmaii* was flavine. This can explain why *Xenorhabdus* species are usually more effective in suppressing fungal phytopathogens than *Photorhabdus* species, as *Photorhabdus* species do not produce flavine. However, the CFSof wild-type bacteria contain several secondary metabolites, which differ among different species and even strains. A recent study demonstrated *that Photorhabdus akhurstii supressed* the mycelial growth of *Colletotrichum gloeosporioides*, and the molecules responsible for antifungal activity were identified as glidobactin A, scopafungin I, and glidobactin C [19]. In some cases, different products with antifungal activity may work synergistically to enhance their activity against fungal pathogens.

IV. CONCLUSION

In conclusion, this study underscores the potential of using bacterial supernatants from *Xenorhabdus* and *Photorhabdus* species as effective biocontrol agents against the fungal phytopathogens *P. infestans* and *M. laxa*. This study demonstrated varying degrees of antifungal efficacy among bacterial species, with *X. cabanillasii* being the most effective species, especially against *M. laxa* whereas *P. kayaii* being the least effective. These findings emphasize the importance of exploring new biocontrol agents, particularly integrated pest management strategies, to mitigate the impact of fungal diseases on agricultural crops. Further studies should be conducted *in vivo* to develop sustainable and nontoxic alternatives to chemical pesticides using bacterial metabolites.

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V. REFERENCES

[1] S. Akino, D. Takemoto, and K. Hosaka, *Phytophthora infestans*: a review of past and current studies on potato late blight, *J. Gen. Plant. Pathol*., vol. 80, no. 1, pp. 24–37, Jan. 2014, doi: 10.1007/s10327-013-0495-x.

[2] N. J. Grünwald and W. G. Flier, "The Biology of *Phytophthora infestans* at its center of origin", *Annu. Rev. Phytopathol*., vol. 43, no. 1, pp. 171–190, 2005, doi: 10.1146/annurev.phyto.43.040204.135906.

[3] I. Larena et al., "Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*", *Biol. Control*, vol. 32, no. 2, pp. 305–310, Feb. 2005, doi: 10.1016/j.biocontrol.2004.10.010.

[4] M. Touray, H. Cimen, E. Bode, H. B. Bode, and S. Hazir, "Effects of *Xenorhabdus* and *Photorhabdus* bacterial metabolites on the ovipositional activity of *Aedes albopictus*", *J. Pest Sci*., Mar. 2024, doi: 10.1007/s10340-024-01760-7.

[5] H. B. Bode, "Entomopathogenic bacteria as a source of secondary metabolites", *Curr. Opin. Chem. Biol*., vol. 13, no. 2, pp. 224–230, Apr. 2009, doi: 10.1016/j.cbpa.2009.02.037.

[6] D. I. Shapiro-Ilan, C. C. Reilly, and M. W. Hotchkiss, "Suppressive effects of metabolites from *Photorhabdus* and *Xenorhabdus* spp. on phytopathogens of peach and pecan", *Arch. Phytopathol. Pflanzenschutz*, 42(8), pp. 715–728, 2009, doi: 10.1080/03235400701390539.bvg

[7] C. H. Bock, D. I. Shapiro-Ilan, D. E. Wedge, and C. L. Cantrell, "Identification of the antifungal compound, trans-cinnamic acid, produced by *Photorhabdus luminescens*, a potential biopesticide against pecan scab", *J. Pest Sci.*, vol. 87, no. 1, pp. 155–162, Mar. 2014, doi: 10.1007/s10340-013-0519-5.

[8] I. Ullah et al., "Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021", *J. Microbiol.*, vol. 53, no. 2, pp. 127–133, Feb. 2015, doi: 10.1007/s12275-015-4632-4.

[9] S. Hazir, D. I. Shapiro-Ilan, C. H. Bock, C. Hazir, L. G. Leite, and M. W. Hotchkiss, "Relative potency of culture supernatants of *Xenorhabdus* and *Photorhabdus* spp. on growth of some fungal phytopathogens", *Eur. J. Plant Pathol.*, vol. 146, no. 2, pp. 369–381, Oct. 2016, doi: 10.1007/s10658-016-0923-9.

[10] D. Shi, R. An, W. Zhang, G. Zhang, and Z. Yu, "Stilbene derivatives from *Photorhabdus temperata* SN259 and their antifungal activities against phytopathogenic fungi", *J. Agric. Food Chem.*, vol. 65, no. 1, pp. 60–65, Jan. 2017, doi: 10.1021/acs.jafc.6b04303.

[11] H. Cimen et al., "Antifungal activity of different *Xenorhabdus* and *Photorhabdus* species against various fungal phytopathogens and identification of the antifungal compounds from *X. szentirmaii*", *Appl. Microbiol. Biotechnol.*, vol. 105, no. 13, pp. 5517–5528, Jul. 2021, doi: 10.1007/s00253-021-11435-3.

[12] A. O. Brachmann and H. B. Bode, "Identification and bioanalysis of natural products from insect symbionts and pathogens", *Adv. Biochem. Eng. Biotechnol.*, vol. 135, pp. 123–155, 2013, doi: 10.1007/10_2013_192.

[13] S. P. Stock, A. Kusakabe, and R. A. Orozco, "Secondary metabolites produced by *Heterorhabditis* symbionts and their application in agriculture: what we know and what to do next", *J. Nematol.*, vol. 49, no. 4, pp. 373–383, Dec. 2017.

[14] S. H. Gulsen et al., "Antiprotozoal activity of different *Xenorhabdus* and *Photorhabdus* bacterial secondary metabolites and identification of bioactive compounds using the easyPACId approach", *Sci. Rep.*, vol. 12, no. 1, Art. no. 1, Jun. 2022, doi: 10.1038/s41598-022-13722-z.

[15] Md. M. I. Mollah and Y. Kim, "Virulent secondary metabolites of entomopathogenic bacteria genera, *Xenorhabdus* and *Photorhabdus*, inhibit phospholipase A2 to suppress host insect immunity", *BMC Microbiol.*, vol. 20, no. 1, p. 359, Nov. 2020, doi: 10.1186/s12866-020-02042-9.

[16] J. Dreyer, A. P. Malan, and L. M. T. Dicks, "Bacteria of the Genus *Xenorhabdus*, a Novel Source of Bioactive Compounds", *Front. Microbiol.*, vol. 9, p. 3177, Dec. 2018, doi: 10.3389/fmicb.2018.03177.

[17] K. K. Ng and J. M. Webster, "Antimycotic activity of *Xenorhabdus bovienii* (Enterobacteriaceae) metabolites against *Phytophthora infestans* on potato plants", *Can. J. Plant Pathol.*, vol. 19, no. 2, pp. 125–132, Jun. 1997, doi: 10.1080/07060669709500540.

[18] J. G. Chacón-Orozco, C. J. Bueno, D. I. Shapiro-Ilan, S. Hazir, L. G. Leite, and R. Harakava, "Antifungal activity of *Xenorhabdus* spp. and *Photorhabdus* spp. against the soybean pathogenic *Sclerotinia sclerotiorum*", *Sci. Rep.*, vol. 10, no. 1, p. 20649, Nov. 2020, doi: 10.1038/s41598-020-77472-6.

[19] P.-W. Tu et al., “Evaluation of the Antifungal Activities of *Photorhabdus akhurstii* and Its secondary metabolites against phytopathogenic *Colletotrichum gloeosporioides*”, *J. Fungi (Basel)*, vol. 8, no. 4, p. 403, Apr. 2022, doi: 10.3390/jof8040403.