lSSN 2709–3662 (Print)
ISSN 2709–3670 (Online)
https://doi.org/10.52587/JAF070105
Journal of Agriculture and Food
2026, Volume 7, No.1, pp. 43-51

Biocontrol Potential of Secondary metabolites of *Aspergillus flavus* Link. Against Some Plant Pathogenic Bacteria

Syed Muhammad Waleed[1], Syeda Nida Fatima[2], Bisma[1], Syed Abdul Ahad[3], *Syeda Kanz-ul-Eman[4] and Mukhtar Hussain Shah[5]

Abstract

Many plant pathogenic bacteria are responsible for important diseases of economically important crops, which produce enzymes that degrade the cell wall. The use of fungal metabolites as biological control is an environment friendly way of replacing the conventional bactericide. Thus, the present study investigated the antibacterial activity of metabolites of *Aspergillus flavus* on three plant pathogenic bacteria namely *Xanthomonas campestris*, *Pseudomonas syringae*, *Serratia rubidaea* in laboratory conditions. Well and disc diffusion assays were used to measure the antibacterial activity. Growth of all three bacterial species was strongly inhibited by all levels of the fungal metabolites. The 100% metabolite concentration showed the most effective antibacterial activity followed by the 50%. The disc diffusion method was found to have comparatively more inhibition while both the methods proved to demonstrate the

[1]Institute of Botany, University of the Punjab, Lahore, Pakistan;[2]Institute of Zoology, University of the Punjab, Lahore, Pakistan;[3]College of Food Science &Technology, Huazhong Agricultural University, China;[4]Faculty of Agriculture Sciences, University of the Punjab, Lahore, Pakistan;[5]Government Graduate College of Science, Wahdat Road, Lahore
**Corresponding Authors' email*: kanzul.res.fas@pu.edu.pk

antibacterial potential of the fungal metabolites. The above results indicate that metabolites of the fungus *A. flavus* have excellent antibacterial activity and could be used for the control of bacterial plant diseases.

Keywords: *Aspergillus flavus*, *Xanthomonas campestris, Pseudomonas syringae, Serratia rubidaea,* Biocontrol, Fungal metabolites

History: **Received:** 29th May 2026**; Revised:** 26th June, 2026; **Accepted:** 4th July, 2026

Introduction

Filamentous fungi are well known for their secondary metabolite, featuring an array of infamous structures and fascinating bioactivities. *Aspergillus flavus* is an obnoxious member of this group contaminating a variety of food material, also produces carcinogenic compounds (Uka et al., 2020). *A. flavus* is the second most common cause of aspergillosis after *A. fumigatus.* It is well known as an abundant saprophyte and opportunistic pathogen and is ubiquitous in warm environments (Yang et al., 2020). *Aspergillus* species are exploited as biocontrol agents against a wide variety of plant pathogens and also promotes growth of many crops. Competition with pathogens, parasitism and the release of compounds with antifungal properties being the most important in biocontrol mechanism (Karthick et al., 2019). Bacterial pathogens are one of the major threats to biosafety and environmental health, and an advanced assessment is a prerequisite for combating bacterial pathogens (Yang et al., 2023). Almost around 100 species of bacteria are able to cause diseases in plants. In order to cause disease in plants bacteria have specific pathogenicity factors like cell wall degrading enzymes, phytohormones, exopolysaccharides, effectors protein and the toxins. *Pseudomonas syringae* is one of the phytopathogenic bacteria associated with several plant species. It is an opportunistic pathogen of a variety of injured woody plants (Arnold & Preston, 2019). Bacteria of this group produces plant diseases like cankers, leaf spot and blight and also soft and brown rots with varying symptoms (Basavand et al., 2020). *Xanthomonas* spp., a large genus of Gram-negative plant pathogenic bacteria that causes disease to a large number of plants/crops and exploits various virulence factors for pathogenicity (Timilsina et al., 2020) and fitness in host. Pathogenic species exhibit host specificity limiting themselves to vascular or mesophyll tissues of the host plant. Early infections transplantation may lead to complete loss of crops. *X. campestris is the* cause of bacterial blight, irregular yellow-necrotic areas, Black rot, citrus canker. Bacteria like *Serratia* spp. occur in a variety of habitats, are gram-negative in nature with the ability to infect vertebrates and humans (Grimont & Grimont, 2006). It has been isolated from fruits and vegetables (Soenens & Imperial, 2020). Plant pathogens are of great concern for crop production throughout the world and demands for an active control mechanism of crop diseases for the provision of food to ever growing population of the world (Ayaz et al., 2021). At present plant diseases are being controlled by growing resistant varieties and applying pesticides with promising results. However, the use of chemicals as pesticides has posed serious threat to the environment thus limiting their scope in agriculture (Gao et al., 2016). Therefore, now a day's scientists are exploiting the beneficial microorganisms as an alternate to the chemical pesticide for management of crop diseases and a variety of bacteria and fungi have successfully been used for this purpose with promising results against important pathogens of economic crops. Recent researches on the use of fungal strains for controlling plant diseases has attracted the scientists worldwide. The antagonistic potential of such fungi make them potential bio-pesticides against a wide range of soil and airborne plant

pathogens both in greenhouse experiments and during field trials (Zubair et al., 2021). Biocontrol agents interact with the plant pathogens in a variety of ways by exploiting one or a combination of processes directly or indirectly to reduce plant disease (Ayaz et al., 2021). The present study was designed to exploit the secondary metabolites of *A. flavus* against devastating bacterial pathogens of economic importance.

Materials and Methods

Procurement of Test Species

Aspergillus flavus (FCBP-PTF-0051) and different species of bacteria viz. *Pseudomonas syringae* (FCBP-NO. 0009), *Serratia rubidaea* (FCBP-NO.045) and *Xanthomonas campestris* (FCBP-NO. 003), were obtained from Fungal Culture Bank Pakistan, Department of Plant Pathology, University of the Punjab, Lahore, Pakistan.

Preparation of Fungal Metabolites

Malt extract agar (2% MEA) was employed for sub culturing of *A. flavus* and Luria–Bertani agar (LBA) media was used for the evaluation of antibacterial activity of fungal metabolites, respectively.

Fungal metabolites were prepared in 2% ME media inoculated with 5 mm disc of the *A. flavus*. These flasks were incubated at 27 ± 2 ^{o}C for 27 days. Double filtration was carried out first with sterilized Whatsman filter paper followed by Millipore (0.5cm) filter paper to obtain the fungal metabolites. Different test concentrations of fungal metabolites i.e 0, 50 and 100% were prepared.

Assessment of Antibacterial Activity of Fungal Metabolites

Antibacterial activity of fungal metabolite's was performed on LBA media plates using well diffusion and disc diffusion methods. For well diffusion method, bacterial suspension was spread over LBA media Plates. Wells were made in center of plates and 50µl of each test concentration of fungal metabolite was loaded to each well, and streptomycin (10 mg/ml) was loaded in control plate well. Plates were incubated at 37 °C for 24 hours.

In disc diffusion method, filter paper discs were soaked in different concentrations of fungal metabolites for 20-25 minutes, and then placed discs on each plate. These Petri plates were then incubated at 37 °C for 24 hours. After incubation period the zone of inhibition for three target species of bacteria was measured.

Fourier Transform Infrared Spectroscopy

The fungal metabolites were subjected to FTIR to identify the functional groups responsible for their antibacterial activity.

Statistical analysis

The collected data was subjected to Analysis of variance (ANOVA) to evaluate the significance of the differences (Steel & Torrie, 1980).

Results and Discussion

The ever-increasing resistance of bacteria against antimicrobial agents being employed presently has posed serious threats in the field of agriculture worldwide. This situation necessitates to address the issue seriously and demands for a suitable substitute approach for bacterial control leading to new sources of compounds with antibacterial properties. Fungi contain a wide variety of such compounds being employed to promote plant growth and as antibiotics in the field of agriculture and medicine (Synytsya et al., 2017). Keeping in view these properties of fungi and its scope of being used as antibacterial agent, the present study was therefore designed to investigate the potential of secondary metabolites of *A. flavus* against *X. campestris, P. syringae* and *S. rubideae* through by well and disc methods. For this purpose, different concentrations of fungal metabolites i.e. 0, 50, and 100% were employed.

The metabolites of the fungus *A. flavus* were found to be very effective in inhibiting bacterial growth in well and disc diffusion assays (Fig. 1-3). The antibacterial activity can be attributed to the production of bioactive secondary metabolites such as phenolic compounds, organic acids, peptides and other antimicrobial compounds which disrupt the integrity of microbial cells and essential metabolic pathways of the bacteria (Maliehe et al., 2022; Hussein et al., 2022). The highest inhibition was obtained by streptomycin which was used as a positive control. The two levels of fungal metabolites were significantly inhibitory to bacteria, with the higher level showing more antibacterial activity, and inhibition similar to streptomycin. The same results were obtained by Mohammed et al. (2016) and Al-Maqtoofi et al. (2019).

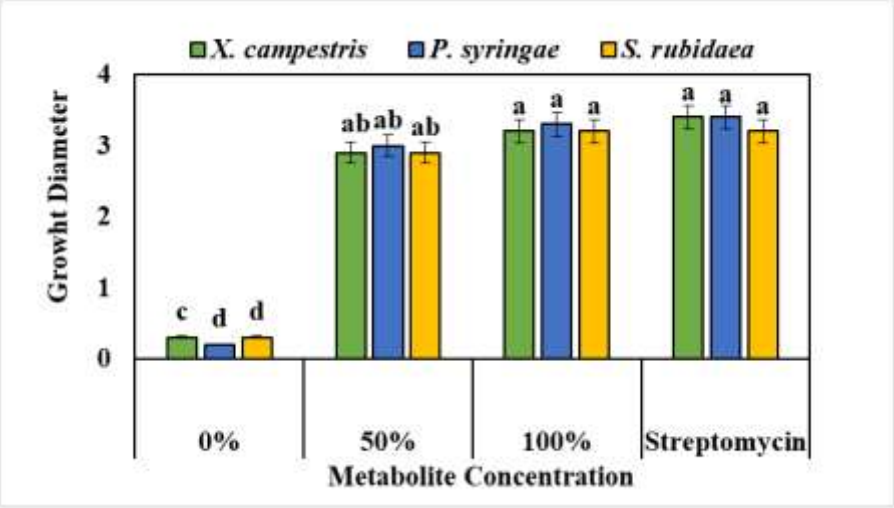

Figure 1. The well method shows inhibition of three bacterial species by metabolites of *A. flavus*. Error bar represents standard error (n = 3). Non-identical letters specify significant differences among the treatments at $p \leq 0.05$

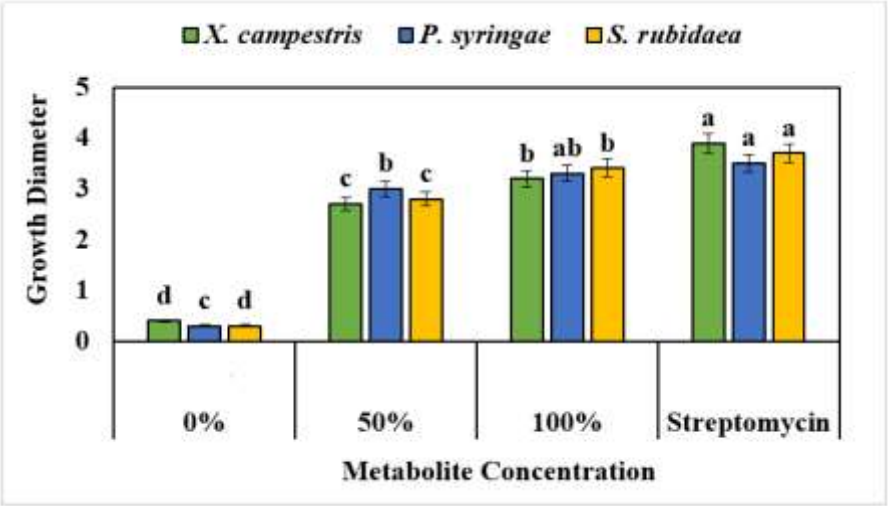

Figure 2. The disc and well methods show inhibition of three bacterial species by metabolites of *A. flavus*. Error bar represents standard error (n = 3). Non-identical letters specify significant differences among the treatments at $p \leq 0.05$

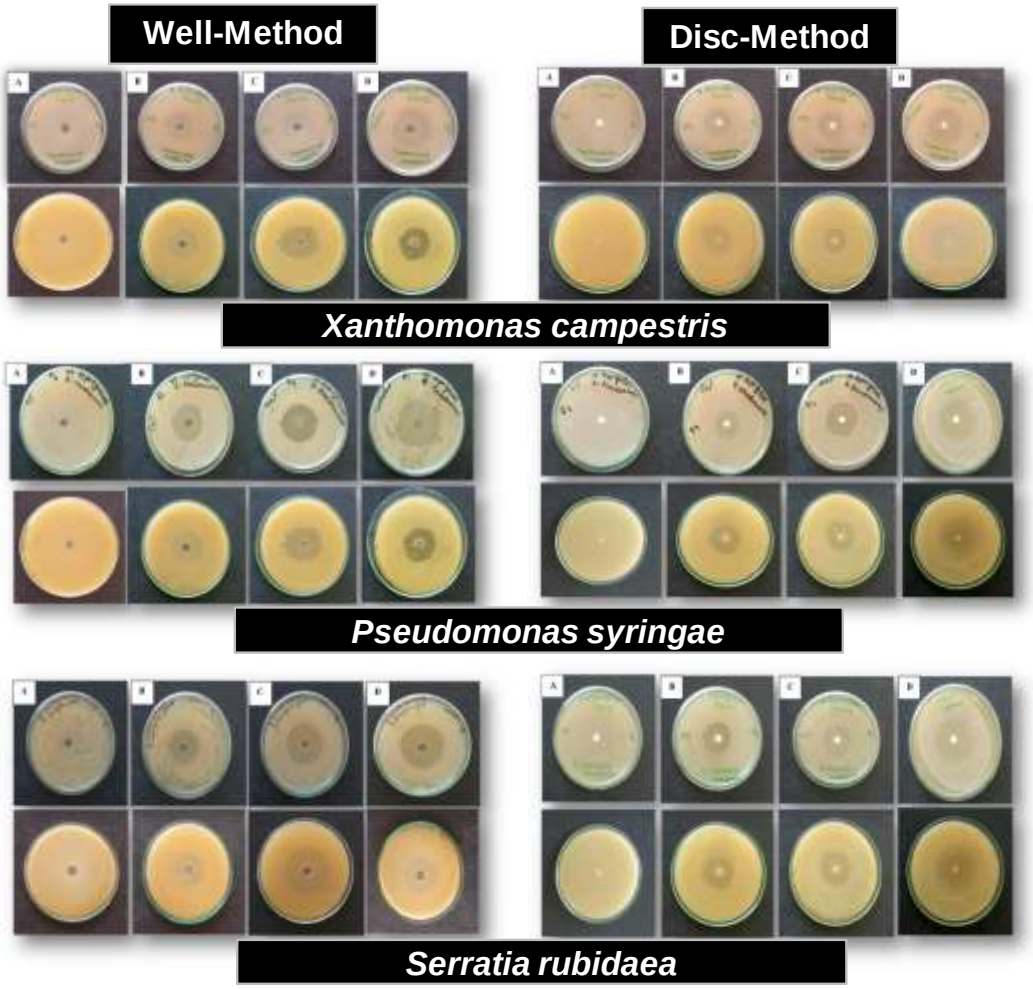

Figure 3. Growth response of *Xanthomonas campestris*, *Pseudomonas syringae*, and *Serratia rubidaea* to metabolites of *Aspergillus flavus* by well- and disc methods. (A) 0% concentration (B) 50% concentration (C) 100% concentration (D), and Streptomycin.

Fourier transform infrared spectrometer of fungal metabolies

Infrared spectral analysis of secondary metabolites of *Aspergillus flavus* revealed presence of functional group (OH) broad stretching at 3281 -3300cm^{-1} Likewise appearance of peak around 1638-1640cm^{-} indicate presence of an amide 1 group in protein in fungal metabolites. These active groups in this region could be responsible for antimicrobial activity against

three target species of bacteria. Similar peaks corresponding to the same groups have been reported previously (Kosa et al., 2017).

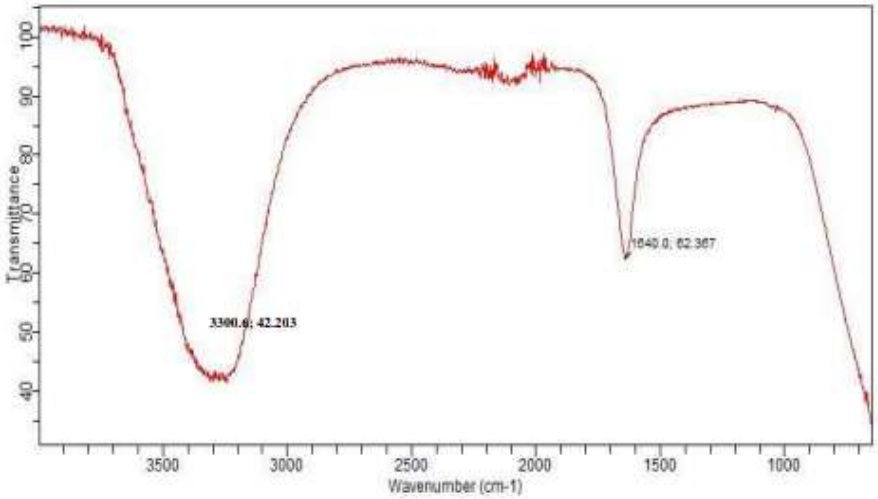

Figure 4. Fourier transform infrared spectrum of Aspergillus flavus fungal metabolites.

Conclusion

The present study, conclude that the secondary metabolites of *Aspergillus flavus* has the potential to be used as antibacterial agents against selected plant pathogenic bacteria.

Acknowledgements: The authors highly acknowledge Govt. Graduate College of Science, Wahdat Road Lahore, Department of Plant Pathology and Fungal Culture Bank of Pakistan, University of the Punjab, Lahore, for providing the necessary facilities, academic support and research environment that significantly contributed to the successful completion of this study.

References

Al-Maqtoofi, M. Y., Burghal, A. A., & Al-Muosawi, A. A. (2019). Screening of Antibacterial Activity from Aspergillus Species Treated with Synthetic Antifungal Agent. *Asian J. Microbiol. Biotechnol. Environmental Sci.*, *21*, 335-338.

Arnold, D. L., & Preston, G. M. (2019). Pseudomonas syringae: Enterprising epiphyte and stealthy parasite. *Microbiology*, *165*(3), 251-253.

Ayaz, M., Ali, Q., Farzand, A., Khan, A. R., Ling, H., & Gao, X. (2021). Nematicidal volatiles from Bacillus atrophaeus GBSC56 promote growth and stimulate induced systemic resistance in tomato against Meloidogyne incognita. *International journal of molecular sciences*, *22*(9), 5049.

Basavand, E., Khodaygan, P., Babaeizad, V., Rahimian, H., & Mirhosseini, H. A. (2020). Soft rot disease caused by Klebsiella aerogenes on Austrocylindropuntia subulata in Iran. *Indian Phytopathology*, *73*, 371-372.

Gao, H., Qi, G., Yin, R., Zhang, H., Li, C., & Zhao, X. (2016). Bacillus cereus strain S2 shows high nematicidal activity against Meloidogyne incognita by producing sphingosine. *Scientific reports*, *6*(1), 28756.

Grimont, F., & Grimont, P. A. (2006). The genus serratia. *Prokaryotes*, *6*(219), 44.

Hussein, M. E., Mohamed, O. G., El-Fishawy, A. M., El-Askary, H. I., El-Senousy, A. S., El-Beih, A. A., ... & Hamed, A. A. (2022). Identification of antibacterial metabolites from endophytic fungus Aspergillus fumigatus, isolated from Albizia lucidior leaves (Fabaceae), utilizing metabolomic and molecular docking techniques. *Molecules*, *27*(3), 1117.

Karthick, N. A., Balamurugan, A., Prakash, N. U., & Bhuvaneswari, S. (2019). ENVIS NEWSLETTER.

Kosa, G., Kohler, A., Tafintseva, V., Zimmermann, B., Forfang, K., Afseth, N. K., Tzimorotas, D., Vuoristo, K. S., Horn, S. J., & Mounier, J. (2017). Microtiter plate cultivation of oleaginous fungi and monitoring of lipogenesis by high-throughput FTIR spectroscopy. *Microbial Cell Factories*, *16*, 1-12.

Maliehe, T. S., Mbambo, M., Nqotheni, M. I., Senzo, N. S., & Shandu, J. S. E. (2022). Antibacterial effect and mode of action of secondary metabolites from fungal endophyte associated with Aloe ferox Mill. *Microbiology Research*, *13*(1), 90-101.

Mohammed, G. J., Hameed, I. H., & Kamal, S. A. (2016). Study of secondary metabolites produced by Aspergillus flavus and evaluation of the antibacterial and antifungal activity. *African Journal of Biotechnology*.

Soenens, A., & Imperial, J. (2020). Biocontrol capabilities of the genus Serratia. *Phytochemistry Reviews*, *19*(3), 577-587.

Steel, R. G., & Torrie, J. H. (1980). Principles and procedures of statistics mcgraw-hill book co. *Inc., New York*, *481*.

Synytsya, A., Monkai, J., Bleha, R., Macurkova, A., Ruml, T., Ahn, J., & Chukeatirote, E. (2017). Antimicrobial activity of crude extracts prepared from fungal mycelia. *Asian Pacific Journal of Tropical Biomedicine*, *7*(3), 257-261.

Timilsina, S., Potnis, N., Newberry, E. A., Liyanapathiranage, P., Iruegas-Bocardo, F., White, F. F., Goss, E. M., & Jones, J. B. (2020). Xanthomonas diversity, virulence and plant–pathogen interactions. *Nature Reviews Microbiology*, *18*(8), 415-427.

Uka, V., Cary, J. W., Lebar, M. D., Puel, O., De Saeger, S., & Diana Di Mavungu, J. (2020). Chemical repertoire and biosynthetic machinery of the Aspergillus flavus secondary metabolome: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 2797-2842.

Yang, G., Cao, X., Ma, G., Qin, L., Wu, Y., Lin, J., Ye, P., Yuan, J., & Wang, S. (2020). MAPK pathway-related tyrosine phosphatases regulate development, secondary metabolism and pathogenicity in fungus *Aspergillus flavus*. *Environmental Microbiology*, *22*(12), 5232-5247.

Yang, X., Jiang, G., Zhang, Y., Wang, N., Zhang, Y., Wang, X., Zhao, F. J., Xu, Y., Shen, Q., & Wei, Z. (2023). MBPD: A multiple bacterial pathogen detection pipeline for One Health practices. *IMeta*, *2*(1), e82.

Zubair, M., Farzand, A., Mumtaz, F., Khan, A. R., Sheikh, T. M. M., Haider, M. S., Yu, C., Wang, Y., Ayaz, M., & Gu, Q. (2021). Novel genetic dysregulations and oxidative damage in Fusarium graminearum induced by plant defense eliciting psychrophilic Bacillus atrophaeus TS1. *International Journal of Molecular Sciences*, *22*(22), 12094.

Citation

Syed, M. W., Fatima, S. N., Bisma, Ahad, S. A., Kanz-ul-Eman, S., & Shah, M. H. (2026). Biocontrol potential of secondary metabolites of *Aspergillus flavus* Link. against some plant pathogenic bacteria. *Journal of Agriculture and Food*, *7*(1), 43–51. https://doi.org/10.52587/JAF070105